Agronomy Research

Established in 2003 by the Faculty of Agronomy, Estonian Agricultural University

Aims and Scope:
Agronomy Research is a peer-reviewed international Journal intended for publication of broad-spectrum original articles, reviews and short communications on actual problems of modern biosystems engineering incl. crop and animal science, genetics, economics, farm- and production engineering, environmental aspects, agro-ecology, renewable energy and bioenergy etc. in the temperate regions of the world.

***Agronomy Research* online:**
Agronomy Research is available online at: http://agronomy.emu.ee/

Acknowledgement to Referees:
The Editors of *Agronomy Research* would like to thank the many scientists who gave so generously of their time and expertise to referee papers submitted to the Journal.

Abstracted and indexed:
SCOPUS, EBSCO, CABI Full Paper and Thompson Scientific database: (Zoological Records, Biological Abstracts and Biosis Previews, AGRIS, ISPI, CAB Abstracts, AGRICOLA (NAL; USA), VINITI, INIST-PASCAL).

Subscription information:
Institute of Technology, EULS
St. Kreutzwaldi 56, 51014 Tartu, ESTONIA
E-mail: timo.kikas@emu.ee

Journal Policies:
Estonian University of Life Sciences, Latvia University of Life Sciences and Technologies, Vytautas Magnus University Agriculture Academy, Lithuanian Research Centre for Agriculture and Forestry, and Editors of *Agronomy Research* assume no responsibility for views, statements and opinions expressed by contributors. Any reference to a pesticide, fertiliser, cultivar or other commercial or proprietary product does not constitute a recommendation or an endorsement of its use by the author(s), their institution or any person connected with preparation, publication or distribution of this Journal.

ISSN 1406-894X

CONTENTS

Agronomy Research **18**(1), 5–17, 2020
https://doi.org/10.15159/AR.20.004

Composting olive pomace: evolution of organic matter and compost quality

H. Ameziane[1,*], A. Nounah[1], M. Khamar[1] and A. Zouahri[2]

[1]Mohammed V University, High School of Technology, Civil Engineering and Environment Laboratory (LGCE), Materials Water and Environment team, MA11060 Sale, Morocco
[2]INRA, Regional Center for Agricultural Research in Rabat, Research unit on Environment and Conservation of Natural Resources, MA10112 Rabat, Morocco
[*]Correspondence: amezianehalima@gmail.com

Abstract. Morocco is one of the major olive-producing countries with an annual production of 1.56 million tonnes, part of which is dedicated to olive oil production. This important production generates, in addition to oil as the main product, a significant amount of waste (pomace and olive mill wastewater). The latter, when released in large quantities into the natural environment, cause fatal pollution. A suitable valuation of this waste will allow a clean and sustainable production for the sector. This work consists of composting olive pomace from the traditional system with two structural agents (poultry droppings and cattle manure) and comparing the two composts in terms of composting process parameters (pH, electrical conductivity, organic matter temperature, etc.), organic matter dynamics and compost quality, with manual aeration of the compost. Despite the high humidity level of the used pomace (80%), the adopted composting conditions have been effective in reducing high levels of organic matter and therefore organic carbon, as well as reducing the extreme phytotoxicity of the pomace. The experiment showed that the stabilization process in all the four treatments studied is comparable, and the final quality of the composts was adequate for agricultural use.

Key words: compost phytotoxicity, compost quality, degradation of organic matter, olive pomace.

INTRODUCTION

The imbalance between population growth in the Mediterranean Basin countries, particularly in the south and east of the Mediterranean, and the capacity of ecosystems to meet human needs, has led to over exploitation of agricultural soils and therefore to the depletion of soils in fertilizing elements, including carbon, which represents a large part of the organic matter. Therefore and in order to combat famine caused by this imbalance, Man has turned to the use of chemical fertilizers to increase productivity. But the irrational use of these fertilizers caused their accumulation, which affected the productivity and quality of the obtained fruit. In order to restore this soil degradation, Man has turned to organic farming, based on fertilizing the soil with organic matter, which present a reservoir of nutrients, and plays a major role in the physical fertility of soil, its aeration and its resistance to degradation and erosion (Girard et al., 2005).

For these reasons, organic matter is essential for fertilization and the base of the production method using biological, sustainable and environmentally friendly techniques. In Morocco, where the practice of organic farming is quite recent (the 1980s), the use of organic matter is becoming increasingly important, especially following the rapid expansion of the areas converted to organic production.

On the other hand, Morocco is one of the major olive oil producing countries with an annual production of 300,000 T (Ministry of Agriculture and Maritime Fisheries, 2013), this production generates a significant quantity of waste (Pomace and olive mill wastewater) which, when released into the natural environment, causes fatal pollution of the latter. The valuation of olive waste is a potential source of additional income that can contribute to improving the profitability of olive farms (Messineo et al., 2019). Olive pomace can be used as fuel, livestock feed, fertilizer and thermal insulation in some construction materials (Chouchene, 2010).

The use of olive pomace as an organic amendment has shown some problems in plant growth, due to their high organic content, the mineral salts, the low pH and the presence of phytotoxic compounds (Del Buono et al., 2011; Gigliotti et al., 2012; Proietti et al., 2015). Therefore, composting is necessary to stabilize the high organic content and benefit from the pomace fertilizing power (Gómez-Muñoz et al., 2012). However, no negative effects were observed when raw pomace was used as an amendment on soil fertility parameters (Ameziane et al., 2019). Similar studies have shown that olive pomace compost improves the physical and chemical characteristics of the soil, and provides the necessary nutrients for plant growth (especially nitrogen, potassium and phosphorus) (Sellami et al., 2008; Del Buono et al., 2011).

Consequently, and within the framework of the sustainable development aimed by the Moroccan kingdom, to guarantee proper agriculture, our work consists in valorizing olive pomace by composting with two structural agents (poultry droppings and cattle manure) and evaluating the quality of the obtained composts, while making a comparison between the studied treatments. This comparative study will determine the performance of each composting pile from the viewpoints matter evolution and different composting parameters (pH, electrical conductivity, temperature, humidity) in order to make the necessary adjustments for a good composting of these substances at the end of the process.

MATERIALS AND METHODS

Experimental design

The experiment consists of composting olive pomace with two structural agents: poultry droppings and cattle manure. The olive pomace compost subjects are from the traditional system and are used under two textures:

- Raw as sampled after trituration.
- Modified: dried at 60 °C to facilitate grinding and then ground.

While poultry droppings and cattle manure are used raw.

Composting is carried out in 30-litre drums, perforated to ensure an aerated environment and placed in a sunny place. This is a comparative study of four composting piles composed of:

- 57% poultry droppings and 43% raw pomace (C1).
- 57% poultry droppings and 43% ground pomace (C2).
- 57% cow manure and 43% raw pomace (C3).
- 57% cow manure and 43% ground pomace (C4).

The composting test lasted about 5 months (from 17/01/2019 to 04/06/2019), the follow-up parameters (pH, electrical conductivity, temperature, humidity, organic matter) were measured once a week. During composting, aeration was done manually using a pitchfork. At the end of the composting process (after maturation) a representative sample was analyzed to characterize the final compost.

Sampling of the raw material

Olive pomace is a by-product of the olive oil extraction process consisting of skins, pulp residues and stone fragments. These olive pomace were collected from an oil mill in the city of Tahla (Tahla, Morocco, latitude 34°02'58" North, longitude 4°25'17" West, altitude above sea level: 606 m). This oil mill crushes the olives traditionally and the paste is pressed manually using the pressing mats (scourtins).

Poultry droppings are all the elements released by the digestive and urinary tract through the poultry cloaca. This waste was collected from a poultry farm located in Tiflet (Tiflet, Morocco, latitude: 33°53′40″ North, longitude: 6°18′23″ West, altitude above sea level: 340 m).

Cattle manure is a mixture of cow dung and straw. It is the most commonly composted manure. Cattle manure is also collected from a cow stable in the city of Tiflet.

Physico-chemical characterization of the raw material and compost

To carry out the physico-chemical characterisation of the raw material and compost, a representative sample of the waste is dried in an oven at 60 °C and then ground and sifted to 2 mm to determine the pH and electrical conductivity (EC), then calcined in the oven at 500 °C for 4 hours. The obtained ashes are dissolved to determine the other mineral elements contained in the organic matter (OM), total organic carbon (TOC), total Kjeldhal nitrogen (TKN), potassium (K) and phosphorus (P).

Moisture is determined by drying the sample at 105 °C until constant weight (Rodier et al., 2009). For organic matter, a dried sample is burned in an oven at 525 °C for 3 hours (Rodier et al., 2009). pH and EC are measured in the aqueous extract using the ratio 2:5 (w/v) for pH (Rodier et al., 2009), and the ratio 1:5 (w/v) for EC (ISO 11265, 1994).

Total nitrogen is measured by the Kjeldahl method (Bremner & Mulvaney, 1982), while potassium and phosphorus are determined by atomic absorption spectrometry (Pinta, 1976). All analyses, which were repeated three times, were carried out in the laboratory of the Regional Centre of Scientific Research in Rabat.

Germination index

Phytotoxicity is estimated using the germination index (GI) as described by Zucconi et al. (1985). It is determined by comparing the root development of each sprouted seed in the compost and deionized water. For this test, 10 g of powdered compost was mixed with 100 mL of deionized water and the solution was shaken for 2 hours. After agitating, the solution was left to stand for half an hour then diluted with distilled water at three different concentrations (25%, 50% and 100%) with clear supernatant. The control concentration (0%) included only distilled water. 5 mL of each compost solution was added to a petri dish containing cotton on which 25 watercress seeds were uniformly placed. The concentrations of each compost sample, including the control, were replicated three times. The Petri dishes were incubated under light for 48 hours at 25 °C. After the incubation period, the root length was measured in each germinated seed and the GI was estimated.

$$GI = \left(\frac{GB}{GT}\right) \cdot \left(\frac{LB}{LT}\right) \cdot 100 \quad (1)$$

where GB – Number of seeds germinated in the compost; GT – Number of seeds germinated in the control; LB – Length of roots in compost; LT – Length of roots in the control.

- If the GI is less than 60%, the compost must be applied 90 days before the crop is planted.
- If the GI is less than 50%, the compost is not yet ripe, it is recommended to continue composting.

Statistical analyzes

The obtained results correspond to the average of 3 repetitions. The experimental data were subjected to unidirectional variance analysis (ANOVA) and mean separations were made by the smallest difference (LSD) at the significance level of $P < 0.05$, using the Statgraphics centurion XVI program for Windows.

RESULTS AND DISCUSSION

Physico-chemical characterization of the primary material

The results of the physico-chemical analyses of the materials to be composted are presented in table (Table 1).

Table 1. Physical and chemical characteristics of the primary material

	Olive pomace	Poultry droppings	Cattle manure
pH	$5.28^{a} \pm 0.05$	$6.48^{b} \pm 0.13$	$8.56^{c} \pm 0.02$
EC (mS cm^{-1})	$1.883^{a} \pm 0.56$	$0.0039^{b} \pm 0.87$	$0.00261^{b} \pm 0.19$
Hum in %	$80.34^{a} \pm 0.072$	$88.6^{b} \pm 0.045$	$90.9 \pm {}^{c}0.083$
OM in %	$77.02^{a} \pm 0.08$	$63.75^{b} \pm 0.28$	$60.23^{c} \pm 0.13$
TOC in %	$49.72^{a} \pm 0.21$	$35.81^{b} \pm 0.18$	$16.36\ {}^{c} \pm 0.03$
TKN in %	$0.861^{a} \pm 2.19$	$1.713^{b} \pm 1.78$	$0.45^{a} \pm 2.57$
C/N	57.74	20.90	36.35

The values obtained represent the average of three repetitions; for each treatment, the averages in each line followed by the same letter are not significantly different at $P < 0.05$.

The pomace has an acid pH (5.28), a high electrical conductivity (1.883 mS cm^{-1}) following the use of salts for the conservation of olives before crushing. They are very humid (80.4%), rich in organic matter (77.02%), as well as in total organic carbon (49.72%). While their total nitrogen concentrations (0.861%) are low, resulting in a high C/N ratio.

Poultry droppings have a low acid pH (6.48), low electrical conductivity (0.00391 mS cm^{-1}), high organic matter content (63.75%), as well as organic carbon (35.81%). Their total nitrogen content is high (1.713%), while their C/N ratio is lower (20.90%) compared to other waste.

Cattle manure has a basic pH (8.56), low electrical conductivity (0.00261 mS cm^{-1}) and high humidity (90.9%). Its organic matter content is high (60.23%) as well as organic carbon (16.36%). These manures have a low total nitrogen concentration (0.45%) and a high C/N ratio (36.35%).

Compost monitoring

pH

The mixture of the four compost piles had a neutral pH between 7.3 and 7.9 (Fig. 1). This pH promotes the development of actinomycetes and bacteria (Mustin, 1987) responsible for the organic matter degradation.

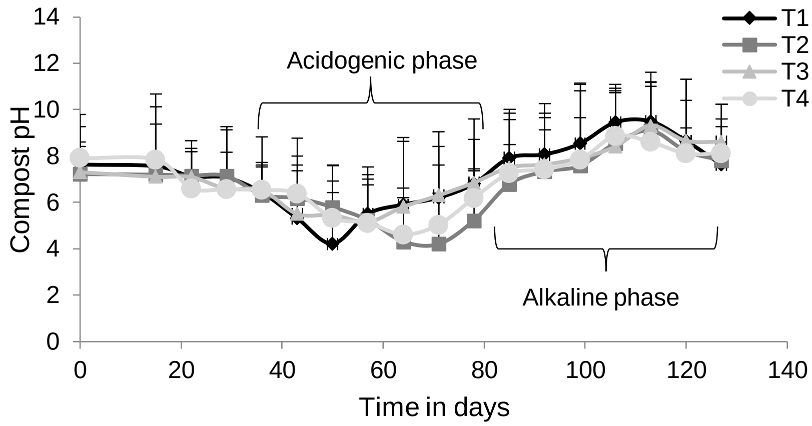


Figure 1. Temporal evolution of pH during composting.

At the beginning of composting, treatments based on raw pomace behave in the same way. Indeed, after 57 days of composting, the pH decreases rapidly to 4.21 for the mixture containing poultry droppings and 5.1 for the mixture of cattle manure. Then the pH of both treatments begins to increase until the end of the process to 8.11 for the treatment containing cattle manure and 7.8 for the treatment containing poultry droppings.

For the treatments based on ground pomace, the acidogenic phase lasted until day 71, reaching 4.2 for compost containing poultry droppings and 4.6 for the one with cattle manure. Thereafter the pH increases to alkalinity. Indeed, the composting process releases ammonia from the mineralization of proteins, amino acids and peptides, which explains this increase in pH (Gigliotti et al., 2012).

At the end of the process and for all four treatments, a slight decrease in pH begins to achieve neutrality: this is the maturation phase. Indeed, the micro-organisms activity responsible for pH variation is complete and pH remains constant (Godden, 1986; Gobat et al., 1998).

According to Mustin (1987), this decrease in pH can be explained by the production of organic acids following the degradation of carbohydrates, fats and other substances. As well as the production of CO_2 during aerobic degradation which contributes to the acidification of the environment by dissolving it in water and producing carbonic acid. This resulting drop in pH promotes fungi growth and the degradation of lignin and cellulose (Paredes et al., 1999).

The apparent phase shift between the treatment of raw and ground pomace can be explained by the fact that ground pomace has a compact environment that makes air circulation difficult and therefore the development of acidogenic bacteria, which somewhat slows down the acidogenic phase in the treatment of ground pomace.

The temperature evolution study during the composting process shows that the initial temperature is almost the same for all four treatments (Fig. 2). From the 22^{nd} day, the temperature of the three composting piles C1, C2 and C3 starts to increase to 50.9 °C for C1 and 54.8 °C for C2 and 55.5 °C for C3, while the maximum temperature reached for the C4 treatment does not exceed 37.9 °C. The increase in temperature is attributed to the activity of thermophilic microorganisms (Mustin, 1987). However, this temperature remains below 70 °C, above which there is destruction of living organisms and therefore degradation of compost quality (Bernal et al., 2009). Aware that heat production by microorganisms is proportional to the mass of the pile (Godden, 1986), the C4 treatment pile was the smallest, which is why the maximum temperature reached remains lower than that of other treatments.

Likewise for pH, the phase shift in the C1–C3 and C2–C4 treatments for maximum tepmerature can be attributed to the compact texture of the ground pomace, which reduces the porosity of the compost (Cayuela et al., 2006;. Sánchez-Arias et al., 2008) and therefore the availability of oxygen and consequently slows down the composting process.

It can be considered that the maturation phase begins from the 113^{th} day of composting since the last turning of the day 99 did not have a significant effect on the temperature increase.

At the end the temperature of the windrows merge with the ambient temperature of 19 °C to 20 °C, which shows that there is no longer any microbial activity, so the compost is ripe and ready for use (Fig. 2).

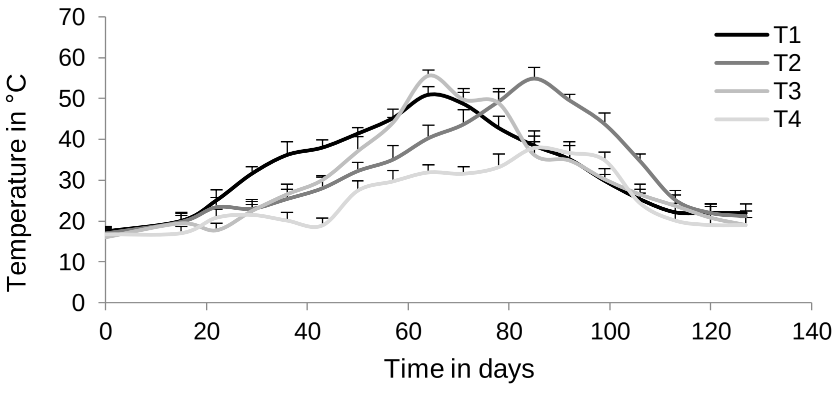


Figure 2. Time evolution of the temperature during composting.

Electrical conductivity

The electrical conductivity evolution (Fig. 3) shows that the different treatments start from high values (A), which can be explained by the high salinity of all the starting materials (Table 1).

During the composting process, the electrical conductivity of the various piles fluctuates due to the degradation of organic complexes. To find stability between 2.02 and 2.21 mS cm^{-1} from the 100^{th} day (C): this is the beginning of the maturity phase. In some pomace composting cases, final EC values were found to be lower (Abid & Sayadi, 2006; Makni et al., 2010). This is related to a leaching effect, which occurred when water was used for irrigation during the maturation phase. It should be noted that the leachate is recovered for possible treatment.

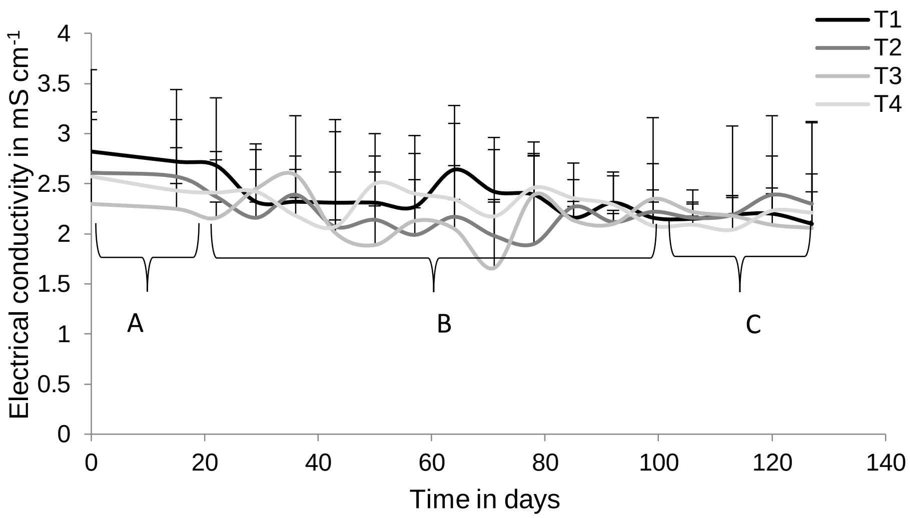


Figure 3. Electrical conductivity evolution during the composting process.

Humidity

The humidity of the four composting treatments is very high, as the pomace from the traditional system has high water content (Table 1). At the end of the 43^{rd} day (A) the composting process is slow and the humidity of the mixtures is almost similar to that of the start (Fig. 4). From the 43^{rd} day (B) the thermophilic phase already begins, and therefore excessive water consumption is expected, which is manifested by a decrease in the humidity level of the composts (Jemali et al., 1996). The humidity curve in the thermophilic phase is in sinusoidal form, it consists of a decrease in humidity resulting in water consumption, followed by an increase in humidity following watering. After the last watering before the 92^{nd} day (C), no decrease in humidity was observed, this is the beginning of maturation.

In terms of comparison between the four treatments, it can be seen that the variation in humidity is remarkable for the C1 and C2 treatments composed of poultry droppings, this can be attributed to the importance of microbial activity in the compost containing poultry droppings. Indeed, poultry droppings contain much more nitrogen than cattle manure (Table 1), which allows intense microbial reproduction and therefore significant activity.

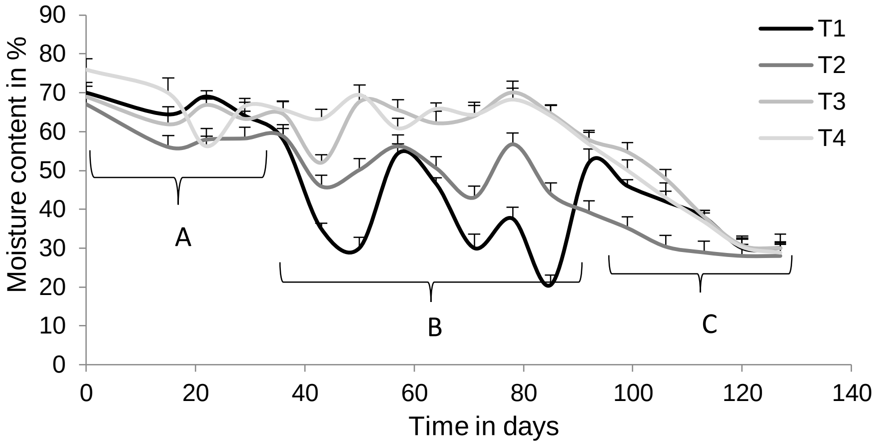


Figure 4. Humidity evolution during the composting process.

Organic matter evolution

The olive pomace subject to composting is rich in organic matter (77.02%) (Table 1), so the four initial treatments have a high concentration of organic matter. And since composting is a degradation of organic matter so a decrease in organic matter concentration is expected as the main result during composting, which is remarkable for all four treatments (Fig. 5). This significant loss of organic matter can be reflected in a decrease in volatile solids and total organic carbon throughout the process (Garcia-Gomez et al., 2003), which is due to the presence of relatively stable organic compounds probably represented by lipids, polyphenols, lignins, cellulose, hemicellulose and pectin (Aviani et al., 2010; Michailides et al., 2011a; Tortosa et al., 2012).

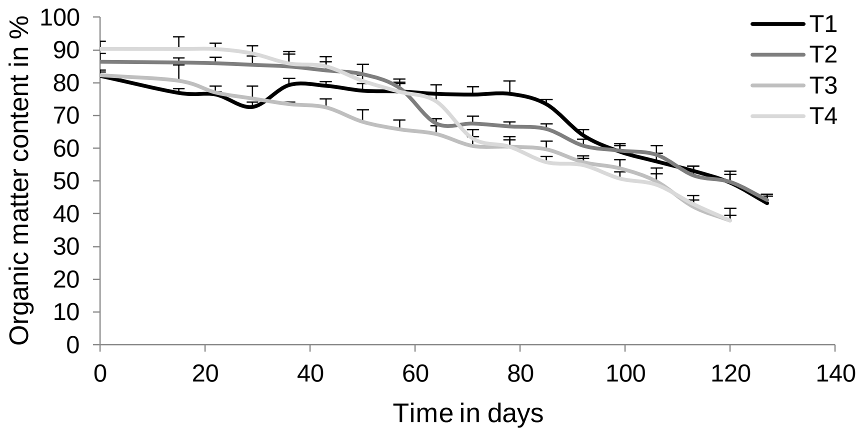


Figure 5. Organic matter evolution during the composting process.

The decrease in organic matter content is ensured by different groups of microorganisms that function according to the temperature and compost mass (Keener et al., 2000). Thus, bacteria conduct the composting phase early, while fungi are present throughout the process, but are very active when water levels are below 35% and inactive at temperatures above 60 °C (Bernal et al., 2009). During the maturation phase actinomycetes predominate, together with fungi, are capable of degrading highly resistant polymers (Bernal et al., 2009; Federici et al., 2011; Agnolucci et al., 2013).

Compost quality parameters

Physico-chemical quality

Compost quality parameters are based on a number of standards that vary considerably from one country to another. However, these standards converge towards a single objective: To produce compost that can be recycled without generating negative impacts on the environment quality and human as well as animal health.

Given the absence of Moroccan Standards in terms of compost quality, we will content with a technical sheet from the monthly information and liaison bulletin of the National Programme for Technology Transfer in Agriculture (NPTTA) elaborated by the Ministry of Agriculture, Rural Development and Maritime Fisheries of Morocco (Soudi, 2005).

The four composts pH is a neutral pH that tends towards basicity (7.63–8.61). This pH is high in relation to the quality objectives; in fact this can be attributed to the variation in composted waste as well as the quantity of ammonia released during the mineralization of proteins, amino acids and peptides (Abu Khayer et al., 2013). However, these values remain appropriate for agricultural applications (Gigliotti et al., 2012).

The abundant minerals in olive pomace increase the electrical conductivity of the final obtained composts, unlike composting of other organic materials in which the final compost conductivity does not exceed 1.5 mS cm^{-1} (Znaidi, 2002). Despite this increase in conductivity, it remains within the range of quality objectives. Given the high organic matter content of olive pomace, the concentration of organic matter remains high at the end of composting. Similar values (36%, 47%) were successively observed by Hachicha et al. (2009b) and Paredes et al. (2002). As well as for nitrogen, significant values were recorded in mature composts, resulting in lower C/N ratios.

The four treatments composts contain high levels of phosphorus (0.12–1.07%) and potassium (2.8–3.5%), which encourages their use as fertilizers (Hachicha et al., 2009c). Differences in mineral concentrations in the different composts could be attributed to the different composition of the initial materials.

Table 2. Physico-chemical properties of mature composts

	C1	C2	C3	C4	Compost quality objectives (Soudi, 2005)
pH	7.63 ± 0.12a	7.8 ± 0.1a	8.61 ± 0.03b	8.11 ± 0.22ab	5–7.5
Humidity (%)	30 ± 0.34a	28.2 ± 0.23b	30.4 ± 0.14a	28.9 ± 0.42ab	30–35
EC in mS cm^{-1}	2.1 ± 0.45a	2.3 ± 0.5a	2.06 ± 0.22a	2.21 ± 0.43a	2–5
OM in %	43.15 ± 0.15a	44.1 ± 0.36a	38.4 ± 0.76b	38 ± 1.27b	30–40
K_2O in %	2.9 ± 1.22a	3.5 ± 0.45b	2.8 ± 0.36a	3.2 ± 0.92ab	nd
TKN in %	1.5 ± 2.43a	1.9 ± 1.67ab	1.3 ± 1.89a	1.7 ± 0.26b	nd
P_2O_5 in %	0.3 ± 0.87a	1.07 ±0.45b	0.42 ± 0.86a	0.12 ± 1.34a	nd
TOC in %	25.09 ± 0.76a	25.65 ± 1.47a	22.32 ± 0.89b	22.09 ± 1.47b	nd
C/N in %	16.72	13.5	17.16	12.99	12–15

nd: Not defined; the values obtained represent the average of three repetitions; for each treatment, the averages in each line followed by the same letter are not significantly different at $P < 0.05$.

Compost phytotoxicity

Maturity is partly influenced by the relative stability of the composting material but it also describes the effect of other physical and chemical properties of compost on plant growth and development. Composts from immature olive pomace may contain high amounts of free ammonia, specific organic acids or other water-soluble compounds that can prevent seed germination and root development (Finstein et al., 1985). The mature compost that will be applied to agricultural land should be free of these potentially phytotoxic substances.

Table 3. Germination index (GI) of the four composts

Type of compost	C1	C2	C3	C4
The germination index (%)	75 ± 0.36a	78 ± 1.2b	73 ± 0.65a	74 ± 0.67a

The values obtained represent the average of three repetitions; for each treatment, the averages in each line followed by the same letter are not significantly different at $P < 0.05$.

The studied composts have optimal germination indices (Table 3), which reflects their maturity. These results corroborate with the physico–chemical analyses of the four composts. Indeed, according to Zucconi et al. (1985) the compost acceptable level of phytotoxicity must not exceed 80% with a minimum of 60%. Similar germination index values (71%, 72%, 74%) were obtained successively by Sánchez-Arias et al. (2008); Altieri& Esposito (2010) and Cayuela et al. (2010).

Summarizing the obtained results, we can say that: As compost will be applied to agricultural land, its physical and chemical parameters (i.e. pH, electrical conductivity and mineral concentrations) should be within a specific range to prevent any adverse effects on soil quality. So the compost of the four treatments initially had acid pH values, so much so that the final pH values are neutral and therefore ideal for agricultural applications (Gigliotti et al., 2012). The initial acid pH of the four treatments promotes the degradation of lignin and cellulose (Paredes et al., 1999). The compost of the four treatments has similar electrical conductivity values (2.1 mS cm^{-1}, 2.3 mS cm^{-1}, 2.06 mS cm^{-1} and 2.21 mS cm^{-1}) and which always remain below 4,000 mS cm^{-1} maximum tolerable values in agriculture (Lasaridi et al., 2006). Although values tend to increase during composting (since compost mass decreases) in our case during composting of olive pomace, electrical conductivity decreased due to mineral leaching a similar result has been observed by other authors (Paredes et al., 2002; Abid & Sayadi, 2006; Hachicha et al., 2009b; Makni et al., 2010; Gigliotti et al., 2012).

In addition to pH and conductivity, high levels of mineral nutrients such as phosphorus, potassium and nitrogen are essential to improve the quality of composts used for agricultural purposes. This is the case for compost in the four treatments, as there is an increase in these elements (Table 2), as is the case in many studies that report that olive-pomace-based composts have high levels of mineral nutrients (phosphorus: 0.1–3%; potassium: 0.12–4.4%; sodium: 0.05–4.1%) (Sanchez-Arias et al., 2008; Hachicha et al., 2009b; Michailides et al., 2011a).

CONCLUSIONS

Despite the olive pomace high moisture content and the presence of toxic chemicals (phenolic compounds), olive pomace composting took about 130 days. The organic matter content of the four mixtures decreased during composting and, at the end of the process, to values consistent with those generally desired for mature compost.

The four composts have optimal pH values, a C/N ratio of 13 to 17 and low phytotoxicity, these parameters fall within the defined limits for the quality of a compost. In addition, despite the compact texture of the ground pomace, and their high moisture content, their final composts are a high quality fertilizer from an agronomic point of view. As a result, these laboratory scale results can be replicated in full-scale installations by adjusting the forced ventilation according to the available equipment.

Finally, it can be said that composting olive pomace is the best method to benefit from its fertilizing power and guarantee farmers a stable, hygienic, less expensive and durable fertilizer.

REFERENCES

Abid, N. & Sayadi, S. 2006. Detrimental effect of olive mill wastewater on the composting process of agricultural wastes.*Waste Management* **26**, 1099–1107.

Abu Khayer, M., Cowdhury, M.B., Akratos, C.S., Vayenas, D.V. & Pavlou, S. 2013. Olive mill waste composting: A re-view. International Biodeterioration& Biodegradation 85, 108–119.

Agnolucci, M., Cristani, C., Battini, F., Palla, M., Cardelli, R., Saviozzi, A. & Nuti, M., 2013. Microbially-enhanced composting of olive mill solid waste (wet husk): bacterial and fungal community dynamics at industrial pilot and farm level. *BioresourceTechnology* **134**, 10–16.

Altieri, R. & Esposito, A. 2010. Evaluation of the fertilizing effect of olive mill waste compost in short-term crops. *International Biodeterioration and Biodegradation* **64**, 124–128.

Ameziane, H., Nounah, A., Khamar, M. & Zouahri, A. 2019. Use of olive pomace as an amendment to improve physicochemical parameters of soil fertility. Agronomy Research **17**(6), 2158–2171.

Aviani, I., Laor, Y., Medina, S., Krassnovsky, A. & Raviv, M. 2010. Co-composting of solid and liquid olive mill wastes: management aspects and the horticultural value of the resulting composts.*Bioresource Technology* **101**, 6699–6706.

Bernal, M P, Alburquerque, J.A. & Moral, R. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology* **100**, 5444–5453.

Bremner, J.M. & Mulvaney, C.S. 1982. *Nitrogen-Total. In: Methods of soil analysis*, part 2. A.L. Page, et al. (eds.). Agronomy Monog. 9 ASA and SSSA, Madison, WI, pp. 595–624.

Cayuela, M.L., Sánchez-Monedero, M.A. & Roig, A. 2006. Evaluation of two different aeration systems for composting two-phase olive mill wastes. *Process Biochemistry* **41**, 616–623.

Cayuela, M.L., Sanchez-Monedero, M.A. & Roig, A. 2010. Two-phase olive mill waste composting: enhancement of the composting rate and compost quality by grape stalks addition. *Biodegradation* **21**, 465–473.

Chouchene Ajmia '*experimental and theoretical study of processes for the valorisation of olive oil by-products by thermal and physicochemical means*'. Doctoral thesis in Energy Engineering-Process Engineering, under the supervision of F. Zagrouba, Monastir, Ecolenationaled' ingénieurs de Monastir, et G. Trouve, Université de haute-alsace, Mulhouse, 2010, 220 pp. (in French).

Del Buono, D., Said-Pullicino, D., Proietti, P., Nasini, L. & Gigliotti, G. 2011. Utilization of olive husks as plant growing substrates: phytotoxicity and plant biochemical responses. *Compost Sci. Util*. **19**, 52–60.

Federici, E., Pepi, M., Esposito, A., Scargetta, S., Fidati, L., Gasperini, S., Cenci, G. & Altieri, R. 2011. Two-phase olive mill waste composting: community dynamics and functional role of the resident microbiota. *Bioresource Technology* **102**, 10965–10972.

Finstein, M.S., Miller, F.C., MacGregor, S.T. & Psarianos, K.M. 1985. The Rutgers Strategy for Composting: Process Design and Control. Project Summary (EPA 600/S2-85/059). EPA, Washington.

Garcia-Gomez, A., Roig, A. & Bernal, M.P. 2003.Composting of the solid fraction of olive mill wastewater with OL: organic matter degradation and biological activity. *Bioresource Technology* **86**, 59–64.

Gigliotti, G., Proietti, P., Said-Pullicino, D., Nasini, L., Pezzolla, D., Rosati, L. & Porceddu, P.R. 2012. Co-composting of olive husks with high moisture contents: organic matter dynamics and compost quality. *International Biodeterioration and Biodegradation* **67**, 8–14.

Girard Michel-Claude, Christian Walter, Jean-Claude Rémy, Jacques Berthelin, Jean-Louis Morel 2005 : *Sols et Environnement*, 2ème édition, Editions DUNOD, p 816.

Gobat, J.M., Aragno, M. & Matthey, W. 1998. *The living soil. Basics of pedology*. Soil biology. Presses Polytechniques and Universitaires Romandes. Manage the Environment Collection N°14. Lausanne, Suisse. 519 pp. (in Frensh).

Godden, B. 1986. *Study of the composting process of cattle manure*. Doctoral thesis in Agronomic Sciences, UniversitéLibre de Bruxelles. Microbiology laboratory, 136 pp. (in Frensh).

Gomez-Munoz, B., Hatch, J.D., Bol, R. & Garcia-Ruiz, R. 2012. 'The compost of olive mill pomace: From a waste to a resource – Environmental benefits of its application in olive oil groves', in: Sustainable Development - Authoritative and Leading Edge Content for Environmental Management, S. Curkovic (ed.), In Tech, Rijeka, Croatia, pp. 459–484.

Hachicha, S., Cegarra, J., Sellami, F., Hachicha, R., Drira, N., Medhioub, K. & Ammar, E. 2009b. Elimination of polyphenols toxicity from olive mill wastewater sludge by its co-composting with sesame bark. *Journal of Hazardous Materials* **161**, 1131–1139.

Hachicha, S., Sallemi, F., Medhioub, K., Hachicha, R. & Ammar, E. 2009c. Biological activity during co-composting of sludge issued from the OMW evaporation ponds with poultry manured-Physico-chemical characterization of the processed organic matter. *Journal of HazardousMaterials* **162**, 402–409.

ISO 11265. Soil quality - Determination of the specific electrical conductivity. October 1994. (in Frensh).

Jemali, B., Soudi, B. & Lhadi, E.K. 1996. Control of composting parameters and assessment of the quality of household waste compost in the Wilaya of Rabat-Salé. *Moroccan Journal of Agronomic and Veterinary Sciences* **16**(2), 43–50 (in Frensh).

Keener, H.M., Dick, W.A. & Hoitink, H.A.J. 2000. Composting and beneficial utilization of composted by-product materials. In: Dick, W.A. (Ed.), Land application of agricultural, industrial, and municipal by-products. *Soil Science Society of America Inc*., Madison, pp. 315–341.

Lasaridi, K., Protopapa, I., Kotsou, M., Pilidis, G., Manios, T. & Kyriacou, A. 2006. Quality assessment of composts in the Greek market: the need for standards and quality assurance. *Journal of Environmental Management* **80**, 58–65.

Makni, H., Ayed, L., Ben Khedher, M. & Bakhrouf, A. 2010.Evaluation of the maturity of organic waste composts.*Waste Management & Research* **28**, 489–495.

Messineo, A., Maniscalco, M.P. & Volpe, R. 2019. Biomethane recovery from olive mill residues through anaerobic digestion: A review of the state of the art technology. *Science of The Total Environment*, 135508.

Michailides, M., Christou, G., Akratos, C.S., Tekerlekopoulou, A.G. & Vayenas, D.V. 2011a. Composting of olive leaves and pomace from a three-phase olive mill plant. *International Biodeterioration and Biodegradation* **65**, 560–564.

Ministry of Agriculture and Maritime Fisheries-Strategy and Statistics Department, Economic Watch-Olive Sector. September 2013. (http://www.agriculture.gov.ma/sites/default/files/130828-veille_huile_dolive-sl.pdf) (in Frensh).

Mustin, M. 1987. *Compost, organic matter management*. F. Dubuse 954 pp. (in Frensh).

Paredes, C., Cegarra, J., Roig, A., Sanchez-Monedero, M.A. & Bernal, M.P. 1999. Characterisation of olive mill wastewater (alpechin) and itssludge for agricultural purposes. *Bioresource Technol.* **67**, 111–115.

Paredes, C., Bernal, M.P., Cegarra, J. & Roig, A. 2002. Bio-degradation of olive mill wastewater sludge by its co-composting with agricultural wastes. *Bioresource Technology* **85**, 1–8.

Pinta, M. 1976. Atomic absorption spectrometry. Application to chemical analysis. 470 pp. (in Frensh).

Proietti, P., Federici, E., Fidati, L., Scargetta, S., Massaccesi, L., Nasini, L., Regni, L., Ricci, A., Cenci, G. & Gigliotti, G. 2015. Effects of amendment with oil mill waste and its derived-compost on soil chemical and microbiological characteristics and olive (*Oleaeuropaea* L.) productivity. *Agric. Ecosyst. Environ*. **207**, 51–60.

Rodier Jean, Bernard Legube & Nicole Merlet. *Water Analysis*.9th edition. Dunod, Paris, 2009. 1511 pp (in Frensh).

Sánchez-Arias, V., Fernández, F.J., Villaseñor, J. & Rodríguez, L. 2008. Enhancing the co-composting of olive mill wastes and sewage sludge by the addition of an industrial waste. *Bioresource Technology* **99**, 6346–6353.

Sellami, F., Jarboui, R., Hachicha, S., Medhioub, K. & Ammar, E. 2008. Co-composting of oil exhausted olivecake, poultry manure and industrial residues of agro-food activity for soil amendment. *Bioresour. Technol*. **99**, 1177–1188.

Soudi, B. 2005. Composting greenhouse crop waste and manure, PNTTA. Technology Transfer in Agriculture, 6 pps. (in Frensh).

Tortosa, G., Alburquerque, J.A., Ait-Baddi, G. & Cegarra, J. 2012.The production of commercial organic amendments and fertilisers by composting of two-phase olive mill waste (‘alperujo’). *Journal of Cleaner Production* **26**, 48–55.

Znaidi A. 2002. Study and evaluation of the composting of different types of organic materials and the effects of organic compost juices on plant diseases.Master of Science Degree n°286. Bari: Mediterranean Organic Agriculture, CIHEAM Mediterranean Agronomic Institute, 94 pp. (in Frensh).

Zucconi, F., Monaco, A., Forte, M. & de Bertoldi, M. 1985. Phytotoxins during the stabilization of organic matter. In: Gasser, J.K.R. (Ed.), Composting of Agricultural and Other Wastes. Elsevier Applied Science Publishers, Barking, Essex, UK, pp. 73–85.

Agronomy Research **18**(1), 18–37, 2020
https://doi.org/10.15159/AR.20.057

Theory of grain mixture particle motion during aspiration separation

V. Bulgakov[1], S. Nikolaenko[1], I. Holovach[1], V. Adamchuk[2], S. Kiurchev[3], S. Ivanovs[4] and J. Olt[5,*]

[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony Str., UA 03041 Kyiv, Ukraine
[2]National Scientific Centre, Institute for Agricultural Engineering and Electrification, 11, Vokzalna Str., Glevakcha – 1, Vasylkiv District, UA08631 Kiev Region, Ukraine
[3]Dmytro Motornyi Tavria State Agrotechnological University, Ukraine
[4]Latvia University of Life Sciences and Technologies, 2 Liela Str., LV-3001 Jelgava, Latvia
[5]Estonian University of Life Sciences, 56 Kreutzwaldi Str., EE51006 Tartu, Estonia
[*]Correspondence: jyri.olt@emu.ee

Abstract. The practice of separating grain mixtures with the use of the difference in the aerodynamic properties of their components has proved that the process of separating mixtures in the aspiration separator is the most promising one with regard to the improvement of quality and intensification of production. The authors have developed a new improved design of aspiration seed separators, in which the work process of separating seed material is performed with the use of vibration processes. In this seed material separator, the constant force air flow that acts on the sail members on the central pipe of the separator, when seeds are fed for processing, generates self-excited oscillations in the pipe, which produces centrifugal forces of inertia in the seed feeding system. As a result of the mentioned effect, the propelling force in the process under study substantially increases, accelerating the seeds of different fractions, which differ in their masses, to different velocities. The motion paths of the seed particles change accordingly, heavier particles moving closer to the vertical axis of the aspiration channel, which provides for increasing the efficiency of separation of the seeds of different fractions from each other. In this paper, a new mathematical model is developed for the motion of a seed mixture material particle in the operating space of the separator's aspiration channel. The mathematical modelling of the process of vibration and aspiration separation has indicated that the separation of the motion paths of the medium and heavy fractions takes place within the range of 20–40 mm; the flying speed of the particles is equal to 3.2–8.0 m s^{-1}, respectively; and their acceleration is equal to 1.8–3.3 m s^{-2}, which provides the necessary conditions for the accurate and high quality separation into the required fractions. In view of the found differences between the kinematic characteristics of the separated fractions of the grain mixture, the diameter of the pipeline for the medium fraction is to be within the range of 90–100 mm, for the heavy fraction – 50–70 mm.

Key words: air flow, aspiration separator, material particle, seed, mathematical model.

INTRODUCTION

The production of grain and oil crops has always been one of the leading industries in the world. Therefore, the issues of harvesting and initial processing of these crops, the methods of producing their seed materials as well as the scientific basis for the storage of these products are topics of current interest.

It should be noted that one of the main stages in the preparation to the storage of food grain and the seed grain for various crops is their postharvest treatment, in particular, the separation and assorting into separate fractions.

Currently, a multitude of methods exists for separating grain crops and oil plant seeds: gravity, vibration-and-centrifugal etc. At the same time, many scientists have for decades been interested in the process of separating agricultural crop seeds with the use of air flows, which allows to avoid damaging the seeds. Generally, in the existing seed-cleaning machines the process of separating seeds with the use of air flows takes place in aspiration channels of various designs, each of the latter having a number of drawbacks.

Basing on the results of the research carried out by many scientists investigating the seed separation processes, in particular, with regard to sunflower seeds, the conclusion can be arrived at that the use of vertical aspiration channels with bottom discharge is the most suitable method for these purposes. At the same time, it is desirable to generate in the discussed system of sorting the seeds into fractions self-oscillatory motion of the separator’s central pipe, which will raise the separation efficiency and stabilise the motion paths of the seed material fractions.

In the early period of developing and engineering separators based on pneumatic dynamics, the separation of seeds into fractions was done in horizontal air streams, from which lighter seeds were collected in the one bin and heavier seeds in the other bin. But the quality of sorting the seeds into light and heavy fractions was low. The principal drawback in the described layout was that the horizontal air flow acted on two seeds of similar sizes and weights with two different forces: in one of the possible positions the force was maximal, in another one – minimal, and it turned out that that circumstance affected the process of good quality separation of the seed mixture. The further studies led to the development of a new seed separation process with the use of a vertical aspiration channel, in which the fed seeds interacted with the opposite-direction air stream and obtained different vertical velocities depending on the geometric shapes and masses of the seeds. As a result of that, all the seeds got into the position of the lowest aerodynamic drag with regard to the vertical airflow and, due to the quasi fluid-drop shape of the surfaces of the particles, the Joukowsky lifting force arose, which improved the conditions for separating the particles onto different motion paths. However, in this case again the precision of the seed separation into fractions on account of the different air flows proved to be insufficient.

Today, a sufficiently large number of various designs exists. Among them, there are designs with a vertical aspiration channel, but the currently available mechanical means for seed separation feature the following major drawbacks: sophisticated adjustment, high power consumption, low quality of separating seeds into fractions and insufficient accuracy in operation with seeds of different moisture content.

The above-mentioned shortcomings are to some extent made even, when using the already developed design of the pneumatic and gravity seed separator with bottom separation of the fractions. In that case, the aspiration separator of a rather simple design delivers high productivity in the separation of loose dry materials and provides for the handling of multiple-fraction mixtures.

The said design is further developed in the new vibrating pneumatic dynamics unit devised by the authors, which provides for the improvement of the separation quality indicators in the processing of oil crop seeds, in particular, sunflower seeds. The process flow diagram of the new design of vibration and aspiration separator is presented in Fig. 1.

The vibration and aspiration separator under consideration comprises the feeding chute 1 of a conical shape, from which the seed material flows from above onto the central pipe 2. The upper end of the central pipe 2 features the conical spreader 3. The central pipe 2 is situated inside the fixed channel 4 (stationary pipe) in such a way that a cylindrically shaped space appears between the pipe 2 and the channel 4 and in the middle of that space the work process of grain mixture separation takes place. The central pipe 2 is connected to the stationary channel 4 with springing members 5, which are installed in the upper and lower areas, aligned radially and retain the pipe 2 in the position shown in Fig. 1. On the outer (cylindrical) surface of the central pipe 2 the sail members 6 are fixed, which have pre-set sizes and are positioned on the pipe 2 in the form of a provisional spiral. The angle of lead of the said spiral is 45° with respect to the longitudinal axis of the central pipe 2. The lower part of the separator features the elbow pipes 7 and 8 for the intake and further carrying away of the heavy and medium seed fractions, respectively. The process flow diagram features the seeds of the light 9, medium 10 and heavy 11 fractions together with the indication of their motion directions (shown by the short arrows).

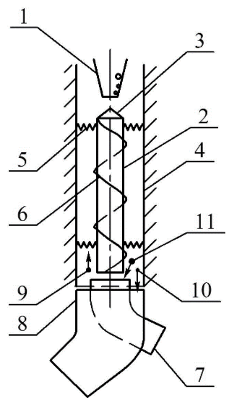


Figure 1. Process flow diagram of vibration and aspiration separator: 1 – feeding chute; 2 – central pipe; 3 – conical spreader of central pipe; 4 – stationary channel; 5 – springing members; 6 – sail members; 7 – heavy fraction discharge pipe; 8 – medium fraction discharge pipe; 9 – light fraction seeds; 10 – medium fraction seeds; 11 – heavy fraction seeds.

During the operation of the aspiration seed separator, the exhaust fan (not shown in the diagram in Fig. 1) generates a stream of air moving from the bottom upwards in the stationary channel 4, which applies pressure to the sail members 6 situated on the outer surface of the central pipe 2. That results in the generation of external forces applied to them and those forces make the pipe 2 rotate about its vertical axis. The springing members 5 arrest, after their respective deformation, the described rotary motion of the central pipe 2 in one direction. Since the air flow varies as a consequence of the variation of the effective cross-section (that is, the space between the outer surface of the central pipe 2 and the internal surface of the stationary channel 4 being filled with the seeds fed from the top downwards), the external forces applied to the sail members 6 vary as well, which results in the change of the direction of rotation of the central pipe 2, which starts rotating in the opposite direction. Thus, the cycle of action of the variable force factors imparted to the seed material fed from above onto the conical spreader 3 of the central pipe 2 recurs. In this way, the devised mechanical system generates the self-oscillatory mode of motion of the principal operating device, i.e. the central pipe 2, onto which seed material is fed for separation from the feeding chute 1 situated above it. Due to the self-oscillatory motions, the conical spreader 3 situated at the top of the central pipe 2 imparts to the seeds (considered as material particles) different kinematic and force parameters (in effect, conditioned by the said self-oscillations), which results in the seeds being evenly distributed along the cone base circumference and then input into the space between the pipe 2 and the channel 4. Further, under the action of the counter-current flow of air that applies forces to every material particle, the separated seeds fall down, but moving along different paths of motion. For example, the stream of the flowing up air immediately carries up and away from the separator the seeds of the light fraction 9 (dust and other light impurities), while the medium fraction seeds 10 in their downward travel eventually concentrate nearer to the internal surface of the stationary channel 4 and, on their arrival to the bottom part of the separator, are carried away via the pipe 8 away from the separator. Finally, the heavy fraction seeds 11 sinking closer to the outer surface of the central pipe 2 are discharged via the pipe 7 in the respective direction away from the aspiration separator.

As a result of the above-mentioned angular self-oscillations, during the rotation of the central pipe 2 about the vertical axis of the separator, the field of centrifugal forces of inertia emerges. As a consequence, the propelling force of the seed medium separation process under consideration increases and accelerates the seeds of different fractions that differ in their masses to different velocities. The motion paths of the particles change respectively, that is, their separation takes place, which facilitates improvement of the efficiency of dividing the seeds into separate fractions. Depending on their masses, the seeds move down either into the pipe 7 for the discharge of the heavy fraction, the diameter of which, in accordance with the results of the experimental research and pilot tests, has to be equal to 70 mm, or into the pipe 8 for the discharge of the medium fraction, also situated at the bottom, the diameter of which has to be equal to at least 160 mm. Meanwhile, the seeds in the light fraction with masses not exceeding 40 mg, as noted above, are quite easily picked up by the stream of air, fly upwards, in the opposite direction, and are separately collected at the top, which fully completes the operating cycle of seed material separation.

Thus, the work process of grain mixture separation takes place under the above-described conditions. At the same time, the self-oscillations of the central pipe 2 together with the conical spreader 3 facilitate the considerable intensification of the process of dividing the seed mixture into separate components.

Basing on the completed engineering developments, experimental studies and production tests, it is possible to infer that the new aspiration-and-vibration separation system offers the following advantages:

– minimised costs due to the fact that the self-oscillatory mode of operation significantly intensifies the separation process and does not require any special mechanism for impelling the self-oscillatory motion;

– only part of the power consumed by the pressure fan is used for maintaining the self-oscillatory mode of operation of the principal operating device that imparts its parameters to the seed mixture components;

– simplicity of design and the minimal costs of modernisation and maintenance, which imply only providing the movability of the central pipe and the possibility to attach sail members of different sizes to it.

The research into the theoretical and practical basis of the separation of grain crop seeds is represented in the papers (Poturayev & Franchuk, 1970; Burkov, 1991; Kotov, 2002; Leshchenko & Vasilkovsky, 2009; Brăcăcescu et al., 2012; Kyurchev & Kolodiy, 2015; Kroulik et al., 2016; Saitov et al., 2016; Saitov et al.,2018; Badretidinov et al., 2019; Brăcăcescu et al., 2019). It is to be noted that the mentioned studies pay attention to the parameters that have effect on the velocity of soaring of the grain particles, that is, the interaction between the seeds and the counter flow of air. The further research has proved that the said velocity depends on quite a number of factors: the masses of the seeds, the aerodynamic drag factor, the maximum cross-section of each type of seeds, the space orientation of the seeds etc. The authors of the above-mentioned studies have arrived to their conclusions basing on the results of research, but they are correct only under specific conditions.

In the paper (Kyurchev & Kolodiy, 2012), the effect of the design parameters of the conical aspiration channel on the kinetic properties of the motion of seeds is examined. In the paper (Bernik & Palamarchuk, 1996), the relations are obtained for the dynamics of the motion of particles in the air flow and the efficiency of air cleaning in the operating space of the vertical aspiration channel. The paper (Vasilkovsky et al., 2007) presents the study and justification of the possibility to separate seeds not only by weight, but also by size with the use of an inclined air flow. After revealing the principal rules that govern the process with the use of the devised pneumatic and centrifugal separator equipped with a spreading disk, it became possible to improve the quality of the seed distribution. In the current paper, the authors propose using a vertical aspiration channel with bottom discharge and generating the self-oscillatory motion of the separator's central pipe in order to increase substantially the propelling force of the process and the separation of the motion paths of the seed material fractions.

The aim of the study is to improve the productivity and quality of the loose seed mixture separation by means of developing a new design of the aspiration separator and theoretically substantiating its rational parameters.

MATERIALS AND METHODS

In order to substantiate theoretically the main parameters of the aspiration sunflower seed separator, it is necessary first of all to examine the process of self-oscillations of the separator's central pipe as one of the principal elements in the discussed method of separating the seeds into the required fractions.

It is to be noted that the self-oscillatory motion of the separator's central pipe is generated under the action of a number of perturbing force factors and the restoring forces of its springing members.

Therefore, it is necessary to start with generating the equivalent schematic model of the central pipe of the vibration and aspiration separator, indicating in it all the perturbing forces and the restoring springing members (Fig. 2).

For the purpose of describing the force interaction between the central pipe and the air stream and the self-oscillatory motion of the central pipe about the vertical axis of the separator, it is necessary to set up the fixed Cartesian reference system *Oxyz*, the origin of which (point *O*) is situated at the centre of the upper base of the central pipe, that is, the lower base of its spreading cone, the *Ox* axis is aligned horizontally to the right, the *Oy* axis is aligned vertically down along the central axis of the aspiration channel and the *Oz* axis is situated in the horizontal plane and directed perpendicularly to the *Oxy* plane (Fig. 2).

The first step is to consider the forces shown in the equivalent schematic model that act on the central pipe and determine their values. The perturbing force factors include the force $\bar{F}_{af}$ of the air flow, which acts on the sail member generating the moment of rotation M_d that rotates the central pipe about the vertical axis *Oy*. The restoring forces of the springing members include the elastic resistance force $\bar{R}_r$ and the moment of rotation $M_{r\varphi}$ of the elastic forces of resistance during the rotation of the central pipe about the vertical axis Oy. In the equivalent schematic model, the springing members that connect the central pipe with the stationary channel of the separator at the top and the bottom of the channel and have elastic coefficients C_x and C_z are shown.

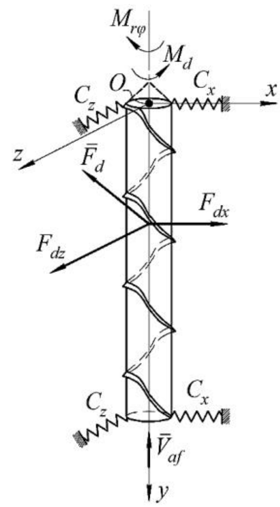


Figure 2. Equivalent schematic model of central pipe of vibration and aspiration separator.

RESULTS

The next step is to find the analytic expressions for the above-mentioned force factors. The moment of rotation M_d applied to the central pipe is determined by the following expression:

$$M_d = F_{af} \cdot r_D \cdot \sin \omega t \qquad (1)$$

where r_d – radius of the central pipe of the separator; ω – angular velocity of rotation of the central pipe about the longitudinal axis (axis *Oy*).

The components of the restoring force $\bar{R}_r$ vectored along the *Ox* and *Oz* axes are equal to, respectively:

$$R_{rx} = C_x \cdot x, \qquad (2)$$

$$R_{rz} = C_z \cdot z, \qquad (3)$$

where C_x and C_z – springing member stiffness factors along the *Ox* and *Oz* axes, respectively; x and z – deformations of the springing members in case of the central pipe's deviation from the vertical axis.

The moment of the elastic forces that act during the rotation of the central pipe about the longitudinal axis (about the *Oy* axis) is equal to:

$$M_{r\varphi} = C_\varphi \cdot \varphi, \qquad (4)$$

where C_φ – total stiffness factor of the springing members in case of the central pipe's rotation about the Oy axis; φ – angular displacement of the central pipe.

When the central pipe rotates (performs angular oscillations) about the vertical axis of the separator, i.e. about the *Oy* axis, the field of centrifugal forces of inertia emerges as a result of the deviation of the pipe's cross-section from the axis of rotation. The said forces of inertia can be represented by the equivalent force of inertia $\bar{F}_d$. In this case, the components of the above-mentioned centrifugal forces vectored along the *Ox* and *Oz* coordinate axes can be represented in the form of their projections on the said axes, which are equal to, respectively:

$$F_{dx} = m_D \cdot l_x \cdot \omega^2 \sin \omega t, \quad (5)$$

$$F_{dz} = m_D \cdot l_z \cdot \omega^2 \sin \omega t. \quad (6)$$

where m_D – mass of the central pipe; l_x and l_x – deviations of the cross-section of the central pipe from the axis of rotation (vertical axis of the separator); ω – angular velocity of the pipe's rotation about the vertical axis of the separator.

The schematic model of the deviation of the central pipe's cross-section that intersects the central pipe's centre of gravity, which occurs under the action of the forces of inertia, is shown in Fig. 3. In this deviation, the pipe's centre and accordingly the origin of the system of coordinates *Oxyz* takes the new position (O_1), shifting along the *Ox* axis by an amount of l_x.

Upon reaching a certain position in the process of the discussed rotation, the moment of rotation M_b becomes counterbalanced by the moment of elastic forces $M_{r\varphi}$, in consequence of which the angular velocity ω of the central pipe's rotation becomes equal to zero, the centrifugal forces disappear and the central pipe stops rotating in this direction, then it starts rotating in the opposite direction also under the action of the moment of elastic forces, thus performing angular oscillatory motion in the other direction. This sequence occurs periodically.

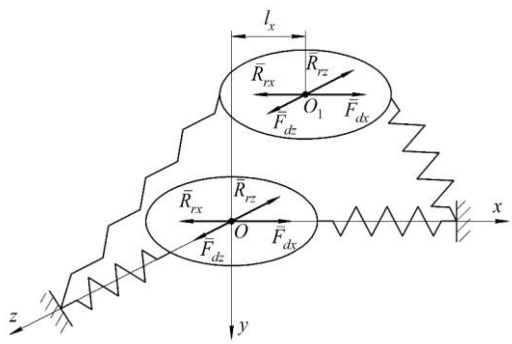


Figure 3. Schematic model of linear displacement of central cross-section intersecting centre of gravity *O* of central pipe: *O*, O_1 – positions of cross-section before and after displacement, respectively.

Thus, consideration has been given to all the force factors causing self-oscillation of the central pipe and, as a result of the self-oscillation, generation of the necessary centrifugal forces of inertia involved in the separation of the seed flow into the required fractions.

Further, it is necessary to analyse the movement of the seeds under the effect of the air flow and the generated centrifugal forces of inertia applied to the seeds, using the same system of coordinates *Oxyz*.

As the seeds moving in the flow are connected via friction forces with the surface of the central pipe and sail members and also connected via internal friction forces with each other in the said flow, the above-mentioned centrifugal forces act on the seeds and impart to them different velocities depending on their masses.

Besides, each individual flow particle (seed) is under the action of the force of gravity $\bar{G}$, the magnitude of which is equal, as is known, to:

$$G = m_S \cdot g, \quad (7)$$

where m_s – mass of the particle (seed), g – acceleration of gravity.

Moreover, the material particle (seed) is under the action of the air flow resistance force $\bar{R}$, the magnitude of which can be determined with the use of the following expression:

$$R = k_c \left(V_S - V_{af}\right)^2 \quad (8)$$

where k_c – coefficient representing the properties of the material particles (seeds) and the medium, in which they move; ξ – coefficient of resistance of the medium, $k_c = \xi \cdot S_M \cdot \frac{\rho}{2}$; S_M – area of the midsection of the particle (seed); ρ – density of the air; V_s – velocity of the material particle (seed); V_{af} – air velocity.

In order to describe the motion of the material particle *M* (sunflower seed) performed under the action of the above-mentioned forces, a separate equivalent schematic model has been generated (Fig. 4). In the equivalent schematic model, the material particle *M* is shown at an arbitrary position. The centrifugal forces of inertia represented by the equivalent force of inertia $\bar{F}_d$ that act on the central pipe are imparted to the material particles as perturbing forces, which can also be represented by their projections $\bar{F}_{dx}$, $\bar{F}_{dz}$ on the respective coordinate axes *Ox* and *Oz*.

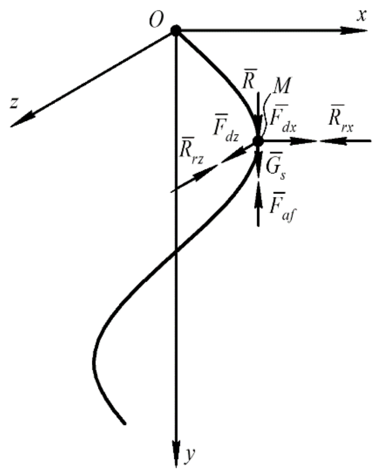


Figure 4. Equivalent schematic model of motion of material particle *M* (sunflower seed) under action of forces applied to it: $\bar{F}_{dx}$, $\bar{F}_{dz}$ – components of perturbing force $\bar{F}_d$, which causes vibrations of central pipe; $\bar{F}_{af}$ – air flow force; $\bar{G}$ – seed gravity force; $\bar{R}$ – resistance of air; $\bar{R}_{rx}$, $\bar{R}_{rz}$ – elastic forces.

Taking into account the obtained schematic model of system forces represented in the above equivalent schematic model and on using Newton's second law of motion, it is possible to generate the system of differential equations that describes the motion of the material particle *M* (seed) in the central part of the aspiration channel. The system of differential equations appears as follows:

$$\left.\begin{aligned} m_S\ddot{x} &= F_{dx} - R_{rx}, \\ m_S\ddot{y} &= G_S - F_{af} + R, \\ m_S\ddot{z} &= F_{dz} - R_{rz}. \end{aligned}\right\} \quad (9)$$

Taking into account the expressions (2), (5) – (8) and performing certain transformations, the system of differential equations (9) can be transformed into the following system:

$$\left.\begin{aligned} &\ddot{x} + \frac{C_x}{m_S} \cdot x = \frac{m_D}{m_S} \cdot l_x \cdot \omega^2 \cdot \sin\omega t, \\ &\ddot{y} = g - \frac{F_{af}}{m_S} + \frac{k_c}{m_S}\left(V_S - V_{af}\right)^2, \\ &\ddot{z} + \frac{C_z}{m_S} \cdot z = \frac{m_D}{m_S} \cdot l_z \cdot \omega^2 \cdot \sin\omega t. \end{aligned}\right\} \tag{10}$$

It is obvious that each differential equation in the system (10) can be solved individually.

The following designations are introduced for solving the first equation of the system (10):

$$\frac{m_D}{m_S} \cdot l_x \cdot \omega^2 = D_x, \quad \frac{C_x}{m_S} = k_x^2. \tag{11}$$

With such designations, the first equation of the system (10) is reduced to the following expression:

$$\ddot{x} + k_x^2 x = D_x \sin\omega t. \tag{12}$$

The general solution of the differential equation (12) is sought in the following form:

$$x = \bar{x} + x^*, \tag{13}$$

where $\bar{x}$ – general solution of the homogeneous equation (without the right member); x^* – partial solution of the differential equation with the right member.

As is known, the general solution of the homogeneous equation appears as follows:

$$\bar{x} = C_1 \cdot \cos k_x t + C_2 \cdot \sin k_x t, \tag{14}$$

where C_1 and C_2 – arbitrary constants.

The partial solution x^* is sought in the following form:

$$x^* = B_1 \cdot \sin\omega t + B_2 \cdot \cos\omega t, \tag{15}$$

where B_1 and B_2 – coefficients that can be found with the use of the method of undetermined coefficients.

Employing the said method, the following is obtained:

$$B_1 = \frac{D_x}{k_x^2 - \omega^2}, \quad k_x^2 \neq \omega^2, \quad B_2 = 0 \tag{16}$$

Hence, the partial solution obtains the following form:

$$x^* = \frac{D_x}{k_x^2 - \omega^2} \cdot \sin\omega t \tag{17}$$

Then, by substituting (14) and (17) into (13), the following is arrived at:

$$x = C_1 \cdot \cos k_x t + C_2 \cdot \sin k_x t + \frac{D_x}{k_x^2 - \omega^2} \cdot \sin\omega t \tag{18}$$

After differentiating the expression (18) with respect to time *t*, the following is obtained:

$$\dot{x} = C_1 \cdot k_x \cdot \sin k_x t + C_2 \cdot k_x \cdot \cos k_x t + \frac{D_x \cdot \omega \cdot \cos \omega t}{k_x^2 - \omega^2} \tag{19}$$

When the initial conditions are taken into account:
at $t = 0$: $x_0 = \alpha,\ \ \dot{x}_0 = V_{x0} = 0.7$ m s^{-1}
the following is derived from the expressions (18) and (19):

$$C_1 = \alpha,$$

$$C_2 = \frac{0.7}{k_x} - \frac{D_x \cdot \omega}{(k_x^2 - \omega^2) \cdot k_x} \tag{20}$$

where *a* – original coordinate of the position of the material particle *M* on the curved path featured in the equivalent schematic model.

After substituting (20) into (18), the resulting expression is:

$$x = a \cdot \cos k_x t + \left[\frac{0.7}{k_x} - \frac{D_x \omega}{\left(k_x^2 - \omega^2\right) k_x} \right] \sin k_x t + \frac{D_x}{k_x^2 - \omega^2} \sin \omega t \tag{21}$$

Under steady-state conditions, the first two components of the expression (21) tend to zero, because they represent the natural oscillations of the system, which are damped oscillations, therefore, the obtained solution takes the following form:

$$x = \frac{D_x \cdot \sin \omega t}{k_x^2 - \omega^2}. \tag{22}$$

In a similar way, after solving the third differential equation of the system (10), the following is arrived at:

$$z = \frac{D_z \cdot \sin \omega t}{k_z^2 - \omega^2} \tag{23}$$

where

$$D_z = \frac{m_D}{m_s} l_z \omega^2, \ \ k_z^2 = \frac{c_z}{m_s}. \tag{24}$$

Further, the angular velocity ω of the central pipe rotation about the vertical axis of the aspiration separator can be expressed in terms of the latter's design and dynamic parameters.

For that purpose, the maximum values of the centrifugal forces arising during the rotation of the central pipe have to be analysed.

Taking into account (5) and (6), the following is obtained:

$$F_{dx_{max}} = m_D \cdot l_{x_{max}} \cdot \omega^2, \ \ F_{dz_{max}} = m_D \cdot \ l_{z_{max}} \cdot \omega^2. \tag{25}$$

On the other hand:

$$F_{dx_{max}} = F_{af} \cdot \sin \alpha, \qquad F_{dz_{max}} = F_{af \cdot \cos \alpha}. \tag{26}$$

where F_{af} – air flow force; α – angle of tilt of the sail element spiral's axis with respect to the longitudinal axis of the pipe.

As stated above, $\alpha = 45°$.

In view of that circumstance, the following is obtained:

$$F_{dx_{max}} = F_{dz_{max}} = \frac{\sqrt{2}}{2} \cdot F_{af} = \frac{F_{af}}{\sqrt{2}} \cdot \qquad (27)$$

Due to the symmetry considerations, it is assumed that:

$$l_{x_{max}} = l_{z_{max}} = l.$$

Subsequently, by equating (25) and (27) and replacing $l_{x_{max}}$ and $l_{z_{max}}$ with l, the following is arrived at:

$$m_D \cdot l \cdot \omega^2 = \frac{F_{af}}{\sqrt{2}},$$

from which follows:

$$\omega^2 = \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l}. \qquad (28)$$

Hence:

$$\omega = \sqrt{\frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l}} \qquad (29)$$

Further, after substituting the expression (28) into the expression (11), the following is obtained:

$$D_x = \frac{m_D}{m_S} l_x \omega^2 = \frac{m_D}{m_S} l_x \cdot \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l} = \frac{F_{af}}{\sqrt{2} \cdot m_S} \cdot \frac{l_x}{l} \qquad (30)$$

Similarly:

$$D_z = \frac{m_D}{m_S} l_z \omega^2 = \frac{m_D}{m_S} l_z \cdot \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l} = \frac{F_{af}}{\sqrt{2} \cdot m_S} \cdot \frac{l_z}{l} \qquad (31)$$

After (30) is substituted into the expression (22), the result is:

$$x = \frac{F_{af}}{\sqrt{2} \cdot m_S} \cdot \frac{l_x}{l} \cdot \frac{\sin \omega t}{k_x^2 - \omega^2} = \frac{F_{af}}{\sqrt{2} \cdot m_S} \cdot \frac{l_x}{l} \cdot \frac{\sin \omega t}{\frac{C_x}{m_S} - \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l}} =$$

$$= \frac{l_x \cdot F_{af} \cdot \sin \omega t}{l \cdot \sqrt{2}\left(C_x - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)}. \qquad (32)$$

Similarly:

$$z = \frac{F_{af}}{\sqrt{2} \cdot m_S} \cdot \frac{l_z}{l} \cdot \frac{\sin \omega t}{k_z^2 - \omega^2} = \frac{F_{af}}{\sqrt{2} \cdot m_S} \cdot \frac{l_z}{l} \cdot \frac{\sin \omega t}{\frac{C_z}{m_S} - \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l}} =$$

$$= \frac{l_z \cdot F_{af} \cdot \sin \omega t}{l \cdot \sqrt{2}\left(C_z - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)}. \qquad (33)$$

The expressions (32) and (33) define the law of the particle (seed) displacement as a function of time t with provisions for the design and dynamic parameters of the aspiration separator.

The complete displacement S of the material particle M (sunflower seed) in the cross-section of the aspiration channel is equal to:

$$S = \sqrt{x^2 + y^2} \tag{34}$$

or, taking into account (32) and (33):

$$S = \frac{F_{af}}{l \cdot \sqrt{2}} \cdot \sin\omega t \cdot \sqrt{\frac{l_x^2}{\left(C_x - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2} + \frac{l_z^2}{\left(C_z - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2}} \tag{35}$$

Further, by substituting the expression (28) into the expressions (5) and (6), the following is obtained:

$$F_{dx} = m_D \cdot l_x \cdot \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l} \cdot \sin\omega t = \frac{l_x \cdot F_{af} \cdot \sin\omega t}{\sqrt{2} \cdot l} \tag{36}$$

$$F_{dz} = m_D \cdot l_z \cdot \frac{F_{af}}{\sqrt{2} \cdot m_D \cdot l} \cdot \sin\omega t = \frac{l_z \cdot F_{af} \cdot \sin\omega t}{\sqrt{2} \cdot l} \tag{37}$$

The centrifugal force F_d is equal to:

$$F_d = \sqrt{F_{dx}^2 + F_{dz}^2} \tag{38}$$

or

$$F_d = \frac{F_{af}}{\sqrt{2} \cdot l} \cdot \sin\omega t \cdot \sqrt{l_x^2 + l_z^2} \tag{39}$$

Finally, after the expressions (32), (33) are substituted into the expressions (2) and (3), the following is arrived at:

$$R_{rx} = \frac{C_x \cdot l_x \cdot F_{af} \cdot \sin \omega t}{l \cdot \sqrt{2} \left(C_x - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)} \tag{40}$$

$$R_{rz} = \frac{C_z \cdot l_z \cdot F_{af} \cdot \sin \omega t}{l \cdot \sqrt{2} \left(C_z - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)} \tag{41}$$

The final value of R_r is equal to:

$$R_r = \sqrt{R_{rx}^2 + R_{rz}^2} \tag{42}$$

or

$$R_r = \frac{F_{af} \cdot \sin \omega t}{l \cdot \sqrt{2}} \cdot \sqrt{\frac{C_x^2 \cdot l_x^2}{\left(C_x - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2} + \frac{C_z^2 \cdot l_z^2}{\left(C_z - \frac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2}} \tag{43}$$

Thus, the analytic expressions have been obtained that allow to determine the perturbing forces and elastic resistance forces expressed in terms of the design and dynamic parameters of the aspiration separator that provide for the performance of the work process under consideration.

The next task is to find out the velocities and accelerations of the material particles (seeds) in the work process under consideration.

The velocity of the seeds can be determined in terms of its projections on the *x* and *z* axes. That is:

$$V_x = \dot{x} = \frac{l_x \cdot F_{af} \cdot \omega \cdot \cos \omega t}{l \cdot \sqrt{2}\left(C_x - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)} = \frac{l_x \cdot F_{af} \cdot \sqrt{\dfrac{F_{af}}{\sqrt{2} \cdot m_D \cdot l}} \cdot \cos \omega t}{l \cdot \sqrt{2}\left(C_x - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)} \tag{44}$$

and

$$V_z = \dot{z} = \frac{l_z \cdot F_{af} \cdot \omega \cdot \cos \omega t}{l \cdot \sqrt{2}\left(C_z - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)} = \frac{l_z \cdot F_{af} \cdot \sqrt{\dfrac{F_{af}}{\sqrt{2} \cdot m_D \cdot l}} \cdot \cos \omega t}{l \cdot \sqrt{2}\left(C_z - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)} \tag{45}$$

As is known, the total velocity is equal to:

$$V = \sqrt{V_x^2 + V_z^2} \tag{46}$$

or

$$V = \frac{F_{af} \cdot \sqrt{\dfrac{F_{af}}{l \cdot m_D \cdot \sqrt{2}}} \cdot \cos \omega t}{l \cdot \sqrt{2}} \cdot \sqrt{\frac{l_x^2}{\left(C_x - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2} + \frac{l_z^2}{\left(C_z - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2}} \tag{47}$$

The acceleration of the seeds is also determined in terms of its projections on the *Ox* and *Oz* axes. That is:

$$a_x = \ddot{x} = -\frac{l_x \cdot F_{af}^2 \cdot \sin \omega t}{2 \cdot l^2 \cdot m_D\left(C_x - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)} \tag{48}$$

and

$$a_z = \ddot{z} = -\frac{l_z \cdot F_{af}^2 \cdot \sin \omega t}{2 \cdot l^2 \cdot m_D\left(C_z - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)} \tag{49}$$

As is known, the total acceleration is equal to:

$$a = \sqrt{a_x^2 + a_z^2} \tag{50}$$

or

$$a = \frac{F_{af}^2 \cdot \sin \omega t}{2 \cdot l^2 \cdot m_D} \cdot \sqrt{\frac{l_x^2}{\left(C_x - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2} + \frac{l_z^2}{\left(C_z - \dfrac{m_S \cdot F_{af}}{m_D \cdot l}\right)^2}} \tag{51}$$

DISCUSSION

On the basis of the obtained relations, the kinematic and force characteristic curves of the researched process have been plotted with the use of the specially developed computation program in the MathCAD environment. When determining the amount of the displacement of sunflower seeds, their different masses were taken into account, that is, the following seed mass limit values were used in the PC-assisted numerical calculations for the heavy and medium fractions:

– medium fraction m_{S1} = 0.040–0.060 g;

– heavy fraction m_{S1} = 0.065–0.080 g.

The kinematic parameters taken into consideration included the displacement *S*, velocity *V* and acceleration *α* of the seeds in the fractions under study.

The above-said parameters were calculated in relation to the angular displacement $\varphi = \omega t$ of the central pipe in the vibration and aspiration separator of sunflower seeds.

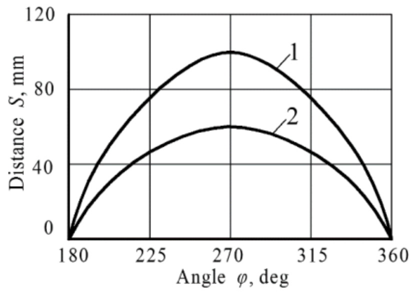


Figure 6. Relation between covered distance and angular displacement *φ* of central pipe for medium (1) and heavy (2) fractions of sunflower seed mixture.

Thus, the diagrams shown in Fig. 6 give evidence of the substantial difference in the distance covered by the seeds of the medium and heavy fractions in the radial (transverse) plane of the aspiration separator, especially for the angular displacement *φ* of the central pipe within the range of 200° to 335°, in which case the said difference reaches 20 to 40 mm, respectively.

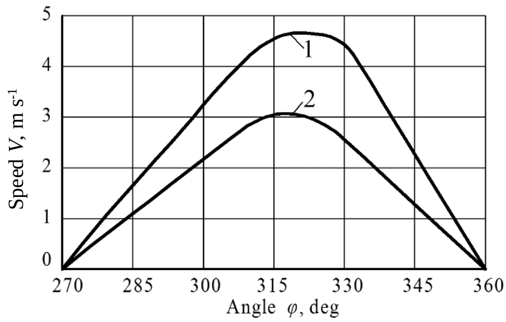


Figure 7. Relations between velocity of seeds of medium (1) and heavy (2) fractions and angular displacement *φ* in radial plane of central pipe.

That indicates the substantial influence of the field of centrifugal forces of inertia on the process of separation of sunflower seeds into fractions within the above-mentioned (main) range of the angular displacement of the separator's central pipe, which translates into the high quality of operation in the examined work process of seed mixture separation. The said influence is especially pronounced at the instant, when the central pipe rotates through an angle of 270°. As is seen from the curves presented in the figure, in the absence of the action of centrifugal forces (when the angular displacement of the central pipe is equal to 180° or 360°) virtually no separation of the seeds into fractions takes place.

Similar diagrams have been plotted for the relations between the maximum velocities of the motion of sunflower seeds in the radial plane and the angular displacement φ of the central pipe for each of the above-mentioned fractions (Fig. 7).

As is seen on the diagrams presented in Fig. 7, the maximum velocity of sunflower seeds in the heavy fraction is equal to 4.5 m s^{-1}, while in case of the medium fraction – 3.0 m s^{-1}. Also, the difference between the velocities of seeds in the medium and heavy fractions is equal to 0.6–1.5 m s^{-1}, when the angular displacement φ of the central pipe varies within the range of 285°–345°. This difference between the velocities of particles in the medium and heavy fractions is exactly what facilitates the separation of the seeds into fractions.

The diagrams in Fig. 8 represent the relations between the acceleration of sunflower seeds in each of the fractions and the angular displacement φ of the central pipe of the vibration and aspiration separator.

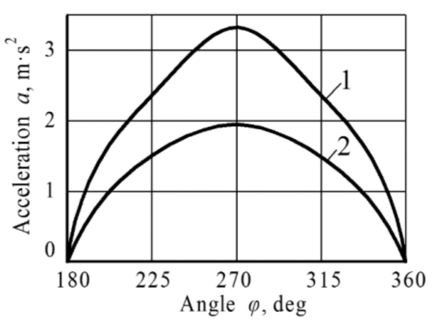


Figure 8. Relation between acceleration of sunflower seeds in heavy (1) and medium (2) fractions and angular displacement φ of central pipe.

As is seen on the diagrams shown in Fig. 8, the maximum acceleration of sunflower seeds in the heavy fraction is equal to 3.3 m s^{-2}, in the medium fraction – 1.9 m s^{-2}. Also, it is evident from the data of the diagrams that for the angular displacement φ of the central pipe within the range of 200°–335° the difference between the accelerations of seeds in the medium and heavy fractions is equal to 0.7–1.4 m s^{-2}, which again contributes to improving the quality of the separation of the seed mixture into fractions.

With the use of PC-assisted calculations, the authors have also determined the power consumption indicators for the discussed work process of separating sunflower seeds with the use of a vibration and aspiration separator.

The relations presented in Fig. 9 specify the variation of magnitude of the perturbing (centrifugal) force that arises, when self-oscillatory motion is generated in the components of the separated mixture. In case of the heavy fraction of sunflower seeds, its maximum value is equal to 100 N, for the seeds in the medium fraction – 68 N. Therefore, the difference in this indicator amounts to 32%.

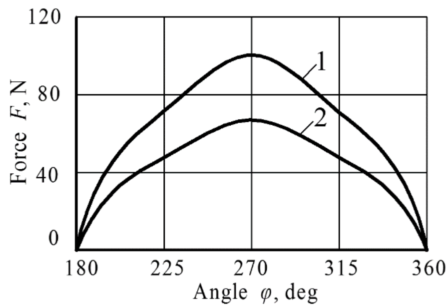


Figure 9. Relations between magnitude of perturbing force and angular displacement φ of central pipe for seeds in heavy (1) and medium (2) fractions, respectively.

It is logical to assume that the centrifugal force acting on the heavier seeds is greater than that acting on the medium fraction seeds, since it depends on the mass of the seed.

The difference between the centrifugal forces acting on the particles (seeds), as shown in the diagrams (Fig. 9), is the exact cause of the separation of the seeds into fractions.

Calculations have also been carried out to determine the magnitude of the moment of rotation M_d and find its relation with the angular displacement φ of the central pipe for the sunflower seeds in each of the fractions (Fig. 10).

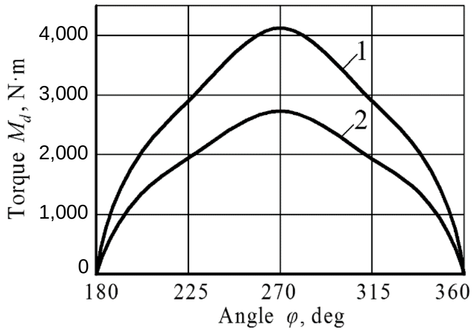


Figure 10. Relations between magnitude of moment of rotation M_d and angular displacement φ of central pipe for seeds in heavy (1) and medium (2) fractions, respectively.

As is seen on the diagrams presented in Fig. 10, the maximum magnitude of the moment of rotation M_d that arises during the rotation of the central pipe is equal to 4,100 Nm for the heavy fraction, for the medium fraction – 2,700 Nm. The difference between the sunflower seed fractions in this parameter amounts to 32.5%. The said difference results in the different motion paths of the fractions under consideration.

Finally, the authors have carried out the PC-assisted numerical calculation of the power required for the performance of the work process of sunflower seed separation. In Fig. 11, the relations are shown for the power required for separating the seeds of the medium and heavy fractions.

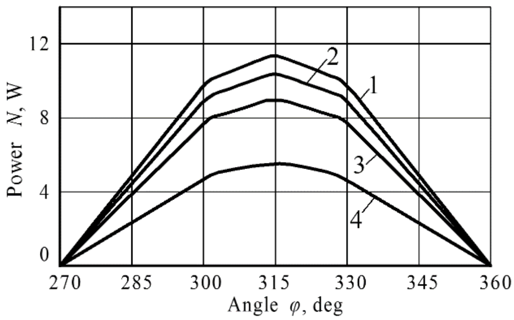


Figure 11. Relations between angular displacement φ of central pipe and maximum (1) and minimum (2) power consumption rates for heavy fraction seeds; maximum (3) and minimum (4) power consumption rates for medium fraction seeds, in process of generating self-oscillatory mode of operation.

As is seen on the diagrams presented in Fig. 11, the maximum power consumed by the pressure air for the respective alteration of the motion paths of seed particles in the heavy fraction amounts to only 11.5 W, in the medium fraction – 9.0 W, i.e. by 22% less. Hence, it is obvious that the power costs of the work process under study are essentially insignificant.

In order to verify the results obtained in the theoretical investigations, the authors have carried out the experimental research into the vibration and aspiration separation of sunflower seeds. For that purpose, an experimental unit has been manufactured. Its general view is shown in Fig. 12, the schematic model of the unit is presented in Fig. 13. The unit is an industrial prototype of the vibration and aspiration separator, which is equipped with the required instruments and sensors.

The operating principle of the laboratory experimental unit is as follows. The grain mixture of sunflower seeds is fed from the feed hopper located above for its separation into fractions. In this process, the slide gate 5 makes up the batch of grain mixture with a pre-set mass and controls its feeding. The said grain mixture batch is fed by gravity via the shaped seed duct onto the top spreader of the central pipe situated in the fixed casing of the aspiration channel. The fan 3 generates the necessary induced air draught inside the fixed casing of the aspiration channel, the flow rate of which is controlled in the process of the experimental investigations by the instrument 2, while the instrument 4 registers the air velocity. Further, the process of vibration and aspiration sunflower seed separation into fractions, as described earlier, takes place, and the results of the process take shape and get fixed below on the horizontal surface 1. The surface 1, onto which the seeds of different (medium and heavy) fractions arrive and on which they get fixed, is a replaceable sheet of paper coated with an adhesive layer, which is fixed in a strictly set position with respect to the central pipe of the vibration and aspiration separator. After separating each pre-set size batch of sunflower seeds, the paper sheet is taken away in order to carry out the corresponding measurements of the displacement of seeds with respect to the central pipe and to weigh the seeds.

Figure 12. General view of experimental unit for research into vibration and aspiration separation of sunflower seeds.

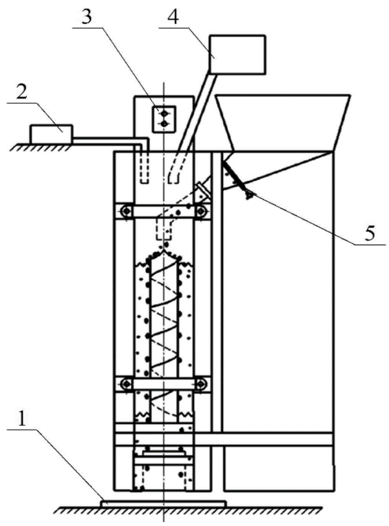


Figure 13. Schematic model of laboratory experimental unit for research into vibration and aspiration separation: 1 – surface, where different fraction seeds arrive and get fixed; 2 – instrument for measuring dynamic pressure of air; 3 – fan for generation of induced air draught; 4 – instrument for measuring air velocity; 5 – slide valve that controls feeding of batch of sunflower seeds.

The operating principle of the laboratory experimental unit is as follows. The grain mixture of sunflower seeds is fed from the feed hopper located above for its separation into fractions. In this process, the slide gate 5 makes up the batch of grain mixture with a pre-set mass and controls its feeding. The said grain mixture batch is fed by gravity via the shaped seed duct onto the top spreader of the central pipe situated in the fixed casing of the aspiration

channel. The fan 3 generates the necessary induced air draught inside the fixed casing of the aspiration channel, the flow rate of which is controlled in the process of the experimental investigations by the instrument 2, while the instrument 4 registers the air velocity. Further, the process of vibration and aspiration sunflower seed separation into fractions, as described earlier, takes place, and the results of the process take shape and get fixed below on the horizontal surface 1. The surface 1, onto which the seeds of different (medium and heavy) fractions arrive and on which they get fixed, is a replaceable sheet of paper coated with an adhesive layer, which is fixed in a strictly set position with respect to the central pipe of the vibration and aspiration separator. After separating each pre-set size batch of sunflower seeds, the paper sheet is taken away in order to carry out the corresponding measurements of the displacement of seeds with respect to the central pipe and to weigh the seeds.

Several sets of experimental investigations with the necessary numbers of test replications have been carried out in accordance with the specially developed programme and technique of a multifactorial experiment and following the respective setup and adjustment of the laboratory unit. The necessary measurements and the weighing of the separated fractions have been performed and their results have been processed on the PC with the use of statistical methods.

As is seen from the presented graphic relations plotted on the basis of the results of the experimental investigations, the motion paths of sunflower seeds depend to a great extent on the masses of the seeds and the velocity of the air flow (Fig. 14).

As can be seen from the presented graphs, the heavy fraction sunflower seeds land virtually in the immediate vicinity of the outer generating line of the central pipe in the aspiration channel, having traversed the shortest distances.

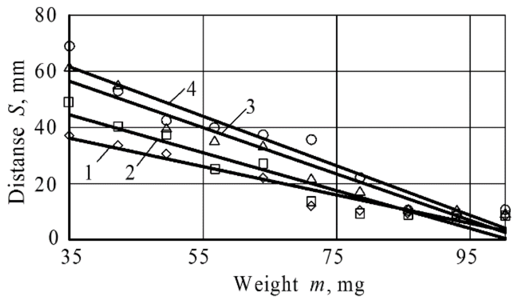


Figure 14. Relation between distance traversed by sunflower seed relative to outer surface of vertical pipe and its mass at different air flow velocities: 1) 4.0 m s^{-1}; 2) 4.5 m s^{-1}; 3) 5.0 m s^{-1}; 4) 5.5 m s^{-1}.

If the theoretically derived distances traversed by the heavy fraction seeds (Fig. 6) are compared to the similar distances obtained as a result of the experimental research (Fig. 14), it becomes obvious that there is a virtually complete agreement with regard to this criterion between the theoretical and experimental results, because in both cases the said distance does not exceed 40 mm. At the same time, the most efficient sunflower seed separation takes place at an air flow velocity of 4.0 m s^{-1}. When the mentioned velocity is increased, the share of the low quality light fraction sunflower seeds (where good quality medium fraction seeds can get as well) carried away together with the air flow beyond the limits of the separator also increases.

The same agreement between the results of the theoretical and experimental investigations is observed in case of the medium fraction sunflower seeds, which are deposited at a greater distances from the central pipe of the aspiration separator.

Thus, when the design and kinematic parameters obtained theoretically are used in the operation of the laboratory and industrial unit, the vibration and aspiration separator demonstrates stable performance providing guaranteed separation of sunflower seeds into the above-mentioned fractions.

CONCLUSIONS

1. The authors have developed a new theory of the motion of a grain mixture particle, i.e. a mathematical model has been generated for describing the motion of a material particle (seed) in the operating space of the vertical aspiration channel of new design under the action of the air flow forces and the centrifugal forces of inertia generated by the angular self-oscillations of the separator's central pipe.

2. Analytical solutions have been obtained for the differential equations of motion of the material particle that represent the laws of motion and variation of velocity and acceleration in time in relation to the design and dynamic parameters of the vibration and aspiration separator.

3. The numerical calculations have indicated a significant difference between the distances covered by the seeds of the medium and heavy fractions. For example, for the central pipe's angular displacement within the range of 200°–335° the said difference amounts to 20–40 mm. That proves the high quality of the separation of seeds into fractions under the action of the centrifugal forces of inertia generated by the oscillating central pipe of the separator.

4. The difference in the velocities of the motion of particles in the cross-section of the central part of the aspiration channel is equal to 0.6–1.5 m s^{-1}, the difference in the accelerations is equal to 0.7–1.4 m s^{-2}.

5. The numerical calculations have also indicated that the maximum magnitude of the perturbing force acting on seeds in the heavy and medium fractions is equal to 100 N and 68 N, respectively, the difference between these values reaching 32%. The maximum magnitude of the rotation moment for the heavy fraction seeds is equal to 4100 Nm, for the medium fraction seeds – 2,700 Nm, the difference between different sunflower seed fractions amounting to 32.5%. The said differences are exactly what causes the fractions under research to move on different motion paths.

6. The maximum power consumed by the pressure air for changing the motion paths of the heavy fraction particles as described amounts to 11.5 W, in case of the medium fraction – 9.0 W, i.e. 22% less. Therefore, the completed analysis has proved that the power consumption in the performance of the researched work process is rather insignificant.

7. The obtained theoretical results have provided a possibility to substantiate a number of design and kinematic parameters for the vibration and aspiration separator: diameter of the seed duct for the medium fraction – within the range of 50–70 mm, the heavy fraction – 90–110 mm; diameter of the separator's central pipe – 200 mm; air flow velocity – 4.5–5.5 m s^{-1}; angular velocity of rotation of the central pipe in its self-oscillatory motions – 50 s^{-1}.

8. The results of the completed experimental investigations fully confirm the validity of the developed theory.

REFERENCES

Badretidinov, I., Mudarisov, S., Tuktarov, M.,, Dick, E. & Arslanbekova, S. 2019. Mathematikcal modelling of the grain material separation in the pneumatic system of the grain cleaning machine. *Journal of Applied Engineering Scienve* **17**(4), 529–534. doi: 10.5937/jaes17-22640

Bernik, P.S. & Palamarchuk, I.P. 1996. *Conveyor vibration machines for finishing and handling treatmen*t. Kiev, Võshaja škola, 237 pp.

Brăcăcescu, C., Pirna, I., Sorica, C., Popescu, S. & Stan, O. 2012. Experimental researchon influence of functional parametrs of gravity separator on quality indicators of separation process with application on cleaning of wheat seeds. *Engineering for Rural Development* **11**, 16–21.

Brăcăcescu, C., Tenu, I., Mircea, C. & Bunduchi, G. 2019. Experimental research on influence of functional parameters of combined installations designed at separating the i mpurities out of cereal seeds. *E35 Web of Conferences, 8th Int. Conf. on Thermal Equipment, Renewable Energy and Rural Development*. Vol. 112, Article no 03004, doi: 10.1051/e3sconf/201911203004

Burkov, A.I. 1991. *Research into work process of closed pneumatic system in seed-cleaning machine*. Thesis for degree of Candidate of Sciences. Kirov, 217 pp.

Kotov, V.V. 2002. Determining velocity of grain in air flow. *Technologies and Mechanical Means in Field Husbandry*, Zernograd, pp. 137–140.

Kroulik, M., Hula, J. & Rybka, A. 2016. Pneumatic conveying characteristics of seeds in a vertical ascending airstream. *Research in Agricultural Engineering* **62**(2), 56–63.

Kyurchev, S.V. & Kolodiy, A.S. 2012. Analysis of methods to improve agricultural crop capacity and requirements to separated material. In: *Proceedings of Scientific Papers*, Vinnitsa National Agrarian University, **11**(2), 322–327.

Kyurchev, S.V. & Kolodiy, A.S. 2015. Multi-criterion analysis of existing seed separators with different operating devices. In: *Bulletin of Kharkiv National Technical University of Agriculture* **156**(1), 86–92.

Leshchenko, S.M., Vasilkovsky, A.N., Vasilkovsky, M.I. & Gontšarov, V.V. 2009. Raising efficiency of pre-treatment of grain mixtures. In: *Agricultural Machinery*, Lutsk, **18**, 230–234.

Poturayev, V.N. & Franchuk, V.P. 1970 *Selected theoretical prerequisites for research into mechanics of motion of thick loose load layer under the action of vibration*. Kiev: Naukova Dumka, pp. 173–181.

Saitov, V.E., Farafanov, V.G., Gataullin, R.G. & Saitov, A.V. 2018. Research of a diametrical fan with suction channel. IOP Conf. Series: *Materials Science and Engineering* **457**, 6 pp. doi: 10.1088/1757-899X/457/012009

Saitov, V.E., Kurbanov, R.F. & Suvorov, A.N. 2016. Assessing the Adequacy of Mathematical Models of Light Impurity Fractionation in Sedimentary Chambers of Grain Cleaning Machines. *Procedia Enginering* **150**, 107–110. doi: 10.1016/j.proeng.2016.06.728

Vasilkovsky, M.I., Goncharova, S.J. & Leshchenko, S.M. 2007. Substantiation of parameters for grain separation in inclined air flow. Design, Production and Operation of Agricultural Machines: *Nationwide Interagency Research and Technology Collected Papers*. Kirovograd National Technical University, Kirovograd, **37**, 132–137.

Agronomy Research **18**(1), 38–52, 2020
https://doi.org/10.15159/AR.20.058

Theory of oscillations performed by tools in spiral potato separator

V. Bulgakov[1], I. Holovach[1], Z. Ruzhylo[1], I. Fedosiy[1], Ye. Ihnatiev[2] and J. Olt[3,*]

[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony Str., UA 03041 Kyiv, Ukraine
[2]Tavria State Agrotechnological University, 18[B] Khmelnytsky Ave, UA 72310 Melitopol, Zaporozhye Region, Ukraine
[3]Estonian University of Life Sciences, Institute of Technology, 56 Kreutzwaldi Str., EE 51006 Tartu, Estonia
[*]Correspondence: jyri.olt@emu.ee

Abstract. The aim of this study is to provide for the intensification of the process of removing impurities from the potato heap by oscillating devices, which takes place, when potato tubers are cleaned in the spiral separator during their lifting. The authors have devised a new design of the spiral separator, which can be used in the process of lifting potatoes from the soil. It comprises cantilever cleaning spiral springs, which in their simultaneously rotary and oscillatory motion clean and transport the potato tubers with good quality. However, the removal of impurities from the cleaning area and the cleaning of the side surfaces of potato tuber bodies from the stuck soil can be significantly more effective, if the spiral springs perform their oscillatory motions in the longitudinal and vertical plane during their operation more intensively. In order to achieve that, the authors have worked out a new theory of the oscillatory motion of the cleaning spiral working under the action of the potato heap. That promotes the intensification of the process of cleaning potato bodies from the stuck soil. The authors have generated a new partial differential equation, which describes the oscillations of the cantilever cleaning spiral. The PC-assisted numerical solving of the said differential equation and further numerical modelling have made it possible to obtain the analytic expressions of the relations between the change in the helix pitch distance of the cleaning spiral and its deformation, in particular, the simultaneous longitudinal extension and transverse bending. That result, in its turn, provided for finding the maximum limit amount of the above-mentioned deformation under the condition that the potato tubers may not fall through the spaces between the spiral turns, taking into account the design and kinematic parameters of the cleaning spiral itself, the material it is made of and the process-dependent mode of operation of the separator. As a result of the PC-assisted numerical modelling, it has been found that the total deflection of the spiral on its length varies within the range of 0 to 0.05 m under the following parameters: angular velocity of rotation of the spiral ω = 30 rad s^{-1}, density of the material of the spiral ρ = 7,700 kg m^{-3}, elastic modulus $E = 2 \cdot 10^{11}$ Pa, cleaning spiral bar stock radius r = 8.5 mm and a uniform load intensity of 1,000 N m^{-1}. Such variation ensures the good quality of cleaning and transportation of potato tubers. According to the results of the PC-assisted numerical calculations, the helix pitch distance of a cleaning spiral with the above parameters and the original helix pitch distance S = 48 mm, due to the spiral's deformation under the proposed transverse oscillations, can change up to 54 mm, which makes impossible for potato tubers to fall out of the cleaning unit. Experimental studies fully support the results from the theoretical calculations to determine the displacement of the ends of the cleaning unit's spiral springs.

Key words: cleaning unit, deformation, oscillatory motion, potato, soil.

INTRODUCTION

One of the principal requirements in the potato harvesting is to ensure the good quality cleaning of the potato heap from soil and plant impurities as well as the cleaning of individual potatoes from the stuck soil, reduction of their damage and loss rates. Meanwhile, the high quality cleaning of potato tubers from impurities is possible only in case a considerable amount of the soil and other components of the lifted potato heap is immediately separated from the tubers at the beginning of the heap transportation, or right after the potato bed is lifted from the soil by the lifting implement. In that case, a significant amount of the soil and plant debris will not enter the further parts of the potato harvester with its cleaning devices, which will provide for the adequate quality of harvesting.

However, the existing potato heap cleaning tools employed in the state-of-the-art potato harvesters insufficiently meet the above-listed requirements. Potato harvesting often takes place on rather damp and soft soils – as a result, it is a frequent occasion in the existing cleaning tools that the separating spaces get clogged with sticky wet soil and plant debris, which significantly reduces the capability of the potato heap cleaning units to reject the impurities and, consequently, the quality of the cleaning of potato tubers.

The machine that is most compliant with the above conditions of cleaning potato tubers from impurities during their lifting is the worked out new separator of the spiral type, the structural layout of which is presented in Fig. 1.

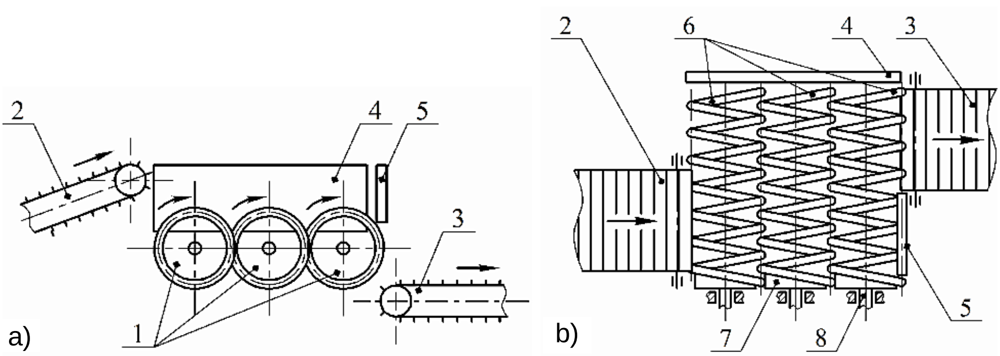


Figure 1. Spiral separator of potato heap lifted from soil: a – side view, b – top view; 1 – spirals; 2 – feeding conveyor; 3 – discharge conveyor; 4 – side protective apron; 5 – face protective apron; 7 – hub; 8 – drive shaft (Bulgakov et al., 2019).

As regards its structure, the cleaning unit is designed in such a way that the process of breaking the potato heap just dug out from the soil, dividing it into separate components and removing the soil impurities and plant debris out of the cleaning space takes place on the three cleaning spirals 1 installed in series. The spirals 1 are cantilevered with their ends on one side attached to the hubs 7 mounted on the drive shafts 8, which generate their rotary motion in the same direction at a pre-set angular velocity of rotation. Their second (free) ends 6 can perform oscillatory motion in the longitudinal and vertical plane under the action of the variable load. The heap of the potato tubers dug out from the soil is fed with the use of the feeding conveyor 2, while the evacuation of cleaned potato tubers from the spirals 1 is performed by the discharge conveyor 3. In order to prevent the loss of potato tubers during the work process of their cleaning from soil impurities and plant debris, the rectangular end face 4 and side 5 protection shields are installed around the spirals 1. The cantilever cleaning spirals 1 are installed with mutual overlapping, which facilitates their self-cleaning from the stuck wet soil.

The efficiency of the self-cleaning of the cleaning spirals 1 is provided by their oscillatory motion, which produces the variable deformation of the spirals 1 themselves, that is, their longitudinal extension and transverse bending. Such conditions result in the spacing between any adjacent turns of the spirals 1, i.e. the helix pitch distance, constantly changing, which promotes detachment (and compaction) of the stuck soil in the turn-to-turn space followed by its sieving down and out of the cleaning unit. Thus, in the proposed potato heap cleaning unit not only the self-cleaning of the cleaning spirals 1 takes place, but also the active separation of soil impurities and plant debris through the spaces between the turns of the spirals 1 as well as between the spirals 1 themselves.

Since the spirals 1 are attached, via the hubs 7, to the drive shafts 8, they perform rotational motions and, therefore, the spirals 1 entrain with their turns the bodies of potato tubers and soil clots and start transporting them in both the longitudinal and radial directions. However, in view of the present large gaps (between the turns of each spiral 1 and also the space between the spirals 1 themselves), the soil particles in great quantities are immediately sieved down and out of the separator.

Hence, the surface formed by the three cleaning spirals in the discussed unit for cleaning potatoes from impurities features significant sieving openings, also the said surface is active in view of the rotary motion of the spirals and the spirals generate the motion of the components of the heap fed for cleaning simultaneously in two directions, that is, the radial and axial ones. Moreover, the cantilever mounting of the cleaning spirals implies that their free ends perform oscillatory motion in the longitudinal and vertical plane, which not only promotes the development of favourable conditions for the efficient agitation of the potato heap, but also generates the periodic changes in the turn-to-turn spacing and that in effect provides the conditions for the positive entrainment of strong soil bodies and plant debris and their removal out of the cleaning area. At the same time, the potato tubers may not become entrapped by the spiral turns or sustain damage. Finally, the mutual overlapping of the spirals and their oscillatory motion under the action of the variable load can result in the eccentricity of the ends of the spirals, which promotes the generation of repetitive potato tuber shock motions and that, in its turn, facilitates their effective cleaning from the stuck soil.

The problem of the development and research of potato heap separators is discussed in the papers (Petrov, G., 1984; Feller et al., 1987; Holland-Batt, 1989; Misener & McLeod, 1989; Peters, 1997; Krause & Minkin, 2005; Bishop et al., 2012; Ichiki et al., 2013; Guo & Campanella, 2017; Wang et al., 2017; Ye et al., 2017; Wei et al., 2017). However, as stated above, the cleaning tools mentioned in those papers have one essential deficiency – their cleaning gaps are clogged by soil and plant debris, when operating on soils with an increased water content, which significantly reduces the separating capacity of such tools. The authors have devised the spiral separator

(Bulgakov et al., 2018a, 2018b, 2018c; 2019) that is capable of not only efficiently cleaning potato tubers from impurities, but self-cleaning from the stuck soil as well. The authors have also carried out its testing and initial research. In order to optimise the design and kinematic parameters of the new cleaning unit, it is necessary to carry out the theoretical investigation of the operation of its tools.

The investigation of the beam deflection curve can be done with the use of the differential equation for the case of loading by a free system of forces and a moment at the end (Vasilenko, 1996) or on the basis of strain compatibility equations (Chelomey, 1981). In a simplified form, such calculations of deflection can also be performed with the use of the equations obtained in (Tymoshenko, 1959). But, in the above cases, beams with constant cross-sections and constant properties are assumed. For the spiral spring, the moment of inertia of which is a function of the axial coordinate and time, these methods do not provide any new information. Therefore, for the purposes of this study, it would be most reasonable to apply the Bernoulli-Euler differential equation of the transverse flexural oscillations of a deformed beam, which comprises the relation between the curvature and the deflection and the differential equation of static beam deflection by J. Bernoulli together with the dynamic member added to the equation by L. Euler to take account of the transverse inertia forces. In case of free oscillations, the right-hand member of the equation is equal to zero. In case of an external perturbing force acting on the beam, the right-hand member of the equation assumes the form of the said perturbing force function. In case of a load uniformly distributed in time and along the axial coordinate, the right-hand member of the equation assumes the form of the uniformly distributed load.

The aim of the study is to provide for the intensification of the process of removing impurities from the heap by vibrating tools in the process of cleaning potato tubers by the spiral separator at the stage of their lifting.

MATERIALS AND METHODS

In the development of the theory of oscillations of the cleaning tools in the spiral potato separator, the methods of the modelling and the strength of materials have been used, in particular, the theory of the oscillations of elastic and deformed beams on the basis of the Bernoulli-Euler theory, the application of the Krylov special functions as well as the methods of programming and numerical modelling.

In view of the fact that the oscillatory motion of cantilever cleaning spirals plays an important part in the work process of cleaning potato tubers from soil and plant debris, the need arises first of all to investigate the said oscillatory process analytically.

For that purpose, it is necessary first of all to generate a mathematical model of the oscillatory process performed by the spirals under the action of a variable load.

The first step is to work out the equivalent schematic model of the oscillations performed by the spiral attached to the drive shaft in cantilever mode, which rotates and is under the action of the external load produced by the potato heap situated on it (Fig. 2).

The equivalent schematic model (Fig. 2) features the cleaning spiral in two positions: unstrained condition, where its longitudinal axis coincides with the *OZ* axis, and the deformed condition, where its longitudinal axis undergoes a deflection under the action of the variable distributed load designated $\tilde{q}(z,t)$. The said load is produced by the potato heap continuously fed by the feeding conveyor onto the surface of the cleaning spirals. In the schematic model under consideration, the parameter *S* is shown, which is the helix pitch distance of the unstrained spiral and it has the same value all along the whole length of the spiral. The schematic model features also $S(z, t)$ – the helix pitch distance of the spiral that undergoes deformation under the action of the of the external load. This deformed spiral pitch distance varies both along the length of the spiral itself and with time due to the spiral's oscillatory motion. The deflection of the spiral's longitudinal axis designated $W(z, t)$ is also variable along the length of the spiral's axis (*z* coordinate) and depends on time (parameter *t*) as a result of the spiral's oscillatory motion. *R* – the spiral's radius. ω – angular velocity of the spiral's rotary motion about its longitudinal axis (sense of rotation is shown by arrow).

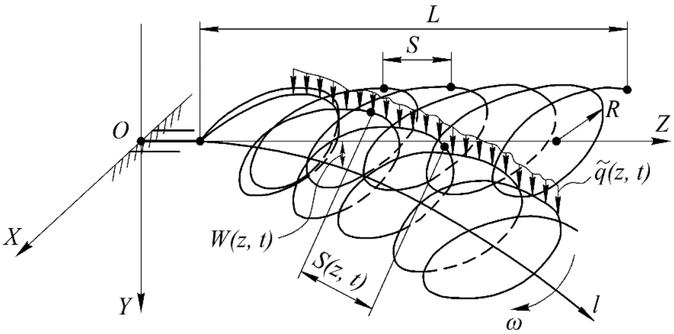


Figure 2. Equivalent schematic model of oscillations of spiral spring in potato heap separator (Nowak et al., 2019).

This investigation of the transverse oscillations of the cantilever cleaning spiral is carried out in the absolute coordinate system *OXYZ*, in which the axis *OZ* is directed along the longitudinal axis of the unstrained spiral, the axis *OY* is directed downwards and the axis *OX* – perpendicular to the plane *OYZ*. The longitudinal axis of the deformed spiral is designated *Ol*.

Thus, there are good reasons to investigate theoretically the process of oscillation of the potato heap cleaning unit spiral considering it as an elastic beam designed in the form of a cylindrical spring with a radius of *R*, a winding pitch of *S* and a helix angle of γ.

It is to be noted already now that under the operating conditions with the load varying along the length of the spiral and in view of the variable properties of the spiral with regard to its compliance to the flexural and torsional deformations, the helix pitch distance parameter *S* becomes variable along the length and this variation is not in accordance with the plane-sections hypothesis.

RESULTS AND DISCUSSION

In order to simplify the investigation at this point and taking into account the rigidity of the spiral, the curved beam is replaced by a cantilever beam with the presented parameters.

Hence, as a first approximation, there is a possibility to describe the flexural oscillations of the spiral (considered, by convention, as a cantilever beam) by the differential equation of the following form (Chelomey, 1981):

$$\frac{\partial^2}{\partial z^2}\left(E \cdot I_R \frac{\partial^2 W}{\partial z^2}\right) + \rho \cdot F \frac{\partial^2 W}{\partial t^2} = \tilde{q}(z,t) \tag{1}$$

where W – deflection of the spiral's longitudinal axis; $\tilde{q}(z,t)$ – distributed and time-variant load of the potato heap on the cleaning spiral; ρ – specific gravity of the spiral's material; F – area of cross section of the cleaning spiral bar stock; E – Young modulus; I_R – reduced moment of inertia of the spiral.

In the classical bending theory, a hypothesis is admitted that plane sections retain their planarity also after the deflection, while the flexural deformation is directed normally to the longitudinal fibres of the beam. That is, for the purpose of simplifying the theoretical investigation in the case under consideration, an assumption is made that pure bending takes place.

The result of solving the differential Eq. (1) is the relations between the deflection of the cantilever beam and its length for the cases of free and forced oscillations (Dolgov, 1996; Grote & Antonsson, 2008).

The next step is to perform the necessary mathematical transformations. For example, the general solution of the differential Eq. (1) can be represented by the following sum of functions:

$$W(z,t) = W^*(z,t) + \tilde{W}(z,t), \tag{2}$$

where $W^*(z,t)$ – general solution of the homogeneous differential equation that has the following form:

$$\frac{\partial^2}{\partial z^2}\left(E \cdot I_R \frac{\partial^2 W}{\partial z^2}\right) + \rho \cdot F \frac{\partial^2 W}{\partial t^2} = 0 \tag{3}$$

$\tilde{W}(z,t)$ – partial solution of the original differential equation with the right-hand member.

In this case, the starting and limit conditions are as follows:
at $z = 0$, $t = 0$,

$$W(z,t) = 0\ ,\quad \frac{\partial^2 W}{\partial z^2} = 0 \tag{4}$$

at $z = L$,

$$E \cdot I_R \frac{\partial^2 W}{\partial z^2} = 0,\quad \frac{\partial}{\partial z}\left(E \cdot I_R \frac{\partial^2 W}{\partial z^2}\right) = 0. \tag{5}$$

The partial solution $\tilde{W}(z,t)$ can be written in the form of a relation between the deflection and the length of the cleaning spiral (Dolgov, 1996; Grote & Antonsson, 2008). Thus, the following is obtained:

$$\tilde{W}(z,t) = \left[q \cdot L^2\left(\frac{z^4}{L^2} - \frac{2z^3}{L} + 6z^2\right)\right] \cdot \left(24E \cdot I_R\right)^{-1} \tag{6}$$

where L – length of the spiral.

The general solution of the homogeneous differential Eq. 3), following the Fourier method, can be written in the form of the product of the longitudinal coordinate function $Z(z)$ and the time function $T(t)$. That is:

$$W^*(z,\ t) = Z(z)\cdot T(t)\cdot \qquad (7)$$

It is assumed that the frequency of the spiral's natural oscillations is *k* times greater than its rotation frequency. In this case, *k* – multiplicity of oscillations. Then, it can be assumed that:

$$T(t) = \cos(\omega k t)\cdot \qquad (8)$$

The first factor in the expression (7) can be written in the form of the Krylov functions (Ananyev, 1965; Dolgov, 1996):

$$Z(z) = AS(\lambda z) + BT(\lambda z) + CU(\lambda z) + DV(\lambda z), \qquad (9)$$

where $S(\lambda z)$, $T(\lambda z)$, $U(\lambda z)$ and $V(\lambda z)$ – special functions introduced into the mechanics by A.N. Krylov; *A*, *B*, *C* and *D* – constants determined by substituting the general solution into the limit conditions; λ – spectrum of oscillation frequency eigenvalues.

Hence, taking into account the expressions (2), (7), (8) and (9), the general solution of the differential Eq. (1) assumes the following form:

$$W = \left[AS(\lambda z) + BT(\lambda z) + CU(\lambda z) + DV(\lambda z)\right]\cdot\cos(\omega\cdot k\cdot t) + \\ + \left[qL^2\left(\frac{z^4}{L^2} - \frac{2z^3}{L} + 6z^2\right)\right]\cdot\left(24E\cdot I_R\right)^{-1}. \qquad (10)$$

Further, it is necessary to determine the reduced second moment of area I_R of the spiral, which is part of the expression (10) and is a value that varies along the length of the spiral, because depends on the angular parameter ψ, which defines the current length of the spiral along its longitudinal axis *z*. In order to find it, the following expression can be used (Svetlinsky, 1967):

$$I_R = \frac{\pi\cdot r^4}{4}\left\{\sin\gamma\left[1 + (1+2\nu)\sin^2\psi\cdot\cos^2\gamma\right]^{-1}\right\}, \qquad (11)$$

where *r* – radius of the bar stock, from which the spiral is wound; ν – Poisson's constant of the material, from which the spiral is made.

In case the angular parameter of the spiral is represented by the following expression:

$$\psi = \psi_o + \omega t = \frac{2\pi\cdot z}{S} + \omega t \qquad (12)$$

where ω – angular velocity of the spiral's rotary motion, the relation between the reduced second moment of area I_R of the spiral cantilever beam, on the one hand, and the length (*z* coordinate) and the time *t*, on the other hand, is obtained at the constant values of the helix pitch distance *S* = const and the angular velocity ω = const, that is:

$$I_R = \frac{\pi\cdot r^4}{4}\left\{\sin\gamma\left[1 + (1+2\nu)\sin^2\left(\frac{2\pi\cdot z}{S} + \omega t\right)\cdot\cos^2\gamma\right]^{-1}\right\}\cdot \qquad (13)$$

Upon carrying out PC-assisted numerical calculations (13), the graphical representation is obtained for the relation between the reduced second moment of area I_R of the spiral and its longitudinal coordinate *z* (Fig. 3).

With the use of the data presented in Fig. 3, the differential Eq. (10) that represents the relation between the process of oscillations of the cantilever spiral and the spiral's design parameters and material properties can be numerically solved.

In view of the fact that the coefficients at the Krylov functions have certain numerical values for each position of the spiral, its PC-assisted solving can be done in the form of diagrams, taking into account the following design, kinematic and force parameters: angular velocity of rotation of the spiral $\omega = 30$ rad s^{-1}; density of the material (spring steel), from which the spiral is made, $\rho = 7{,}700$ kg m^{-3}; elastic modulus $E = 2 \cdot 10^{11}$ Pa; spiral bar stock radius $r = 8.5$ mm; under the action of a uniformly distributed load with an intensity of 1,000 N m^{-1} at the points of time: 1) 0 s; 2) 0.05 s; 3) 0.25 s (Fig. 4, 5).

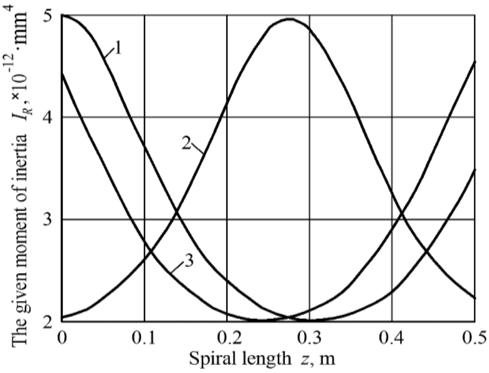


Figure 3. Variation of reduced second moment of area I_R of cantilever beam along length of spiral with spring diameter d = 17 mm, helix pitch distance S = 48 mm, helix angle γ = 7° and angular velocity of rotation ω = 30 rad s^{-1} at points of time: 1) t = 0 s; 2) t = 1 s; 3) t = 2 s.

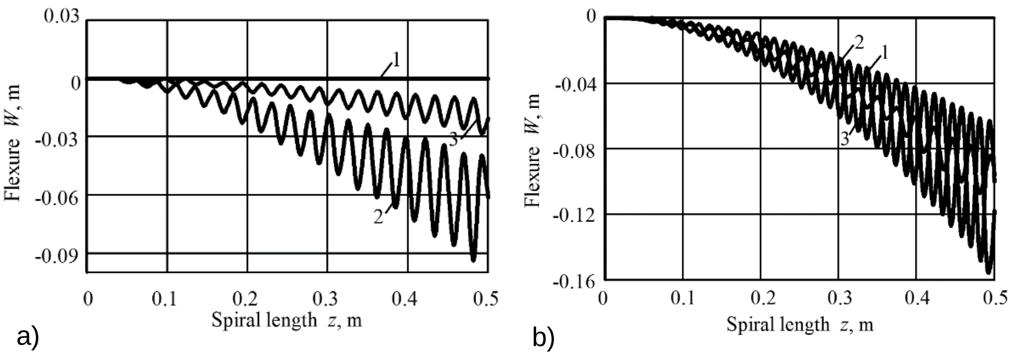


Figure 4. Relation between deflection W of axis at free end of separator spiral and its length z at points of time: 1) t = 0 s; 2) t = 0.05 s; 3) t = 0.25 s: a – in case of free flexural oscillations; b – under action of uniformly distributed load.

The diagram in Fig. 4 a represents the transverse oscillations of the unloaded spiral, which take place under the action of solely the weight of the spiral itself distributed along the longitudinal axis. In this case, the deflection varies from 0 to 0.1 m. Under the action of the distributed load produced by the potato heap (Fig. 4 b), the oscillations take place about the bent axis of the spiral. In this case, the deflection of the spiral axis changes within the range of 0 to 0.16 m.

When both the oscillations of the unloaded spiral and the oscillations under the action of the uniformly distributed load are taken into account, the spiral axis deflection varies from 0 to 0.25 m.

In order to determine the variation of the cleaning spiral pitch distance *S* in the process of its deformation during oscillations, the spiral's flexure geometry is to be analysed in detail. For that purpose, the equivalent schematic model of the spiral axis bending is to be generated (Fig. 6). It is assumed that, conventionally, the spiral flexure can be analysed considering it as an arc with a respective curvature.

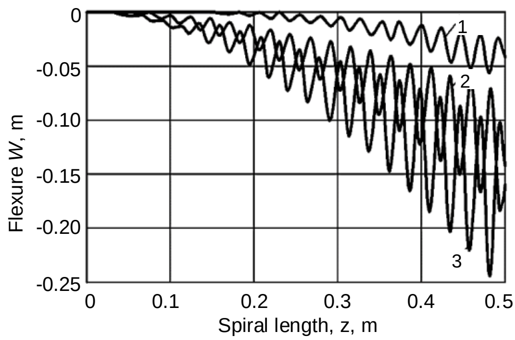


Figure 5. Relation between total deflection *W* of axis of spiral and its length *z* in case of natural flexural oscillations and under action of uniformly distributed load: at points of time: 1) $t = 0$ s; 2) $t = 0.05$ s; 3) $t = 0.25$ s.

For the purpose of describing the longitudinal and transverse deformation of the spiral, the plane Cartesian coordinate system *OZY* is introduced with its origin (point *O*) situated at cantilever spiral attachment point, its *OZ* axis directed horizontally to the right and the *OY* axis directed downwards. At the same time, it is assumed that the longitudinal axis of the unstrained (straight) spiral is aligned with the axis *OZ*, the longitudinal axis of the bent spiral is aligned with the curve *Ol*. In order to investigate the longitudinal (extension) and transverse (bending) deformation of the spiral axis, two arbitrary adjacent turns of the straight spiral can be taken. The turn on the left is assigned the number *i*, the turn on the right – the number $i + 1$, while the centre of the *i*-th turn is the point A_0, the centre of the $i + 1$-th turn – the point B_0, both situated on the *OZ* axis with the coordinates $A_0(z_i; 0)$ and $B_0(z_{i+1}; 0)$, respectively. It is obvious that the distance between the points $A_0$and B_0 is equal to $A_0B_0 = z_{i+1} - z_i = S$, where *S* – helix pitch distance of the straight spiral, S = const.

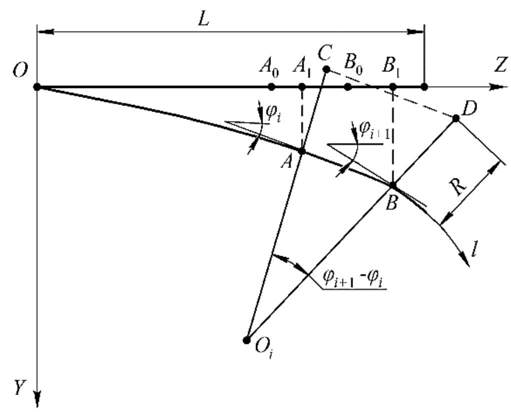


Figure 6. Equivalent schematic model of cantilever elastic spiral bending.

Further, the longitudinal deformation and then the transverse deformation of the spiral have to be analysed. Following the longitudinal deformation of the spiral, the point A_0 will move along the *OZ* axis to some point A_1, the point B_0 – to some point B_1 with the coordinates $A_1(z_i + \Delta_i); 0$ and $A_1(z_{i+1} + \Delta_{i+1}; 0)$, respectively, where Δ_i and Δ_{i+1} – displacements of the points A_0 and B_0, respectively, caused by the longitudinal deformation of the spiral. Following the transverse deformation of the spiral, the point A_1 will move, due to the deflection, to the point *A*, the point B_1 – to the point *B*. These points are situated on the curved axis *Ol*, and also, the point A_1 is displaced along the axis *OY* by the amount of deflection W_A, the point B_1 – by the amount of deflection W_B.

Thus, the points A and B will become the centres of the turns with the numbers i and i + 1, respectively, after the simultaneous longitudinal and transverse deformations, and their coordinates will be $A(z_i + \Delta_i;\ W_A)$ and $B(z_{i+1} + \Delta_{i+1};\ W_B)$, respectively.

The distance between the points A and B is determined as follows:

$$AB = \sqrt{(Y_B - Y_A)^2 + (Z_B - Z_A)^2} = \sqrt{(W_B - W_A)^2 + (S + \Delta_{i+1} - \Delta_i)^2}. \quad (14)$$

The curve that passes through the points A and B, represents the flexure of the spiral's longitudinal axis. Tangents to the curve have to be drawn at these points. The angles between the tangents and the horizontal are φ_i and φ_{i+1}, respectively, and they represent the angular displacement of the cross-sections with respect to their initial positions. The angular displacements of the cross-sections are found with the use of the following expressions (Ananyev, 1965):

$$\varphi_i = \arctan\frac{\partial W}{\partial z} \text{ at } z = Z_A; \quad \varphi_{i+1} = \arctan\frac{\partial W}{\partial z} \text{ at } z = Z_B. \quad (15)$$

Assuming that the normal lines drawn through the points A and B meet at the point O_i, the distances from that point to the points A and B are the curvature radii of the curve *AB* at the points A and B, respectively.

Further, the following designations are introduced: $O_iA = \rho_i$, $O_iB = \rho_{i+1}$. Then, according to (Ananyev, 1965), the following is arrived at:

$$\rho_i = \frac{1}{\dfrac{\partial^2 W}{\partial z^2}} \text{ at } z = Z_A, \qquad \rho_{i+1} = \frac{1}{\dfrac{\partial^2 W}{\partial z^2}} \text{ at } z = Z_B \quad (16)$$

As is seen in Fig. 6, the angle between the above-mentioned curvature radii is equal to $\varphi_{i+1} - \varphi_i$.

If the normal lines O_iA and O_iB are extended by the length of the spiral's radius $R = AC = BD$, that will result in obtaining the final (upper) points C and D of the deformed i-th and i + 1-th turns, respectively, of the operating (separating) surface, on which the process material is situated. As is seen in Fig. 6, the coordinates of the points C and D in the assumed frame of reference are equal to:

$$\begin{aligned} Y_D &= W_B - R\cos\varphi_{i+1}, & Y_C &= W_A - R\cos\varphi_i, \\ Z_D &= Z_B + R\sin\varphi_{t+1}, & Z_D &= Z_B + R\sin\varphi_t. \end{aligned} \quad (17)$$

The distance between the points C and D represents the distance between the adjacent turns, i.e. the pitch distance between the turns in the upper part of the spiral after the deformation of the spring.

Then, the said pitch distance *CD* is equal to:

$$CD = \sqrt{(Y_D - Y_C)^2 + (Z_D - Z_C)^2}, \quad (18)$$

or, taking into account the expression (17), the following is obtained:

$$CD = \sqrt{\left[W_B - W_A - R(\cos\varphi_{i+1} - \cos\varphi_i)\right]^2 + \left[S + \Delta_{i+1} - \Delta_i + R(\sin\varphi_{i+1} - \sin\varphi_i)\right]^2}. \quad (19)$$

The problem under investigation presumes the following requirement to the acceptable values of the winding pitch: the potato tubers may not fall through the turn-to-turn spaces. Therefore, it is necessary to ensure that $CD \le [S_{max}]$, where $[S_{max}]$, –

maximum acceptable value of the winding pitch after the deformation of the spiral, which is defined by the geometric parameters of the potato tubers.

Taking into account the above-mentioned in equation and the expression (19), the criterion is obtained for the potato tubers not falling through the turn-to-turn space with due account for the design and kinematic parameters of the cleaning spiral, the material, from which it is made, and the process modes of operation of the potato heap cleaning unit.

By employing the cosine theorem and analysing the triangle O_iCD (Fig. 6), the equivalent expression can be formulated for determining the winding pitch of the spiral during its deformation of flexure with simultaneous extension. Then, taking into account the above-mentioned in equation, the equivalent criterion is obtained for the potato tubers not falling into the turn-to-turn space:

$$CD = \sqrt{(\rho_i + R)^2 + (\rho_{i+1} + R)^2 - 2(\rho_i + R)(\rho_{i+1} + R)\cos(\varphi_{i+1} - \varphi_i)} \le [S_{\max}]. \qquad (20)$$

Thus, the obtained analytic expression (20) makes it possible to model the dynamics of the cantilever tool (cleaning spiral) during its performance of the work process with the chosen design parameters and modes of operation, subject to the requirement of the potato tubers not falling from the separating surface, under the action of a variable load and taking into account the variation of the second moment of area of the elastic cantilever beam with time and along its length.

Taking into account the obtained expressions (4), (5), (10), (15) and (16), it is possible to generate the formulae for determining the angular displacement φ_i of the spiral's cross-section at an arbitrary point z_i on the length of the spiral and the respective curvature radius ρ_i of the spiral's axis at the said point. Thereafter, on the basis of these formulae, it is possible to obtain the specific values of the angular displacement φ_i of the spiral's cross-section and the curvature radius ρ_i at a specific point along the length of the spiral.

Then, at the pre-set radius R of the spiral, it is possible to determine with the use of the formula (20) the pitch distance of the spiral during its flexural deformation simultaneously with the extension along its longitudinal axis. However, it is to be noted that the expressions for finding the first-order and especially the second-order derivatives of the expression (10) that determines the deflection at an arbitrary point along the length of the spiral are rather bulky, therefore, they are better calculated with the use of the PC.

Still, as a result of the completed analysis, it is possible to use the formula (20) for obtaining the maximum permissible helix pitch distance S between the adjacent turns of the spiral subject to the requirement of the potato tubers not falling through the spaces between those turns.

But, in case only a modest load is fed onto the cleaning spiral or its stiffness is sufficiently high, the variation of its pitch distance will be insignificant (due to the small deflection of the spiral's longitudinal axis). In such cases, the oscillations of the spiral will take place without any entrapping of the potato tubers or their falling inside the spiral spring.

Using the potato cleaner that was designed and developed by us, we have conducted experimental field studies involving the new spiral separator for potato tubers on a specially-developed and manufactured potato separator. Fig. 7 shows the overall view of the experimental potato separator.

The experimental studies were carried out in the course of harvesting potatoes from one row of the Ukrainian potato, *Lugovskaya*. The potato field's physical and technological characteristics were as follows: the yield for the potato crop was 40.35 t ha^{-1}. The potatoes were planted in rows with a row interval of 0.7 m. The soil in which the potatoes were planted was black earth with a medium humus content and a medium loam content; the soil's humidity level was between 6–8% in the upper layer, and 12–16% at a depth of 10 cm and deeper; soil hardness at the tuber-carrying level was 0.3–0.5MPa.

Figure 7. The potato separator during the field experiments.

The structural parameters of the spiral separator when the experimental studies were being conducted were as follows: the separator spiral's outer diameter was 133 mm; the pitch of the spiral's helix line was 25°; the diameter of the spiral wire was 17 mm; the step of the spiral's helix was 48 mm; the overlap of the adjacent spirals was 6–8mm; the minimum number of spirals was three; and the calculated length of the spiral for a row of those tubers that were being harvested was 500 mm.

The movement mode for the experimental potato separator when digging out potato tubers was chosen in the form of several variants: 0.53; 0.67; 0.83; and 1.11 m s^{-1} (or 1.91; 2.41; 2.99; and 4.0 km h^{-1}, respectively). At each speed of movement, experimental observations were carried out in five repetitions.

In the field experiments, second feeds of potato tubers were determined for the cleaning spirals, taking the depth from which to lift the tubers to be 27 cm; the width of the processed field strip was to be 55 cm, and the total mass of the potato heap was set as 1,300 kg m^{-3}.

During the course of the experimental potato separator's movement along the planting row of potatoes, the tuber-carrying layer of the soil was fed into the cleaner's spiral surfaces. Under the influence of the lifted tubers being fed into the spirals, the console ends of the cleaning spirals angled downwards, ie. the longitudinal axes of each cleaning spiral were displaced downwards from their horizontal position by the value of *W*. These displacements, *W*, were measured during the experimental studies and the limits of the movements of the spiral ends were documented for each test.

To conduct the experimental field study in question, a multifactor experiment of type 2^{4-1} was implemented, with a repetition of three for each test.

The experimental tests provided an opportunity to study the effect of several factors on the outcomes of the spiral separator's operation. These involved the angular speed of its cleaning spirals, the eccentricity of its spirals on their drive shafts, the number of lifted tubers that were fed into the cleaner, and also the effect of the downward displacement, *W*, on the ends of the cleaning spirals. The output parameter was the amount of soil that was separated through the cleaning spiral, evaluated as a percentage. For each test in these experimental studies, the experimental potato separator and the tractor pulling it were reset to the initial parameters that corresponded to those factors that were being monitored, and also to the movement speed that was being used. In view of this, samples were taken each time of the planted soil and other additives in the soil mixture that were passing through the cleaning spirals into prepared containers. The samples that were taken were then weighed on electronic scales with a precision of up to 1.0 g. The weighing results were entered into a table.

On the basis of the multifactor experiment that has been conducted, utilising the new spiral potato tuber separator that has been developed by us, statistical calculations were carried out on a PC using the data from the potato separator's performance results, and employing the main positions for correlation and regression analysis, and graphs were drawn up showing the dependencies between the relevant quality indicators.

It was in this way that dependencies were determined for the percentage of soil separation through the separator's cleaning spirals on the value of the downwards displacement *W* of the cleaning spirals (Fig. 8).

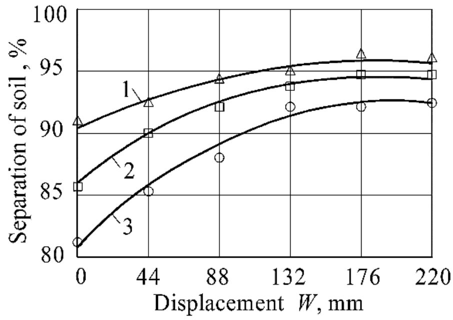


Figure 8. The dependence of soil separation on the displacement *W* of the cleaning spiral ends upon the following moisture contents of the mass that was fed into the separator: 1) 7.0%; 2) 9.5%; and 3) 12.5%.

As can be seen from the graphs of dependencies that are presented here, an increase in the displacement *W* leads to a significant increase of the percentage of soil that is separated through the spiral. This is especially observable when increasing the moisture content in the soil layer that is having its potato tubers fed into the cleaner. In this way, if the soil layer's moisture content is 12.5% and the displacement *W* is at its maximum of 220 mm, the percentage of soil that can be separated through the cleaning spiral increases by 11%. This is explained by the fact that an increase of the displacement *W* in the cleaning spiral ends - and therefore also in the step *S* of those spirals - leads to the effective forced grabbing of soil additives and other soil clumps and their transfer outside the separator's borders. And yet such changes in the step *S* of the cleaning spirals do not lead to any crushing or breaking of the tubers, so there is practically no loss of or damage to potatoes. In the case of the tuber-carrying soil layer being drier, a significant spreading take place of the soil components through the cleaning spirals, along their longitudinal axes if these are horizontal. But if the aforementioned axes are inclined to the maximum displacement *W*, then the percentage of soil that can be separated through the cleaning spirals increases on average by 4–5%.

Therefore the values of the displacements of the cleaning spiral ends as determined by the experimental studies practically coincide with data for the theoretical calculations that was displayed in Fig 5. This means that the theoretically-calculated maximum value for the displacement of a cleaning spiral's end is 0.23m, which corresponds to the experimental data. Consequently, the theory of oscillations that is carried out by the cleaning spirals on a new spiral potato separator corresponds to the actual oscillation process.

CONCLUSIONS

1. The analytical mathematical model of the oscillations performed by the tools of the spiral potato heap separator has been developed, which has allowed to generate the differential equation of the transverse flexural oscillations of its cantilever cleaning spiral.

2. On the basis of solving the differential equation of the transverse flexural oscillations of the cleaning spiral, the analytic expressions that describe the law of the oscillatory process and the flexure of the spiral at an arbitrary point of time for any point on its longitudinal axis have been obtained.

3. Also, analytical dependences have been obtained for determining the variable pitch distance of the bent spiral at an arbitrary point of time and for any turn-to-turn space during the above-mentioned oscillatory process.

4. When the angular velocity of rotation of the spiral is equal to ω = 30 rad s^{-1}, the density of the material, from which the spiral is made, is equal to ρ = 7,700 kg m^{-3}, the elastic modulus is $E = 2 \cdot 10^{11}$ Pa, the radius of bar stock is r = 8.5 mm and the uniformly distributed load of the potato heap on the spiral has an intensity of 1,000 N m^{-1}, the total deflection of the spiral along its length varies within the range of 0 to 0.25 m.

5. The analytic expressions have been obtained for the limitation of the maximum variation of the helix pitch distance of the cleaning spiral during its oscillations subject to the condition that potato tubers may not fall through the turn-to-turn spaces of the spiral, taking into account the design and kinematic parameters of the cleaning spiral, the material it is made of, the process modes of operation and the tuber sizes.

6. The PC-assisted numerical calculations have established that the helix pitch distance of the cleaning spiral with the above-listed parameters and the original helix pitch distance S = 48 mm can vary within the range of up to 54 mm due to the deformation during the considered transverse oscillations, which ensures the potato tubers not falling out of the cleaning unit.

7. The results of the experimental studies that have been conducted in regards to the oscillation process of the cleaning spirals on a new spiral potato separator all serve to confirm the calculated theoretical data.

REFERENCES

Ananyev, I.V. 1965. *Oscillations of elastic systems in airplane structures and their damping*. Moscow, Mechanical Engineering, 526 pp. (in Russian).

Bishop, C., Rees, D., Cheema, M.U.A., Harper, G. & Stroud, G. 2012. *Potatoes.* Crop Post-Harvest: Science and Technology: Perishables. Book Chapter, pp. 179–189.

Bulgakov, V., Nikolaenko, S., Adamchuk, V., Ruzhylo, Z. & Olt, J. 2018a. Theory of retaining potato bodies during operation of spiral separator. *Agronomy Research* **16**(1), 41–51. doi: 10.15159/AR.18.036

Bulgakov, V., Nikolaenko, S., Adamchuk, V., Ruzhylo, Z. & Olt, J. 2018b. Theory of impact interaction between potato bodies and rebounding conveyor. *Agronomy Research* **16**(1). 52–64. doi: 10.15159/AR.18.037

Bulgakov, V., Nikolaenko, S., Adamchuk, V., Ruzhylo, Z. & Olt, J. 2018c. Mathematical model of cleaning potatoes on surface of spiral separator. *Agronomy Research* **16**(4), 1590–1606. doi: 10.15159/AR.18.173.

Bulgakov, V., Pascuzzi, S., Nikolaenko, S., Santoro, F., Anifantis, A.S. & Olt, J. 2019. Theoretical study on sieving of potato heap elements in spiral separator. *Agronomy Research* **17**(1), 33–48. doi: 10.15159/AR.19.073

Chelomey, V.N. 1981. *Vibrations in Technology. Handbook*, 6. Part. Moscow, Mechanical Engineering, 356 pp. (in Russian)

Dolgov, N.M. 1996. Elements of dynamics of systems on movable deformable bases. Kiev: Tekhnika Publishers, 92 pp. (in Russian).

Feller, R., Margolin, E., Hetzroni, A. & Galili, N. 1987. Impingement angle and product interference effects on clod separation. *Transactions of the American Society of Agricultural Engineers* **30**(2), 357–360.

Grote, K.-H. & Antonsson, E. 2008. Springer Handbook of Mechanical Engineering, 1580 pp.

Guo, W. & Campanella, O.H. 2017. A relaxation model based on the application of fractional calculus for describing the viscoelastic behavior of potato tubers. *Transactions of the ASABE* **60**(1), 259–264.

Holland-Batt, A.B.1989. Spiral separation: theory and simulation. *Transactions of the Institution of Mining and Metallurgy, Section C: Mineral Processing and Extractive Metallurgy*, **98**, 46–60.

Ichiki, H., Nguyen Van, N. & Yoshinaga, K. 2013. Stone-clod separation and its application to potato in Hokkaido. *Engineering in AgricultureEnvironment and Food* **6**(2), 77–85.

Krause, F. & Minkin, A. 2005. Research on shaftless screw conveyors. *Bulk Solids Handling* **25**(2), 92–100.

Misener, G.C. & McLeod, C.D. 1989. Resource efficient approach to potato-stone-clod separation. *AMA, Agricultural Mechanization in Asia, Africa and Latin America* **20**(2), 33–36.

Nowak, J., Bulgakov, V., Holovach, I., Olt, J., Arak, M., Ruzhylo, Z., Nesvidomin, A. 2019. Oscillation theory of the free ends of the spiral separator for a potato heap. In: X International Scientific Symposium FMPMSA 2019: *Farm Machinery and Process Management in Sustainable Agriculture*, 157–162. doi: 10.24326/fmpmsa. 2019.1.

Peters, R. 1997. Damage of potato tubers: A Review. *Potato Research* **39**(Spec. Issue), 479–484.

Petrov, G. 1984. *Potato harvesting machines*. Mashinostroenije (Mechanical Engieering), Moscow, 320 pp. (in Russian).

Svetlinsky, V.A. 1967. Mechanics of the rods. 2nd Part: Dynamics, Moscow, Nauka (Science), 304 pp. (in Russian).

Tymoshenko, S.P. 1959. *Oscillations in engineering*. Moscow: Fismatgiz, 327 pp. (in Russian).

Vasilenko, P.M. 1996. *Introduction to agricultural mechanics*. Kiev, Agricultural Education, 252 pp.

Wang, X., Sun, J., Xu, Y., Li, X. & Cheng, P. 2017. Design and experiment of potato cleaning and sorting machine. *Nongye Jixie Xuebao/Transactions of the Chinese Society for Agricultural Machinery* **48**(10), 316–322 and 279.

Wei, Z., Li, X., Sun, C., Liu, W. & Zhang, Y. 2017. Analysis of potato mechanical damage in harvesting and cleaning and sorting storage. *Journal of Agricultural Science and Technology* **19**(8), 63–70. doi: 10.6041/j.issn.1000-1298.2019.01.014

Ye, G., Ma, L., Huang, G., Yu, Y., Sun, X. & Wu, L. 2017. Dynamic and optimization analysis of spiral separator. Meitan Xuebao/*Journal of the China Coal Society* **42**, 479–485. doi: 10.13225/j.cnki.jccs.2017.0271

Agronomy Research **18**(1), 53–62, 2020
https://doi.org/10.15159/AR.20.015

Influence of row spacing on canopy and seed production in grain amaranth (*Amaranthus cruentus* L.)

P. Casini* and G. Biancofiore

University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Piazzale delle Cascine 18, IT50144 Florence, Italy
*Correspondence: paolo.casini@unifi.it

Abstract. A new crop recently introduced in Italy is amaranth. Studies involving agronomic techniques on this plant are limited. The aim of the present research was to assess the effect of distance between rows on both seed yield and ground cover in *Amaranthus cruentus* L. Sowing treatments included two single row spacing designs (18 and 60 cm) and one double row spacing design (18 + 60 cm). At the six true leaf stage, in the single row design of 60 and 18 cm row spacing, ground cover was 16% and 47% respectively. An intermediate coverage of 31% was evident in the double rows. At the ten true leaf stage, plants cultivated in single rows at 18 cm covered the ground early, thereby attaining a ground cover of 85%. Regarding yield, a seed production of 0.92 t ha^{-1} was obtained from plants in the double row design compared to the respective single row spacing designs of 18 and 60 cm, where yields were 0.85 and 0.70 t ha^{-1} respectively. The selection of one mode of sowing over another will largely depend on the type of equipment available to the farm. Whilst single row spacing distances of 18 cm displayed a net of advantage against weeds, difficulties were encountered in the case of managing weeds by mechanical equipment. The use of double rows permitted taking advantage of a slightly better ground cover than single rows, together with the possibility of mechanical intervention for the control of weeds, and importantly also provided a higher yield.

Key words: grain amaranth, *Amaranthus cruentus*, row spacing, Central Italy.

INTRODUCTION

The globalization and industrialization of agriculture on a worldwide scale has led to a number of deep changes in the sector, many of which have negative effects. These include the development of technologies geared towards crops with a high demand for fertilizers, water and pesticides (herbicides, fungicides and insecticides) and, therefore, a high-energy demand. Moreover, the widespread diffusion of monocultures over time has resulted in a significant reduction in biodiversity, also within the agricultural sector, as well as problems of soil fertility and weed- and pathogen-induced resistance.

Within the framework of challenges facing Italian agriculture, particularly that of the hilly areas of Central Italy, traditional industrial food crops are increasingly less attractive from an economic point of view (Casini & La Rocca, 2012). Farmers are faced with difficulties in selecting crops that secure an income. Moreover, given that the average size of the farms is limited, the choices and interests of farmers are oriented towards to 'new crops' to secure a market outlet. These market outlets are not only reflected in products for celiac patients, but also in pharmaceutical, herbal and nutraceutical products in general. Currently, research interest in these crops, relating to both resurgence and enhancement, is now a strongly acquired trend in both scientific and economic sectors.

New seed crops, recently introduced in Italy, include quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus cruentus* L. and *Amaranthus hypochondriacus* L.). Although a small supply chain production has already been initiated for quinoa, the situation for grain amaranth is still at the starting point (Lovelli et al., 2005; Rivelli et al., 2008; Casini & La Rocca, 2015). The most common studies conducted thus far are related to the adaptability of different species of amaranth, as well as genotype characterization (Ercoli et al., 1987; Massantini et al., 1987; Alba et al., 1997; Casini et al., 2012; El Gendy et al., 2018). Studies involving agronomic techniques are limited (Ercoli et al., 1987; Casini & La Rocca, 2014; Pulvento et al., 2015).

Research conducted on different row space distances and in different climatic conditions has produced contrasting results. In some cases, variable distances ranging from 30 and 76 cm did not produce any significant effect on seed yield (Robinson 1986; Putnam 1990; Kauffman, 1992; Tracey et al., 2000; Nurse et al., 2016). In other studies, production increases and decreases were attained depending on row space differences, often also in relation to density or the cultivation environment (Misra et al., 1985; Svirskis, 2003; Rotich et al., 2017). Yield increases were reported with narrow row spacing ranging between 30 and 36 cm (Endres 1986; Jamriška 1998; Chaudhari et al., 2009; Olofintoye et al., 2011), whereas a decrease was reported by Misra et al. (1985).

No specific studies have yet been carried out in Italy. Initial research included the use of variable row space distances ranging from 50 to 70 cm. For the distance set at 50 cm, different plant densities were investigated and preliminary results indicated that the best yields were obtained with 30 and 60 plants m^{-2} (Casini & La Rocca, 2014).

Unlike in conventional agriculture, the use of double row spacing is very common in organic agriculture. In the latter, particularly for winter cereals, double row spacing is technique that facilitates the mechanical control of weeds, and it derives from an ancient technique, widespread in Italy when weeds were removed manually.

The aim of the present research was to assess the effect of row spacing (single or double) using the best plant density as observed by previous trial (Casini & La Rocca, 2014) either seed yield or ground cover in *Amaranthus cruentus* L. in Central Italy.

MATERIALS AND METHODS

A field experiment was carried out in 2019 in Tuscany, Central Italy at the ‘Centro per il Collaudo ed il Trasferimento dell’Innovazione di Cesa (Arezzo)’ (43°18’ north; 11°47’ east, 246 m asl), on a neutral, loamy-sandy soil. The physical and chemical characteristics of the soil (depth of 20 cm) were as follows: sand 36.2%, loam 37.9%, clay 25.9%, total N 0.121% and P (Olsen) 13 ppm. Exchangeable Ca, Mg and K, were 4,180, 641 and 142 ppm, respectively. Meteorological data were recorded trough SIAP automatic equipment controlled and validated by the Regional Hydrological and Geological Sector.

The experiment was carried out in rain fed conditions according to a Randomized Complete Block (RCB) design with five replicates. Sowing was performed on May 8. Sowing treatments (Fig. 1) included two single row spacing designs (18 and 60 cm) and one double row spacing design (18 + 60 cm). Seeding rate was 8.3 kg ha^{-1}. In order to attain a planting density of 25 plants m^{-2} (close to the optimal evaluated in previosus trials in the same area) seedlings were thinned at the two true leaf stage. Plots were 7 m long and the number of rows differed according to the different row spacing designs. Plots with 18 and 60 cm single row spacing had sixteen and four rows, respectively. The plots with double rows had eight rows: four pairs of rows spaced 18 cm apart and separated by 60 cm. Harvested rows were the middle 12 rows within the 18 cm plots and two rows in the both the 60 cm and the 18+60 cm plots, respectively. For the purpose of collecting the seed from an identical surface for all layouts of row spacing equivalent to 7.8 m^{-2}, the length of the rows in the test area corresponded to 3.5, 6.6 and 5.0 m, respectively for single rows, and 18 and 60 cm for double rows.

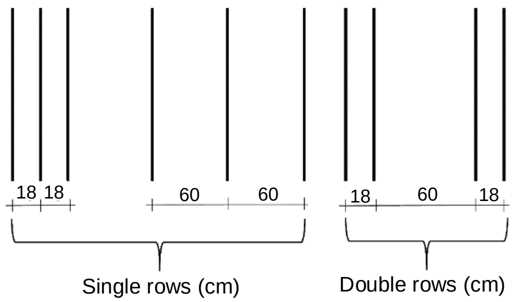


Figure 1. Layout of the row spacing utilized in the experiment.

A-61, a new breeding line of *Amaranthus cruentus* L., obtained by the University of Florence, was used. The line was obtained through a poly-cross between Mexican genotypes: PI-614882 as the female parent, and AMES-13729 and AMES-13734, respectively, as the male parents.

Fertilizer treatment before seeding was as follows: 76 kg ha^{-1} of N as ammonium nitrate, and 100 kg ha^{-1} of P_2O_5 as superphosphate. Plots were hand-weeded twice (38 and 52 Days After Emergence [DAE]) during the growth cycle. The incidence of sugar beet flea beetle (*Chaetocnema tibialis* (Illiger, 1807), was estimated at the two, four, and six true leaf stage. Immediately after the last estimation, the seedlings were treated with the insecticide, deltamethrine (50 mL 100 L $water^{-1}$).

Visual ground cover and the date of phenological plant stages, expressed as DAE, were recorded corresponding to emergence of the two, four, and six true leaf stages, respectively. Early panicle appearance, full panicle appearance, early flowering, milky maturation, waxy maturation and maturation at 75% were similarly recorded. For the maturation stage, seed consistency was taken in consideration together with complete filling (non-translucent endosperm). The final plant density was determined by counting plants in the sample area. The harvest was performed manually starting from September 18. Seed humidity at harvest was recorded on a 100 g sample for sampling area. After drying the seeds to a standard humidity of 12%, (airflow at 35 °C for 48 h), yield calculations were performed. Row spacing were considered as a factor with fixed effects in the ANOVA model. Differences between means were tested utilized Tukey test at $P \leq 0.05$, $P \leq 0.01$ or $P \leq 0.001$. COSTAT 6.45 software was used for the statistical analisys.

RESULTS AND DISCUSSION

Fig. 2 shows the climatic trends during the test period. Average minimum and maximum temperatures were 11.8 and 26.6 °C, respectively. Rainfall, that occurred in both the second and third ten days of April led to a delay in the sowing period compared to that considered optimal for the area. During the month of May, when the crop was at the two-four true leaf stage, temperatures ranged from 6.5 and 18.0 °C (below average monthly levels) with a rainfall of 127 mm. These environmental conditions led to a delay in plant development, thereby resulting in a delay of the physiological parameters characterizing the species in this phase of development. The last 10 days of July was characterized by both heavy and abundant rainfall, attaining a level of 233 mm of the total of 552 mm recorded in April-September period.

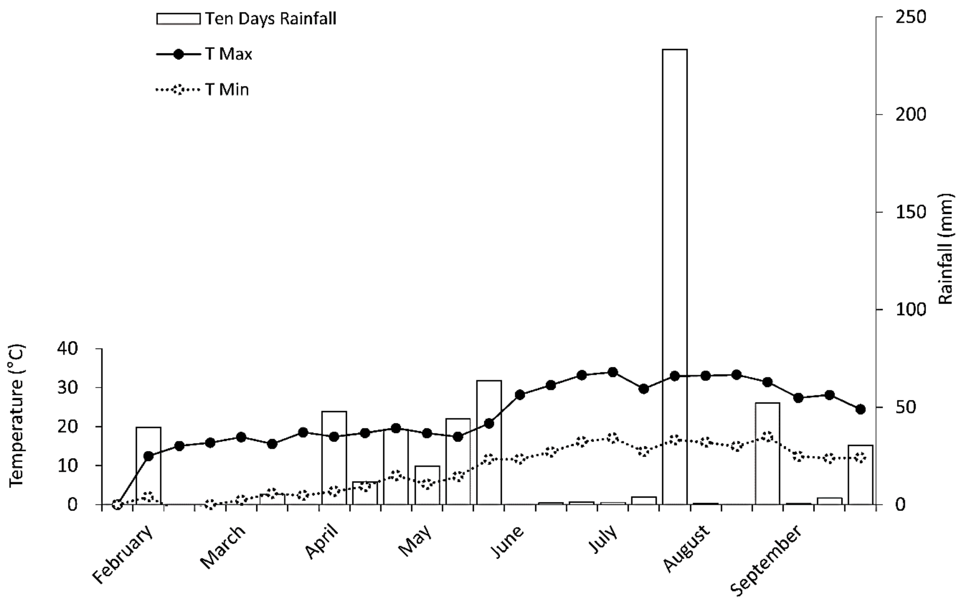


Figure 2. Temperature and rainfall recorded during the field experiment.

The analysis of variance (ANOVA) highlighted significant differences between the sowing designs, both in terms of different phenological phases and ground cover, respectively (Tables 1 and 2).

By analyzing in detail the phenological phases (Fig. 3), significant differences ($P \leq 0.05$) were reported from the beginning of panicle formation, i.e. coinciding with the initiation of the fastest growth phase in amaranth. This phase, characterized by physiology of the plant and the increase in average daily temperatures, occurred with a slight delay of about 5 d for all developmental phases in plants sown in single rows with a spacing of 18 cm. Evidently, intraspecific competition delayed development to full maturity in accordance with Henderson et al. (2000). The maturation of plants in the single row design of 18 cm, occurred at 127 DAE compared to 122 DAE for the remaining two experimental designs, respectively.

Table 1. Analysis of variance of the growth stages

Source of variation	DF	Four true leaves	Six true leaves	Ten true leaves	Early panicle	Panicle	Flowering	Milky maturity	Waxy maturity	Maturity
Blocks	4	0.40 ns	1.06 ns	1.07 ns	6.67 ns	6.67 ns	6.66 ns	6.40 ns	4.40 ns	3.73 ns
Row spacing	2	0.13 ns	1.73 ns	1.73 ns	32.93*	32.93*	32.93*	19.1*	21.73*	48.53***
Error	8	1.20	2.93	2.93	23.73	23.74	23.73	12.80	17.60	7.47
Total	14	1.73	5.74	5.74	63.33	63.33	63.34	38.4	43.73	59.73

ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$.

Table 2. Analysis of variance of the ground cover recorded at different growth stages

Source of variation	DF	Four true leaves	Six true leaves	Ten true leaves	Early panicle	Panicle	Flowering	Milky maturity	Waxy maturity	Maturity
Blocks	4	10.01 ns	56.67 ns	106.67 ns	93.33 ns	93.34 ns	6.66 ns	6.40 ns	4.40 ns	3.73 ns
Row Spacing	2	13.34 ns	2403.33***	5063.34***	2653.34***	2653.33***	32.93*	19.1*	21.73*	48.53***
Error	8	20.00	63.33	353.33	146.67	146.66	23.73	12.80	17.60	7.47
Total	14	43.33	2523.33	5523.32	2893.33	2893.34	63.34	38.4	43.73	59.73

ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$.

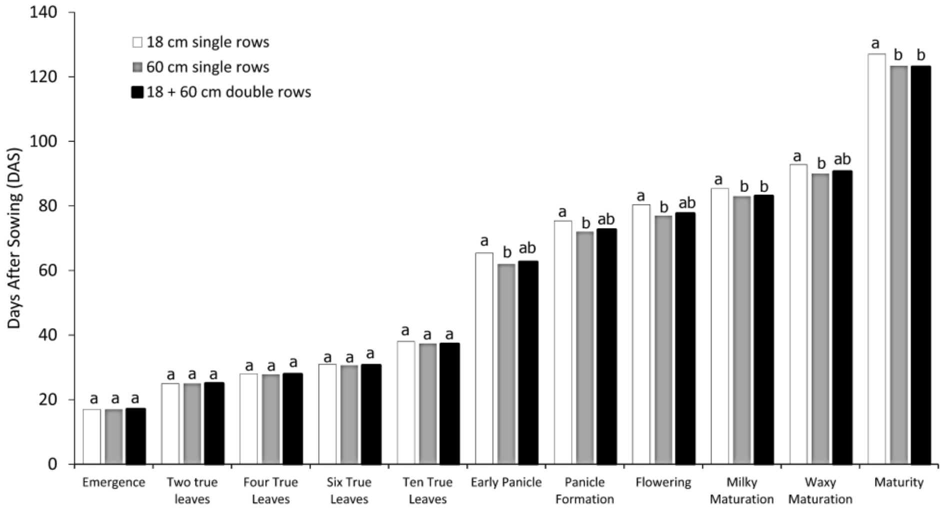


Figure 3. Date of the main phenological phases according to row spacing. Means within followed by same letter(s) are not different for $P \leq 0.05$ for the Turkey test.

The results pertaining to the ground cover (Fig. 4) permitted us to make some interesting observations. Starting from the six true leaf stage, prior to the rapid growth phase, there was a significant difference in ground cover between the different experimental row spacing designs. In the single row design of 60 and 18 cm row spacing, ground cover was 16% and 47% respectively. An intermediate coverage of 31% was evident in the double rows. In the subsequent growth phases of the crop, ground cover of the plants was similar in both the double row design and single rows with a spacing of 60 cm. In contrast, plants in the 18 cm row spaces exceeded an average of 28% ground cover up until maturity.

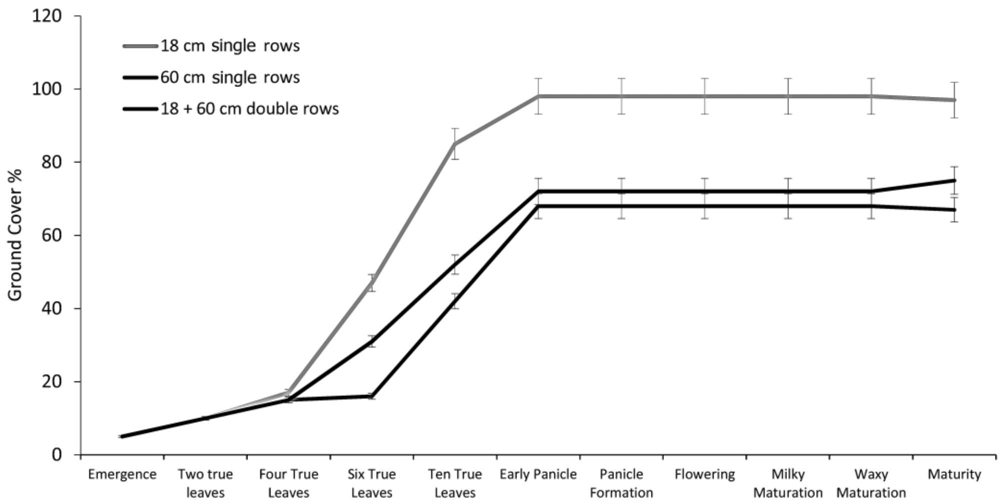


Figure 4. Ground cover according to the row spacing. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $P \leq 0.05$.

This behavior may be of particular importance to an early successful competition of amaranth against weeds, as was also reported by Gelinas & Seguin (2008). Prior to the ten true leaf stage, plants cultivated in single rows at 18 cm covered the ground early, thereby attaining a ground cover of 85% by the ten true leaf stage. This was significantly higher than the ground cover attained by plants in the double rows and single rows at 60 cm spacing, which was 52% and 42%, respectively. The present results corroborated previous findings (Gelinas & Seguin, 2008; Nurse et al., 2016), which also highlighted how reduced row spacing distances, despite the excellent competition exerted against weeds, manifested problems in the use of mechanical equipment as well as lodging where plant density was too high.

The different sowing designs did not influence diversification of sugar beet flea beetle attack (Table 3) which affected approximately 7–8% of the plants in the early stage of the crop (two true leaf stage) and then 50% at the six true leaf stage, at which point we intervened with deltamethrine treatment. The incidence rates of this insect are in accordance with that reported previously in different amaranth trials conducted in the same area (Casini & La Rocca, 2012; 2014; 2015). In Table 3, a significant effect on the height of amaranth at harvest was reported. Aside from the general reduction in crop development, attributable to environmental conditions, plants in the single rows of 18 cm were 20% shorter than those in the remaining two seeding designs. This effect is assumed to be due to the intraspecific competition mentioned previously, which reduced panicle development by 34%, compared to the remaining experimental designs.

Table 3. Incidence of sugar beet flea beetle, plant height, panicle length and plant density at harvest as affected by row spacing

	Sugar Beet Flea Beetle Incidence, %					
Row Spacing	Two true leaves	Four true leaves	Six true leaves	Plant height, cm	Panicle length, cm	Plant density, n m^{-2}
18 cm Single Rows	8 [a]	49 [a]	48 [a]	85.6 [b]	21.2 [b]	11.6 [b]
60 cm Single Rows	7 [a]	50 [a]	47 [a]	113.4 [a]	26,2 [b]	17.9 [a]
18+60 cm Double Rows	7 [a]	49 [a]	49 [a]	110.9 [a]	25.0 [ab]	17.3 [a]

Means followed by the same letter(s) are not different for $P \leq 0.05$ according to the Tukey test.

At harvest, a lower plant density of 11.6 plants m^{-2} in single row spacing of 18 cm was observed compared to the other two treatments reporting a density of 17.3–17.9 plants m^{-2}. The present results corroborated previous work by Myers (1996) using lower row spacing distances. That author attributed the low density to a 'self-thinning' put in place by amaranth when cultivated under conditions with more reduced row spacing distances.

The average seed moisture content at harvest (Fig. 4) was higher (23%) that the standard (12%) required for proper storage. The values obtained in the present study are similar to the averages observed in previous years in other amaranth trials carried out in the same period and area (Casini & La Rocca, 2012; 2014; 2015). However, seed humidity, derived from plants cultivated at a single row spacing of 18 cm (23.5%), was significantly higher than that recorded for the double rows. This may be attributable to the better distribution of plants in double rows with 60 cm spacing, which permitted improved air circulation.

Regarding yield, a seed production of 0.92 t ha^{-1} was obtained from plants in the double row design compared to the respective single row spacing designs of 18 and 60 cm, where yields were 0.85 and 0.70 t ha^{-1} respectively. Given that experiments using close double rows in amaranth were not found in the literature, it was not possible to make comparisons with the present results. A row spacing similar to the one used in this experiment, was adopted by Svirskis (2003) in Lithuania. This author reports that grain yield is higher in double rows spaced 50 cm apart at 100 cm compared to wider rows or arranged in four row strips 50 cm always spaced 100 cm apart.

Nonetheless, it has been noted that better yields have been obtained when row spacing was reduced (Jamriška 1998; Chaudhari et al.; 2009 and Olofintoye et al., 2011). In the present experiment, the double rows, though spaced by 60 cm, were very close (18 cm). Within the single row experiments, yield was higher at a row spacing of 18 cm than 60 cm.

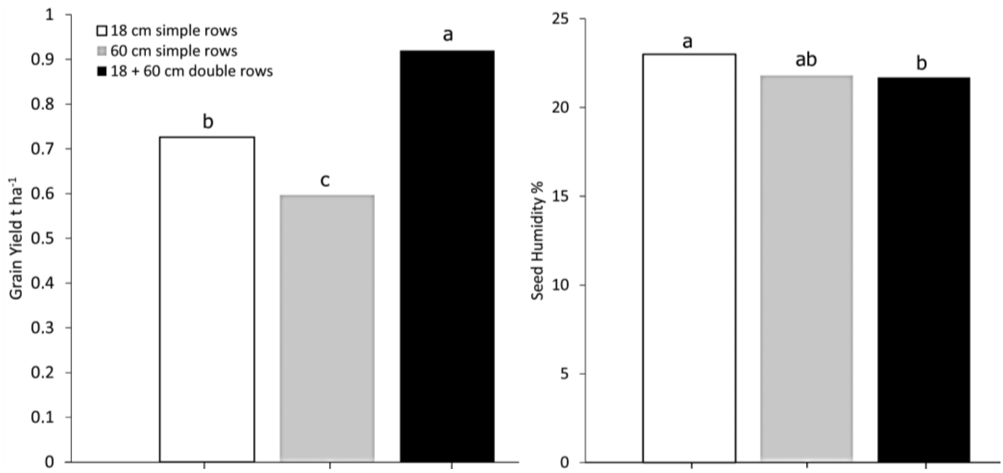


Figure 5. Seed humidity at harvest and grain yield production. Means followed by the same letter(s) are not different for $P \leq 0.05$ according to Tukey test.

CONCLUSIONS

The results of the present trial, together with previous scientific reports, confirm amaranth as a species with considerable morphological plasticity. This feature enables the plant to manifest physiological and yield responses in function of row spacing despite environmental factors that also induce variable responses to different agronomic techniques (Gelinas & Seguin, 2008). Although yield is a major factor worthy of consideration, in order to be able to objectively assess the results of our research, additional aspects also require consideration. Firstly, the rapid ground cover manifested in the 18 cm single row spacing distance is interesting for a species like amaranth, characterized by a slow growth up until the six to ten true leaf stage. In the latter seeding design, a ground cover of 85% was attained early in the development, ensuring not only high competition against weeds, but also better potential soil erosion control (Weber, 1990). In some environments, this feature also facilitated faster dry-down and seed yield

(Wall, 1986). In fact, amaranth, even at the end of its reproductive cycle, has a tendency to dry very slowly. This behavior often forces to harvest seeds with high humidity (> 20%). In turn, additional costs must be incurred to dry the seeds to the standard 12%, considered suitable for safe storage.

The use of reduced row spacing distances requires careful selection in plant density. A high plant density may be associated with an excessive intraspecific competition and an increase of lodging (Myers, 1996; Henderson et al., 2000).

Currently no herbicides use on amaranth has been authorized; therefore, weed control must be performed mechanically. The use of very narrow row spacing greatly hinders mechanical interventions and, in order to overcome this difficulty, wider row distances must be employed (Sooby et al., 2005). Alternatively, it could be carry out a ‘false seeding’ to significantly reduce the weed seed stock before carrying out the amaranth sowing at proper date. However, the use of single row spacing distances of 60 cm resulted in a lower seed yield than that of the rows of 18 cm, while the use of double rows (18 + 60 cm) allowed the attainment of the best yield (0.92 t ha^{-1}).

Whilst single row spacing distances of 18 cm displayed a net of advantage against weeds, difficulties were encountered in the case of managing weeds by mechanical equipment. In this regard, the use of double rows appears to be a good compromise for farmers who can then also use equipment that can be used for other crops sown in rows (corn, sunflower, etc.). The use of double rows permitted taking advantage of a slight better ground cover than single rows, together with the possibility of mechanical intervention for the control of weeds, and importantly also produced a higher yield. The influence of climatic trend on both production and weed competition remains to be verified through additional experimentation.

ACKNOWLEDGMENTS. A special thanks to all the staff of the ‘Centro per il Collaudo ed il Trasferimento dell’Innovazione di Cesa (Arezzo)’ for the competent and enthusiastic cooperation in conducting the experimental trail.
FUNDING. This study was supported by Tozzi Green S.p.A., Italy.

REFERENCES

Alba, E., Polignano, G.B. & Notarnicola, L. 1997. Yield stability in a set of *Amaranthus* entries in Souther Italy. *Ital. J. Agron*. **1**, 65–71.

Casini, P. & La Rocca, F. 2012. Amaranto: pseudocereale dai molteplici utilizzi. *L’Informatore Agrario* **39**, 57–60.

Casini, P. & La Rocca, F. 2014. *Amaranthus cruentus* L. is suitable for cultivation in Central Italy: field evaluation and response to plant densities. *Ital. J. Agron*. **9**, 602, 166–175. doi: 10.4081/ija.2014.602

Casini, P. & La Rocca, F. 2015. Amaranto, manuale per la coltivazione, 1st ed., LoGisma, Vicchio, Italy, pp. 104.

Chaudhari1, J.H., Raj, V.C., Srivastava, R.K. & Ahir, M.P. 2009. Effect of varying sowing date and row spacing on yield attributes and yield of *rabi* grain amaranth (*Amaranthus hypochondriacus* L.) under south Gujarat conditions. *Agric. Sci. Digest* **2**(29), 65–68.

El Gendy, A.N.G., Tavarini, S., Conte, G., Pistelli, L., Hendawy, S.F., Omer, E.A. & Angelini, L.G. 2018. Yield and qualitative characterisation of seeds of *Amaranthus hypochondriacus* L. and *Amaranthus cruentus* L. grown in Central Italy. *Ital. J. Agron*. **13**(1), 63–73.

Endres, C.S. 1986. Influence of production practices on yield and morphology of *Amaranthus cruentus* and *Amaranthus hypochondriacus*. *M.S. Thesis, Univ. of Arkansas*, Fayetteville.

Ercoli, L., Masoni, A. & Massantini, F. 1987. Influenza della fertilizzazione azotata sulla produzione di proteine da amaranto e chenopodio attraverso il frazionamento umido. *Agric. Med.* **117**, 131–139.

Gélinas, B. & Seguin, P. 2008. Evaluation of management practices for grain amaranth production in Eastern Canada. *Agron. J.* **2**(100), 344–351.

Henderson, T.L., Burton, L.J. & Schneiter, A.A. 2000. Row spacing, plant population, and cultivar effects on grain amaranth in the Northern Great Plains. *Agron. J*. **92**, 329–336.

Jamriška, P. 1998. The effect of variety and row spacing on seed yield of Amaranth (*Amaranthus* ssp.). *Rostlinna Vyroba* **2**(44), 71–76.

Kauffman, C. S. 1992. The status of grain amaranth for the 1990s. *Food Rev. Int*. **8**, 165–185.

Lovelli, S., Pizza, S., Capolnio, T., Gherbin, P. & Perniola, M. 2005. Analisi di crescita e valutazione agronomica di alcune specie erbacee a basso tenore di glutine. Proceedings of 34th Congress of the Italian Society of Agronomy. Foggia, Italy, 20–22 September, 298–299.

Massantini, F., Masoni, A. & Ercoli, L. 1987. Potenzialità agronomiche dell'amaranto e del chenopodio come specie adatte al frazionamento umido: prove biennali. *Agric. Med.* **117**, 31–41.

Misra, P.M., Pal, M. & Pandey, R.M. 1985. Studies on the effect of varying plant density on the growth and yield of grain amaranth (A*maranthus hypochondriacus* L.). *Amaranth Newsl*. **3**, 2–4.

Myers, R.L. 1998. Nitrogen fertilizer effect on grain amaranth. *Agron. J*. **90**, 597–602.

Nurse, R.E., Obeid, K. & Page, E. R. 2016. Optimal planting date, row width, and critical weed-free period for grain amaranth and quinoa grown in Ontario, Canada. *Can. J. Plant Sci*. **96**, 360–366.

Olofintoye, J.A.T., Adeniyi, H.A. & Olagorite, A.A. 2011. Effects of phosphorus fertilizer and intra row spacing on the growth and yield on grain amaranth (*Amaranthus cruentus*). *Agric. J*. **6**(6), 366–368.

Pulvento, C., Lavini, A., Riccardi, M., D'Andria, R. & Ragab, R. 2015. Assessing amaranth adaptability in a Mediterranean area of South Italy under different climatic scenarios. *Irr. and Drain*. **64**, 50–58.

Putnam, D.H. 1990. Agronomic practices for grain amaranth. In *Proc. Natl. Amaranth Symp., 4th Minneapolis*, MN 23-25 Aug. 1990. Minnesota Ext. Serv., Univ. of Minnesota, St. Paul, 151–162.

Rivelli, A.R., Gherbin, P., De Maria, S. & Pizza, S. 2008. Field evaluation of *Amaranthus* Species for Seed and Biomass Yields in Southern Italy. *Ital. J. Agron*. **3**, 225–229.

Robinson, R.G. 1986. Amaranth, quinoa, ragi, tef, and niger: Tiny seeds of ancient history and modern interest. Stn. Bull. AD-SB 2949. *Agric. Exp. Stn.*, Univ. of Minnesota, St. Paul.

Rotich, A.N., Gweyi-Onyango, J.P. & Korir, N.K. 2017. Diagonal offset arrangement and spacing architecture effect on growth and yield components of grain amaranth in Kenya. *Asian Res. J. of Agric.* **6**(1), 1–8.

Sooby, J., Myers, R., Baltensperger, D., Brenner, D., Wilson, R. & Block, C. 2005. Amaranth, production manual for the central United States. *Univ. of Nebraska Coop. Ext.*, Lincoln, NE., pp. 7.

Svirskis, A. 2003. Investigation of amaranth cultivation and utilization in Lithuania. *Agron. Res.* **1**(2), 253–264.

Wall, A.D. 1986. The effects of narrow row planting, high crop densities, and several post-emergence herbicides on the agronomic qualities of four grain amaranth cultivars. *M.S. Thesis, Univ. of Maryland,* College Park.

Weber, L.E. (ed.). 1990. Amaranth grain production guide. Rodale Press, Emmaus, P.A., pp. 13.

Tracey, L.H., Burton, L.J. & Schneiter, A.A. 2000. Row spacing, plant population, and cultivar effects on grain amaranth in the Northern Great Plains. *Agron. J.* **92**, 329–336.

Agronomy Research **18**(1), 63–76, 2020
https://doi.org/10.15159/AR.20.033

Hairy fleabane (*Conyza bonarienis*) response to saflufenacil in association with different formulations of glyphosate subjected to simulated rainfall

G. Dalazen*, A. Pisoni, C. Menegaz and A. Merotto Jr.

Federal University of Rio Grande do Sul, Faculty of Agronomy, Crop Sciences Department, Av. Bento Gonçalves 7712, 91.540-000, Porto Alegre, RS, Brazil
*Correspondence: giliardidalazen@gmail.com

Abstract. Saflufenacil has been used to control glyphosate resistant weeds, including hairy fleabane. There are several glyphosate formulations, which are related to different salts and adjuvants. Differences between these formulations may result in variations in efficacy, especially in the face of unfavorable environmental conditions, such as the occurrence of unexpected rainfall after application. The objective of this study was to evaluate the control of hairy fleabane with saflufenacil in tank mix with different formulations of glyphosate subjected to simulated rainfall after application. The treatments evaluated were salts of glyphosate (isopropylamine, potassium and ammonium salt), different periods of simulated rainfall after herbicide application (30, 120 and 240 min, and no rainfall), and the presence or absence of saflufenacil and a non-ionic adjuvant. Absorption of saflufenacil by hairy fleabane is rapid, since simulated rainfall after 30 min after application did not result in loss of efficiency when the herbicide is applied with adjuvant. The association of isopropylamine and ammonium salts of glyphosate with saflufenacil increases the control of hairy fleabane and prevents the occurrence of regrowth when rainfall occurs at 30 min after the application. The occurrence of simulated rainfall after 240 min reduces the glyphosate efficiency by 30%, 15% and 60% for the isopropylamine, potassium and ammonium salt formulations, respectively. The addition of adjuvant improves the efficiency of glyphosate salt of potassium by 40%. The response of the mixture of glyphosate and saflufenacil is variable, mainly in situations of rainfall after application.

Key words: EPSPS, herbicide absorption, PPO, tank mix, weed resistance.

INTRODUCTION

Hairy fleabane [(*Conyza bonariensis* L. Cronquist (Asteraceae)] is one of the most important weeds in both spring and summer crops, such as soybean and corn. This specie is originated from South America, with annual or perennial life cycle and a great reproductive capacity. Each plant can produce approximately 600,000 seeds (Kaspary et al., 2017), that are easily dispersed by the wind. In addition to *C. bonariensis*, other important *Conyza* species, such as *C. canadensis*, *C. sumatrensis* and *C. albida*, are important weeds in several countries (Mylonas et al., 2014; Okada et al., 2014; Santos et al., 2014).

In addition to their great adaptive capacity to diverse environments and great capacity of reproduction, several populations of *Conyza* are resistant to glyphosate (Heap, 2020). Glyphosate is an inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSPS), which is responsible for the synthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan (Holländer & Amrhein, 1980). It is a non-selective, systemic herbicide used mainly in pre-sowing burndown or post-emergence of glyphosate resistant crops (GR), and also in integrated weed management programs (Vanaga et al., 2006; Duke & Powles, 2008). The glyphosate resistance in species of *Conyza* genus mainly involves non-target-site mechanisms, including reduction of translocation and sequestration of the herbicide in the vacuole, which reduce the herbicide concentration in the chloroplasts of meristem cells (Ge et al., 2011; Kleinman & Rubin, 2017; Moretti & Handson, 2017). However, mechanisms related to the target site have already been verified (Dinelli et al., 2008; Sammos & Gaines, 2014).

Although the occurrence of glyphosate resistant weeds is common, this herbicide remains the most widely used in the world (Viirlaid et al., 2015; Benbrook, 2016), since most of the weed community is mostly sensitive to this herbicide. In addition, glyphosate contributes to the control of resistant weeds, when associated with other herbicides, which, when applied alone, have lower efficiency in comparison with the application in mixture. However, some associations may cause antagonism, reducing herbicide efficiency. This response usually occurs when a contact herbicide, which causes oxidative stress, is associated with glyphosate (Eubank et al., 2013). This is due to the limited absorption and translocation of glyphosate, which is impaired by the rapid action of the contact herbicide (Werlang & Silva, 2002). Combinations of glyphosate with inhibitors of protoporphyrinogen oxidase (PPO), photosystems I and II, or glutamine synthetase (GS), usually result in antagonism (Starke & Oliver, 1998; Vidal et al., 2016).

Saflufenacil has been considered as an alternative for the management of hairy fleabane and other glyphosate-resistant or -tolerant weeds (Geier et al., 2009; Dennis et al., 2016). Although saflufenacil is a PPO inhibitor, its physic-chemical characteristics allow some mobility by xylem and phloem, allowing its association with glyphosate (Ashigh et al., 2010; Grossmann et al., 2010). Even though the hairy fleabane is a glyphosate resistant species, the addition of glyphosate to saflufenacil is necessary to prevent plant regrowth in later stages of development (Dalazen et al., 2015). However, while there are several studies that evaluate the effect of the glyphosate mixture with saflufenacil on the control of several weed species, including *Conyza* (Waggoner et al., 2011; Eubank et al., 2013; Dalazen et al., 2015), information about the effect of the different formulation of glyphosate in mixture with saflufenacil are restricted in the literature.

Glyphosate is commercially available in various formulations, which vary in acid equivalent concentrations, adjuvants and salt type. The addition of a salt to the glyphosate molecule is necessary because of the low solubility of this compound. Glyphosate in its acid form has solubility of 1.54% at 25 °C, whereas formulations with addition of different salts increase the water solubility to more than 90% (Shaner, 2014). The main salts used are isopropylamine, ammonium and potassium, and the efficiency of these formulations varies according to the weed species (Molin & Hirase, 2004; Li et al., 2005; Mueller et al., 2006; Mahoney et al., 2014).

The several formulations of glyphosate have distinct absorption and translocation patterns, which may influence the control efficiency under adverse conditions. The occurrence of rainfall after herbicide application can compromise the weed control, making reapplications necessary in some situations (Pacanoski & Mehmeti, 2019). In addition, the use of adjuvant and variations in formulation may alter the weed control response (Monquero & Silva, 2007; Souza et al., 2014). For glyphosate, periods between one and six hours are required, depending on the factors mentioned previously (Pedrinho Júnior et al., 2002). For PPO inhibiting herbicides, such as saflufenacil, absorption is faster, requiring one to two hours of rain free periods in order to maintain the herbicide efficacy (Grossmann et al., 2010).

In this way, the objectives of this study were: i) to evaluate the effect of different formulations of glyphosate in association with saflufenacil on hairy fleabane control; ii) to determine the effect of simulated rainfall after the application of glyphosate and saflufenacil on hairy fleabane control; iii) to evaluate the effect of adjuvant to the mixture of glyphosate and saflufenacil on hairy fleabane control.

MATERIAL AND METHODS

Plant Material. A hairy fleabane seeds were collected in RS state, Brazil (27° 66' 37" S; 53° 44' 29" W), in a soybean production area with a glyphosate resistant hairy fleabane population, with a resistance factor (RF) of 3.98 (Dalazen et al., 2019). It was decided to use resistant plants, because in Brazil the vast majority of the populations of hairy fleabane are resistant to glyphosate. F2 generation seeds were used in this experiment. Although dormancy in hairy fleabane seeds is not common, to improve germination seeds were immersed in water and kept at temperature of 6 °C, during four days. After, seeds suspended in water were placed in the surface of trays, containing organic substrate, which was sieved and moistened close to the field capacity. The trays were kept at a temperature of 24 °C and a 12/12 hours photoperiod (day/night) until the stage of two leaves. The seedlings (three-leaf stage) were transplanted individually to 250 mL punctured pots, containing substrate produced from the mixture of haplic gleisoil and organic compound (10:1), in addition to NPK fertilizer 5-20-20 at 2.5 g kg^{-1} substrate. The plants were kept in a greenhouse until they reached 15 cm height, at which moment they were submitted to treatments. The environmental conditions inside the greenhouse were 25 °C ± 3 °C temperature and 14/10 hours photoperiod (day/night). The pots were kept in trays with a 0.5 cm layer of water, to keep the substrate moist.

Treatments and Experimental Design. The experiment was repeated twice, during the spring of 2014 and 2015 years, under the same conditions. The experimental design was completely randomized in a factorial scheme, with four replications. The factor A was the different formulations of glyphosate: isopropylamine salt (Roundup Original®, 360 g ae L^{-1}, Monsanto Brazil, São Paulo); potassium salt (Roundup Transorb R®, 480 g ae L^{-1}, Monsanto Brazil, São Paulo); and ammonium salt (Roundup WG®, 720 g ae kg^{-1}, Monsanto Brazil, São Paulo). All the formulations were applied at the dose of 720 g ae glyphosate ha^{-1}. The factor B was formed by 20-mm-rainfall applied at different periods after application of the herbicides (30, 120 and 240 minutes, and no rainfall). The factor C was the presence or absence of saflufenacil (Heat®, 700 g ai kg^{-1}, Basf, São Paulo, Brazil) at 35 g ia ha^{-1}. And factor D was the presence or absence of non-ionic adjuvant at 0.5% v/v (Dash®, mixture of methyl esters, aromatic hydrocarbon, unsaturated fatty acid and 933 g L^{-1} surfactant, Basf, São Paulo, Brazil).

The spraying of herbicides was carried out by an automated spray chamber (Greenhouse Spray Chamber, model Generation III, Devries Manufacturing, Hollandale, MN), pressurized with compressed air at 32 psi, with a Teejet® 8002E nozzle, at velocity of 1.16 m s^{-1}, resulting in a spray volume of 150 L ha^{-1}.

Data Analysis. The evaluations of control were performed at 7, 14, 21 and 28 days after the application of the treatments (DAT) using Frans et al. (1986) scale, in which zero means no control and 100 means total control of the plants. At 28 DAT, the aerial part was harvested to determinate the shoot dry mass (SDM). Plants were maintained in a dryer at 60°C until reaching a constant mass. Data were analyzed for normality (Shapiro-Wilk test) and then subjected to ANOVA by the *F test* ($P < 0.05$) using the softwere Assistat (version 7.7) (Silva & Azevedo, 2006). Percentage control data has been transformed to arcsen (x+1). There was no significant difference between both experiments and data were pooled.

RESULTS AND DISCUSSION

ANOVA indicates the occurrence of interaction among the variation factors (Table 1). A unique pattern of effect was not identified, and the focus of the analysis was related with the target objectives.

Table 1. Summary of variance analysis table (mean squares) for control of *Conyza bonariensis* at 7, 14, 21 and 28 days after the application of treatments (DAT) and shoot dry mass (SDM)

Mean squares						
VF [a]	DF[b]	7 DAT [c]	14 DAT	21 DAT	28 DAT	SDM [d]
Rainfall (R)	3	534 ***	26.71 **	54.65 **	59.33 **	2.15 **
Formulation (F)	3	7718 ***	163.84 **	122.35 **	108.65 **	3.67 **
Saflufenacil (S)	1	227112 ***	1640.87 **	2187.30 **	2503.34 **	77.09
Adjuvant (A)	1	8453 ***	2.93 **	7.31 **	11.62 **	0.80 **
R × F	9	724 ***	3.80 **	8.07 **	8.57 **	0.78 **
R × S	3	817 ***	24.51 **	53.17 **	52.37 **	1.12 **
F × S	3	3701 ***	0.07 [ns]	0.62 [ns]	0.36 [ns]	0.23 [ns]
R × A	3	1311 ***	166.55 **	121.77 **	102.82 **	6.41 **
F × A	3	1146 ***	0.38 [ns]	3.63 **	8.27 **	2.27 **
S × A	1	1575 ***	0.22 [ns]	0.61 [ns]	0.68 [ns]	0.01 [ns]
R × F × S	9	314 ***	3.27 **	7.15 **	7.16 **	0.81 **
R × F × A	9	223 ***	0.34 [ns]	0.65 [ns]	0.59 [ns]	0.36 **
R × S × A	3	138 ***	0.19 [ns]	0.36 [ns]	0.87 [ns]	0.37 **
F × S × A	3	2594 ***	2.12 **	5.32 **	11.93 **	1.92 **
F × R × S × A	9	735 ***	0.31 [ns]	0.81 *	1.15 *	0.57 **
Treatments	63	39.91 **	45.50 **	54.46 **	58.83 **	2.46 **
Residue	192	0.22	0.24	0.35	0.57	0.10
Mean		48.80	61.07	55.44	52.00	1.31
CV (%) [e]		7.27	6.83	8.56	11.27	20.20

** significant at the 1% probability level ($p < 0.01$); * significant at the 5% probability level ($p < 0.05$); [ns] not significant ($p > 0.05$).

[a] VF: variation factor; [b] DF: degrees of freedom; [c] DAT: days after application of treatments; [d] SDM: shoot dry mass; [e] CV (%): coefficient of variation.

Hairy fleabane control at 7 DAT demonstrates the rapid effect of saflufenacil (Fig. 1). Even with the occurrence of rain 30 min after the application, hairy fleabane control was higher than 70%. The addition of adjuvant improved saflufenacil efficiency by 12 to 20%, except when rainfall occurred at 30 min after application (Fig. 1, A). The addition of glyphosate in both isopropylamine (IPA) and potassium (K) salts to saflufenacil increased the control by about 10% (Fig. 1, B, C). However, the addition of the ammonium (NH_4) salt of glyphosate resulted in a control reduction of approximately 15%, when the simulated rainfall occurred at 30 and 120 min after application (Fig. 1, D). For the treatments with absence of saflufenacil, the application of glyphosate K salt resulted in better hairy fleabane control (Fig. 1, C), with greater effect as the interval between the application and the occurrence of simulated rainfall increased. The addition of adjuvant was positive only in this glyphosate formulation, with an increase of 10 to 20% in relation to the application without adjuvant.

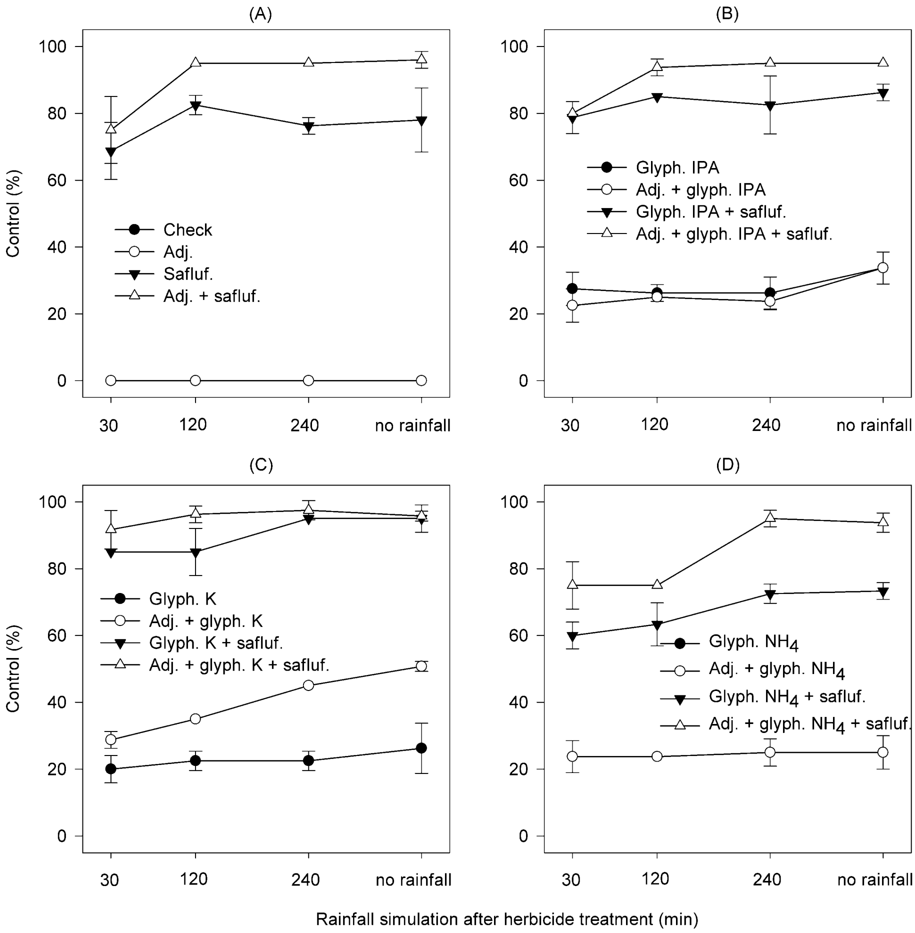


Figure 1. Hairy fleabane control at 7 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

At 14 DAT, the application of saflufenacil with adjuvant resulted in 100% control, regardless the simulation of rainfall after application (Fig. 2, A). However, without the addition of adjuvant, the control was 5 to 10% lower, according to the time of simulated rainfall. The addition of glyphosate IPA (Fig. 2, B) and K (Fig. 2, C) salts to saflufenacil, even without adjuvant, also resulted in satisfactory control, approaching 100%, despite the occurrence of rainfalls after herbicide application. In the case of glyphosate NH_4 salt in mixture with saflufenacil, the control reached 100% only with the addition of adjuvant, except in the occurrence of rain at 30 min after spraying, in which the control was close to 90% (Fig. 2, D). The addition of adjuvant to glyphosate, as at 7 DAT evaluation, only increased the control in the plants treated with glyphosate K salt (Fig. 2, C). The occurrence of simulated rainfall significantly reduced glyphosate efficiency, regardless its formulation. The control reached approximately 65% with absence of simulated rainfall, and it was reduced 10 to 25% with the occurrence of rain 30 minutes after application.

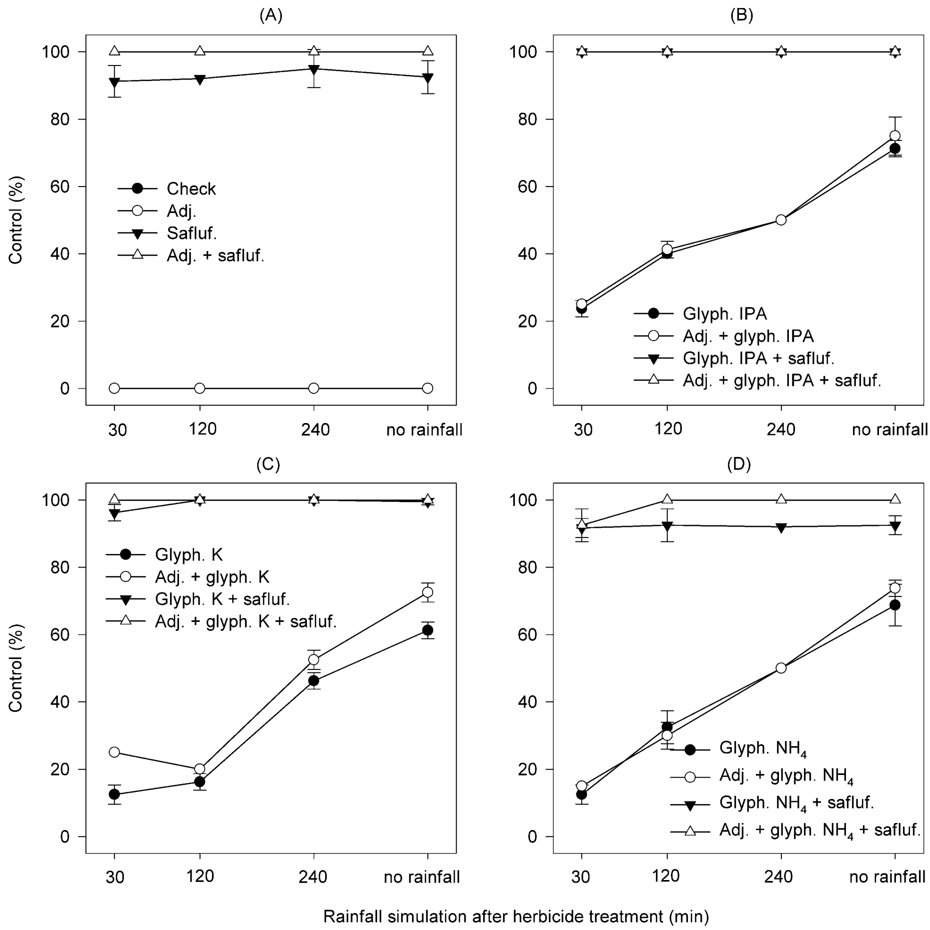


Figure 2. Hairy fleabane control at 14 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

At 21 DAT the control was similar to the observed on 14 DAT (Fig. 3). The addition of adjuvant to saflufenacil resulted in total control of the plants, even with rainfall simulation at 30 min after herbicides spraying (Fig. 3, A). In the treatments that combined saflufenacil with glyphosate, the control was total, independent of glyphosate formulation, addition of adjuvant, and simulated rainfall occurrence (Figs 3, B, C, D).

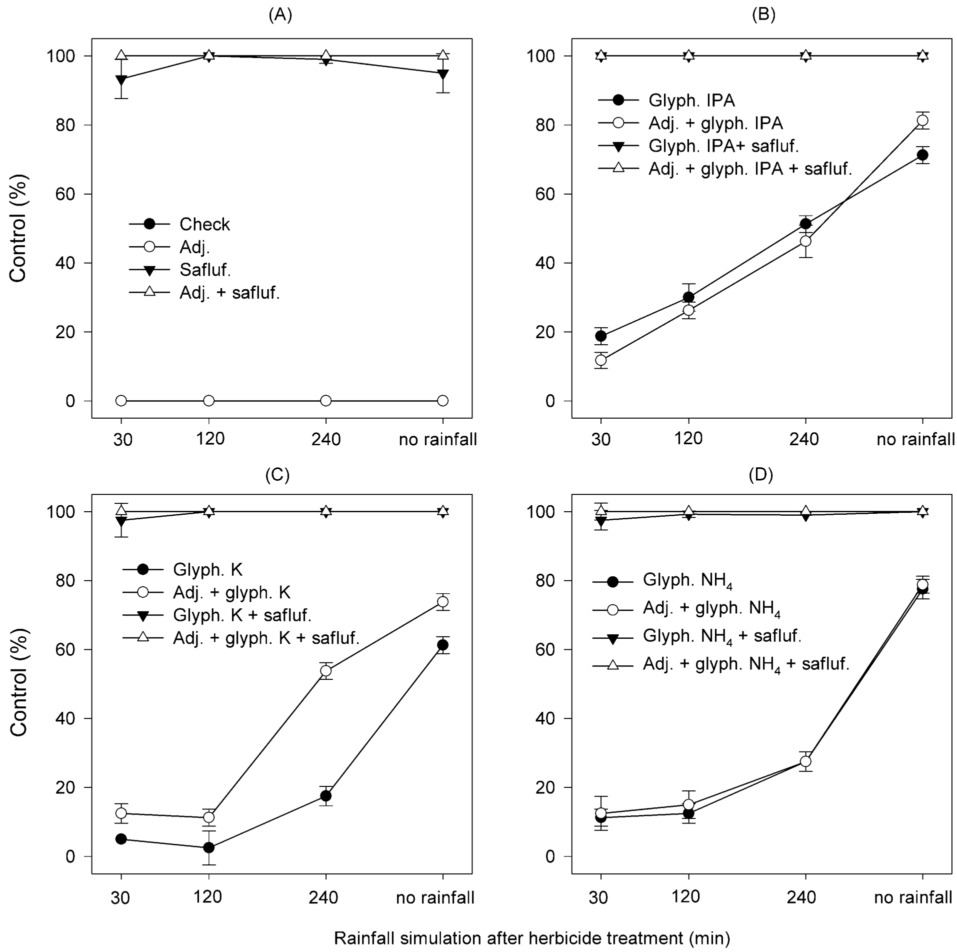


Figure 3. Hairy fleabane control at 21 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall occurrence. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

The evaluation at 28 DAT (Fig. 4) was the most important, because provides information about complete plants death or regrowth after herbicide application. The results showed that the addition of adjuvant is essential when saflufenacil is applied alone (Fig. 4, A) or in combination with glyphosate K salt (Fig. 4, C), and occurrence of rainfall 30 min after application. Plants that received these treatments without addition of adjuvant have survived and were able to regrowth. For other glyphosate formulations, the control was 100%, regardless the addition of adjuvant and simulated rainfall occurrences after application (Figs 4, B, D). As in other evaluations, the addition of adjuvant significantly increased the control over plants that were treated with glyphosate K salt (Fig. 4, C). With the occurrence of simulates rainfall at 240 min after the application of this herbicide, the addition of adjuvant resulted in increase of approximately 40% in the hairy fleabane control. For other glyphosate formulations, the addition of adjuvant increased the control at 10 to 15%, varying according with rainfall occurrences after application.

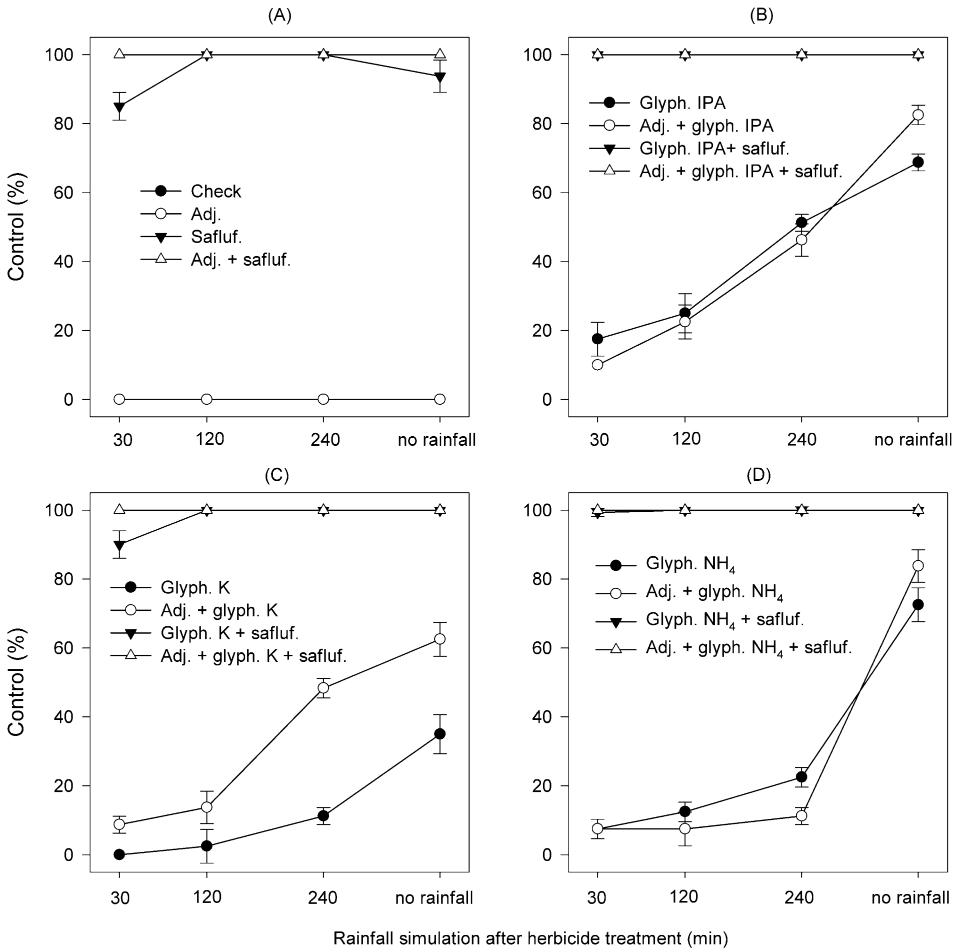


Figure 4. Hairy fleabane control at 28 days after treatment, in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall occurrence. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

Rainfall simulation significantly reduced glyphosate efficiency in all evaluated formulations (Fig. 4). When rainfall was simulated 30 min after glyphosate spraying, the control was less than 20%, regardless glyphosate formulation and addition of adjuvant. In plants that did not receive rainfall after application, the controls were approximately 80%, 60% and 85% for IPA, K and NH_4 salts, respectively, considering the treatments that contained adjuvant. With the simulation of rainfall after 240 min, controls were reduced to 50%, 50% and 20% for the same treatments. These results indicate that it takes more than 4 hours between its application and the occurrence of rainfall for maintaining glyphosate efficiency in hairy fleabane. Glyphosate NH_4 salt was the most sensitive to the rainfall occurrences after application (Fig. 4, D).

The reduction of shoot dry mass (SDM) (Fig. 5) corroborates the visual control evaluation. The application of saflufenacil, with or without adjuvant, reduced significantly the SDM, except in plants that were exposed to simulated rainfall 30 min after herbicides application, reflecting the occurrence of regrowth in these conditions.

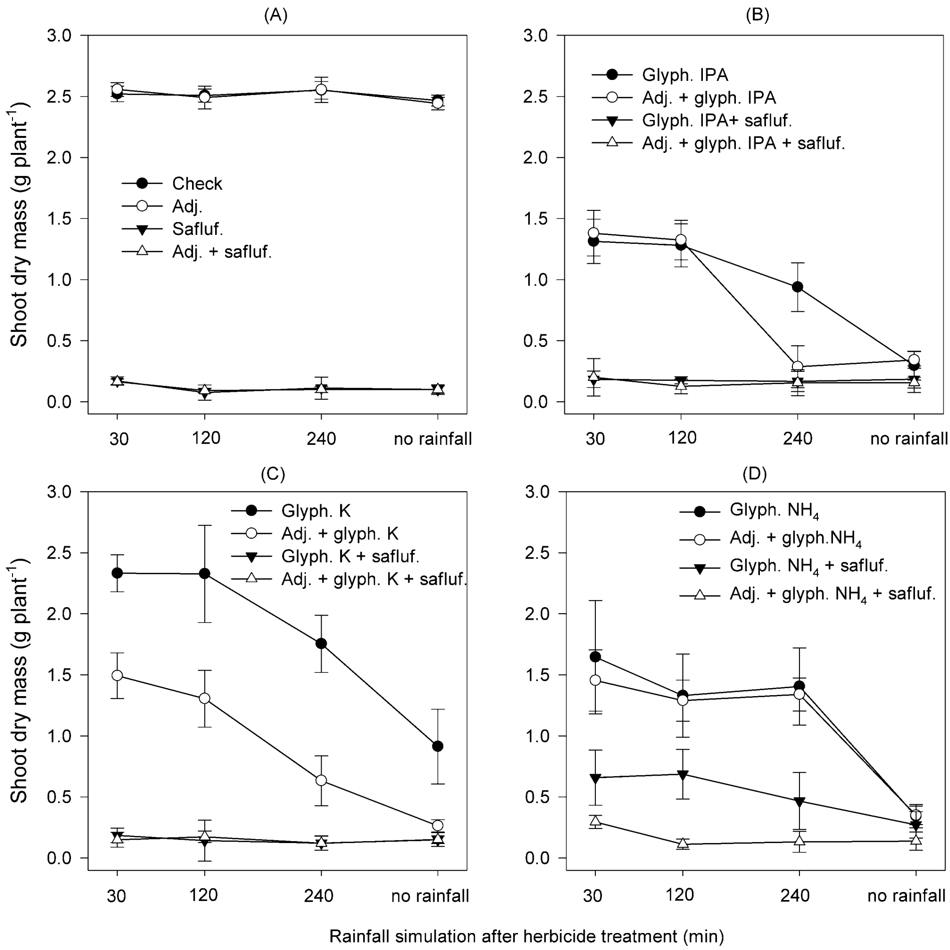


Figure 5. Shoot dry mass (SDM) of hairy fleabane in response to saflufenacil in association with glyphosate formulations, adjuvants and simulated rainfall occurrence. A: without glyphosate; B: glyphosate isopropylamine salt (IPA); C: glyphosate potassium salt (K); and D: glyphosate ammonium salt. Error bars smaller than the size of the symbol are not shown.

When glyphosate IPA (Fig. 5, B) or K salts (Fig. 5, C) were added to saflufenacil, the reduction of SDM occurred independently of the simulated rainfall after application. However, the addition of glyphosate NH_4 salt to saflufenacil resulted in smaller reduction of SDM, compared to the application of saflufenacil alone or in combination with the other glyphosate formulations (Fig. 5, D). It was observed for all simulated rainfall periods after the application of herbicides without adjuvant, and when the simulated rainfall occurred at 30 min after herbicide treatment when the adjuvant was added to the spray solution. These results reflect the lower control observed in the first evaluation, at 7 DAT (Fig. 1, D), in relation to other treatments. The lower initial control allowed the growth of the plants for a longer period and, consequently, the higher accumulation of SDM. The highest accumulations of SDM, considering treatments that received herbicides (excluding check and adjuvant), were observed in plants treated with glyphosate K salt (Fig. 5, B), especially in the absence of adjuvant and rainfall simulation at 30 min after spraying.

The results demonstrated that saflufenacil herbicide has efficiently controlled 15-cm-height hairy fleabane, even with the occurrence of simulated rainfall 30 min after its application. However, to obtain total control, when simulating rainfall occurred at 30 min after application, it was necessary to add adjuvant or use glyphosate IPA or NH_4 salts (Fig. 4). Adjuvants, especially surfactants, are compounds that accelerate and increase the absorption of post-emergent herbicides through the plant cuticle (Liu, 2004). In addition, these compounds reduce the surface tension of the droplets and improve their contact and adhesion with the leaf surface, improving the herbicide absorption and efficiency (Castro et al., 2018).

Besides the presence of adjuvants in adequate proportions, two other factors are important in the herbicide absorption by the leaves: the physical-chemical characteristics of the herbicides and the foliar surface characteristics of the weeds (Liu, 2004). The absorption of saflufenacil was less impaired by rainfall occurrence compared to that observed for glyphosate herbicide, regardless its formulation. Saflufenacil has a log Kow (octanol/water partition coefficient) value higher than glyphosate (saflufenacil: 2.60; glyphosate: -2.56) (Shaner, 2014). The log Kow indicates the affinity of the active ingredients with the cuticle of the leaves, which normally presents an apolar character. The higher the log Kow value, the faster the herbicide will be absorbed and the lower the losses by rainfall occurrences after application. This may explain the reduction of glyphosate herbicide efficiency, even with rainfall occurring after 240 min (4h).

The addition of adjuvant and glyphosate to saflufenacil had little effect on hairy fleabane final control in absence of rainfall (Fig. 4). It does not mean that adding these compounds into the spray solution is not important. When applied at high doses or in small plants, saflufenacil alone was able to efficiently control *Conyza* plants without the need for adjuvant and glyphosate (Mellendorf et al., 2015; Castro et al., 2018). However, in plants at an advanced stage of growth, or at lower doses of saflufenacil, the addition of adjuvant and glyphosate is important, because they can both increase the efficiency of control and reduce the occurrence of regrowth (Eubank et al., 2013; Dalazen et al., 2015).

The glyphosate application reached a control of 60 to 85%, depending on the formulation (Fig. 4). These levels of control can even be considered high for a resistant population. However, the resistance factor (RF) of the population used in the study can be considered low (FR: 3.98) (Dalazen et al., 2019) and, probably, the effect of glyphosate would be lower in populations with higher RF. Therefore, the addition of saflufenacil to glyphosate has been reported as a good strategy for manage weed species that are difficult to control, including species of *Conyza* (Eubank et al., 2013; Dalazen et al., 2015; Budd et al., 2016). However, the mixture of glyphosate with contact-action PPO inhibitors, such as fomesafen and sulfentrazone, results in antagonism, reducing the efficiency of both herbicides (Shaw & Arnold, 2002). Saflufenacil, however, has some peculiar physicochemical characteristics (pKa of 4.41 and log Kow of 2.6) compared to other PPO inhibitors, which confer to it a systemic character, with translocation via xylem and phloem (Bromilow et al., 1990; Grossmann et al., 2011). In addition, the translocation capacity of saflufenacil may also be related with its lower affinity with PPO enzyme, in comparison with other PPO inhibitors. Therefore, other PPO herbicides rapidly affect vascular tissue, making difficult the translocation. The translocation capacity of saflufenacil makes possible their association with systemic herbicides, such as glyphosate (Dalazen et al., 2015).

The glyphosate salts which were used in this study showed different hairy fleabane control in some situations. Considering all evaluated factors, the association of glyphosate IPA salt and saflufenacil presented the best results, in relation to both plant control at 28 DAT and SDM evaluations (Figs 4, 5, respectively). Although the addition of glyphosate NH_4 salt resulted in 100% control, the lower initial control and higher accumulation of SDM would result in higher interference on the culture. The simulation of rainfall after 30 min for the glyphosate K salt resulted in plant regrowth (Fig. 4, C). This would require an additional herbicide application in order to obtain complete weed control.

Several studies evaluate the effect of different glyphosate salts on weed control. Recently, Travlos et al. (2017) have done an extensive review on the efficiency of different glyphosate salts formulations and adjuvants on various weed species. In most cases, the final control provided by the formulations becomes equal, since glyphosate's acid equivalent amounts are the same. However, their absorption and translocation rates vary according to each one's formulation and species, which may be important in adverse conditions, such as rainfall occurrence after herbicides application. Li et al. (2005) reported that, although the final control was not affected, the initial absorption of glyphosate IPA salt was higher in common waterhemp (*Amaranthus rudis*) compared to di-ammonium salt formulation. However, in morningglory (*Ipomoea lacunose*), the initial uptake of glyphosate was higher for the di-ammonium salt. These differences are associated with the cuticle composition of each weed species, which may vary, as well as its polarity and affinity with the herbicides.

The occurrence of rainfall after application, even after 240 min, reduced the efficiency of all glyphosate formulations, although it was more harmful to both K and NH_4 salts formulations, compared to IPA (Fig. 4). In another study, the occurrence of rainfall at 6 h after application reduced the efficiency of glyphosate (Pedrinho Júnior et al., 2002). However, the authors did not report which salt formulation was used in this research. Similarly, using the same salts of glyphosate which were used in the present study, Costa et al. (2017) observed higher control of alexandergrass (*Urochloa decumbens*) in plants that were treated with glyphosate IPA salt in comparison to K and di-ammonium after rainfall simulation. Even with rainfall only after 15 min, the IPA formulation resulted in 100% control at 28 DAT, whereas, for both K and di-ammonium formulations, the controls were approximately 20 and 85%, respectively. In the case of sicklepod (*Senna obtusifolia*), both the formulation and the rainfall occurrence after 15 min did not interfere in the control (Souza et al., 2014), confirming the variation in the responses according to the weed species. Moreover, these responses may also to vary according to the sensitivity of different populations of the same weed species, as well as the occurrence of resistance should be considered.

In addition to the type of glyphosate salt, it is important to emphasize that the type of adjuvants and other compounds of the formulation are also fundamental to the prediction of absorption of this herbicide. However, these information are many times not provided in the technical reports or herbicide labels. Thus, although the active ingredient is the same among trademarks, they may have formulation differences, which may result in variations in uptake, translocation and amount of molecules that will reach the EPSPS enzyme. These considerations may explain the increased efficiency only of for glyphosate K salt when the adjuvant was added (Fig. 5, C).

CONCLUSION

In summary, the absorption of saflufenacil by hairy fleabane occurs rapidly and the simulated rainfall from 30 min after application does not result in loss of efficiency, when the herbicide is applied with adjuvant. The association of glyphosate isopropylamine (IPA) and ammonium salts (NH_4) to saflufenacil provides increased control and prevents the occurrence of regrowth when rainfall occurs soon after the application. The occurrence of simulated rainfall after 240 min considerably reduces glyphosate efficiency, regardless its salt formulation. In addition, the use of adjuvant improves the efficiency of glyphosate potassium (K) salt.

REFERENCES

Ashigh, J.J. & Hall, C. 2010. Bases for interactions between saflufenacil and glyphosate in plants. *Journal of Agriculture Food and Chemistry* **58**, 7335–7343.

Benbrook, C.M. 2016. Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe* **28**, 1–15.

Bromilow, R.H.K., Chamberlain, K. & Evans, A.A. 1990. Physicochemical aspects of phloem translocation of herbicides. *Weed Science* **38**, 305–314.

Budd, C.M., Soltani, N., Robinson, D.E., Hooker, D.C., Miller, R.T. & Sikkema, P.H. 2016. Glyphosate-resistant horseweed (*Conyza canadensis*) dose response to saflufenacil, saflufenacil plus glyphosate, and metribuzin plus saflufenacil plus glyphosate in soybean. *Weed Science* **64**, 727–734.

Castro, E.B., Carbonari, C.A., Velini, E.D., Gomes, G.L.G.C. & Belapart, D. 2018. Influence of adjuvants on the surface tension, deposition and effectiveness of herbicides on fleabane plants. *Planta Daninha* **36**, e018166251.

Costa, A.C.R., Gonçalves, C.G., Silva Junior, A.C., Cardoso, L. & Martins, D. 2017. Action of rain on formulations of glyphosate mixed with saflufenacil for the control of *Brachiaria decumbens*. *Arquivos do Instituto Biológico* **84**, 1–8.

Dalazen, G., Kruse, N.D., Machado, S.L.O. & Balbinot, A. 2015. Synergism of the glyphosate and saflufenacil combination for controlling hairy fleabane. *Pesquisa Agropecuária Tropical* **45**, 249–256 (in Portuguese).

Dalazen, G., Pisoni, A., Menegaz, C. & Merotto Jr, A. 2019. Low temperature reverses the resistance to glyphosate in hairy fleabane (*Conyza bonariensis*). *Journal of Plant Protection Research* **59**, 433–440.

Dennis, M., Hembree, K.J., Bushoven, J.T. & Shrestha, A. 2016. Growth stage, temperature, and time of year affects the control of glyphosate-resistant and glyphosate-paraquat resistant *Conyza bonariensis* with saflufenacil. *Crop Protection* **81**, 129–137.

Dinelli, G., Marotti, I., Bonetti, A., Catizone, P., Urbano, J.M. & Barnes, J. 2008. Physiological and molecular bases of glyphosate resistance in *Conyza bonariensis* biotypes in Spain. *Weed Research* **48**, 257–265.

Duke, S.O. & Powles, S.B. 2008. Glyphosate: a once-in-a-century herbicide. *Pest Management Science* **64**, 319–325.

Eubank, T.W., Nandula, V.K., Reddy, K.N., Poston, D.H. & Shaw, D.R. 2013. Saflufenacil efficacy on horseweed and its interaction with glyphosate. *Weed Biology and Management* **13**, 135–143.

Frans, R., Talbert, R., Marx, D. & Crowley, H. 1986. Experimental design and techniques for measuring and analyzing plant responses to weed control practices. *In:* Research methods in weed science. Champaign: Southern Weed Science Society Pages 29–46.

Ge, X., d'Avignon, D.A., Ackerman, J.A., Duncan, B., Spaur, M.B. & Sammons, R.D. 2011. Glyphosate-resistant horseweed made sensitive to glyphosate: low-temperature suppression of glyphosate vacuolar sequestration revealed by 31P NMR. *Pest Management Science* **67**, 1215–1221.

Geier, P.W., Sthlman, P.W. & Charvat, L.D. 2009. Dose responses of five broadleaf weeds to saflufenacil. *Weed Technology* **23**, 313–316.

Grossmann, K., Niggeweg, R., Christiansen, N., Looser, R. & Ehrhardt, T. 2010. The herbicide saflufenacil (Kixor^{TM}) is a new inhibitor of protoporphyrinogen IX oxidase activity. *Weed Technology* **58**, 1–9.

Grossmann, K., Hutzler, J., Caspar, G., Kwiatkowski, J. & Brommer, C.L. 2011. Saflufenacil (Kixor^{TM}): biokinetic properties and mechanism of selectivity of a new protoporphyrinogen IX oxidase inhibiting herbicide. *Weed Science* **59**, 290–298.

Heap, I. 2020. The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: 11.03.2020.

Holländer, H. & Amrhein, N. 1980. The Site of the inhibition of the shikimate pathway by glyphosate: I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Gagopyrum esculentum* Moench). *Plant Physiology* **66**, 823–829.

Kaspary, T.E., Lamego, F.P., Cutti, L., Aguiar, A.C.M., Rigon, C.A.G. & Basso, C.J. 2017. Growth, phenology and seed viability between glyphosate-resistant and glyphosate-susceptible hairy fleabane. *Bragantia* **76**, 92–101.

Kleinman, Z. & Rubin, B. 2017. Non-target-site glyphosate resistance in *Conyza bonariensis* is based on modified subcellular distribution of the herbicide. *Pest Management Science* **73**, 246–253.

Li, J., Smeda, R.J., Sellers, B.A. & Johnson, W.G. 2005. Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Science* **53**, 153–159.

Liu, Z. 2004. Effects of surfactants on foliar uptake of herbicides – a complex scenario. *Colloids and Surfaces B: Biointerfaces* **35**, 149–153.

Mahoney, K.J., Shropshire, C. & Sikkema, P.H. 2014. Comparison of glyphosate formulations for weed control and tolerance in maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. *Agricultural Sciences* **5**, 1329–1337.

Mellendorf, T.G., Young, J.M., Matthews, J.L. & Young, B.G. 2015. Influence of application variables on the foliar efficacy of saflufenacil on horseweed (*Conyza canadensis*). *Weed Science* **63**, 578–586.

Molin, W.T. & Hirase, K. 2004. Comparison of commercial glyphosate formulations for control of prickly sida, purple nutsedge, morningglory and sicklepod. *Weed Biology and Management* **4**, 136–141.

Monquero, P.A. & Silva, A.C. 2007. Effect of rainfall on *Euphorbia heterophylla* and *Ipomoea purpurea* control by glyphosate and sulfosate. *Planta Daninha* **25**, 399–404 (in Portuguese).

Moretti, M.L. & Hanson, B.D. 2017. Reduced translocation is involved in resistance to glyphosate and paraquat in *Conyza bonariensis* and *Conyza canadensis* from California. *Weed Research* **57**, 25–34.

Mueller, T.C., Main, C.L., Thompson, M.A. & Steckel, L.E. 2006. Comparison of glyphosate salts (isopropylamine, diammonium, and potassium) and calcium and magnesium concentrations on the control of various weeds. *Weed Technology* **20**, 164–171.

Mylonas, P.N., Giannopolitis, C.N., Efthimiadis, P.G., Menexes, G.C., Madesis, P.B. & Eleftherohorinos, I.G. 2014. Glyphosate resistance of moleculary identified *Conyza albida* and *Conyza bonariensis* populations. *Crop Protection* **65**, 207–215.

Okada, M., Hanson, B.D., Hembree, K.J., Peng, Y., Shrestha, A., Stewart, C.N., Wright, S.G. & Jasieniuk, M. 2014. Evolution and spread of glyphosate resistance in *Conyza bonariensis* in California and a comparison with closely related *Conyza canadensis*. *Weed Research* **55**, 175–185.

Pacanoski, Z. & Mehmeti, A. 2019. Pre-emergence grass weed control in winter wheat (*Triticum aestivum* L.) with soil applied premixed herbicides influenced by precipitations. *Agronomy Research* **17**, 2386–2398.

Pedrinho Junior, A.F.F., Piva, F.M., Martini, G., Felici, G.V. & Durigan, J.C. 2002. Influence of rain on the efficacy of glyphosate in mixture with adjuvants in weed desiccation. *Planta Daninha* **20**, 263–271 (in Portuguese).

Sammons, R.D. & Gaines, T.A. 2014. Glyphosate resistance: state of knowledge. *Pest Management Science* **70**, 1367–1377.

Santos, G., Oliveira Junior, R.S., Constantin, J., Francischini, A.C., Machado, M.F.P.S., Mangolin, C.A. & Nakajima, J.N. 2014. *Conyza sumatrensis*: A new weed species resistant to glyphosate in the Americas. *Weed Biology and Management* **14**, 106–114.

Shaner, D.L. 2014. *Herbicide Handbook.* 10th ed. Lawrence, KS: Weed Science Society of America. 513 pp.

Shaw D.R. & Arnold J.C. 2002. Weed control from herbicide combinations with glyphosate. Weed Technol 16, 1–6

Silva, F.A.S. & Azevedo, C.A.V. 2006. A new version of the Assistat-statistical assistance software. In: *Proceedings of the 4th World Congress on Computers in Agriculture and Natural Resources.* Orlando, Florida: American Society of Agricultural and Biological Engineers, pp 393–396.

Souza, G.S.F., Martins, D., Pereira, M.R.R. & Bagatta, M.V.B. 2014. Action of rain on the efficiency of herbicides applied post-emergence in the control of *Senna obtusifolia*. *Rev. Ciên. Agron.* **45**, 550–557.

Starke, R.J. & Oliver L.R. 1998. Interaction of glyphosate with chlorimuron, fomesafen, imazetaphyr and sulfentrazone. *Weed Science* **46**, 652–660.

Travlos, I., Cheimona, N. & Bilalis, D. 2017. Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. *Agronomy* **7**, 1–9.

Vanaga, I., Gurkina, J., Treikale, O., Priekule, I. & Pugacova, J. 2006. Influence of Heracleum sosnowskyi control measures on weed diversity in agricultural fields in Latvia. *Agronomy Research* **4**, 433–436.

Vidal, R.A., Queiroz, A.R.S., Trezzi, M. & Kruse, N. 2016. Association of glyphosate with other agrochemicals: the knowledge synthesis. *Revista Brasileira de Herbicidas* **15**, 39–47.

Viirlaid, E., Riiberg, R., Mäeorg, U. & Rinken, T. 2015. Glyphosate attachment on aminoactivated carriers for sample stabilization and concentration. *Agronomy Research* **13**, 1152–1159.

Waggoner, B.S., Mueller, T.C., Bond, J.A. & Steckel, L.E. 2011. Control of glyphosate-resistant horseweed (*Conyza canadensis*) with saflufenacil tank mixtures in no-till cotton. *Weed Technology* **25**, 310–315.

Werlang, R.C. & Silva, A.A. 2002. Glyphosate - carfentrazone-ethyl interaction. *Planta Daninha* **20**, 93–102 (in Portuguese).

Agronomy Research **18**(1), 77–87, 2020
https://doi.org/10.15159/AR.20.001

Genetic components for fodder yield and agronomic characters in maize lines

O. Gayosso-Barragán[1,7], S.A. Rodríguez-Herrera[2,*], C.D. Petroli[3], O. Antuna-Grijalva[4], A. López-Benítez[2], A. Mancera-Rico[5], M.P. Luévanos-Escareño[6] and A.J. Lozano-del Río[2]

[1]Postgrado en Ciencias Agrarias, Universidad Autónoma Agraria Antonio Narro UL, Periférico Raúl López Sánchez, MX27054 Torreón, Coahuila, México
[2]Departamento de Fitomejoramiento, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, MX25315 Saltillo, Coahuila, México
[3]International Maize and Wheat Improvement Center (CIMMYT), El Batán Km. 45, MX56237, Texcoco, Estado de México, México
[4]Departamento de Fitomejoramiento, Universidad Autónoma Agraria Antonio Narro UL, Periférico Raúl López Sánchez, MX27054 Torreón, Coahuila, México
[5]Centro de Capacitación y Desarrollo en Tecnologías de Semillas, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, MX25315 Saltillo, Coahuila, México
[6]Facultad de Ciencias Biológicas, Universidad Autónoma de Coahuila, Carretera Torreón-Matamoros Km. 7.5, MX27275 Torreón Coahuila, México
[7]Centro Nacional de Investigación Disciplinaria en Agricultura Familiar (CENID AF), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Carretera Ojuelos-Lagos de Moreno km 8.5, MX47540 Ojuelos, Jalisco, México
[*]Correspondence: sarh50@live.com.mx

Abstract. In maize hybrid development, it is essential to know the parent's performance per se in different environments as well as the genetic base in populations in order to facilitate selection of superior lines. The objective of this work was to estimate the genetic variance and heritability while assessing agronomic behavior in 237 maize fodder lines featuring different inbred levels and origin in two different locations. Traits such as plant height, cob height, stem diameter, and fodder yield were taken. A combined variance analysis was carried out using this information. The variable mean squares showing significant differences were used to estimate the genetic variance components. Significant differences were observed ($p \leq 0.01$) for the line variation source and location × line interaction in all assessed characteristics. The genetic parameter estimation provided information on the existing genetic variance and heritability among the population which is important for the progress of the selection process. Lines CLWN701, CLWN345, CML476, CML216, CLWQ232, and CML 528 measured 74.8, 72.2, 67.7, 65.8, 63.8 and 62.5 t ha^{-1}, respectively showed the highest yield potential for green fodder.

Key words: heritability, additive variance, green fodder, inbred lines, coefficient of variation genetic.

INTRODUCTION

In Mexico, maize is cultivated in diverse environments ranging from sea level to upper valleys (above 2,200 m.a.s.l.) with a wide variety of thermal rainfall systems from very drastic to mild ones. From self-consumption to highly commercial production schemes (Preciado et al., 2005), maize cultivated area in Mexico, accounted for 7.3 million hectares for grains and 602617 hectares for green fodder with an average 36.12 t ha^{-1} yield (SADER-SIAP, 2018).

Because there are no highly productive maize lines, double or three-way hybrids are used in commercial hybrid production. Besides, high costs of simple hybrid-seed have limited its commercial production (Luna et al., 2012). Parents are of underlying importance in maize hybrid formation in both behavior per se in different environments and their combining ability. Knowledge of combining ability allows for recognition and identification of parents with desirable characteristics for seed production and hybrid formation (Pérez et al., 1992). According to Marquez (1988), in order to obtain high-yielding maize lines, it is necessary to have big-size, variable, and high-yield populations. Hallauer (1970) reported that yield may be increased through selection and enhancement of existing lines.

In genetic enhancement programs, it is desirable to be genetically acquainted with base populations in order to facilitate ideal line selection. In order to select the enhancement scheme, it is necessary to first know the type of heredity or specific gene action for quantitative characters of agronomic concern (Peyman et al., 2009).

The genetic parameters estimate in maize populations provide information on the existing genetic variation among the population and supports progress on the selection process. The heritable genetic variance ratio is therefore broken down into an additive portion associated to the average output of genes effect, a dominance portion resulting from the allelic interaction of a genetic locus and epistasis (Hallauer, 1970). Current variations among the population resulting from genetic additive effects are measured by additive variance (Rovaris et al., 2011).

During genetic enhancement by selection and hybridization, the plant breeder besides knowing the agronomic aspects of the species in question, must also know the genetic characteristics of the populations to be enhanced (Moreno et al., 2002). According to Marquez (1985), the additive effects are used in the selection process both intra locus and inter loci for population improvement. In addition, it was further stated that the general procedure consists of: 1) Selection of the best individuals among the population; 2) Utilization of the selected individuals as parents for the next generation; 3) Inception of a selection cycle in the population resulting from the selected individuals mating; and 4) Realization of various additional cycles up to reduction of the additive variance.

Estimation of the genetic variability extent shown by the different quantitative characters in any vegetal species is essential to begin their genetic enhancement. Answer to the selection being performed in any individual depends primarily genetic variability, heritability and gene frequency present in the population to be enhanced.

The objective of this work was to estimate both the genetic variance and heritability as well as to evaluate the agronomic behavior in 237 fodder maize lines at two locations. This was with a view to select those lines that consistently manifest high yield potential for fodder.

MATERIALS AND METHODS

Location of the experiment

The current investigation was carried out in two locations: The Dr. Ernest W. Sprague Experimental Station from the International Maize and Wheat Improvement Center in Agua Fria, Puebla (812 m.a.s.l.), 20º 27' Lat. N, 97º38' Long. O) and Rancho Santa Margarita, Puerto Vallarta, Jalisco (731 m.a.s.l.), 20º 44'Lat. N, 105º10' Long. O). Cultural practices on typical handling were carried out for each region in such a way that cultivation was kept free of pests and diseases. Sowing and harvesting were performed manually.

Genetic material

In this study, 237 maize lines of different origins and inbreeding levels were evaluated. These lines came from tropical and subtropical genetic enhancement programs and the germplasm bank from the International Maize and Wheat Improvement Center.

Experiment initiation and agronomic management

Sowing at the Agua Fria and Puerto Vallarta location took place on December 19 and 21, 2018 respectively. Planting density was 83,000 plants per hectare. Experimental unit was made up of two 5-meter-long furrows with 0.80 distance in between furrows and 0.15 meter space in between plants. Irrigation water supply was achieved by means of a trickle irrigation ribbon with 15 cm separation. Approximately 20% urea was applied at sowing, two additional applications (40%) were carried out at bloom period and grain filling stage. Weeds were controlled through the application of Callisto ® at 300 cm^3 ha^{-1} dose. Insect pests were controlled with Denim® 19 CE and Lorsban™ 4 EC applications at 100 mL ha^{-1} and 1.0 L ha^{-1} dose accordingly.

Evaluated variables

Cob Height (CH) in five randomly selected plants was taken from the soil surface to the point where the first ear was inserted.

Plant height (PH) was measured when the plants reached harvest maturity. It was recorded in five plants taken at random from the plot, measuring from the point of the union of the root and stem to the base of the male inflorescence.

Stem diameter (ST) in five central plants was measured with a vernier with results reported in centimeters.

Forage yield (FY) was taken when the plants reached physiological maturity and the weight of five plants per experimental unit was recorded, and extrapolated to green forage yield per hectare.

Statistical analysis

A double-repetition, random combined-analysis following a full-block design was carried out. Two hundred and thirty-seven (237) lines were sowed and split into 26, 9-line groups by repetition in two locations. The significant agronomic characteristics ($p \leq 0.01$) were used to estimate variance components.

The model used for phenotype dissection in different environments was $y_{ijkl} = \mu+\alpha_i+\beta_j+\gamma_k+\delta_l+(\gamma_k)j+\alpha\beta_{ij}+(\delta_l)j+(\alpha\delta_{il})j+\varepsilon_{ijkl}$, where y_{ijkl} – line phenotypic value;

μ – target feature mean; α_i – effect of the i-th location; β_j – effect of the j-th group; γ_k – effect of the k-th repetition; δ_l – effect of the l-th line; $(\gamma_k)_j$ – k-th repetition nested in the j-th group; $\alpha\beta_{ij}$ – interaction of the i-th location and the j-th group; $(\alpha\delta_{il})_j$ – interaction of the i-th location and the l-th line nested in the j-th group; ε_{ijkl} – experimental error. The combined-variance analysis was carried out using the R 3.6.0 statistical program (R Core Team, 2013).

Genetic variability estimates

Data were recorded for each genotype. The variance components estimate was calculated taking the mean squares from the line variation sources as well as from the lines × location obtained from the mixed variance analysis. Variance components were used to make estimates of genetic variance.

The genotypic variance is the part of that total variation explained by the genotypes effect evaluated in different environments. As for lines S_1, it provides a direct estimate of the additive genetic variance (σ^2_A). This occurs under the assumption that dominant effects and epistasis are minimized during inbred. Likewise, the reference population from which study lines were developed is supposed to be in Hardy-Weinberg equilibrium.

Genetic variance (σ^2_g), phenotypic variance (σ^2_f), variance interaction (σ^2_{ga}) and broad sense heritability (H^2) was determined by the method of the mean squares of the variance components (Barriga et al., 1983). Phenotypic (CV_F), genotypic (CV_G) and environmental (CV_A) variation coefficients were calculated, considering the methodology proposed by Pistorale et al. (2008).

Best linear unbiased predictor (BLUP)

In order to eliminate the environmental effect, a mixed linear model was adjusted to calculate the best linear unbiased predictor (BLUP) value for each line. The model used for phenotype dissection in different environments was: $yijk = \mu + gi + ej + \varepsilon ijk$. In this equation, *yi* represents the "*i*" line phenotype, μ represents the great mean value for the environment target feature, *gj* represents the genetic effect, *ej* represents the environmental effect and *εijk* represents the random error. All effects were considered as random except for the mean (Li et al., 2016). The BLUP value estimate was therefore denoted as the sum of the great mean and the genetic effect of each line. Such values were obtained using the lme4 statistical package (Linear Mixed-Effects Model version 1.1-21) from the R 3.6.0 statistical program (Bates et al., 2015).

RESULTS AND DISCUSSION

Variance analysis

The mixed variance analysis results from the 237 maize inbred lines evaluated at Agua Fria and Puerto Vallarta, Mexico are shown in Table 1. Highly significant differences ($p \leq 0.01$) are observed in lines and interactions line × location in fodder yield variables (FYG), plant height (PH), cob height (CH) and stem diameter (ST). Upon detection of such differences, these were used to estimate variance components. The mean for the evaluated agronomic variables is also shown. As for fodder yield, the mean value was 37.77 t ha^{-1}. Variation coefficient ranged from 3.4 (plant height) to 16.06 (stem diameter).

Table 1. Mean squares for the mixed variance analysis for yield and agronomic characters in 237 maize lines evaluated at Agua Fria and Puerto Vallarta, Mexico

SV	DF	Fodder yield (t ha^{-1})	Plant height (cm)	Cob height (cm)	Stem diameter (mm)
Loc	1	17,001.28**	22,363.88 **	42,153.78**	324.10**
Groups	25	1,083.00*	6,908.48*	3,093.72*	244.70*
Repetitions (Groups)	26	0.9914 ns	21.25 ns	21.773 ns	13.75 ns
Loc*Groups	25	170.81**	388.19**	249.33**	16.88 *
Lines (Groups)	208	500.85**	1245.59**	721.91**	40.14**
Loc*Lines(Groups)	208	107.69**	226.66**	116.87**	13.91**
Error	454	11.81	41.03	8.53	11.58
R^2		0.95	0.95	0.96	0.79
CV(%)		9.1	3.8	3.4	16.06
Mean		37.77	168.97	86.21	21.18

*, ** significant at 5% and 1% level, respectively; ns = Non-significant; SV = Variation source; DF = Degree of freedom; Loc = Locations; CV = Variation coefficient.

The frequency distribution of agronomic characters (Fig. 1) shows the way in which variable data were distributed in both environments. As for fodder yield, distribution frequency ranged from 10 to 80 t ha^{-1}, thus showing the differences of the genotypes under study in which the highest frequency fell to within 30 to 40 t ha^{-1}.

According to the mixed variance analysis results, highly significant values for lines variation sources and lines × location were present across both locations. Thus, showing that at least one out of the 237 lines was different compared to the rest, highlighting the differences in the lines' genetic origin besides the effect caused by climate conditions during cultivation development. Garcia & Villa (1995) pointed out that growth and development directly depend on the plant's genetic structure, soil and climate conditions in which evaluation had been conducted. The statistical differences observed from one line to another, are the result of the genetic variability relevant to the germplasm used in the process.

The significant line interaction per location is noteworthy as it shows that production was not kept relatively equal in the two evaluation environments by the parents. Due to random environmental fluctuations, maize germplasm agronomic evaluation needs to be carried out in different environments allowing for a more accurate estimate of the genetic components value thus, detaching the environmental genetic effect (Gutiérrez-Del Rio et al., 2004).

As for the plant and cob height variables, mean values noted were 168.97 and 86.21 centimeters, respectively. In general, lines evaluated in this study are considered as low height due to the inbreeding depression occurring when lines are self-fertilized successively with the purpose to achieve homogeneity and consistency. The inbreeding depression depends directly on the level of dominance, so that no changes in the mean are expected under inbreeding when genes controlling the trait follow a strictly additive model (Falconer, 1984).

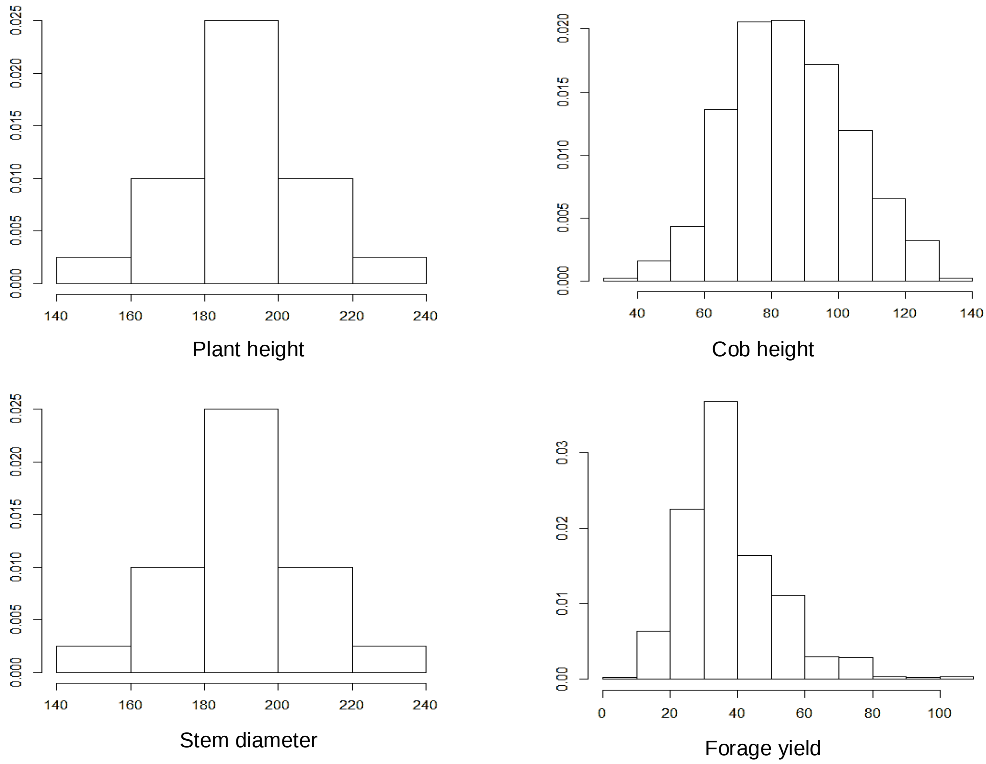


Figure 1. Frequency distribution for agronomic characters; Plant height (cm), cob height (cm), stem diameter (cm) and fodder yield (t ha^{-1}) from 237 maize lines evaluated at Agua Fria and Puerto Vallarta, Mexico.

Similar results for the variables plant height and cob height were reported by Alfaro & Segovia (2009), when maize cultivars were characterized in the Southern part of Venezuela. Bejarano et al. (2000) reported significant differences regarding the environmental effects for PH and CH evaluated in simple breeds of maize coming from lines featuring different inbred levels. Silva et al. (2009) also noted significant differences in maize lines for these characteristics in each evaluated environment. In a study to estimate the genetic variability degree in maize genotypes, Sabiell et al. (2014) found highly significant differences among plant height (PH) genotypes in two locations. A similar conclusion was reported by Salami et al. (2007) and Hajibabaee et al. (2012).

As for fodder yield, the significant differences observed among the lines suggests the presence of a vast genetic diversity in the evaluated genotypes despite the yield mean in both evaluated environments (37.84 t ha^{-1}). Values of 74.8, 72.2, 67.7, 65.8, 63.8 and 62.5 t ha^{-1} for CLWN701, CLWN345, CML476, CML216, CLWQ232, CML528 lines, respectively correspond to the highest yield potential and up to 21.46, 21.31, 21.30, 21.26, 20.92, 17.42 t ha^{-1} for CSL1619, CML527, CML327, CML506, CLYN531, CML529 lines were observed as the lines with the lowest yield, respectively.

Yield values in higher potential lines exceeded the 36.12 t ha^{-1} national average (SADER-SIAP, 2018) and are similar to those recorded by Peña-Ramos et al. (2008) and Castillo et al. (2009), with yields ranging between 70 and 95 t ha^{-1}. With the purpose to identify outstanding fodder cultivars for upper valleys, Franco-Martinez et al. (2015) assessed 29 maize cultivars with 86.96 and 88.28 t ha^{-1} average fodder yields for SBA-470 and AS-722 cultivars. Sánchez-Hernandez et al. (2011) reported 44.2 and 32.5 t ha^{-1} average fodder yields in maize genotypes evaluated in Oaxaca, Mexico. On the other hand, Nunez et al. (2001) evaluated fodder production in early and intermediate tropical and mild weather maize under irrigated conditions, cultivated in the Northern part of Mexico. The green fodder yield found ranged from 52.8 to 75.6 t ha^{-1}.

Table 2. BLUP values applicable to the agronomic characteristics from the 237 maize lines fraction evaluated at Agua Fria and Puerto Vallarta, Mexico

Variables	Fodder yield		Plant height		Cob height		Stem diameter	
Lines	(t ha^{-1})		(cm)		(cm)		(mm)	
CLWN345	71.0	a	209.8	bcd	110.2	b	25.6	cd
CLWN701	68.5	b	239.5	a	127.7	a	28.3	a
C LRCW96	62.1	c	190.0	degf	91.5	cdef	25.3	cd
CML563	56.5	d	198.0	bcde	97.0	c	25.5	cd
CLWN509	55.3	e	196.5	cdef	91.7	cdef	20.9	ghi
CLWN379	55.2	e	203.3	bcde	106.0	b	24.4	de
CLQRCWQ8	54.2	f	220.1	ab	104.5	b	26.3	bc
CSL1676	52.9	g	156.4	ijk	70.0	jk	22.1	fgh
CLWN630	52.3	g	221.5	ab	109.2	b	27.5	ab
CLWN775	52.1	g	219.0	abc	111.5	b	22.8	ef
CLWN750	38.59	h	191.5	defg	82.5	gh	26.6	cd
CLRCW97	38.25	hi	165.5	hij	70.0	j	22.7	efg
CLRCW100	38.06	hi	157.9	ijk	83.8	fgh	20.8	hi
CLYQ266	37.81	hi	198.8	bcde	92.0	cde	17.9	klm
CLWN603	37.75	i	204.8	bcde	94.0	cd	22.0	fgh
CLYN619	37.65	i	183.3	efgh	87.0	defgh	12.6	n
CLYN460	37.50	ij	172.0	ghi	70.0	jk	20.5	hij
CLWN496	37.50	ij	172.6	ghi	72.0	j	20.5	hij
CML444	36.81	lk	155.4	ijk	88.2	defg	18.8	jkl
CLYN548	36.62	k	199.0	bcde	85.5	efgh	11.3	n
CSL1673	23.28	l	175.1	fghi	73.0	ij	17.1	lm
CSL1656	23.28	l	165.8	hij	82.6	gh	19.8	ijk
CSL1643	22.96	l	138.1	lm	70.0	jk	20.3	hij
CSL1671	22.65	lm	147.4	jkl	62.2	k	19.2	ijk
CLYN631	22.62	lm	174.3	fghi	94.5	cd	18.2	klm
CSL1669	22.03	mn	153.9	ijk	65.0	jk	17.03	m
CML537	21.40	n	144.5	jkl	80.0	hi	19.7	ijk
CSL1612	20.06	o	129.8	lm	49.0	l	12.8	n
Mean	37.77		168.9		86.2		21.1	

Different superscripts denote statistical significance at $p \leq 0.05$ by Tukey's post-hoc test.

Best linear unbiased predictor (BLUP) values for yield and concerned agronomic characteristics were obtained. The upper fraction values for the 237 lines evaluated in two environments are shown in Table 2. As for fodder yield, the general mean was 37.84 t ha^{-1} while an important group of lines showed values higher than the mean. This is probably as a result of the vast genetic diversity present in the assessed line population.

Variance components estimate

All evaluated agronomic variables showed significant differences ($p \leq 0.05$), therefore the variance components were estimated. Under the assumption that dominant effects and epistasis are minimized during the inbred process and taking into consideration that the lines under study showed a high inbred level (S_{10}), the genetic variance provides a direct estimate of the additive genetic variance. The genetic parameter estimates for the population under study are shown in Table 3. It was observed that phenotypic variance estimate, in all evaluated characters; showed a greater scale with regard to the additive variance estimate. Characters heritability showed values ranging from 34% (stem diameter) up to 65% (plant height).

Table 3. Variance components estimate and genetic, phenotypic and environmental coefficient of variation (%) for different agronomic characters in 237 maize lines evaluated at Agua Fria and Puerto Vallarta, Mexico'

Variance components	Fodder yield	Plant height	Cob height	Stem diameter	Plant average weight
σ^2g	98.29	254.73	151.26	6.55	26,606.01
σ^2f	158.04	388.57	268.13	19.29	4,2075.77
σ^2ga	47.94	92.81	108.34	1.16	12,447.23
σ^2e	11.81	41.03	8.53	11.58	3,022.53
H^2	0.62	0.65	0.56	0.34	0.63
CV_G	26.25	9.44	14.26	12.08	27.01
CV_F	33.28	11.66	18.99	20.74	33.88
CV_A	18.34	5.7	12.07	5.08	18.43
CV_G/CV_A	1.4	1.6	1.1	2.3	1.3

σ^2_g = Genetic variance; σ^2_f = Phenotypic variance; σ^2_{ga} = Genotype interaction variance × location; σ^2_e = Error variance; H^2= Heritability in broad sense; CV_G (%) = Coefficient of variation genetic; CV_F (%) = Coefficient of variation phenotypic; CV_A (%) = Coefficient of variation environmental.

Variance components allowed for different genetic parameters estimate. Genotypic variance (σ^2_g) provided for 98.29 additive variance (σ^2_A) direct estimate in fodder yield variance. This represents sufficient line genetic variance that, when combined with each other, showed good yields in the single crossbreeding that may take place (Maphumulo et al., 2015). The magnitude of genetic variance will lead to a rapid genetic advance during selection processes (Bertoia & Aulicino, 2014).

Both phenotypic and additive variance components turned out to be according to expectations because phenotypic variance besides the genetic effect, composed of those environmental deflections as well as of genotype × environment interaction component. Therefore, it is expected that phenotypic variance magnitudes in genetic studies exceed those of additive variance. A similar behavior of the aforementioned parameters was found in a tropical maize segregating population evaluated in Morelos, Mexico (Rebolloza-Hernandez et al., 2016).

On the other hand, additive genetic variance in intra-population genetic enhancement programs, significantly contributes to the selective response among the population. For this reason, heritability estimates are essential to predict the response of a population during the selection process. In consequence, the existence of high genetic-additive variations justifies continuance of a population enhancement program (Rovaris et al., 2011).

Heritability values observed in agronomic variables represent a good indicator to perform genetic enhancement selection. Heritability of the characters showed values ranging from 34% (stem diameter) to 65% (plant height). Heritability expresses total variance ratio attributable to the mean effects from the genes and this, in turn, determines the resemblance level among relatives (Falconer, 1984). The study of those genetic mechanisms governing the various significant characters in fodder maize indicated that fodder yield character was highly heritable (62%) thus making recurring selection effective.

The relationship between the coefficient of genetic and environmental variation (CV_G/CV_A) was high for the characteristics evaluated (Table 3). According to Vencovsky (1987), there is a very favorable situation for the selection gain when the CV_G/CV_A tends or is higher than one, since in these cases, the genetic variation is greater than the environmental variation, which indicates that the selection for these characters has the best conditions in terms of immediate genetic gain. Together, the CV_G and heritability are indicative of the potential to make any selection in genotypes analyzed.

CONCLUSIONS

The genetic parameters estimate provided information on the genetic variance and heritability existing among the population, which are essential for the progress of the selection process. In this study, the fodder yield character was highly heritable thus rendering recurring selection as effective. Lines CLWN701, CLWN345, CML476, CML216, CLWQ232, CML528 with 74.8, 72.2, 67.7, 65.8, 63.8 and 62.5 t ha^{-1}, respectively showed the highest green fodder yield. These lines may be potentially used in genetic enhancement programs for this characteristic in maize.

ACKNOWLEDGEMENTS. We would like to thank Consejo Nacional de Ciencia y Tecnologia (Science and Technology National Board –CONACYT) for the graduate studies' scholarship granted to the lead author.

FUNDING. This research was funded by Universidad Autónoma Agraria Antonio Narro (UAAAN), project 2498. The MasAgro program: Sustainable modernization of traditional agriculture through the resources managed by the CIMMyT and the Secretary of Agriculture and Rural Development (SADER).

REFERENCES

Alfaro, Y. & Segovia, V. 2009. Formation, evaluation and description of single-cross yellow maize (*Zea mays* L.) INIA 21. *Revista UDO Agrícola* **9**, 499–508.

Barriga, B., Fuentes, P., Carrillo, L. & Jobet, F. 1983. Heritability and coefficients of phenotypic and genetic variation of agronomic characters in triticale (*Triticosecale Wittmack*). *Agrosur* **11**(1), 43–48.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1–48.

Bejarano, A., Segovia, V. & Marín, C. 2000. Evaluation of simple maize crossings from lines with three different levels of inbreeding. *Agronomía Trop.* **50**, 461–476.

Bertoia, L.M. & Aulicino, M.M. 2014. Maize forage aptitude: Combining ability of inbred lines and stability of hybrids. *The Crop Journal* **2**(6), 407–418

Castillo, J.M., Rojas, B.A. & Wing, J.R. 2009. Nutritional value of silage made with a mixture of corn and mung bean (*Vigna radiata*). *Agron. Costarric*. **33**(1), 133–146.

Falconer, D.S. 1984. Introduction to quantitative genetics. Editorial CECSA. 4° edition. México, 430 pp.

Franco-Martínez, J.R., González-Huerta, A., Pérez-López, D.J. & González-Ronquillo, M. 2015. Phenotypic characterization of hybrids and varieties of forage maize in high valley state of México, México. *Rev. Mex. Cienc. Agríc*. **6**(8), 1915–1927.

García, T. & Villa, N. 1995. Corn planting times due to soil water deficiencies in Cambara. *Pesq. Agropec. Bras*. **30**, 505–514.

Gutiérrez-Del Rio, E., Espinoza-Banda, A., Palomo-Gil, A., Lozano-García, J. & Antuna-Grijalva, O. 2004. Combining ability of maize hybrids for the comarca lagunera. *Rev. Fitotéc. Mex*. **27**, 7–11.

Hajibabaee, M., Azizi, F. & Zargari, K. 2012. Effect of drought stress on some morphological, physiological and agronomic traits in various foliage corn hybrids. *Am-Euras. J. Agric. & Environ Sci*. **12**(7), 890–896.

Hallauer, A.R. 1970. Genetic variability for yield after four cycles of reciprocal recurrent selections in Maize. *Crop Sci*. **10**(5), 482–485.

Li, K., Wang, H., Hu, X., Liu, Z., Wu, Y. & Huang, C. 2016. Genome-wide association study reveals the genetic basis of stalk cell wall components in Maize. *PLoS ONE* **11**(8), e0158906.

Luna, M., Hinojosa, R., Ayala, G., Castillo, G. & Mejía, C. 2012. Perspectives of the maize seed industry development in México. *Rev. Fitotec. Mex*. **35**(1), 1–7.

Márquez, S.F. 1985. Plant genetics. Methods, theory and results. *AGT Editor, S. A.* México, 357 pp.

Márquez, S.F. 1988. Plant genetics. Methods, theory and results. AGT editor, S.A. México, pp. 472–473.

Maphumulo, S.G., Derera, J., Qwabe, F., Fato, P., Gasura, P. & Mafongoya, P. 2015. Heritability and genetic gain for grain yield and path coefficient analysis of some agronomic traits in early-maturing maize hybrids. *Euphytica* **206**(1), 225–244.

Moreno, M.M., Peña, L.A., Sahagún, C.J., Rodríguez, P.E. & Mora, A.R. 2002. Additive variance, heritability and correlations in the m1-fitotecnia variety of husk tomato (*Physalis ixocarpa* Brot). *Rev. Fitotec. Mex*. **25**(3), 231–237.

Núñez, H.G., Faz, R.C., Tovar, G.M. & Zavala, G.A. 2001. Corn hybrids for forage production with high digestibility in northern Mexico. *Técnica Pecuaria en México* **39**, 7–88.

Peña-Ramos, A., González-Castañeda, F., Núñez-Hernández, G., Preciado-Ortiz, R., Terrón-Ibarra, A. & Luna-Flores, M. 2008. H-376, corn hybrid for forage and grain production in el bajío and the north central part of México. *Rev. Fitotec. Mex*. **31**(1), 85–87.

Preciado, O.R., Terrón, I.A., Gómez, M.N. & Robledo G.E. 2005. Genetic components in heterotically contrasting maize populations of tropical y subtropical. *Agronomía Mesoamericana* **16**(2), 145–151.

Pérez, C., Larios, L., Quemé, JL., Fuentes, M. & Castellanos, C. 1992. Evaluation of yellow grain corn lines in six environments of Central America, 1992. *Synthesis of experimental results of prm. 1992* **4**, 61–62.

Peyman, S., Hamid, D., Ali, M. & Mohammad, M. 2009. Genetic and genotype x enviroment interaction effects for appearance quality of rice. *Agric. Sci. China* **8**(8), 891–901.

Pistorale, M., Abbott, A. & Andrés, A. 2008. Genetic diversity and broad sense heritability in tall wheatgrass (*Thinopyrum ponticum*). *Cien. Inv. Agr*. **35**(3), 259–264.

R Core Team 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Rebolloza-Hernández, H., Castillo-Gutiérrez, A., Carapia-Ruíz, V., Andrade-Rodríguez, M., Villegas-Torres, O.G., Núñez-Valdés, M., Suárez-Rodríguez, R. & Perdomo-Roldán, F.

2016. Estimation of genetic parameters and selection of S_1 lines in a segregating population of tropical maize. *Revista Mexicana de Ciencias Agrícolas* **7**, 1893–1904.

Rovaris, S.R., Araújo, P.M., Garbuglio, D.D., Prete, C.E., Zago, V. & da Silva, L.J. 2011. Estimates of genetic parameter in maize commercial variety IPR 114 at Paraná State, Brazil. *Acta Scientiarun. Agron*. **33**(4), 621–625.

SADER-SIAP. 2018. Closure of agricultural production by crop. Secretariat of Agriculture and Rural Development– Agricultural and Fisheries Information Service. Online document: http:// infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ResumenProducto.do

Silva Díaz, W.R., Alfaro Jiménez, Y.J. & Jiménez Aponte, R.J. 2009. Evaluation of morphological and agronomical characteristics of five yellow-maize inbred lines in different sowing dates. *Revista UDO Agrícola* **9**(4), 743–755.

Sabiell, A.I., Abdelmula, A.A., Bashir, E., Jamali, K.D., Baloch, S.K., Baloch, S.U., Muhammed, A. & Bashir, W. 2014. Genetic variations of leaf trait in maize (*Zea mays* L.) under drought stress in different growth stages. *Journal of Biology, Agriculture and Healthcare* **4**, 108–112.

Salami, A.E., Adegoke, S.A. & Adegbite, O.A. 2007. Genetic variability among maize cultivars grown in Ekiti-State, Nigeria. *Middle-East. J. Sci. Res*. **2**, 9–13.

Sánchez-Hernández, M.A., Aguilar-Martínez, C.U., Valenzuela-Jiménez, N., Sánchez-Hernández, C., Jiménez-Rojas, M.C. & Villanueva-Verduzco, C. 2011. Plant density and growth of forage maize genotypes. *Agronomía mesoamericana* **22**(2), 281–295.

Vencovsky, R. 1987. Quantitative genetics. *In*: Corn breeding and production in Brazil (Herança quantitativa. *In*: Paterniani, E. (ed). Melhoramento e Produção do Milho no Brasil). Edição da Fundação Cargill. Instituto de Genética, Esc. Sup. Agricultura Luis de Queiroz, Universidad de São Paulo. Piracicaba. São Paulo, pp. 122–199.

Agronomy Research **18**(1), 88–99, 2020
https://doi.org/10.15159/AR.20.017

Biomass yield of silage maize, fertilizers efficiency, and soil properties under different soil-climate conditions and fertilizer treatments

L. Hlisnikovský[1], P. Barlog[2], E .Kunzová[1], M. Vach[1] and L. Menšík[3]

[1]Division of Crop Management System, team Management of Nutrients in Agrosystems, Crop Research Institute, Drnovská 507, CZ161 01 Prague 6, Czech Republic
2Poznan University of Life Sciences, Department of Agricultural Chemistry and Environmental Biogeochemistry, Wojska Polskiego 71F, PL60-625 Poznan, Poland
[3]Division of Crop Management System, team Sustainable Management and Utilization of Permanent Grassland, Crop Research Institute, K.H. Borovského 461, CZ569 43 Jevíčko, Czech Republic

Abstract. We evaluated the efficiency (the netto agronomic efficiency – NAE, the physiological efficiency – PE, and the apparent recovery efficiency – ARE) of farmyard manure (FYM) applied alone, and together with mineral N (FYM+N), and NPK (FYM+NPK), on the biomass production of silage maize at three localities (Caslav, Ivanovice, Lukavec) in the Czech Republic, characterised by different soil-climate conditions. The effect of fertilizer treatment on soil chemical properties was also analyzed. After four years of evaluation, the application of FYM resulted in comparable biomass production as in the FYM+N, and FYM+NPK treatments, showing the good ability of the mineralized FYM to provide enough nutrients during the growing season. Increasing doses of applied nutrients were connected with higher biomass production. However, no significant differences were recorded between fertilizer treatments. The efficiency of applied nutrients was higher on soils of worst quality (sandy loamy Cambisol – Lukavec), while lower on naturally fertile loamy degraded Chernozem (Ivanovice). But again, no significant differences between the selected parameters were recorded. Although the application of mineral fertilizers has not increased maize biomass yield significantly, they positively affected soil chemical properties, mainly the soil concentration of P, K, Mg, and soil organic carbon content. This shows the beneficial effect of the application of mineral fertilizers, especially in the Czech Republic, where the application of mineral P and K decreased drastically during the last thirty years.

Key words: *Zea mays* L., organic manure, NPK, soil–climate conditions, fertilizer efficiency.

INTRODUCTION

The silage maize represents one of the most important strengthening feed for ruminant livestock. Maize is widely used and popular among livestock farmers due to its positive characteristics, such as high yields, palatability, high energy content, and low labour and machinery inputs. Maize also serves as a base material for agricultural biogas stations and source material for ethanol production (Klopfenstein et al., 2013; Yu et al., 2016; Kuglarz et al., 2019).

Every farmer tries to achieve high yields of cultivated crops. For that reason, farmers select suitable cultivars (Mandić et al., 2017), environmental conditions (Peichl et al., 2019), planting techniques (Novák et al., 2019), and fertilizers (Fageria, 2001; Hirzel et al., 2007; Černý et al., 2012; Kuglarz et al., 2019). The selection of fertilizers and their doses represents a crucial step in the farm's management. The choice of fertilizer type and the dose is influenced by the availability of funds and fertilizers in order to optimize yields, maintain soil fertility, and minimize negative environmental impacts. This leads to a specific situation in the Czech Republic because many farmers have literally abandoned the application of mineral P and K fertilizers due to their high prices, and the entire conventional agricultural sector is dependent on mineral nitrogen (Fig. 1).

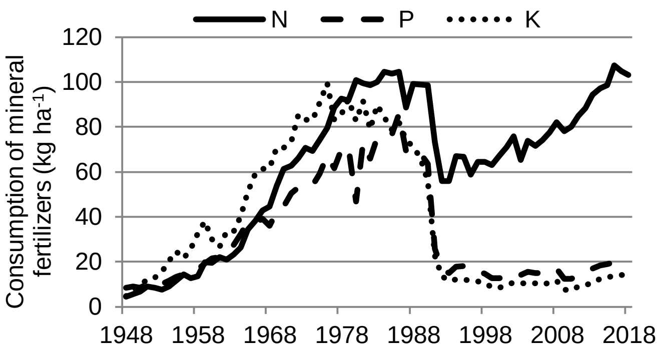


Figure 1. The mean consumption of mineral N, P, and K in the Czech Republic since 1948.

Silage maize is usually fertilized with organic manures. According to Lamptey et al. (2017), application of organic manure applied together with chemical fertilizers positively affects the leaf area index and leaf chlorophyll content, which results in higher dry matter accumulation and nitrogen content. The recommended dose of farmyard manure applied in autumn is approximately up to 40 t ha^{-1} in the Czech Republic. Application of slurries in the spring is even more efficient way of delivering the nutrients, because the mineralization process of manures with low C:N ratio is quicker, the amount of released nitrogen is higher, and the timing of nitrogen release meets the increased requirements of maize for that element. Maize is also considered as a plant requiring high doses of phosphorus and potassium. If no organic manures were applied, the application of mineral NPK in autumn and NP during the planting is recommended.

The efficiency of fertilizers varies significantly depending on soil type and season's weather conditions. According to Berenguer et al. (2008), the minimum rates for achieving maximum yields in northeast Spain (Petrocalcic Calcixerept soil type) are 30 m^3 ha^{-1} of pig slurries (PS) applied together with 100 kg N ha^{-1}, or 60 m^3 of PS per ha without any addition of mineral fertilizers. In Belgium, where sandy loam soil occurs, is the recommended dose of cattle slurry 180 kg ha^{-1}, applied together with 90 kg of inorganic N ha^{-1} (Nevens & Reheul, 2005). Higher nitrogen inputs are associated not only with unnecessary financial costs, but also closely affect the environment and long-term sustainability. According to several papers, many farmers in China apply high doses of fertilizers, mainly nitrogen and phosphorus, to obtain high yields due to rising maize prices. This leads to nutrient accumulation in the soil, causing environmental problems if concentrations exceed critical limits (Zhao et al., 2006; Xu et al., 2014). The situation is particularly sensitive in the case of maize because slow growth and occurrence of yellow whirls in spring can be misinterpreted as nitrogen deficiency, which is followed by the application of further dressings (Nevens & Reheul, 2005). Another risk of over-fertilization raises from the fact, that maize is predetermined for slurry application due to its late sowing date. Many farmers think about manures as a waste product and don't reduce the amount of mineral nutrients applied on the field together with manures (Schröder et al., 2000; Berenguer et al., 2008).

According to Černý et al. (2012) the amount of nutrients uptake in the Czech Republic is higher than their inputs to the soil via the applied fertilizers. As shown in Figure 1, most companies in the Czech Republic apply only mineral nitrogen and the application of mineral phosphorus, potassium, and organic manures is significantly restricted, which may negatively affect the soil properties in the long term. The aim of this work is to find out whether the application of manure can provide comparable conditions to maize as mineral fertilizers and what the farmer will benefit from, or will bear the consequences of the application of individual types of fertilizers. For that reason, we evaluated the maize biomass yield (BY), nutrients uptake (N, P, K), netto agronomic efficiency (NAE), physiological efficiency (PE), apparent recovery efficiency (ARE), and soil chemical properties at three localities with different soil-climate conditions and over four years (2012–2015).

MATERIAL AND METHODS

Site description

Three long-term experiments were established in three locations with different soil-climate conditions in the Czech Republic in 1955. The long-term experiments aim is to analyze the effect of different fertilizer treatments and soil-climate conditions on the yield of arable crops and soil chemical parameters. The experiments are located in Caslav, Ivanovice na Hané, and Lukavec. The basic description of the experimental sites is given in Table 1.

Table 1. The basic description of the experimental localities between 2012 and 2015

Experimental station	Caslav	Ivanovice	Lukavec
Altitude (m a.s.l.)	263	225	620
Mean temperature 1956–2006 (°C)	8.9	8.4	6.8
Mean temperature 2012 (°C)	9.8	9.6	8.1
Mean temperature 2013 (°C)	9.4	9.2	7.3
Mean temperature 2014 (°C)	11.4	10.5	8.8
Mean temperature 2015 (°C)	11.0	10.4	8.7
Mean precipitation 1956–2006 (mm)	556	555	686
Mean precipitation 2012 (mm)	637	482	747
Mean precipitation 2013 (mm)	621	551	876
Mean precipitation 2014 (mm)	618	520	936
Mean precipitation 2015 (mm)	442	387	576
Soil type	Greyic Phaeozem	Loamy degraded Chernozem	Sandy loamy Cambisol

Experiment methodology

The long-term experiments in Caslav, Ivanovice, and Lukavec have the same standardized design. The experiment consists of four fields in each location. Together twelve different fertilizer treatments with four replications are evaluated on each field, arranged in a completely randomized block design (12×4 = 48 experimental plots per field). The size of the experimental plot is 8×8 m^2, but to eliminate the edge effect only the 5×5 m central area is used to get the samples for analysis.

Out of twelve fertilizer treatments, we evaluated four treatments in this paper: 1) Control (unfertilized since 1955), 2) cattle farmyard manure (FYM), 3) cattle farmyard manure combined with mineral nitrogen (FYM+N), and 4) cattle farmyard manure combined with mineral NPK (FYM+NPK). The cattle farmyard manure was each year plowed into the soil in the autumn at a dose of 40 t ha^{-1} (typical dose of manure applied to maize by farmers in the Czech Republic). The content of N, P, and K in the FYM was approximately 200, 56, and 236 kg ha^{-1}, respectively. Mineral nitrogen was applied as ammonium nitrate with lime. Mineral phosphorus was applied as triple superphosphate. Mineral potassium was applied as potassium chloride. Mineral N was applied in two dressings (½ of the dose was applied before planting, ½ was applied at BBCH 16 – 6 leaves unfolded). Mineral P and K were applied in the autumn. The dose of mineral N, applied in the FYM+N and FYM+NPK treatments, was 80 and 120 kg ha^{-1}, respectively. The dose of mineral P, and K, applied in the FYM+NPK treatment, was 80 and 100 kg ha^{-1}, respectively. The total amount of clear nutrients, applied in the analyzed fertilizer treatments, shows Table 2. The influence of fertilizer treatment on the pH value, and concentration of N, P, K, Mg, Ca, $C_{org.}$, and $N_{tot.}$ over the evaluated period is shown in Table 3.

Table 2. The doses of N, P, and K applied in experimental fertilizer treatments

Fertilizer treatment	Nutrient (kg ha^{-1})		
	N	P	K
Control	0	0	0
FYM	200	56	236
FYM+N	280	56	236
FYM+NPK	320	136	336

Table 3. Soil chemical properties in the Control, FYM, FYM+N, and FYM+NPK treatments in Caslav, Ivanovice, and Lukavec in 2012–2015. Assessment of the P, K, and Mg (mg kg^{-1}) soil concentrations were made according to Budňáková et al. (2004)

Treatment	pH	P	P assess.	K	K assess.	Mg	Mg assess.	Ca	$C_{org.}$ (%)	$N_{tot.}$ (%)
Caslav										
Control	7.47a	44a	Low	97a	Low	110a	Suitable	3610a	1.26a	0.13a
FYM	7.48a	64a	Suitable	131a	Suitable	130b	Suitable	3497a	1.38ab	0.14a
FYM+N	7.35a	54a	Suitable	124a	Suitable	145c	Suitable	3083a	1.47bc	0.15b
FYM+NPK	7.29a	138b	High	195b	Good	148c	Suitable	3085a	1.53c	0.17c
Ivanovice										
Control	7.55a	97a	Good	199a	GSuitable	195a	GSuitable	4409a	1.87a	0.19a
FYM	7.50a	144b	High	280b	Good	216ab	GSuitable	4466a	1.97a	0.20ab
FYM+N	7.56a	136b	High	293b	Good	235bc	Good	4435a	2.17b	0.22c
FYM+NPK	7.43a	192c	Very high	364c	High	244c	Good	4342a	2.14b	0.21bc
Lukavec										
Control	6.45b	37a	Low	111a	Suitable	104a	Low	2016a	1.61a	0.17a
FYM	6.43b	72b	Suitable	148bc	Suitable	116a	Suitable	2114a	1.84b	0.20b
FYM+N	6.28ab	39a	Low	125ab	Suitable	104a	Low	2038a	1.90b	0.21b
FYM+NPK	6.20a	176c	High	156c	Suitable	103a	Low	2114a	1.93b	0.21b

The crop rotation of the long-term experiments consists of eight crops: *Hordeum vulgare* L., *Trifolium pratense* L., *Triticum aestivum* L., *Zea mays* L., *Hordeum vulgare* L., *Brassica napus* L., *Tritticosecale* W., *Solanum tuberosum* L. Maize was each year planted during the April (Caslav, Ivanovice), and up to May, 10th (Lukavec). The maize cultivar used in the experiment was LG 32.58. The planting rate was 95–100 thousand seeds ha^{-1}, the row spacing was 0.70×0.15 m.

Nitrogen efficiency of applied fertilizers

The efficiency of applied fertilizers was calculated according to Fageria et al. (2010). The netto agronomic efficiency (NAE, kg) was calculated as:

$$\left(\frac{Y_T - Y_C}{N_T}\right) \qquad (1)$$

where Y_T is the biomass yield of fertilized treatment; Y_C is the biomass yield of the Control treatment; N_T represents the dose of applied nitrogen.

The physiological efficiency (PE, kg) was calculated as:

$$\left(\frac{Y_T - Y_C}{N_{TU} - N_{CU}}\right) \qquad (2)$$

where N_{TU} represents N uptake of the fertilizer treatment; N_{CU} represents N uptake of the Control treatment.

The apparent recovery efficiency (ARE, %) was calculated as:

$$\left(\frac{N_{TU} - N_{CU}}{N_T}\right) \cdot 100 \qquad (3)$$

Soil properties

Soil samples were collected at two depths (0.3 and 0.3–0.6 m) in spring, before N application. The pH of the soil was measured after shaking for 2 h in the suspension of 0.2 M KCl_2 (soil/solution ratio 2.5:1; w/v). The $C_{org.}$ was determined by combustion analysis using a Vario Max analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Content of plant-available forms of phosphorus, potassium, magnesium, and calcium was established by the extraction in Mehlich 3 reagent (Mehlich, 1984), followed by ICP-OES analysis (Thermo Jarrell Ash, Trace Scan, Franklin, USA). Mineral nitrogen ($N_{tot.}$) was determined in field-fresh soil samples by using 1% K_2SO_4 (soil/solution ratio 5:1; w/v). Concentrations of NH_4-N and NO_3-N were determined by the colorimetric method using the flow injection analyses (SAN plus SYSTEM, SKALAR, De Breda, The Netherlands).

Statistical analysis

The biomass yield (BY – t ha^{-1}) was analyzed by one-way ANOVA and factorial ANOVA (MANOVA). All statistical analyses were performed using Statistica 13.3 (Tibco Software, Inc.).

RESULTS AND DISCUSSION

The BY was significantly affected by locality ($p < 0.001$, 11%), fertilizer treatment ($p < 0.001$, 25%), weather conditions during the year ($p < 0.001$, 37%), and by the interaction between all analyzed factors, mainly by locality and year ($p < 0.001$, 25%).

Ivanovice was the most productive area with the average BY 19.62 t ha^{-1}, followed by Caslav (17.71 t ha^{-1}), and Lukavec (17.51 t ha^{-1}). Ivanovice has the best combination of conditions for growing the maize, including the soil type and climate. These conditions (fertile Chernozem, lower altitude, higher mean temperature, decent precipitation) are optimal for C4 plants and create a beneficial natural background for maize production in this area. Čáslav has climatic conditions comparable with Ivanovice, but has a different soil type. Both Phaeozems and Chernozems, represent the most fertile soil types in the Czech Republic (Kozák et al., 2003), and are almost comparable, but Phaeozems are more prone to leaching during the wet seasons and do not contain carbonates in the topsoil layer (European Commission, 2005). Finally, Cambisols in Lukavec are characteristic of low natural fertility and high nutrient depletion (Hejcman & Kunzová, 2010), with the climate conditions more suitable for growing the C3 plants.

The highest yields were recorded in the FYM+NPK treatment at all sites (Table 4). Generally, the increasing dose of nutrients is connected with increasing biomass production (Fig. 2). This is supported by other studies (Kunzová & Hejcman, 2009; Wei et al., 2016; Xin et al., 2017). However, in comparison with the FYM, the application of FYM+N and FYM+NPK was not connected with higher yields (Table 4, the last column). In this respect, the application of mineral P and K fertilizers to farmers is unnecessarily expensive and does not outweigh the resources invested in the purchase and application of fertilizers. A comparison of the uptake of N, P, and K from one hectare (kg ha^{-1}), and the uptake of N, P, and K, for the production of one tone of the BY (kg t^{-1}), as affected by locality and fertilizer treatment, is shown in Table 5. These data were used for calculation of the NAE, the PE, and the ARE (Table 6). The mean BY as affected by locality, fertilizer treatment, and year are shown in Table 4.

Table 4. Biomass yield (t ha^{-1}) as affected by locality, fertilizer treatment, and year

		BY (t ha^{-1})				
Locality	Fertilizer treatment	2012	2013	2014	2015	
Caslav	Control	17.4 ± 0.4^{Ab}	13.0 ± 0.2^{Aa}	13.8 ± 0.8^{Aa}	12.6 ± 0.3^{Aa}	14.2 ± 0.5^{A}
	FYM	22.6 ± 0.3^{Bc}	17.9 ± 0.3^{Bb}	17.8 ± 0.7^{Bb}	13.5 ± 0.3^{ABa}	18.0 ± 0.9^{B}
	FYM+N	23.5 ± 0.2^{Bd}	17.4 ± 0.4^{Bb}	20.8 ± 0.6^{Cc}	14.0 ± 0.3^{BCa}	18.9 ± 0.9^{B}
	FYM+NPK	24.9 ± 0.3^{Cc}	18.9 ± 1.6^{Bb}	20.4 ± 0.3^{Cb}	15.0 ± 0.3^{Ca}	19.8 ± 1.0^{B}
		22.1 ± 0.7^{c}	16.8 ± 0.7^{b}	18.2 ± 0.8^{b}	13.8 ± 0.3^{a}	
Ivanovice	Control	18.0 ± 0.4^{Aa}	21.2 ± 0.5^{Ab}	16.0 ± 0.3^{Aa}	17.0 ± 0.9^{Aa}	18.0 ± 0.6^{A}
	FYM	19.7 ± 0.4^{Bb}	22.5 ± 0.4^{Ac}	15.9 ± 0.5^{Aa}	17.3 ± 0.2^{Aa}	18.8 ± 0.7^{AB}
	FYM+N	20.1 ± 0.3^{Bb}	24.7 ± 0.3^{Bc}	18.3 ± 0.4^{Ba}	18.2 ± 0.3^{Aa}	20.3 ± 0.7^{AB}
	FYM+NPK	20.8 ± 0.4^{Ba}	26.3 ± 0.6^{Bb}	19.0 ± 0.2^{Ba}	19.1 ± 0.5^{Aa}	21.3 ± 0.8^{B}
		19.6 ± 0.3^{c}	23.7 ± 0.5^{d}	17.3 ± 0.4^{a}	17.9 ± 0.3^{b}	
Lukavec	Control	18.0 ± 0.4^{Ac}	10.2 ± 0.4^{Aa}	18.6 ± 0.1^{Ac}	12.2 ± 0.4^{Ab}	14.7 ± 1.0^{A}
	FYM	21.7 ± 1.7^{ABb}	11.9 ± 0.9^{ABa}	20.3 ± 1.4^{Ab}	12.7 ± 0.7^{Aa}	16.7 ± 1.3^{AB}
	FYM+N	25.1 ± 0.5^{Bc}	12.9 ± 1.2^{ABa}	19.1 ± 0.3^{Ab}	16.3 ± 0.5^{Bb}	18.4 ± 1.2^{AB}
	FYM+NPK	25.6 ± 0.9^{Bb}	14.2 ± 0.7^{Ba}	24.8 ± 1.1^{Bb}	16.6 ± 1.0^{Ba}	20.3 ± 1.4^{B}
		22.6 ± 0.9^{b}	12.3 ± 0.5^{a}	20.7 ± 0.7^{b}	14.4 ± 0.6^{a}	

Mean values with the standard error of the mean followed by the same letter (A vertically, a horizontally) are not statistically significantly different ($\alpha < 0.05$).

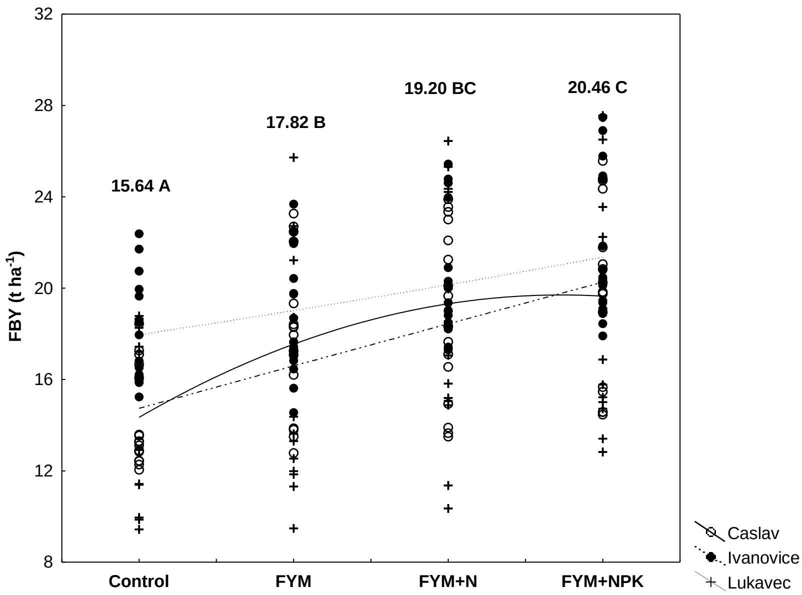


Figure 2. The mean BY (t ha^{-1}) as affected by the locality and fertilizer treatment.

Table 5. The uptake of N, P, and K from 1 ha (kg ha^{-1}), and the uptake of N, P, and K (kg t^{-1}) per production of 1 t of BY

		N (kg ha^{-1})	N (kg t^{-1})	P (kg ha^{-1})	P (kg t^{-1})	K (kg ha^{-1})	K (kg t^{-1})
Caslav	Control	160	10.8	23	1.6	79 a	5.4 a
	FYM	215	12.0	31	1.7	127 b	7.1 ab
	FYM+N	252	12.1	34	1.6	137 b	6.7 ab
	FYM+NPK	259	12.3	41	1.9	157 b	7.4 b
Ivanovice	Control	156	8.6	19	1.0	131	7.4
	FYM	176	9.3	23	1.1	191	10.3
	FYM+N	222	10.8	22	1.2	201	10.1
	FYM+NPK	242	11.9	33	1.7	195	9.4
Lukavec	Control	150	10.3	25	1.7	101	7.0
	FYM	190	11.4	29	1.8	123	7.7
	FYM+N	236	12.9	33	1.8	151	8.5
	FYM+NPK	253	12.8	40	2.0	199	9.8

The NAE is defined as the economic production obtained per unit of nutrient applied (Fageria et al., 2010). In other words, it represents the kg yield increase per kg N applied (Černý et al., 2012). The NAE values were highest at Caslav experimental station (Table 6), ranging from 24 kg in 2012 to 6 kg in 2015. This shows that agronomic efficiency, the use of nitrogen from nitrogen fertilizers, is strongly influenced by the season's conditions (Černý et al., 2012). The year 2015 was the first year characterized by the starting wave of droughts, which continues up today. During the dry years, the mineralization process of manures is significantly inhibited, and the use of mineral fertilizers is also negatively affected. It was very well documented by Hlisnikovsky et al. (2014), when warm weather front from east Europe strongly affected the South Moravian Region in the Czech Republic in 2012, significantly reducing the yields of winter wheat, no matter what kind of fertilizers and doses were applied. The lowest NAE values were recorded at Ivanovice, ranging from 4 kg (2015) to 12 kg (2013) per 1 kg of applied N. These values are very low, but relatively stable during the evaluated period. For example, Černý et al. (2012) recorded values ranging from 26.7 to 27.5 kg in treatments where 60 and 120 kg of N were applied to maize before sowing. In our case, these values were recorded at Caslav (2012, 2013, 2014), and Lukavec (2012). Comparable NAE values as in Ivanovice were recorded at Lukavec experimental station (except 2012). This is interesting because these two localities stand on two opposite poles in terms of soil-climate conditions. It shows that both localities have comparable N utilization efficiency, but soil-climate conditions represent natural borders limiting the BY production. In other words, the same N utilization can provide different BY, as the mean BY was higher at Ivanovice. It also shows that growing maize plants extracted N from other sources at Ivanovice, because the highest yields were obtained here together with the lowest NAE values. The source was naturally highly fertile Chernozem, which means that the amount of applied fertilizers could be lowered here below the doses applied in our experiment, saving farmer's financial and material inputs. A more detailed analysis will be the subject of further work.

Table 6. Netto agronomic efficiency (NAE, kg) per production of BY as affected by fertilizer treatment and year

		NAE				
Locality	Fertilizer treatment	2012	2013	2014	2015	
Caslav	FYM	26 ± 2Ab	25 ± 1Ab	20 ± 7Ab	4 ± 3Aa	19 ± 3A
	FYM+N	22 ± 2Abc	16 ± 2Ab	25 ± 2Ac	5 ± 2Aa	17 ± 2A
	FYM+NPK	23 ± 1Ab	18 ± 5Ab	20 ± 2Ab	8 ± 2Aa	17 ± 2A
		24 ± 1b	20 ± 2b	22 ± 2b	6 ± 1a	
Ivanovice	FYM	8 ± 3Aa	7 ± 2Aa	1 ± 3Aa	2 ± 4Aa	4 ± 2A
	FYM+N	8 ± 2Aa	13 ± 1Ba	8 ± 2Ba	4 ± 3Aa	8 ± 1AB
	FYM+NPK	9 ± 1Aab	16 ± 1Bb	10 ± 1Bab	7 ± 3Aa	10 ± 1B
		8 ± 1ab	12 ± 1b	6 ± 2a	4 ± 2a	
Lukavec	FYM	19 ± 10Aa	9 ± 6Aa	9 ± 7ABa	3 ± 6Aa	10 ± 4A
	FYM+N	25 ± 1Ac	10 ± 3Aab	2 ± 1Aa	15 ± 2Ab	13 ± 2A
	FYM+NPK	24 ± 3Aa	13 ± 3Aa	19 ± 4Ba	14 ± 2Aa	17 ± 2A
		23 ± 3b	10 ± 2a	10 ± 3a	10 ± 3a	

Mean values with the standard error of the mean followed by the same letter (A vertically, a horizontally) are not statistically significantly different ($\alpha < 0.05$).

The physiological efficiency (PE) is defined as the biological yield obtained per unit of nutrient uptake (Fageria et al., 2010). In other words, it represents a kg yield increase per kg increase in N uptake from fertilizer (Černý et al., 2012). The apparent recovery efficiency (ARE) represents the quantity of nutrient uptake per unit of nutrient uptake (Fageria et al., 2010). The PE values varied from 51 to 84 at Caslav, from 46 to 60 at Lukavec, and from 34 to 41 at Ivanovice (Table 7). Not any statistical differences were recorded between the treatments at all localities. These results show that the efficiency of all treatments was comparable, slightly higher at Caslav (FYM treatment). The ARE values ranged between 28–33% in Caslav, from 10–27% in Ivanovice, and 20–32% in Lukavec (Table 7). The lowest values were recorded in the FYM treatment, which means that plant N demands were worse satisfied by N released from the mineralized manure. But again, the results were not statistically different. Comparing all localities, both indicators were not statistically different, but the lowest values were recorded at Ivanovice experimental station, indicating a strong effect of the soil-climate conditions, recorded as well as in the case of the BY and NAE.

The application of all fertilizer treatments significantly affected soil chemical composition (Table 3). The lowest value of pH was recorded at Lukavec, which is the only locality, where the application of mineral fertilizers significantly decreased the pH. No effect of treatment on the value of pH was recorded at Caslav and Ivanovice, although the decreasing pattern of pH with an increasing dose of mineral nutrients was recorded. The lower pH at Lukavec is generally a natural property of the soil type. The significant effect of mineral fertilizers on decreasing pH at Lukavec is caused by lighter soil and lower sorption capacity (Vašák et al., 2015). On the other hand, the high buffering capacity of Chernozems against the soil acidification helps the soil to resists the negative effects of mineral fertilizers (Vašák et al., 2015), such as at Ivanovice.

Table 7. Physiological efficiency (PE), and apparent recovery efficiency (ARE) as affected by locality and fertilizer treatment over the whole time of the experiment

Locality	Fertilizer treatment	PE (kg)	ARE (%)
Caslav	FYM	84 ± 36A	28 ± 5A
	FYM+N	51 ± 12A	33 ± 3A
	FYM+NPK	58 ± 12A	31 ± 3A
		64 ± 10a	31 ± 1a
Ivanovice	FYM	-	10 ± 6A
	FYM+N	34 ± 5A	23 ± 3A
	FYM+NPK	41 ± 8A	27 ± 6A
		38 ± 4a	20 ± 5a
Lukavec	FYM	53 ± 11A	20 ± 7A
	FYM+N	46 ± 18A	31 ± 12A
	FYM+NPK	60 ± 9A	32 ± 9A
		53 ± 4a	28 ± 4a

Mean values with the standard error of the mean followed by the same letter (A vertically for a particular locality, a for comparison of mean values between localities) were not significantly different ($\alpha < 0.05$).

Phosphorus concentration in the soil was positively affected by the application of all fertilizers (Table 3). The higher concentration of soil P was recorded in FYM and FYM+NPK treatments, showing higher utilization of P in the FYM+N treatment for the production of higher BY (in comparison with the FYM treatment). According to our results, the application of FYM with mineral N only can lead to a higher biomass yield (in comparison with the FYM applied without mineral N), but also the one-way soil P depletion can occur. The decrease of the soil P concentration at FYM+N treatment was significant only at Lukavec, but the pattern is visible at all stations and could become significantly evident in a longer period. A similar situation was recorded in the case of potassium (Tables 3).

Concentration of Mg and Ca exhibited a reciprocal relationship at Caslav (r = -0.9) and Ivanovice (r = -0.4), while a strong and positive relationship at Lukavec (r = 0.8). The concentration of Mg significantly increased with the application of mineral N and NPK, showing the enhancing effect of mineral nitrogen on availability of Mg. Similar results of a positive effect of N on other nutrients availability were published by Adeniyan et al. (2011). The concentration of Ca was not affected by the application of any fertilizer at all localities.

The $C_{org.}$ was positively influenced by the application of all fertilizer treatments (Table 3). The highest concentrations were recorded in FYM+N and FYM+NPK treatments. The lowest C_{org}. content was recorded in the unfertilized Control treatment at all localities. As the aboveground biomass of the preceding was not incorporated back to the soil, the increase of $C_{org.}$ is presumably caused by a positive effect of applied organic manures via its content of organic matter, increased proliferation in the upper soil layers, improved soil physical properties (Macholdt et al., 2019), and increased nutrient and water availability (Mosaddeghi et al., 2009). Application of mineral fertilizers also increases the root biomass and crop productivity, especially in wheat (Hirte et al., 2018), which was the preceding crop to maize in our experiment. The same positive effect of fertilizer treatment on the soil content of $C_{org.}$ was published by Kanchikerimath & Singh (2001), Cai & Qin (2006), Zhao et al. (2013), and Xin et al. (2016). The $N_{tot.}$ content was significantly affected by fertilizer application, and locality (Table 3). The application of mineral fertilizers positively affected the $N_{tot.}$ at Caslav (Control, FYM < FYM+N < FYM+NPK), but the different pattern was recorded at Ivanovice (the highest content was recorded in FYM+N treatment), and no differences were recorded between the fertilizer treatments, except the Control, at Lukavec (Table 3). Our results show that the response of the soil on the applied fertilizers is not uniform, strongly depending on the soil type and climate conditions of the locality.

CONCLUSIONS

The BY of silage maize strongly depends on the soil type and climate conditions of the site, where maize is grown. In our case, the most productive site was Ivanovice, offering naturally fertile Chernozem and the most suiTable climate conditions for growing the maize. In comparison with Lukavec, offering higher altitude, lower temperature, higher precipitation, and sandy loamy Cambisol soil type, the BY was 11% higher at Ivanovice. By comparing the effect of fertilization on the BY, we have found that the application of the FYM+NPK achieved the highest yields. With the decreasing quality of soil (Chernozem<Phaeozem<Cambisol) the efficiency of applied fertilizers increased. However, the application of mineral fertilizers together with manure did not bring a statistically significant increase in yield. This shows the good ability of manure to provide maize with sufficient nutrients during the growing season, and additional application of mineral fertilizers can save farmer's finance. This is confirmed by the results of the analyzed fertilizer effectiveness (NAE, PE, ARE), which was not significantly influenced by the fertilization variant but by the locality. However, the application of mineral fertilizers affected soil properties. Application of mineral fertilizers slightly decreased the soil pH value at Caslav, and Ivanovice, and increased the concentrations of P, and K, in the soil. The soil $C_{org.}$ content was also positively affected by the application of manure together with mineral fertilizers. This is very important for the future sustainability of conventional farming, especially under conditions of the Czech Republic, where the application of mineral phosphorus and potassium is very low since 1990.

ACKNOWLEDGEMENTS. The writing of the paper was supported by the Ministry of Agriculture of the Czech Republic by project MZe-RO0418.

REFERENCES

Adeniyan, O.N., Ojo, A.O., Akinbode, O.A. & Adediran, J.A. 2011. Comparative study of different organic manures and NPK fertilizer for improvement of soil chemical properties and dry matter yield of maize in two different soils. *J. Soil Sci. Environ. Manage*. **2**, 9–13.

Berenguer, P., Santiveri, F., Boixadera, J. & Lloveras, J. 2008. Fertilisation of irrigated maize with pig slurry combined with mineral nitrogen. *Eur. J. Agron*. **28**, 635–645.

Budňáková, M., Čermák, P., Hauerland, M. & Klír, J. 2004. Zákon o hnojivech a navazující vyhlášky [Fertilizers Act and related edicts]. ÚZPI, Prague.

Cai, Z.C. & Qin, S.W. 2006. Dynamics of crop yields and soil organic carbon in a long-term fertilization experiment in the Huang-Huai-Hai plain of China. *Geoderma* **136**, 708–715.

Černý, J., Balík, J., Kulhánek, M., Vašák, F., Peklová, L. & Sedlář, O. 2012. The effect of mineral N fertiliser and sewage sludge on yield and nitrogen efficiency of silage maize. *Plant Soil Environ.* **58**, 76–83.

Fageria, V.D. 2001. Nutrient interactions in crop plants. *J. Plant. Nutr*. **2498**, 1269–1290.

Fageria, N.K., de Morais, O.P. & dos Santos, A.B. 2010. Nitrogen use efficiency in upland rice genotypes. *J. Plant. Nutr*. **33**, 1696–1711.

Hejcman, M. & Kunzová, E. 2010. Sustainability of winter wheat production on sandy-loamy Cambisol in the Czech Republic: Results from a long-term fertilizer and crop rotation experiment. *Field Crop. Res*. **115**, 191–199.

Hirte, J., Leifeld, J., Abiven, S. & Mayer, J. 2018. Maize and wheat root biomass, vertical distribution, and size class as affected by fertilization intensity in two long-term field trials. *Field Crop. Res*. **216**, 197–208.

Hirzel, J., Matus, I., Novoa, F. & Walter, I. 2007. Effect of poultry litter on silage maize (Zea mays L.) production and nutrient uptake. *Span. J. Agric. Res*. **5**, 102–109.

Hlisnikovský, L., Kunzová, E., Hejcman, M. & Dvořáček, V. 2014. Effect of fertilizer application, soil type, and year on yield and technological parameters of winter wheat (Triticum aestivum) in the Czech Republic. *Arch. Agron. Soil Sci*. **61**, 33–53.

Kanchikerimath, M. & Singh, D. 2001. Soil organic matter and biological properties after 26 years of maize-wheat-cowpea cropping as affected by manure and fertilization in a Cambisol in semiarid region of India. *Agr. Ecosys. Environ*. **86**, 155–162.

Klopfenstein, T.J., Erickson, G.E. & Berger, L.L. 2013. Maize is a critically important source of food, feed, energy and forage in the USA. *Field Crop. Res*. **153**, 5–11.

Kozák, J., Borůvka, L. & Němeček, J. 2003. Degradation of soils in the Czech Republic. In: Jones, R.J.A., Montanarella, L. (Eds.), Land Degradation in Central and Eastern Europe. European Soil Bureau Research Report No. 10, EUR 20688 EN. Office for Official Publications of the European Communities, Luxembourg, pp. 177–192.

Kuglarz, K., Bury, M., Kasprzycka, A. & Lalak-Kańczugowska, J. 2019. Effect of nitrogen fertilization on the production of biogass from sweer sorghum and maize biomass. *Environ. Technol*. **40**, 1–34.

Kunzová, E. & Hejcman, M. 2009. Yield development of winter wheat over 50 years of FYM, N, P and K fertilizer application on black earth soil in the Czech Republic. *Field Crop. Res.* **111**, 226–234.

Lamptey, S., Yeboah, S., Li, L. & Zhang, R. 2017. Dry matter accumulation and nitrogen concentration in forage and grain maize in dryland areas under different soil amendments. *Agron. Res*. **15**, 1646–1658.

Macholdt, J., Piepho, H.P. & Honermeier, B. 2019. Mineral NPK and manure fertilisation affecting the yield stability of winter wheat: Results from a long-term field experiment. *Eur. J. Agron.* **102**, 14–22.

Mandić, V., Bijelić, Z., Krnjaja, V., Muslić, D.R., Petrović, V.C., Đorđević, S. & Petričević, M. 2017. Effect of different nitrogen fertilization levels on maize forage yield and quality. Proceeding of the 11th International Symposium Modern Trends in Livestock Production. October 11–13.

Mehlich, A. 1984. Mehlich 3 soil extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plan.* **15**, 1409–1416.

Mosaddeghi, M.R., Mahboubi, A.A. & Safadoust, A. 2009. Short-term effects of tillage and manure on some soil physical properties and maize root growth in a sandy loam soil in western Iran. *Soil. Til. Res.* **104**, 173–179.

Nevens, F. & Reuhel, D. 2005. Agronomical and environmental evaluation of a long-term experiment with cattle slurry and supplemental inorganic N applications in silage maize. *Eur. J. Agron.* **22**, 349–361.

Novák, P., Kovaříček, P., Hůla, J. & Buřič, M. 2019. Surface water runoff of different technologies for maize. *Agron. Res.* **17**, 754–760.

Peichl, M., Thober, S., Samaniego, L., Hansjürgens, B. & Marx, A. 2019. Climate impacts on long-term silage maize yield in Germany. *Sci. Rep.* **9**, 7674

Schröder, J.J., Neeteson, J.J., Oenema, O. & Struik, P.C. 2000. Does the crop or the soil indicate how to save nitrogen in maize production? Reviewing the state of the art. *Field Crops Res.* **66**, 151–164.

Vašák, F., Černý, J., Buráňová, Š. & Kulhánek, M. 2015. Soil pH changes in long-term field experiments with different fertilizing systems. *Soil Water Res.* **10**, 19–23.

Yu, C.L., Hui, D., Deng, Q., Wang, J., Reddy, K.C. & Dennis, S. 2016. Responses of corn physiology and yield to six agricultural practices over three years in middle Tennessee. *Sci. Rep.* **6**, 1–9.

Wei, W., Yan, Y., Cao, J., Christie, P., Zhang, F. & Fan, M. 2016. Effects of combined application of organic amendments and fertilizers on crop yield and soil organic matter: An integrated analysis of long-term experiments. *Agr. Ecosys. Environ.* **225**, 86–92.

Xin, X., Zhang, J., Zhu, A. & Zhang, C. 2016. Effects of long-term (23 years) mineral fertilizer and compost application on physical properties of fluvo-aquic soil in the North China Plain. *Soil. Til. Res.* **156**, 166–172.

Xin, X., Qin, S., Zhang, J., Zhu, A., Yang, W. & Zhang, X. 2017. Yield, phosphorus use efficiency and balance response to substituting long-term chemical fertilizer use with organic manure in a wheat-maize system. *Field Crop. Res.* **208**, 27–33.

Xu, X., He, P., Pampolino, M.F., Johnston, A.M., Qiu, S., Zhao, S., Chuan, L. & Zhou, W. 2014. Fertilizer recommendation for maize in China based on yield response and agronomic efficiency. *Field Crop. Res.* **157**, 27–34.

Zhao, R.F., Chen, X.P., Zhang, F.S., Zhang, H., Schroder, J. & Römheld, V. 2006. Fertilization and nitrogen balance in a wheat–maize rotation systém in north china. *Agron. J.* **98**, 938–945.

Zhao, B.Z., Chen, J., Zhang, J.B., Xin, X.L. & Hao, X.Y., 2013. How different long-term fertilization strategies influence crop yield and soil properties in a maize field in the North China Plain. *J. Plant. Nutr. Soil Sci.* **176**, 99–109.

Agronomy Research **18**(1), 100–112, 2020
https://doi.org/10.15159/AR.20.007

Ozone treatment of stored potato tubers

P. Hutla[1,*], M. Kolaříková[1], D. Hájek[1], P. Doležal[2], E. Hausvater[2] and B. Petráčková[1]

[1]Research Institute of Agricultural Engineering, p. r. i., Drnovská 507, CZ161 01 Praha 6 – Ruzyně, Czech Republic
[2]Potato Research Institute, Inc., Protection Department, Dobrovského 2366, CZ580 01 Havlíčkův Brod, Czech Republic
[*]Correspondence: petr.hutla@vuzt.cz

Abstract. During storage, potato tubers are susceptible to different pathogen, which can attack the skin and flesh of the tubers. The most serious damage can be caused by rot inducing bacteria and fungi. A possible way to prevent microbial damage may be the use of ozone in the air ventilated through the stored tubers. However, the tubers can undergo qualitative changes, e.g. dehydration and loss of starch content. This article presents the results of a five-month experiment in which ozone concentration of 5 mg m^{-3} was periodically introduced in some of the stored potato tubers of the cultivar 'Dali'. All potato tubers were stored in closed storage boxes with a metal frame and wood panels in the floor and walls (ground area 1.6×1.2 m, height 0.95 m) which were continuously aerated using the ambient air in a potato warehouse. There was 900 kg of tubers stored in the box. At the end of the experiment, the ozonated variant was compared with the control (not treated). The ozone-treated tubers had 2.95 times lower incidence of infection by rot and the number of microorganisms on healthy tubers was lower than the control. The ozone-treated tubers were less frequently dehydrated. The water loss was higher in control by 0.86%. There was no significant difference in silver scurf manifestation or in the starch content between the two variants.

Key words: cold plasma, potato diseases, tuber quality, vegetable storage.

INTRODUCTION

At present, potatoes are mostly stored in large-capacity warehouses in bulk aerated through floor grates, with controlled environments mostly using outdoor air rather than active cooling. Another option is storage in boxes which are with air ventilation through the ambient air (Schuhmann & Gottschalk, 2012). Bacterial and fungal diseases can cause significant losses during storage. However, during growth, potato diseases should be minimised as well, especially potato late blight (*Phytophthora infestans*), because they may further manifest in storage (Runno-Paurson et al., 2016). Therefore, seed potatoes are treated before planting (Loit et al., 2018). For storage, it is important to prevent mechanical damage which is the entry point for infection. Furthermore, all mechanisms and surfaces in contact with tubers have to be maintained clean and regularly disinfected (Schuhmann & Gottschalk, 2012). Traditionally considered storage diseases are dry rot (caused by *Fusarium* and *Phoma* fungi), soft rot and potato blight on tubers. Other diseases common during potato storage are not as destructive and usually do not cause rot, but only attack the skin or thin layer of flesh beneath it (Hausvater & Doležal, 2011). In the Czech Republic, fungicides are not used and are not allowed during the potato storage period. Therefore, alternative methods are being investigated to prevent potato diseases to reduce storage losses. A possible option is the inclusion of ozone in the storage environment.

In general, ozone has germicidal effects similar to ultraviolet radiation (Gibson et al., 2019; Gündüz & Korkmaz, 2019). For this nature, it is used for air sterilization, e.g. in food industry, (Nguyen et al., 2019) or for drinking water (Wanqing et al., 2019). There is research studying the effects of ozone on the quality of agricultural crops during storage. Ozone is considered an alternative to chemical pesticides and its advantage is, among others, the absence of chemical residues in stored products (Isikber & Athanassiou, 2015), and thus can be used with organic food products.

Most relevant works focus on the application of ozone during storage of fruits and vegetables, especially since it leaves no residue on the treated product (Horvitz & Cantalejo, 2014). The need for further research, in particular into desirable O_3 concentration and exposure time, has been emphasized (Horvitz & Cantalejo, 2014). Ozone in concentration of 0.085 mg m^{-3} has been shown to significantly prolong the shelf life of broccoli and cucumbers stored at 3 °C. At the same time, ozone significantly reduces by oxidation reactions the amount of ethylene which is produced e.g. by metabolic activity in apples and pears in fruit stores (Skog & Chu, 2001). Liew & Prange (1994) have found fungistatic effects of ozone in concentrations ranging from 15.9 to 127 mg m^{-3} for carrot storage, while monitoring post harvest pathogen effects. The effect of the application of high ozone concentrations (0–5 g m^{-3}) in laboratory conditions did not affect the quality of the carrot and at the same time prevented a sharp increase in soluble solids, thereby increasing the shelf life (Souza et al., 2018).

When monitoring the effect of ozone on the properties of stored strawberries, an increased content of vitamin C was found, however, with a negative effect on strawberry flavour (Pérez et al., 1999). Similar results were obtained using infected strawberries stored in an atmosphere with ozone concentration of 3.2 mg m^{-3}. A positive effect on quality has been reported, while decay incidence, weight loss and fruit softening were reduced. (Nadas et al., 2003). When using ozone at concentration of 0.5 mg m^{-3} during orange storage, a positive effect on the reduction of rot on fruit surface was found, while aging and weight loss were also reduced (Di Renzo et al., 2005). More detailed tests with several varieties of mandarins and oranges were carried out in a storage with ozone doses of 1.6 to 60.0 mg kg^{-1}. The application of ozone did not reduce the quality of the fruit and prolonged its subsequent shelf life. Decay of fruits was delayed and weight losses were reduced (García-Martín et al., 2018).

Positive effects were also found when ozone was applied in cereals. When dosing ozone in wheat grain 0.22 mg g^{-1} min^{-1}, 96.9% of fungal spores were inactivated within 5 min. Increased temperature of the wheat grain also increased the effectiveness of the ozone treatment. These ozone concentrations did not reduce germination of the grains. (Wu et al., 2006). With application of 0.16 a 0.10 mg g^{-1} min^{-1} in barley, 96.0% of spores and a small amount of mycelium were inactivated. The effectiveness in barley also increased with temperature (Allen et al., 2003). When passing through a layer of grain, there is a phenomenon of ozone demand of the medium. Therefore, for large scale applications higher concentrations of ozone have to be used to counteract these losses (Isikber & Athanassiou, 2015).

Spencer (2003) used ozone dose 20.0 mg kg^{-1} h^{-1} for 1.7 and 21 days immediately after potato harvest, which did not show a significant effect on the incidence of diseases. A high dose of ozone was also applied once during storage. The results show that ozone reduced potato tuber diseases, but its effect was limited. Hamm (2004) monitored the effect of ozone during potato storage on silver scurf disease. In a two-year trial, a positive effect on this disease prevention could not be verified. Waterer et al. (2003) conducted tests to verify ozone application before and during storage of potatoes for disease control. They used high ozone concentration 1,000 mg m^3 for 16 s and low concentration 5 mg m^{-3} for 6 months of storage. High concentration significantly reduced the infection by bacterial soft rot (BSR), however, it did not affect Fusarium dry rot (FDR), Rhizoctonia black scurf (RBS) and silver scurf (SS). Application of low concentration during storage resulted in an increase in FDR infection. Other types of infections were not affected. Both potato samples were not stored in the same environment which could have resulted in skewed results. Reported works indicate only a limited possible utilization of ozone in potato stores. More recent studies have not been found.

Since the published results on the potato topic are ambiguous the present research aimed to verify the utility of ozone in pilot conditions. Therefore, the aim of this trial was to investigate the effect of ozone treatment, together with microclimatic conditions, on quality of potato tuber cultivar 'Dali' during 133 days in air at temperature of 4.5 °C.

MATERIALS AND METHODS

The cultivar 'Dali' of potato was used to investigate the effect of ozone during the storage period. Trials were conducted on field run potatoes obtained from commercial growers in the Vysočina Region, Czech Republic.

An amount of 900 kg of potato was stored in a closed wooden pallet box with a steel frame, whose ground plan dimensions were 1.6×1.2 m. The height of the potato layer was 0.88 m. The box was sealed using plastic film to assure a better control over the aeration of the box. Another box of the same type was stacked on top, holding bagged potatoes samples. At the top, there was an outlet hole sized 20×20 cm (Fig. 4). The stored potatoes were permanently aerated with ambient air by an air conditioning duct fan TUBE 100WK (supplier Kanlux, s.r.o., Czech Rep.) so that the air speed above the layer of potatoes was 0.012 m s^{-1} (thermal anemometer AIRFLOW TA4, Airflow, United Kingdom), which corresponded to 85 m^3 h^{-1}.

For ozone generation, the PROFIZON-X generator (Guangzhou Chuanghuan Ozone Electric Appliance Co., Ltd, China) was used (Fig. 1).

The ozone concentration was measured by a WASP-XM-E (Guangzhou Chuanghuan Ozone Electric Appliance Co., Ltd, China) (Fig. 2), under the bottom of the box, near the bottom of the potato layer. The ozone generator supplied 0.4 g h^{-1} of ozone, creating a concentration of ozone in the inlet air to the pallet of 5 mg m^{-3} pallets with a deviation of 0.5 mg m^{-3}. The ozone generator was turned on once every 48 h during 8 h.

The complete experimental setup is shown in Fig. 3. The assembly was placed within one section of a potato warehouse with computer-controlled ventilation.

An identical experimental setup was used also for the treatment in which no ozone was applied during the storage period. It was located in a different section of the warehouse to avoid cross-contamination between the treatments. The size of each section was 6 m×26 m with positive pressure aeration through underfloor channels. The sections were partially loaded each with 150 t potatoes of the same cultivar 'Dali'. For each section, a fan APR 800 (Janka Radotín, s.r.o., Czech Rep.) with a 5.5 kW motor was used.

Figure 1. Ozone generator PROFIZON-X.

Figure 2. Ozone meter WASP-XM-E.

During the storage period the parameters of the air passing through the boxes and the ambient air were monitored continuously, i.e. its temperature and the relative humidity (RH). Data loggers R 3120 (COMET SYSTEM, s.r.o., Czech Rep.) were used for monitoring the temperature and the RH. The accuracy of the temperature measurement with the data logger was ± 0.4 °C. They were placed at two locations in each storage condition: In the top box above the potatoes and outside the boxes at 4.0 m height above the floor.

Figure 3. Storage boxes with ventilator and ozone generator during the storage period of 'Dali' potato tuber.

At the end of the experiment, the health (quality) of the potatoes was evaluated. From both boxes, 12 samples were taken into 20 L container (sample weight 10–15 kg). In each sample, the number of tubers infected by mixed infection of dry rot (*Fusarium* spp.), potato gangrene (*Phoma foveata*), and soft rot (*Pectobacterium* spp.), the number of partially dehydrated tubers as well as their weight were determined.

The occurrence of skin scurf (*Helminthosporium solani*) was also evaluated. The scurf rating was performed both prior to the experiment and at the end of the experiment. Each time, the determination was done using 100 randomly selected tubers free of rot and dehydration, where the degree of infection was evaluated on a scale from 1 (non-infected) to 9 (over 90% of the skin affected). The share of infected tubers was evaluated as well as the intensity of the disease manifestation on the tubers calculated as a weighted average of the infection degree from the sample.

A sprouting test was carried out to test for a negative effect of ozone. Samples of 100 tubers for each treatment were divided into boxes containing 25 tubers and stored in the dark at 20–25 °C and 70% relative humidity for 10–12 days (Tuček et al., 1988). Tubers with 2 or more sprouts were considered to be able to germination.

The potato samples were further examined for differences in their microbiological contamination. For this purpose, tubers weighing 95–100 g were selected. The total number of microorganisms and the amount of yeast and mould were determined for 3 ozone treated and 3 control tubers. Physiological saline solution was used to rinse the tubers and subsequently dilute the samples and inoculate growth media by smear technique (ISO 6887-1, 2017).

Figure 4. Datalogger placement in a storage box.

For determination of the total number of microorganisms, an agar medium with enzymatically hydrolysed casein, yeast extract and glucose were used (standard methods agar). To determine the total number of microorganisms, 0.1 mL of the fourth and fifth ten-fold dilutions of the sample were inoculated on a medium in Petri dishes and incubated for 72 h at 30 °C.

The amount of yeast and mould was determined by horizontal method, aerobic cultivation at 25 °C on a selective agar medium with Bengal red and chloramphenicol (ISO 21527-1, 2008). For the determination of yeast and fungi, 0.1 mL of the second and third ten-fold dilutions were inoculated, followed by incubation at 25 °C for 120 h. The number of microorganisms on the tubers was derived from the number of colony-forming units (CFU).

For the quality assessment of the tubers, the dry matter content and the starch content were determined. Dry matter content was measured gravimetrically by drying at 105 °C. The content of starch was found using a Hošpes-Pecold balance. It was calculated from the weight of tubers in air and in water.

RESULTS AND DISCUSSION

The experiment took place from 1.11.2018 to 14.3.2019. Microclimatic conditions, i.e. temperature and relative air humidity, were monitored in the storage boxes and in the ambient air in the warehouse sections. These data are shown in Tables 1 and 2 during the testing period.

The differences between average monthly temperatures inside both storage boxes were 0.2 °C at most, the minimum as well as the maximum temperatures. The ambient temperatures inside the warehouse sections were close in terms of average temperatures, the difference being 0.3 °C. At the same time, the minimum and maximum temperatures differ at most 1.0 °C during some months in minimum temperature and maximum temperature. Temperature fluctuations inside the boxes were influenced primarily by the temperature and humidity of the ambient air ventilated into the box, as can be seen in Fig. 5.

Table 1. Temperatures in ozone treated and control treatments

Month	Temperatures in ozone treated box						Temperatures in non ozonated box					
	Storage box			Ambient air			Storage box			Ambient air		
	Min (ºC)	Mean (ºC)	Max (ºC)	Min (ºC)	Mean (ºC)	Max (ºC)	Min (ºC)	Mean (ºC)	Max (ºC)	Min (ºC)	Mean (ºC)	Max (ºC)
11.2018	3.4	6.5	9.4	2.3	5.9	10.3	3.4	6.4	9.4	2.8	6.2	11.3
12.2018	3.5	4.2	5.0	2.4	3.8	4.9	3.4	4.1	5.0	2.8	4.0	5.0
01.2019	2.8	3.7	4.4	1.4	3.2	4.1	2.7	3.6	4.3	2.3	3.4	4.2
02.2019	3.0	3.8	4.6	1.4	3.4	4.6	3.0	3.7	4.5	2.3	3.6	4.7
03.2019	4.4	5.3	6.1	3.6	5.2	6.1	4.2	5.1	5.9	3.5	5.2	6.1
Mean	4.6			4.2			4.5			4.3		
Standard deviation	1.4			1.5			1.4			1.5		

In Table 2, humidity in the test and control portions of the experiment are given as monthly average, minimum and maximum values for each measurement. Differences in humidity in the sections for both variants are very small - the average humidity differs by a maximum of 2.1%, the minimum humidity differed by 2.3% at most, even at the maximum humidity the differences were 5.3% at most.

Table 2. Air humidity in ozone-treated and control treatments.

Month	Air humidity in ozone treated box						Air humidity in non ozonated box					
	Storage box			Ambient air			Storage box			Ambient		
	Min (%)	Mean (%)	Max (%)	Min (%)	Mean (%)	Max (%)	Min (%)	Mean (%)	Max (%)	Min (%)	Mean (%)	Max (%)
11.2018	72.4	87.6	96.5	60.3	87.4	99.1	67.4	81.6	88.3	58.0	85.5	93.8
12.2018	75.2	88.9	95.1	67.2	85.9	95.4	68.6	80.4	85.4	67.3	84.3	92.6
01.2019	80.4	91.1	97.3	66.7	84.0	93.7	69.8	79.1	84.8	67.5	81.9	91.7
02.2019	86.0	95.5	100.0	62.8	83.5	95.1	71.5	79.3	83.4	63.5	82.0	90.6
03.2019	83.1	97.9	100.0	60.6	81.7	91.6	66.3	79.7	84.0	62.4	80.9	89.0
Mean	91.4			84.9			80.1			83.2		
Standard deviation	5.1			5.0			3.3			4.8		

The humidity in the non-ozonated pallet started at lower values than the humidity in the box, but this difference decreased during storage. The humidity in the boxes showed a considerable difference in values. This difference increased over time, from 6.0% at the start of the experiment to 18.2% at the end of storage time. High humidity in the ozonated storage box was confirmed by visual observation by formation of condensation on the inner walls of the storage box during February and March. The trends of temperature can also be observed in Fig. 5 (T_{ie} – air temperature in ozone-treatment box, T_{ic} – air temperature in non-ozonated box) and Fig. 6 (T_{ee} – air temperature in ozone-treatment section, T_{ec} – air temperature in non-ozonated section). Humidity can be watched in Fig. 7 (H_{ie} – humidity in ozone-treatment box, H_{ic} – humidity in non-ozonated box), which also confirms condensation in the period.

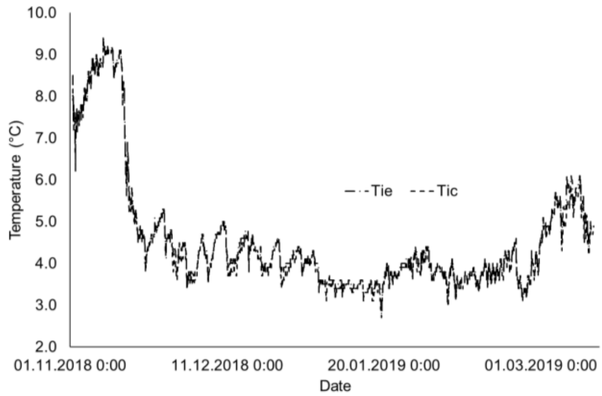


Figure 5. Air temperature in of ozone treated and non ozonated boxes.

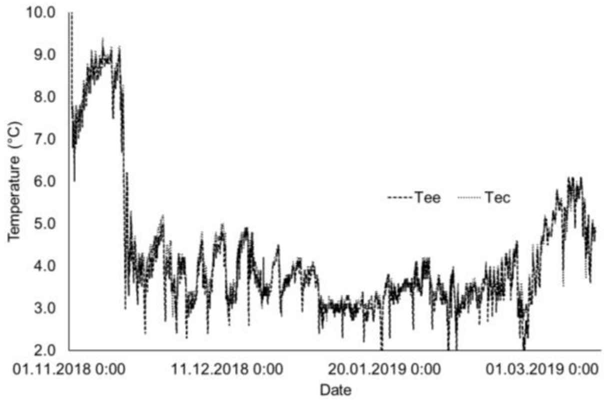


Figure 6. Air temperature in ozone-treated and non ozonated boxes.

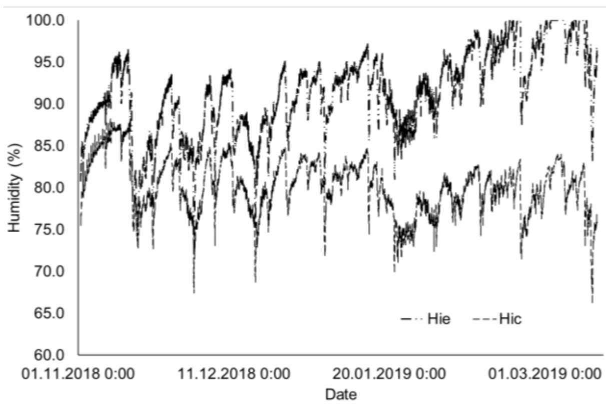


Figure 7. Humidity in ozone treated and non ozonated boxes.

Health state (quality) of the tubers was evaluated at the end of the experiment. All values are shown in Table 3 and 4, where they are also converted to a percent values.

Table 3. Quality of ozone treated potato tuber sample

Sample no.	Infected tubers				Dehydrated tubers				Healthy tubers		Sum	
	Amount		Mass		Amount		Mass		Amount	Mass	Amount	Mass
	pcs	%	kg	%	pcs	%	kg	%	pcs	kg	pcs	kg
1	4	2.60	0.208	1.58	20	12.99	1.256	9.55	130	11.694	154	13.158
2	1	0.65	0.016	0.13	14	9.15	0.794	6.47	138	11.470	153	12.280
3	2	1.72	0.178	1.51	17	14.66	1.330	11.28	97	10.280	116	11.788
4	3	2.22	0.156	1.29	16	11.85	1.010	8.35	116	10.930	135	12.096
5	2	1.54	0.076	0.59	12	9.23	0.792	6.19	116	11.924	130	12.792
6	2	1.63	0.094	0.75	9	7.32	0.658	5.27	112	11.742	123	12.494
7	2	1.37	0.084	0.63	26	17.81	1.596	12.02	118	11.600	146	13.280
8	2	1.47	0.146	1.12	16	11.76	1.192	9.11	118	11.742	136	13.080
9	0	0.00	0.000	0.00	16	13.33	1.404	10.86	104	11.524	120	12.928
10	2	1.48	0.042	0.32	23	17.04	1.636	12.49	110	11.424	135	13.102
11	1	0.60	0.056	0.42	26	15.66	1.532	11.39	139	11.864	166	13.452
12	2	1.42	0.159	1.11	16	11.35	0.994	6.96	123	13.135	141	14.228

Table 4. Quality of untreated potato tuber samples

Sample no.	Infected tubers				Dehydrated tubers				Healthy tubers		Sum	
	Amount		Mass		Amount		Mass		Amount	Mass	Amount	Mass
	pcs	%	kg	%	pcs	%	kg	%	pcs	kg	pcs	kg
1	14	9.40	1.402	12.76	6	4.03	0.304	2.77	129	9.278	149	10.984
2	8	4.94	0.490	3.93	17	10.49	1.172	9.40	137	10.812	162	12.474
3	13	7.65	1.272	10.12	26	15.29	1.334	10.62	131	9.960	170	12.566
4	3	1.85	0.394	3.46	7	4.32	0.308	2.71	152	10.678	162	11.380
5	5	2.53	0.420	3.19	21	10.61	1.208	9.19	172	11.520	198	13.148
6	12	8.05	0.524	4.41	25	16.78	1.572	13.22	112	9.794	149	11.890
7	3	2.07	0.104	0.85	21	14.48	1.272	10.37	121	10.890	145	12.266
8	7	3.68	0.322	2.55	21	11.05	1.024	8.12	162	11.264	190	12.610
9	2	1.10	0.066	0.56	26	14.29	1.124	9.56	154	10.562	182	11.752
10	6	3.26	0.314	2.46	21	11.41	0.784	6.13	157	11.692	184	12.790
11	6	2.96	0.366	3.06	16	7.88	0.866	7.23	181	10.746	203	11.978
12	3	1.70	0.108	0.91	17	9.66	0.936	7.85	156	10.878	176	11.922

Differences between ozone treated and non ozonated samples were statistically evaluated by testing the null hypothesis of equal mean values. The results are shown in Tables 5 and 6.

Table 5. Statistical comparison of infected tuber shares between variants

	Ozone treated Amount, %	Non ozonated Amount, %	Ozone treated Mass, %	Non ozonated Mass, %
Mean	1.39	4.10	0.79	4.02
Standard deviation	0.705	2.789	0.530	3.717
F-test	0.0000704		$1.97 \cdot 10^{-7}$	
t-test	0.0066		0.012	
Difference of means	2.71		3.23	

Table 6. Statistical comparison of dehydrated tuber shares between variants

	Ozone treated Amount, %	Non ozonated Amount, %	Ozone treated Mass, %	Non ozonated Mass, %
Mean	12.68	10.86	9.16	8.10
Standard deviation	3.23	4.04	2.49	3.09
F-test	0.4708		0.4908	
t-test	0.2358		0.3632	
Difference of means	1.82		1.06	

The differences between the means of the number and weight of the infected tubers in the ozone treated samples and in the non ozonated (control) samples were found to be statistically higher. The differences between the mean frequency and weight of dehydrated tubers in the ozone treated samples and in the control samples were found to be statistically nonsignificant.

The sprouting values are listed in Table 7. The results of statistical evaluation are shown in Table 8. The difference between variants being statistically significant.

Table 9 shows the numbers of microorganisms in non ozonated and ozonated tuber samples.

The results showed a significant reduction of the total number of microorganisms by about 1 order of magnitude. The fungicidal activity of ozonation was manifested by a reduction in the number of yeast and mould by 2 to 4 orders.

Table 7. Comparison of sprouting between variants

	Sample no. (25 pcs)	Sprouted tubers (at least 2 sprouts) pcs	Share of sprouted tubers, %
Ozone treated	1	25	100
	2	24	96
	3	25	100
	4	24	96
Non ozonated	1	22	88
	2	24	96
	3	22	88
	4	22	88

Table 8. Statistical comparison of sprouting

	Ozone treated, %	Non ozonated, %
Mean	98	90
Standard deviation	2.31	4.00
F-test	0.39	
t-test	0.0134	
Difference of means	8	

The effect of ozone treatment on the degree of silver scurf infestation is shown in Table 10.

From Table 10, it is clear that the ozone treatment did not affect the degree of silver scurf in tubers.

In addition, the dry matter and starch content of potato samples were determined. For this purpose, 8 ozone treated samples and 8 control samples were used. The values obtained are shown in Table 11. The measured values were evaluated statistically and the results are shown in Table 12.

Table 9. Microbiological contamination on tubers

Sample	Total number of microorganisms (CFU on 1 tuber)	Yeast and mould (CFU on 1 tuber)
Non ozonated - 1	4.5×10^5	2.7×10^4
Non ozonated - 2	7.3×10^5	9.1×10^3
Non ozonated - 3	3.6×10^5	9.1×10^3
Ozone treated - 1	1.0×10^5	4.8×10^2
Ozone treated - 2	9.1×10^4	< 10
Ozone treated - 3	9.3×10^4	< 10

Table 10. Infection of tubers by silver scurf

Sample	Weighted average of the infection degree		Difference
	Before storage	After storage	
Non ozonated - 1	1.62	2.18	0.56
Non ozonated - 2	1.62	2.42	0.8
Ozone treated - 1	1.58	2.30	0.72
Ozone treated - 2	1.52	2.56	1.04

Table 11. Content of dry matter and starch in tuber samples

	Sample no.	Dry matter content % wt.	Starch content % wt.
Ozone treated variant	1	19.98	14.7
	2	20.73	14.7
	3	19.87	14.9
	4	19.95	14.5
	5	20.77	14.5
	6	20.30	14.7
	7	18.80	14.7
	8	20.32	14.7
Non ozonated variant	1	21.63	14.7
	2	20.92	14.7
	3	20.18	14.5
	4	21.90	14.9
	5	21.27	14.9
	6	20.74	14.7
	7	20.22	14.7
	8	20.71	14.7

Table 12. Statistical comparison of dry matter and starch contents in tubers

	Dry matter content, % wt.		Starch content, % wt.	
	Ozone treated	Non ozonated	Ozone treated	Non ozonated
Mean	20.09	20.95	14.68	14.73
Standard deviation	0.62	0.62	0.128	0.128
F-test	0.9934		1.0	
t-test	0.0155		0.4483	
Difference of means	0.86		0.05	

The differences between the mean values of dry matter content were statistically significant, while the differences between the mean values of starch content were nonsignificant. The higher dry matter content in the control tubers is probably due to the loss of water during the experiment. The loss of water in control tubers was 0.86% higher. This is also in agreement with the measured values of air humidity, which were significantly higher in the ozone treated variant than in the control variant.

The results showed a significant impact of ozone treatment on the quality of potato tubers during storage, i.e. reducing the infestation of potato tubers by mixed infection from fungal and bacterial diseases (*Fusarium* spp., *Phoma foveata*, *Pectobacterium* spp.). This was also evidenced by the reduction of possible bacterial and rot sources of infection. This was not in complete agreement with results by Waterer et al. (2003). On the other hand, the ineffectiveness of ozone on the silver scurf on potatoes (*Helminthosporium solani*) was confirmed. This is in agreement with Waterer et al. (2003) and Hamm (2005).

At the same time, the possibility of a negative influence of ozone on the utility properties of potatoes, especially on their sprouting, was ruled out. This issue would be very sensitive when storing seed potato tubers.

An important finding was the effect of ozone on reducing the weight losses in tubers. That corresponds to findings in other types of produce, e.g. oranges (Di Renzo et al., 2005), strawberries (Nadas et al., 2003) and mandarins (Garcia-Martin et al., 2018).

CONCLUSIONS

The results showed the positive effect of ozone application on the reduction of the spread of potato diseases during storage and verified the option of using air ozonation technology in potato warehouses. This technology can significantly reduce losses due to bacterial and fungal diseases. In the case of large-scale warehouses, where bulk potatoes are aerated through floor grates, the generated ozone can be supplied to the inlet air behind the fan. However, the fan speed must be adjustable so that the air velocity exiting from the stored layer of potatoes can be set between 0.01 and 0.015 $m\ s^{-1}$. In the practice, the fans were designed for an air speed exiting the layer 0.1 $m\ s^{-1}$. Therefore, their speed had to be reduced to about 10 to 15%. This way, the passage time through a typical 4 m layer of stored tubers is several minutes. This time is sufficiently short to assure a sufficient ozone concentration.

Ozone application is possible at predetermined intervals when the fan is not used for its main purpose, i.e. climate control. This could be e.g. for 8 h once every 48 h which was tested in the present study.

In practical applications, attention should be paid to operator safety, as ozone is toxic in higher concentrations. The permissible permanent concentration at workplaces is under 100 $\mu g\ m^{-3}$ or 200 $\mu g\ m^{-3}$ in the short term. When these values are reached in the warehouse service rooms, the ozone source, including the main fan, must be switched off. To do this, it is necessary to install safety sensors connected to the control systems.

ACKNOWLEDGEMENTS. The above results have been achieved within the project TH 02020036 'Research and development energy-saving technology and arrangement for potato storage' funded by Technology Agency of the Czech Republic.

REFERENCES

Allen, B., Wu, J. & Doan, H. 2003. Inactivation of fungi associated with barley grain by gaseous ozone. *J. Environ. Sci. Health B-Pestic Food Contam. Agric. Wastes* **38**, 617–630.

Di Renzo, G.C., Altieri, G., D'Erchia, L., Lanza, G. & Strano, M.C. 2005. Effect of gaseous ozone exposure on cold stored orange fruit. *Acta Hortic.* **682**, 1605–1610.

García-Martín, J.F., Olmo, M. & García, J.M. 2018. Effect of ozone treatment on postharvest disease and quality of different citrus varieties at laboratory and at industrial facility. *Postharvest Biology and Technology* **137**, 77–85.

Gibson, K.E., Almeida, G., Jones, S.L., Wright, K. & Lee, J.A. 2019. Inactivation of bacteria on fresh produce by batch wash ozone sanitation. *Food Control* **106**, art. no. 106747.

Gündüz, G.T. & Korkmaz, A. 2019. UV-C treatment for the inhibition of molds isolated from dried persimmons (*Diospyros kaki L.*) and modeling of UV-C inactivation kinetics. *LWT* **115**, art. no. 108451.

Hamm, P.B. 2005. The failure of ozone to control silver sours in stored Russet Norkotah potatoes. *Amer. J. of Potato Res.* **82**, 72.

Hausvater, E. & Doležal P. 2011. Storage diseases of potatoes. https://www.agromanual.cz/cz/clanky/sklizen-a-skladovani/skladovani/skladkove-choroby-brambor. (in Czech) Accessed 29.7.2019

Horvitz, S. & Cantalejo, M.J. 2014. Application of ozone for the postharvest treatment of fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **54**, 312–339.

Isikber, A.A. & Athanassiou, Ch.G. 2015. The use of ozone gas for the control of insects and micro-organisms in stored products. *J. of Stored Products Res.* **64**, 139–145.

ISO 21527-1. 2008. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0,95: Geneva, Switzerland.

ISO 4833-2. 2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 2: Colony count at 30 degrees C by the surface plating technique: Geneva, Switzerland.

ISO 6887-1. 2017. Microbiology of the food chain – Preperation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions: Geneva, Switzerland.

Liew, C.L. & Prange, R.K. 1994. Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (*Daucus carota L.*). *Am. Soc. Hortic.* Sci. **119**, 563–567.

Loit, K., Soonvald, L., Kukk, M., Astover, A., Runno-Paurson, E., Kaart, T. & Őpik, M. 2018. The indigenous arbuscular mycorrhizal fungal colonisation potential in potato roots is affected by agricultural treatments. *Agronomy Research* **16**(2), 510–522.

Nadas, A., Olmo, M. & García. J.M. 2003. Growth of *Botrytis cinerea* and strawberry quality in ozone-enriched atmospheres. *J. Food Sci.* **68**, 1798–1802.

Nguen, H.N., Le, C.C., Tran, V.D., Doan, T.Y.O. 2019. Air desinfection and food preservation by ozone gas. *Vietnam J. Chem.* **57**, 70–74.

Pérez, A.G., Sanz, C., Rios, J.J., Olías, R. & Olías, J.M. 1999. Effects of ozone treatment on postharvest strawberry quality. *J. Agric. Food Chem.* **47**, 1652–1656.

Runno-Paurson, E., Kiiker, R., Aav, A., Hansen, M. & Williams, I.H. 2016. Distribution of mating types, metalaxyl sensitivity and virulence races of *Phytopthora infestans* in Estonia. *Agronomy Research* **14**, 220–227.

Schuhmann, P. & Gottschalk, K. 2012. *Storage and air conditioning of potatoes*. Agrimedia Verlag, **272** pp. (in German)

Skog, L.J. & Chu, C.L. 2001. Effect of ozone on qualities of fruits and vegetables in cold storage. *Canadian Journal of Plant Science* **81**, 773–778.

Souza, L.P., Faroni, L.R., Heleno, F.F., Cecon, P.R., Goncalves, T.D.C., Silva, G.J. & Prates, L.H.F. 2018. Effect of ozone treatment on postharvest carrot quality. *LWT-Food Science and Technology* **90**, 53–60.

Spencer, R.G.M. 2003. *Ozone as a post-harvest treatment for potatoes.* University of Saskatchewan, Saskatoon, 139 pp.

Tuček, V., Bečka, K. & Vokál, B. 1988. *Methodology of seed potatoes preparation*. OSEVA, Havlíčkův Brod, 13 pp. (in Czech)

Wanqing, D., Wenbiao, J., Song, C, Xu, Z., Changping, W., Qijun, J., Hui, H., Renjie, T., Song-Fang, H. & Qilin, W. 2019. Ozone disinfection of chlorine-resistant bacteria in drinking water. *Water Research*. **160**, 339–349.

Waterer, D., Thomson, J. & Spencer, R. 2003. *Ozone as an improved method to control disease in stored potatoes*. University of Saskatchewan, 63 pp.

Wu, J., Doan, H. & Cuenca, M.A. 2006. Investigation of gaseous ozone as an anti-fungal fumigant for stored wheat. *J. Chem. Technol. Biotechnol*. **81**, 1288–1293.

Agronomy Research **18**(1), 113–126, 2020
https://doi.org/10.15159/AR.20.075

Sorbitol and lithovit-guano25 mitigates the adverse effects of salinity on eggplant grown in pot experiment

D.B. Issa[1], S.M. Alturki[2], T.K. Sajyan[1,3,*] and Y.N. Sassine[1,4]

[1]Department of plant production, Faculty of Agriculture, Lebanese University, Beirut, Lebanon
[2]Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 400, Al Ahsa 31982, Kingdom of Saudi Arabia
[3]University of Forestry, 10 Kliment Ohridski blvd, BG1797 Sofia, Bulgaria
[4]Department of Agricultural Biotechnology, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al Ahsa 31982, Kingdom of Saudi Arabia
[*]Correspondence: tony.sajyan@st.ul.edu.lb

Abstract. This trial aimed to study the separate effects of nano-fertilizers and sugar alcohols in mitigating salt-stress on eggplant (*Solanum melongena* L) crop. For this purpose, two different concentrations of lithovit®-guano25 (A1:0.5 g L^{-1} and A2:1 g L^{-1}) and sorbitol (B1:5 g L^{-1} and B2:10 g L^{-1}) were sprayed on eggplant irrigated by three NaCl solutions (EC1:1.5 dS m^{-1}, EC2:3 dS m^{-1} and EC3 6 dS m^{-1}). Control plants were salt-stressed without any product. Results revealed an inhibitory effect of increasing in salt-stress on vegetative traits (plant height, leaf number, weights of plant parts and root mass fraction), reproductive traits (fruit number, fruit weight, yield $plant^{-1}$, fruit diameter) and photosynthetic pigments. Control plants at EC6 had the highest cell electrolyte leakage (51.26%). Plant height and fruit number were maximized by A1 at all salinity levels. Additionally, A2 increased fruit weight by 89.98g, 85g and 92.3g compared to control respectively at 1.5, 3 and 6 dS m^{-1}. Yield $plant^{-1}$ increased by this treatment at all EC levels. At 3 and 6 dS m^{-1}, A2-treated plants had the highest chlorophyll a (respectively 1.67 and 1.4mg g^{-1} fresh weight), total chlorophyll (respectively 2.38 and 1.9mg g^{-1} fresh weight) and carotenoids (respectively 193 and 172µg g^{-1} fresh weight) contents. A2-treated plants had the lowest cell electrolyte leakage at 1.5 dS m^{-1} (14.27%), 3 dS m^{-1} (25.31%) and 6 dS m^{-1} (37.78%). Treating plants with B1 and B2 maximized respectively fruit diameter at 1.5 dS m^{-1} and water content in all plant parts at 3 dS m^{-1}. Both products helped plants reducing the adverse effects caused by salinity.

Key words: growth, nano-fertilizers, physiology, salinity, sugar alcohols, S. *melongena*.

INTRODUCTION

The degradation of soil due to salinization is a main constraint faced worldwide (Qadir et al., 2008). Salinity and drought are considered as major factors affecting crop productivity, undesirable in front of high food demand for growing human population (Arshadi et al., 2018; Martinez et al., 2018; Zargar et al., 2018). Based on the report of the United Nations' Food and Agriculture Organization (FAO & ITPS, 2015), soil salinization expanding in an accelerated rate is causing food insecurity in many countries. This abiotic stress inhibits crop growth by reducing water potential in roots and preventing water uptake, leading to physiological and nutritional disorders (Negrão et al., 2017). According to Pessarakli & Szabolcs, (2010) NaCl is the most dominant salt, adversely affecting morphology and physiology of cell and whole plant. Sodium cation accumulated under salt-stress prevent the accumulation of the remaining vital nutrient such as potassium, magnesium, iron etc, leading to a decrease in the ratio of K^+ over Na^+ (Keutgen & Pawelzik, 2009). This ratio is considered as an indicator of salt-tolerance (Munns & Tester, 2008).

Eggplant (*Solanum melongena* L.) crop is one of the solanaceous crop affected by salinity. It is cultivated in tropical, subtropical and Mediterranean areas. This crop is classified as moderately sensitive to sensitive to salinity based on conflictin g Literature (Bresler et al., 1982; Maas, 1984). It ranked among the top most important vegetable crop in Asia in general and the Mediterranean in specific (Frary et al., 2007). Lebanon ranked number 34 in the year 2017 for the production of eggplant among all countries, producing approximately 43,606 tons (FAOSTAT, 2017). Salinity caused previously, reductions in yields, an inhibition in vegetative growth, and a decrease in germination rate and seedling dry weights of stressed eggplants (Heuer et al., 1986; Akinci et al., 2004; Assaha et al., 2013).

The use of nano-fertilizers on salt-stressed crop has proved to be highly efficient method. Many manufactories are nowadays producing nano-fertilizers with different forms and combinations of nutrients. Lithovit® products are among nano-particles based on limestone and supplemented with diverse compounds (amino25, guano25, urea50 etc) (Bilal, 2010). In agriculture, these products were previously used under non-stressed and stressed conditions; lithovit®-standard was applied previously on salt-stressed tomato crop (Sajyan et al., 2018, 2019a, 2019b). In such trials, lithovit®-standard improved salt-stress of tomato due to, a better nutrient and water uptake, and a well-developed vegetative and reproductive growth. Lithovit-amino25 showed a stimulatory effect when sprayed on grapevines by improving quality and quantity of the targeted crop (Sassine et al.,2019). Moreover, the biological efficiency and production of *Pleurotus ostreatus* was also enhanced by the application of lithovit-urea on the substrate (Naim et al., 2020). Lithovit®-guano25 is another formulation of lithovit (28% CaO; 5% MgO; 4.5% SiO_2; 1.5% N; 0.6% P_2O_5; 0.6% K_2O; 0.5% Fe). Based on the presence of carbonate, this nano-fertilizer have the ability to increase atmospheric CO_2 concentrations. Thus, improving photosynthesis activity and slowing respiration in plants. The composition of this product seems to be highly efficient under salt-stress especially due to its richness in vital elements such as N, P, K, Si, Ca, Mg and Fe. In previous works, these elements which were applied in different forms and combinations on stressed vegetables, improved growth and production of the targeted crops (El-Fouly et al., 2002; Tuna et al., 2007; Tantawy et al., 2009; Siddiqui et al., 2014; Sadak et al., 2015).

On the other hand, Sorbitol is an alditol found in higher plants. It has been considered a non-metabolite, because it is metabolically more inert character than other saccharides (Lambers et al., 1981). Biosynthesis of sorbitol is restricted mainly to source leaves whereas metabolic utilization is restricted to sink tissues. In all cases, sorbitol accumulation is considered as an adaptative response of plants to drought, salinity or chilling stress. Endogenous accumulation of many solutes and compounds as a result of abiotic-stress has been studied intensively as being among the natural mechanisms of stress-tolerance in plants (Munns & Tester, 2008). Previous studies pointed out the exogenous application of these compounds such as melatonin and glycine-betaine on salt-stressed crops such as tomato or others (Sajyan et al., 2019c; Zhan et al., 2019). Accordingly, ameliorative effects following the application of these compounds were observed. Exogenous application of sorbitol was less tested on stressed or non-stressed vegetables. On maize, sorbitol induced accumulation in dry matter and inhibition in biochemical and photosynthetic traits (Jain et al., 2010). Under salt-stress, sorbitol promoted the tolerance of spinach crop by improving photosynthetic pigments, carbohydrates and proteins (Gul et al., 2017). Therefore, its exogenous application could be highly efficient as a method to counteract the adverse effects of salinity.

Accordingly, the current study aimed to test the effect of separate foliar spraying of lithovit-guano25 and sorbitol in different concentrations on salt-stressed eggplant crop irrigated by different NaCl solutions.

MATERIALS AND METHODS

Plant growth, treatments and experimental design

Seeds of eggplant (Var Black Knight) were sown in plastic trays in late April-2019 in a greenhouse located at 33°52'24.9"N 35°31'44.0"E / Lebanon. Thirty days after sowing, uniform plants of 3–4 leaves were transplanted into pots of 40 cm in diameter. Pots were filled by a mixture prepared on volume basis of 33% peat moss and 67% soil (Table 1). During the experiment, plants were kept under ambient light conditions, a day-night temperature of 20–25 ± 5 °C and a relative humidity of 60–70%. Based on substrate test, monopotassium phosphate (52% P_2O_5 and 34% K_2O) and NPK (20% N, 20% P_2O5 and 20% K_2O) were added with a rate of respectively 5 g plant^{-1} and 3 g plant^{-1} at 5 days after transplantation (DAT).

Table 1. Soil physico-chemical characteristics

Soil characteristics	
Sand	75.55
Silt	4.07
Clay	20.38
Texture (USDA Texture Triangle)	Sandy Clay Loam
pH (1:5 soil water suspension)	6.89
Organic matter content (%)	5.09
Total $CaCO_3$ (%)	5.3
ECe (dS m^{-1}, soil paste extract)	0.264
Organic N (%)	0.31
Available P_2O_5 (ppm)	40.25
Exchangeable K_2O (ppm)	117.33
Exchangeable CaO (ppm)	7,019.15
Exchangeable MgO (ppm)	1,315.5
Exchangeable Na (ppm)	28.53

The experiment was arranged in a randomized completely design (CRD) based on NaCl irrigations considered as main factor and product-treatments as sub-factor. NaCl irrigations were divided on three levels of three concentrations namely 1.5 dS m^{-1}, 3 dS m^{-1} and 6 dS m^{-1}, while product treatments were divided into two products namely lithovit-guano25 (A) and sorbitol (B) applied separately in two concentrations at eachNaCl solution. Product concentrations were as follows: A1:0.5 g L^{-1}; A2: 1 g L^{-1}, B1:5 g L^{-1} and B2: 10 g L^{-1}. Control plants were salt-stressed eggplant crop irrigated by three NaCl solution with no product application. Spraying of different concentrations of both products was done four times during growth cycle starting at 15DAT with an interval of 2 weeks. Saline irrigation started at 21DAT with an interval of 2–3 days.

Vegetative and yielding traits

During growth cycle of eggplant (100DAT), vegetative traits were measured: plant height and leaf number. At the end of growth cycle, plants were removed carefully from pots, washed and separated into roots, stems and leaves. Fresh weights (FW) of different plant parts were recorded. For the determination of dry weights (DW), parts were oven dried at 100 °C until a constant weight was obtained. Afterwards, water content (WC) and root mass fraction were calculated as follows: WC = FW-DW/FW, RMF = DW of roots/ DW of total plant parts. In addition, fruits number was counted in all treatments. After fruit harvesting, diameter and weight were measured using a sliding caliper and a digital balance. Yield per plant was calculated by multiplying fruit number at harvesting by average weight of individual fruits of each treatment.

Cell electrolyte leakage

Leaves were sampled at 70DAT for the determination of cell electrolyte leakage as described by Lutt et al. (1996). Fresh leaves were excised into discs of 1cm^2 and water bathed at ambient temperature. After 24 hours, conductivity (EC1) of distilled water was measured. Afterwards, tubed were autoclaved at 120 °C for 20 min and conductivity (EC2) of the solution was measured again. Cell electrolyte leakage was calculated as follows: CEL = (EC1/EC2) ×100.

Chlorophyll and carotenoids content

Fresh leaves were sampled at 75DAT for the determination of leaf chlorophyll and carotenoid content. Photosynthetic pigments were determined by spectrophotometry as described and quantified by Arnon (1949) and expressed as mg g^{-1} FW of leaves.

Statistical analysis

Data was subjected to analysis of variance by using Statistical Package for Social Sciences (SPSS) software version 25® software. Means were compared by Duncan's multiple range tests at $p \leq 0.05$. All graphs and were performed on Microsoft Excel Software. The correlation between yield and some growth attributes was tested by regression analysis which was done on SPSS software.

RESULTS AND DISCUSSION

Vegetative and yielding traits

Data of vegetative and reproductive growth (Table 2) showed that increasing in salt-stress caused reductions in plant height, leaf number, fruit number, yield plant^{-1} and fruit diameter. In fact, in non-treated plants, plant height, leaf number and fruit weight were reduced from 38.89 cm, 20.78 leaves and 25.02 g at 1.5 dS m^{-1} to reach a minimum of 24.44 cm, 5.44 leaves and 18 g respectively at 6 dS m^{-1}. Plant height and fruit number were significantly enhanced the most by A1 at all salinity levels. The remaining indicators were also significantly improved under all EC levels by different products. In specific, spraying of A2 increased fruit weight by 89.98 g, 85 g and 92.3 g compared to control respectively at 1.5, 3 and 6 dS m^{-1}. Accordingly, yield plant^{-1} was maximized by A2 spraying at all EC levels. Moreover, treating plant with B1 enhanced significantly fruit diameter with the best effect observed at 1.5 dS m^{-1} compared to the remaining treatments. When comparing between both products, it was observed that product A was better mainly at higher EC levels compared to product B. Similarly, to previous indicators, salinity caused reductions in weights of plant parts (Table 3) peaking at 6 dS m^{-1}. Foliar spraying of various products with both concentrations enhanced significantly all indicators. Among all treatments, it was observed that foliar spraying of A2 at EC1.5, EC3 and EC6 dS m^{-1} maximized fresh weight of roots (respectively of 8.97 g, 7.16 g and 4.83 g), stems (respectively 24.72 g, 18.72 g and 15.38 g) and leaves (respectively 29.95 g, 30.45 g and 23.12 g) compared to all the remaining treatments including control at all EC levels. Accordingly, dry weights of A2-treated plants were also the highest among all treatments. On the contrary, it was observed that the application of sorbitol improved water content in roots, stems and leaves better that lithovit-guano25 with the best effect observed following B2 application.

Table 2. Effects of lithovit®-guano25 and sorbitol on vegetative and yielding traits of salt-stressed eggplant

Treatments	Plant Height (cm)	Leaf number	Fruit number	Fruit weight (g)	Yield plant^{-1} (g)	Fruit diameter (cm)
EC1.5/Control	38.89cde	20.78bc	1.00e	25.02j	25.02i	2.00j
EC1.5 / A1	46.78ab	25.56bc	6.00a	79.33e	476.00b	3.68hi
EC1.5 / A2	44.56bc	26.89ab	6.00a	115.24c	691.44a	6.44b
EC1.5 / B1	41.11bcd	27.44ab	3.00c	154.45a	463.35b	7.22a
EC1.5 / B2	43.89bcd	34.67a	2.00d	149.03b	298.07cd	6.61b
EC3 / Control	27.11f	11.33de	1.00e	20.00kl	20.00i	2.00j
EC3 / A1	50.67a	25.56bc	4.00b	70.13f	280.52d	5.01d
EC3 / A2	43.00bcd	26.89ab	3.00c	105.00d	315.00c	4.52ef
EC3 / B1	41.11bcd	27.44ab	4.00b	64.15g	256.59e	4.46f
EC3 / B2	37.22de	23.00bc	2.00d	79.25e	158.50f	5.21c
EC6 / Control	24.44f	5.44e	1.00e	18.00l	18.00i	1.50k
EC6 / A1	38.44cde	20.44bc	3.00c	23.47jk	70.40h	3.82gh
EC6 / A2	38.00cde	17.44cd	2.00d	55.15h	110.30g	4.70e
EC6 / B1	33.44e	11.67de	3.00c	20.06kl	60.17h	3.60i
EC6 /B2	38.33cde	17.44cd	2.00d	32.19i	64.39h	3.92g

A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means *(n = 10*) followed by different letter within each column are significantly different according to Duncan's multiple range tests.

Table 3. Effects of lithovit®-guano25 and sorbitol on weights and water content of plant parts of salt-stressed eggplant

Treatments	FWR (g)	DWR (g)	WaR (%)	FWS (g)	DWS (g)	WaS (%)	FWL (g)	DWL (g)	WaL (%)
EC1.5/Control	5.2gh	3.9b	25.5j	16.6e	5.0b	69.7h	10.1j	4.7h	53.3k
EC1.5 / A1	7.4b	3.8b	48.5c	20.0c	5.5a	72.5g	27.9b	7.9c	71.8ef
EC1.5 / A2	9a	4.6a	49.2c	24.7a	5.4a	78.1c	30a	10a	66.8j
EC1.5 / B1	6.1e	2.6f	57.3a	21.0b	5.4a	74.1f	25.9c	6.9e	73.2d
EC1.5 / B2	6.6d	3.3d	50.6b	17.1e	2.9g	82.8a	20.3f	4.8h	76.6b
EC3 / Control	3.0j	2.4g	20.0k	12.0h	3g	75.3e	10.3j	5.0gh	51.5l
EC3 / A1	5.4gh	3.2de	40.7e	15.5f	3.8e	75.2e	19.5g	5.9f	70h
EC3 / A2	7.2c	3.9b	45.9d	18.8d	4.7c	74.7ef	30.5a	8.8b	71.2fg

Table 3 (continued)

EC3 / B1	5.6f	3.6c	36.6g	16.4e	4.4d	73.1g	25d	7.2d	70.8g
EC3 / B2	5.0hi	2.6f	48.6c	14.4g	3.6f	75.3e	15.8i	4i	74.9c
EC6 / Control	1.8l	1.5i	17.9l	9.2j	3.4f	63.1i	3.5k	2.4j	32.5m
EC6 / A1	3.1j	1.9h	39.9e	13.7g	2.9g	79.1b	18.5h	5.2g	72.2e
EC6 / A2	4.8i	3.1e	35.6h	15.4f	3.5f	77.3c	23.1e	7.3d	68.2i
EC6 / B1	2.3k	1.5i	34.4i	14.0g	3.3f	76.3d	15.1i	4.2i	72.5de
EC6 /B2	3.1j	1.9h	39.0f	11.1i	2.9g	74.1f	10.3j	2.1j	79.7a

FWR: fresh weight of roots; DWR: dry weight of roots; WaR: water in roots; FWS: fresh weight of stems; DWS: dry weight of stems; WaS: water in stems; FWL: fresh weight of leaves; DWL: dry weight of leaves; WaL: water in leaves. A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means (*n = 10*) followed by different letter within each column are significantly different according to Duncan's multiple range tests.

Root mass fraction (Fig. 1) decreased by 0.08 g g^{-1} with increasing in salt-stress from 1.5 dS m^{-1} to 6 dS m^{-1} in non-treated plants. However, treated plants despite the product and concentration of application had lower root mass fraction as compared to control at all EC levels.

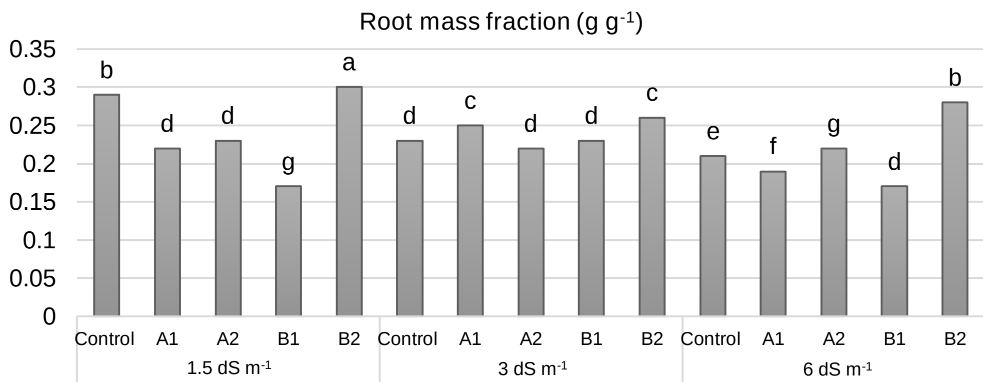


Figure 1. Effects of lithovit®-guano25 and sorbitol on root mass fraction of salt-stressed eggplant. A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means (*n = 10*) followed by different letter are significantly different according to Duncan's multiple range tests.

Cell electrolyte leakage

Cell electrolyte leakage (Fig. 2) was increased drastically with increasing in salt-stress from 1.5 dS m^{-1} (21.18%) to 6 dS m^{-1} (51.26%) reflecting a low membrane stability in stressed plants. All treatments reduced significantly cell electrolyte leakage from leaves. A2-treated plants had the lowest cell electrolyte leakage at 1.5 dS m^{-1}, 3 dS m^{-1} and 6 dS m^{-1} with respectively 14.27%, 25.31% and 37.78%. When comparing between both products, it was observed that spraying of lithovit-guano25 reduced cell electrolyte leakage more than sorbitol. Depending on the concentration, the application of the former reduced this indicator by a range of 3 to 10% compared to the application of the latter.

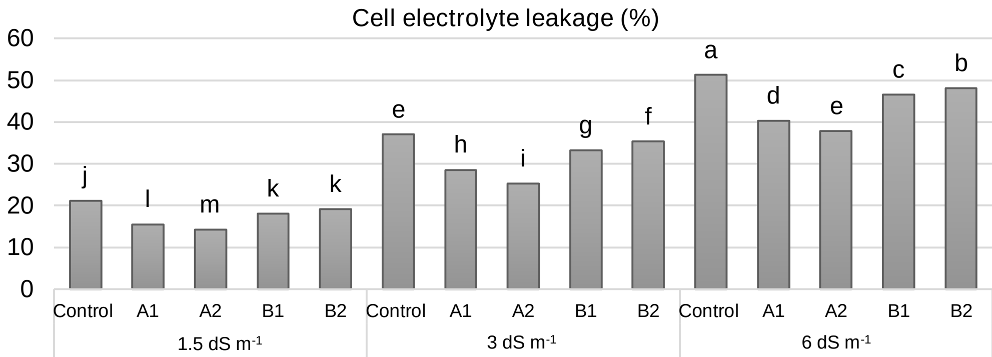


Figure 2. Effects of lithovit®-guano25 and sorbitol on cell electrolyte leakage of salt-stressed eggplant. A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means (*n=10*) followed by different letter are significantly different according to Duncan's multiple range tests.

Chlorophyll and carotenoids content

Photosynthetic pigments including chlorophyll a, b and carotenoids were reduced with increasing in salt-stress with the lowest values obtained at non-treated plants at 6 dS m^{-1}. Chlorophyll a (Fig. 3) was enhanced by all treatments at EC1.5, EC3 and EC6 except B2 treatments. It was maximized by treatments includin g Lithovit-guano25 especially in high concentration (A2: 1 g L^{-1}). A2-treated plants had significantly the highest chlorophyll a content at EC3 and EC6 with respectively 1.67 and 1.4 mg g^{-1} fresh weight. Chlorophyll b and consequently total chlorophyll content were similarly affected by salinity and various treatments. In fact, A1-treated plants caused maximization of chlorophyll contents only at EC1.5. Consequently, the application of lithovit-guano25 seemed to be positively affected by increasing in the applied concentration. On the contrary, the application of sorbitol in lower concentration seemed to be better in enhancing chlorophyll contents more than its application in high concentrations. Carotenoids content (Fig. 4) was also improved the most by lithovit-guano25-treated plants and optimized by A2 application. This latter treatment improved carotenoids

Table 4. Effects of lithovit®-guano25 and sorbitol on the ratio of chlorophyll a over b and total chlorophyll over carotenoids

Treatments	Chlorophyll a/b	Total chlorophyll/ carotenoids
EC1.5/Control	2.57de	11.52b
EC1.5 / A1	2.33e	12.45a
EC1.5 / A2	2.41efg	11.37b
EC1.5 / B1	2.75bc	11.46b
EC1.5 / B2	2.60cd	11.26bc
EC3 / Control	2.55de	9.94d
EC3 / A1	3.09a	10.99bc
EC3 / A2	2.35de	12.33a
EC3 / B1	2.52def	10.64c
EC3 / B2	2.77bc	11.28bc
EC6 / Control	2.15f	8.71e
EC6 / A1	2.58de	11.42b
EC6 / A2	2.80b	11.05bc
EC6 / B1	2.52def	9.68d
EC6 /B2	2.76bc	8.16e

A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means (*n = 3*) followed by different letter within each column are significantly different according to Duncan's multiple range tests.

at EC1.5, EC3 and EC6, by respectively 72, 22 and 20 mg g^{-1} fresh weight compared to control. Again, sorbitol spraying with high concentration (B2) did not improve this pigment as compared to control at E3 and EC6. On the contrary, B1-treated plants showed more or less some ameliorative effects compared to control regarding carotenoids content.

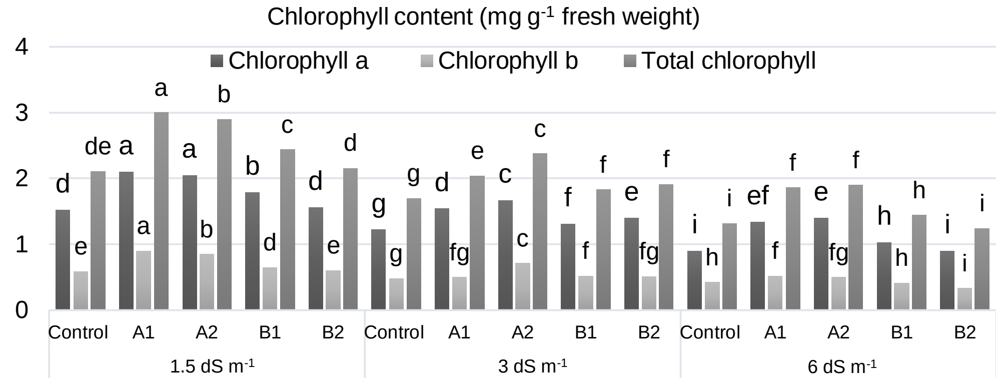


Figure 3. Effects of lithovit®-guano25 and sorbitol on chlorophyll content of salt-stressed eggplant. A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means ($n = 3$) followed by different letter for each indicator are significantly different according to Duncan's multiple range tests.

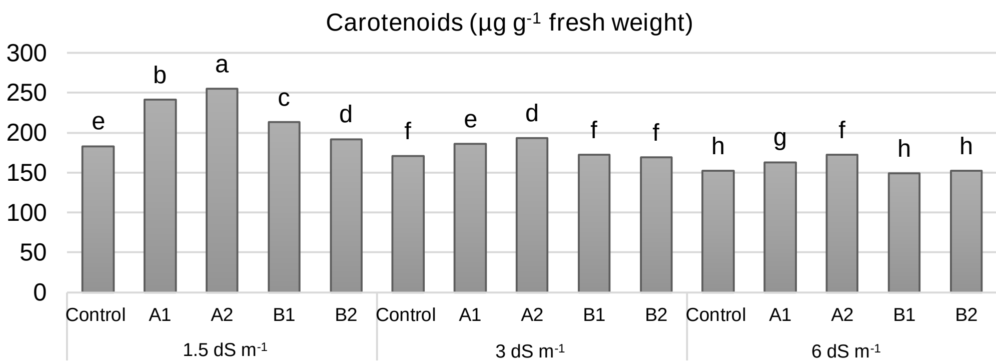


Figure 4. Effects of lithovit®-guano25 and sorbitol on carotenoids content of salt-stressed eggplant. A1 and A2 respectively 0.5 g L^{-1} and 1 g L^{-1} of lithovit®-guano25, B1 and B2 respectively 5 g L^{-1} and 10 g L^{-1} of sorbitol. Means ($n = 3$) followed by different letter are significantly different according to Duncan's multiple range tests.

The ratio of chlorophyll a over chlorophyll b (Table 4) was also reduced by salinity in control plants. However, plants treated with B1 (2.75), A1 (3.09) and A2 (2.8) had respectively the highest ratio respectively at EC1.5, EC3 and EC6 dS m^{-1}. Finally, the ratio of total chlorophyll over carotenoids, which was also lowered in control plants with increasing in salinity stress from 1.5 to 6 dS m^{-1}, was maximized in plants treated respectively with A1 (12.45), A2 (12.33) and A1 (11.42) at EC1.5, EC3 and EC6 dS m^{-1}.

Regression analysis

Results of regression analysis showed that yield plant^{-1} was significantly correlated the most with some traits (Fig. 5) and less with others. However, a linear relationship was observed between yield plant^{-1} and respectively fruit number ($R^2 = 0.673$; $Y = 1.04E^2x-77.05$) and fruit weight ($R^2 = 0.632$; $Y = 3.43x-12.49$). Moreover, a quadratic relationship was found between yield plant^{-1} and respectively fresh weight of roots ($R^2 = 0.847$; $Y = 12.05x^2-36.93x+52.42$), fresh weight of stems ($R^2 = 0.858$; $Y = 2.09x^2-24.79x+52$), fresh weight of leaves ($R^2 = 0.722$; $Y = 0.72x^2-5.66x+22.53$) and total chlorophyll content ($R^2 = 0.768$; $Y = 1.17E^2x^2-1.59E^2x+35.49$). The positive significant, regression coefficients of such independent variables indicate that increasing in their amount will promotes yield plant^{-1}. In other terms, the application of various treatments in the current study under different salinity level enhanced yielding capacity of stressed eggplant due to an improve in, fruit number and weight, fresh weights of plants parts and photosynthetic pigments.

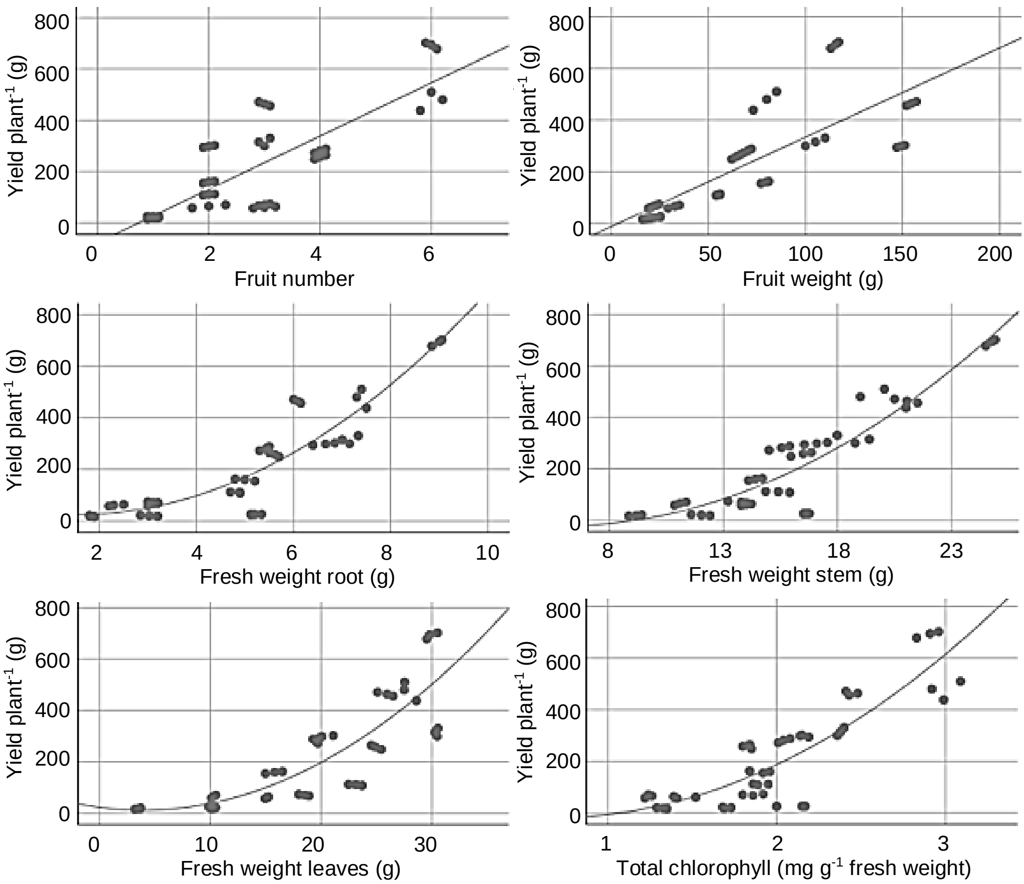


Figure 5. Relationship between yield plant^{-1} and plant traits.

In the current experiment, increasing in salt-stress from 1.5 dS m^{-1} to 6 dS m^{-1} caused severe reductions in the majority of the measured traits mainly yielding capacity, photosynthetic pigments and vegetative growth. Previously, several authors reported the inhibitory effects of salinity on vegetables including eggplants, eggplant, tomato etc. (Cabãnero et al., 2014; Machado & Serralheiro, 2017; Sajyan et al., 2019d). Based on such findings, the accumulation of sodium in the roots zone causes an osmotic stress, preventing the accumulation of important nutrients (K^+, Ca^{2+}, and NO_3^-) (Paranychianakis & Chartzoulakis, 2005). Reductions in yielding capacity of eggplant was previously reported by Gül & Sevgican (1992) due to inhibition in water flow towards fruits, leading to reductions in fruit weight and number. Taiz & Zeiger (2002) stated that the accumulation of detrimental ions leads to, a damage in chloroplast membrane and an inhibition in protein synthesis. Similar findings were observed in the current study were salinity increased cell electrolyte leakage and reduced photosynthetic pigments in stressed non-treated plants.

The application of lithovit®-guano25 and sorbitol showed ameliorative effects on stressed eggplant. lithovit®-guano25 applied in high concentration (A2: 1 g L^{-1}) maximized yielding capacity and traits of eggplant more than sorbitol. The former product was not previously tested on vegetables under salt-stress. In fact, lithovit is available in many formulations (such as urea 50%; boron; guano 25%; amino acids 25%, etc.). The mutual point among all formulations is the presence of carbonate coupled with calcium and magnesium. Such products have the ability to slow down respiration and promotes photosynthesis process though increase in atmospheric CO_2 (Bilal, 2010). This promoting effect was observed following the application of lithovit®-guano25 through an increase in photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids). This product also improved weights and water content of plant parts reflecting a better water movement and potential in stressed plants compared to control. In previous study, the application of lithovit product on salt-stressed tomato did not improved fresh weight of plant parts (Sajyan et al., 2019a). Contradictory findings were reported on such effects. However, one of the important factors of lithovit®-guano25 product is its richness in nutrients in nanoscale (nitrogen, potassium, phosphorus, calcium, iron, silicon, magnesium). Its nano-particle size makes easier the contact with pores of leaves and faster its translocation in plant vessels (xylem and phloem) (Rico et al., 2011). According to Taiz & Zeiger (2002), foliar spraying of a nano-fertilizer is highly efficient for cations (mainly iron, calcium and magnesium and manganese), it increases the availability of nutrient. As mentioned previously, foliar spraying of lithovit-guano was not tested previously on stressed and non-stressed vegetables. However, compounds that are found in lithovit-guano were separately applied and promoted stimulatory effects. For instance, foliar spraying of Ca improved fruit number, decreased Na and increased Ca and K contents of salt-stressed tomato crop (Tuna et al., 2007; Nizam et al., 2019). In addition, the separate application of magnesium also maximized plant growth and production of tomato and strawberry under salt-stress (Carvajal et al., 1999; Yildirim et al., 2009). The application of iron nanoparticles improved yielding components of drought-stressed safflower and salt-stressed sunflower by promoting photosynthesis activity and reducing Na accumulation (Davar et al., 2014; Torabian et al., 2017). In fact, iron play a role in chlorophyll synthesis where it enters in many mechanisms related to photosynthesis and respiration such as oxido-redox reactions (Curie & Briat, 2003). In addition, the presence of Si in oxide form seemed to activate the defense mechanism facing salt-stress. similarly, Siddiqui et al. (2014) stated the effect of silicon oxide on squash plants under salt-stress. Si application also improved water use efficiency and photosynthesis of salt-stressed tomato (Romero-Aranda et al., 2006). Basically, similar results were observed in the current study where it seemed that the amelioration in photosynthetic pigments (Chlorophyll and carotenoids) was due to a sufficient supply by Mg, Ca, Fe and Si.

On the other hand, sorbitol product which showed significant effects when applied in low concentrations, had adverse effects when applied in high concentrations. Sorbitol accumulation is considered as an adaptative response of plants to drought, salinity or chilling stress. Biosynthesis of sorbitol is restricted mainly to source leaves whereas metabolic utilization is restricted to sink tissues (Escobar Gutiérrez & Gaudillère, 1996). The restricted positive effect of sorbitol is due to a protecting role played on cytoplasmic proteins and cell membranes from desiccation. Such effects are equally observed by sugar alcohols (sorbitol and mannitol) and osmoregulators (proline, betaines etc.) (Balal et al., 2012). As a result, low concentration of sorbitol enhanced cell membrane stability and improved yielding capacity of stressed eggplant rather than high concentrations. Sorbitol treatment was less efficient compared to lithovit-guano. However, compared to control, its application caused stimulatory effects (mainly with low concentrations) on photosynthetic pigments, yielding traits and vegetative attributes. Similar findings were reported by Gul et al. (2017) on stressed spinash. On maize, sorbitol induced accumulation in dry matter and inhibition in biochemical and photosynthetic traits (Jain et al., 2010). Additionally, on salt-stressed rice seedlings, sorbitol application improved more or less tolerance of the crop by reducin g Lipid peroxidation (malondialdehyde) and H_2O_2 (Theerakulpisut & Gunnula, 2012). Noteworthy, sugar alcohols including sorbitol, mannitol are endogenously accumulated in stressed plants and confer tolerance to abiotic stress (Williamson et al., 2002). This ability is due to the effect of polyols on osmotic balance, water movement in the apoplast and sodium sequestration to the vacuole (Kanayama et al., 2006). This ability protects provide protection to cell membrane. This was reflected in the current study by a reduction in cell electrolyte leakage following sorbitol spraying. Conclusively, both products had different mechanisms by which they helped plants in counteracting the adverse effect of salinity.

CONCLUSIONS

From the current study, it could be concluded that lithovit®-guano25 was more beneficial on such stress more than sorbitol. The rate of application was a limiting factor for sorbitol where increasing in its application rate caused adverse effects. For lithovit®-guano25, the opposite was observed; its application in higher rates was the most efficient treatment and helped in mitigating the negative effects of salinity on eggplant.

ACKNOWLEDGEMENTS. Authors deeply thank the TRIBOdyn AG Company for providin g Lithovit®-guano25 product and ensuring its shipping to Lebanon.

REFERENCES

Akinci, I., Akinci, S., Yilmaz, K. & Dikici, H. 2004. Response of eggplant varieties (Solanum melonena) to salinity in germination and seedling stages. *New Zeal J Crop Hort*. **32**, 193–200.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiology* **4**, 1–15.

Arshadi, A., Karami, E., Sartip, A., Zare, M. & Rezabaksh, P. 2018. Genotypes performance in relation to drought tolerance in barley using multi-environment trials. *Agronomy research* **16**(1), 5–21

Assaha, D., Ueda, A. & Saneoka, H. 2013. Comparison of growth and mineral accumulation of two solanaceous species, *Solanuum scabrum* Mill. (huckleberry) and *S. melongena* L. (eggplant) under salinity stress. *Soil Sci Plant Nutr*. **59**, 912–920.

Balal, R.M., Khan, M.M., Shahid, M.A., Mattson, N.S., Abbas, T., Ashfaq, M., Garcia-Sanchez, F., Ghazanfer, U., Gimeno, V. & Iqbal, Z. 2012. Comparative studies on the physiobiochemical, enzymatic, and ionic modifications in salt tolerant and salt sensitive Citrus rootstocks under NaCl stress. *J Am Soc Hor Sci*. **137**, 1–10.

Bilal, B.A. 2010. Lithovit®: an innovative fertilizer. Paper presented at: 3rd e-Conference on Agricultural Biosciences (IeCAB 2010)

Bresler, E, B.L., McNeal, D.L. & Carter, D.L. 1982. *Saline and sodic soils*. Springer Verlag, Berlin, Germany, 236 pp.

Cabãnero, F.J., Martínez, V. & Carvaja, M. 2004. Does calcium determine water uptake under saline conditions in eggplant plants, or is it water flux which determines calcium uptake?. *Plant Sci* **166**, 443–450.

Carvajal, M., Martineze, V. & Cerda, A. 1999. Influence of magnesium and salinity on tomato plants growing hydroponics culture. *J. Plant Nutr*. **22**, 177–190.

Curie, C. & Briat, J.F. 2003. Iron transport and signalling in plants. *Annu Rev Plant Biol.* **54**, 183–206.

Davar, F., Zareii, Arash, R. & Amir, H. 2014. Evaluation the effect of water stress and foliar application of Fe nanoparticles on yield, yield components and oil percentage of safflower (Carthamus tinctorious L.). *In.t. J Adv. Biol. Biom. Res.* **2**(4), 1150–1159.

El-Fouly, M.M., Moubarak, Z.M. &Salama, Z.A. 2002. Micronutrient Foliar Application Increases Salt Tolerance of Tomato Seedlings. *Acta Hort. ISHS*, 573.

Escobar Gutiérrez, A.J. & Gaudillère, J.P. 1996. Distribution, metabolism and role of sorbitol in higher plants. A review. *Agronomie* **16**(5), 281–298.

FAO & ITPS .2015. Status of the World's Soil Resources (SWSR) – Main Report. Rome: Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel

FAOSTAT. 2017. Retrieved 10 March 2019

Frary, A., Doganlar, S. & Daunay, M.C. 2007. Eggplant, In: Kole C. (eds) *Vegetables. Genome Mapping and Molecular Breeding in Plants*, (vol **5**). Springer, Berlin, Heidelberg, pp. 287–313.

Gül, A. & Sevgican, A. 1992. Effect of growing media on glasshouse tomato yield and quality. *Acta Horti*. **303**, pp.145–150.

Gul, H., Farman, M., Hussain, A., Irshad, L.M & Azeem, M. 2017. Exogenously applied sorbitol alleviates the salt stress by improving some biochemical parameters in Spinach (*Spinacia oleracea* l.). *Int. J. Biol. Biotech*. **14**(4), 677–686.

Heuer, B., Meiri, A. & Shalhevet, J. 1986. Salt tolerance of eggplant. *Plant Soil*. **95**, 9–13.

Keutgen, A.J. & Pawelzik, E. 2009. Impacts of NaCl stress on plant growth and mineral nutrient assimilation in two cultivars of strawberry. *Environ. Exp. Bot*. **65**, 170–176.

Jain, M., Tiwary, S. & Gadre, R. 2010. Sorbitol-induced changes in various growth and biochemical parameters in maize. *Plant Soil Environ*. **6**:263–267.

Kanayama, Y., Watanabe, M., Moriguchi, R., Deguchi, M., Kanahama, K. & Yamaki, S. 2006. Effects of low temperature and abscisic acid on the expression of the sorbitol-6-phosphate dehydrogenase gene in apple leaves. *J Jpn Soc Hortic Sci*. **75**, 20–25.

Lambers, H., Blacquiere, T. & Stuiver, B. 1981. Interactions between osmoregulation and the alternative respiratory pathway in *Plantago coronopus* as affected by salinity. *Physiologia Plantarum*. **51**(1), 63–68.

Lutts, S., Kinet, J.M. & Bouharmont, J. 1996. NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. *Ann. Bot*. **78**, 389–398.

Machado, R.M. & Serralheiro, R.P. 2017. Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae* **3**, 30–42.

Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T.C., Garcia-Sanchez, F., Rubio, F., Notes, P.A., Mittler, R. & Rivero, R.M. 2018. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules*. **23**, 535.

Maas, E.V. 1984. Salt tolerance of plants. In: B.R. Christie (Ed.), *The handbook of plant science in agriculture*, CRC Press, Boca Raton, FL, pp. 57–75.

Munns, R. &Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*. **59**, 651–681.

Negrão, S., Schmöckel, S.M. & Tester, M. 2017. Evaluating physiological responses of plants to salinity stress. *Annals of Botany*. **119**, 1–11.

Naim, L., Alsanad, M.A., El Sebaaly, Z, Shaban, N, Abou Fayssal, S & Sassine, Y.N., 2020. Variation of *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm. (1871) performance subjected to different doses and timings of nano-urea. *Saudi Journal of Biological Sciences* (2020).

Nizam, R., Hosain, M.T., Hossain, M.E., Islam, M.M. & Haque, M.A. 2019. Salt stress mitigation by calcium nitrate in tomato plant. *Asian J. Med. Biol. Res*. **5**(1), 87–93.

Paranychianakis, N.V. & Chartzoulakis, K.S. 2005. Irrigation of Mediterranean crops with saline water: From physiology to management practices. *Agric. Ecosyst. Environ*. **106**, 171–187.

Pessarakli, M. & Szabolcs, I. 2010. Soil salinity and sodicity as particular plant/crop stress factors. In Pessarakli, M. (Ed.), *Handbook of Plant and Crop Stress*, CRC Press, Boca Raton, Florida, pp. 3–21.

Qadir, M., Tubeileh, A., Akhtar, J., Larbi, A., Minhas, P.S. & Khan, M.A. 2008. Productivity enhancement of salt-affected environments through crop diversification. *Land Degrad. Dev*. **19**, 429–453.

Rico, C.M., Majumdar, S., Duarte-Gardea, M., Perlata-Videa, J.R. & Gardea-Torresdey, J.L. 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J. Agric. Food Chem*. **59**, 3485–3498.

Romero-Aranda, M.R., Jurado, O. & Cuartero, J. 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *J. Plant Physiol*. **163**, 847–855.

Sadak, M., Abdelhamid, M.T. &Schmidhalter, U. 2015. Effect of foliar application of aminoacids on plant yield and some physiological parameters in bean plants irrigated with seawater. *Acta Biol. Colomb*. **20**, 141–152.

Sajyan, T.K., Shaban, N., Rizakallah, J. & Sassine, Y.N. 2018. Effects of monopotassium-phosphate, nano-calcium fertilizer, acetyl salicylic acid and glycinebetaine application on growth and production of tomato (*Solanum lycopersicum*) crop under salt-stress. *Agronomy research* **16**(3), 872–883.

Sajyan, T.K., Shaban, N., Rizakallah, J. & Sassine, Y.N. 2019a. Performance of salt-stressed tomato crop as affected by nano-$CaCO_3$, glycine betaine, MKP fertilizer and aspirin application. *Agriculture and Forestry* **65**(1), 19–27.

Sajyan, T.K., Naim, L., Sebaaly, Z., Rizkallah, J., Shaban, N. & Sassine, Y.N. 2019b. Alleviating the adverse effects of salinity stress on tomato crop (*Solanum lycopersicum*) using nano-fertilizer as foliar application. *Acta Hortic*. **1253**, 33–40.

Sajyan, T.K., Allaw, W., Shaban, N. & Sassine, Y.N. 2019c. Effect of exogenous application of glycine betaine on tomato plants subjected to salt stress. *Acta Hortic*. **1253**, 41–48.

Sajyan, T.K., Rizkallah, J., Sebaaly, Z., Shaban, N. & Sassine, Y.N. 2019d. Investigating the potential use of mono-potassium phosphate (MKP: 0-52-34) applied through fertigation as a method to improve salinity tolerance of tomato plants. *Acta Hortic*. **1253**, 1–8.

Sassine, Y.N., Al Turki, S.M., El Sebaaly, Z., Bachour, L. & El Masri, I.Y. 2019. Finding alternatives for dormex (hydrogen cyanamid) as dormancy breaking agent. *Fresenius Environmental Bulletin*. **28**, 10214–10224.

Siddiqui, M.H., Al-Whaibi, M.H., Faisal, M. & Al Sahli, A.A. 2014. Nano-silicon dioxide mitigates the adverse effects of salt stress on *Cucurbita pepo* L. *Env. Toxicol. Chem*. **33**, 2429–2437.

Taiz, L. & Zeiger, E. 2002. *Plant Physiology.* 3rd ed. Sinauer, Sunderland, United Kingdom, 690 pp.

Tantawy, A.S., Abdel-Mawgoud, A.M.R., El-Nemr, M.A. & Chamoun, Y.G. 2009. Alleviation of salinity effects on tomato plants by application of amino acids and growth regulators. *Eur. J. Sci. Res*. **30**, 484–494.

Theerakulpisut, P. & Gunnula, W. 2012. Exogenous sorbitol and trehalose mitigated salt stress damage in salt-sensitive but not salt tolerance rice seedlings. *Asian J Crop Sci.* **4**, 165– 170.

Torabian, S., Zahedi, M. & Khoshgoftar, A.H. 2017. Effects of foliar spray of nano-particles of FeSO4 on the growth and ion content of sunflower under saline condition. *J Plant Nutr.* **40**(5), 615–623.

Tuna, A.L., Kaya, C., Ashraf, M., Altunlu, H., Yokas, I. & Yagmur, B. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environmental and Experimental Botany.* **59**, 173–178.

Williamson, J.D., Jennings, D.B., Guo, W.W., Pharr, D.M. & Ehrenshaft, M. 2002. Sugar alcohols, salt stress and fungal resistance: polyols: multifunctional plant protection? *J Am Soc Hortic Sci.* **127**, 467–473.

Yildirim, E., Karlidag, H. & Turan, M. 2009. Mitigation of salt stress in strawberry by foliar K, Ca and Mg nutrient supply. *Plant Soil Environ.* **55**, 213–221.

Zargar, M., Bodner, G., Tumanyan, A., Tyutyuma, N., Plushikov, V., Pakina, E., Shcherbakova, N & Bayat, M. 2018. Productivity of various barley (Hordeum vulgare L.) cultivars under semi-arid conditions in southern Russia. *Agronomy Research* **16**(5), 2242–2253.

Zhan, H., Nie, X., Zhang, T., Li, S., Wang, X., Du, X., Tong, W. & Song, W. 2019. Melatonin: A small molecule but important for salt stress tolerance in plants. *Int. J. Mol. Sci*. **20**(3), 709–726.

Agronomy Research **18**(1), 127–136, 2020
https://doi.org/10.15159/AR.20.045

Morphological variability of *Botrytis cinerea* – causal agent of Japanese quince grey mould

I. Jakobija[1,2,*], B. Bankina[1] and A. Klūga[1,2]

[1]Latvia University of Life Sciences and Technologies, Liela iela 2, LV–3001 Jelgava, Latvia
[2]Latvia University of Life Sciences and Technologies, Institute for Plant Protection Research, Paula Lejina iela2, LV–3001 Jelgava, Latvia
[*]Correspondence: inta.jakobija@llu.lv

Abstract. *Botrytis cinerea* is a causal agent of grey mould that damages many species of plants including Japanese quince (*Chaenomeles japonica* (Thumb.) Lindley ex Spach). Grey mould has been found on *Chaenomeles* spp. flowers, fruits in all stages as well as on twigs. Morphological variability within *Botrytis* species has been previously reported in the literature, but no information is available about *B. cinerea* isolated from *Chaenomeles* spp. The aim of this study was to describe the symptoms of grey mould and clarify the morphological variability of *B. cinerea* isolates obtained from samples collected in commercial plantations of Japanese quince. Samples of plant parts with different fungal disease symptoms were collected in eight commercial plantations of Japanese quince during vegetation seasons of 2017 and 2018. Some samples were taken in Japanese quince plantations in Lithuania and Estonia. A total of 286 isolates of fungi were isolated from damaged shoots, leaves and fruits of Japanese quince plants, using potato dextrose agar. *Botrytis cinerea* isolates (39) were separated depending on the morphological characteristics and were proved by using methods of molecular biology. *B. cinerea* was isolated from shoots, leaves and fruits. The isolates of *B. cinerea* were described and classified into distinct morphological types depending on the characteristics of mycelia, sclerotia, reverse side of media and the presence or absence of sporulation.

Key words: *Chaenomeles* spp., fruit rot, sporulation, morphological type, sclerotia.

INTRODUCTION

Japanese quince *Chaenomeles japonica* (Thumb.) Lindley ex Spach belongs to the family *Rosaceae* subfamily *Maloidae* together with other wide growing fruit species such as apple, pear, soft berries, etc. (Weber, 1964; Phipps et al., 1990). Fruits of Japanese quince (hereinafter – quince) are an important source of minerals, ascorbic acid, phenols and a lot of other valuable nutrients (Mierina et al., 2011; Baranowska-Bosiacka et al., 2017). The use of quince fruits is confined not only to food production. Quince fruits are a potential source for the medical and cosmetic production (Nahorska et al., 2014; Banaś & Korus, 2016). The first commercial plantation of quince for fruit production in Latvia was established in the 1950s (Kaufmane et al., 2013). Since then, the area of quince plantations in Latvia has repeatedly increased and decreased. Based on the information provided by the Ministry of Agriculture of the Republic of Latvia (2018), since 2013, the total area of Japanese quince plantations in Latvia has rapidly increased from 102 ha to 200 ha in 2015, and reached 326 ha in 2017.

A significant development of diseases was observed in eight commercial Japanese quince fields in Latvia in the vegetation periods of 2017–2018. During observations, several symptoms of diseases as leaf spots, shoot damages and fruit rot were detected and fungi from damaged plant parts were isolated. An essential proportion of them could be caused by *Botrytis cinerea.*

Only few reports describing symptoms of damages caused by *B. cinerea* on several parts of *Chaenomeles japonica* are available. In most cases, *B. cinerea* is associated with shoot die-back (dead shoots at the beginning of vegetation affected either by unfavourable environmental conditions or by fungal pathogen) and with wilted flowers. The first symptoms – small spots with a dark margin – of fruit rot caused by *B. cinerea* appeared mostly on fruits from the stem or calix side or around a wound. During the development of the disease, spots enlarged and the whole or part of quince fruit became brown (Norin & Rumpunen, 2003). In moisture conditions, grey wooly mycelium appeared on the surface of rotted fruit. Damaged areas can be surrounded by a red margin (Fedulova et al., 2017).

Rumpunen (2002) and Norin & Rumpunen (2003) reported that *B. cinerea* has been found on flowers and fruits at all stages of *Chaenomeles* spp. in Sweden, England, and Romania. Norin & Rumpunen (2003) isolated *B. cinerea* from cankers in shoots. Also, *B. cinerea* has been identified on the fruits of *Chaenomeles japonica* in the Vilnius Botanical Garden (Grigaliūnaitė et al., 2012) and on flowers and fruits in Tambow region of Russia (Fedulova et al., 2017). Jakobija & Bankina (2018) had previously reported the results of research about fruit rot incidence in plantations of Japanese quince in Latvia.

Botrytis cinerea is a worldwide distributed and well-described pathogen. *B. cinerea* belongs to the phylum *Ascomycota,* family *Sclerotiniaceae* (Williamson et al., 2007). This pathogen has a necrotrophic lifestyle (Williamson et al., 2007). *B. cinerea* causes grey mould on 589 species of plants (Elad et al., 2016), including Japanese quince (Hennebert, 1973). *B. cinerea* can cause important damages on all above-ground parts of Japanese quince, which results in serious yield losses, especially in fruit growing. The pathogen overwinters as mycelium, conidia, and, for a long-lasting period, with sclerotia in or on host tissues and soil surface (Elmer & Michailides, 2007; Williamson et al., 2007).

Sclerotia (dense formation of mycelium with a reserve of nutrients for survival) are the main source of inoculum in the life cycle of *B. cinerea* (Elmer & Michailides, 2007). Sclerotia develop on infected and dying host tissues. Fully formed sclerotia are black (Rasiukevičiūtė et al., 2017) and can have different shapes and length (Williamson et al., 2007). The apothecia or sexual stage of *B. cinerea* were detected in rare cases in orchards and are not considered an important part of disease life cycle (Beever & Weeds, 2007).

Conidia of *B. cinerea* can be produced on mycelium and sclerotia on the remains of hosts and on soil surface. It is the most important source of infection at the beginning of vegetation period (Elmer & Michailides, 2007). Conidia spread mainly with air flows and rain splashes (Jarvis, 1962), and start to germinate after 6 h of soaking in free water at the temperature of 20 °C (Hawker & Hendy, 1963). Results of the research of Xu et al. (2009) and Mehra et al. (2019) also showed that optimal temperatures for the development of *B. cinerea* conidia are 20°C and 22–25 °C respectively. Mehra et al. (2019) recognized that both air temperature and humidity are important factors for the development of grey mould. *B. cinerea* can directly penetrate host tissues or infect the plant through wounds and/or develop on senescent and dead plant parts (Elad, 1997). Many repeating generations of conidia from the infected plant parts are observed during vegetation (Elmer & Michailides, 2007).

Morphological variability among *Botrytis* isolates obtained from apple and strawberry in Lithuania (Rasiukevičiūtė et al., 2017), from blackberry, strawberry, grapevine and raspberry in Serbia (Tanović et al., 2009; Tanovic et al., 2014), from several species of ornamentals in South Spain (Martínez et al., 2008) and from many other hosts in Iran (Mirzaei et al., 2009) and India and Nepal (Kumari et al., 2014) has been previously reported in the literature. Chang et al. (2001) have shown that the amount, shape and size of sclerotia of *B. cinerea* on natural or culture media are very variable. Despite the comprehensive investigations in this research area, no information is available about similar studies on *B. cinerea* isolated from *Chaenomeles* spp.

Clarification of the morphological variability of *Botrytis* isolates obtained from different parts of Japanese quince could be used in the identification of the pathogen in Latvia and other countries where quince is cultivated. Moreover, the ability of *Botrytis cinerea* to form mycelium, sclerotium and spores, determine the potential for the spread and infection process of the pathogen (Rasiukevičiūtė et al., 2017). Information about the morphological and genetic diversity could be important for the control and forecast of the disease. According to the results of several studies (Kumari et al., 2014; Zhou et al., 2014; Tanovic et al., 2014; Isaza et al., 2019), the susceptibility of the genetical groups of *B.cinerea* to fungicides could be different.

The aim of this study was to describe the symptoms of Japanese quince diseases caused by *Botrytis cinerea* and clarify the morphological variability of *B. cinerea* isolates obtained from the samples collected in commercial plantations of Japanese quince.

MATERIALS AND METHODS

Collection of fungi isolates

Leaf, shoot, inflorescence and fruit samples with visible damages (associated with symptoms caused by fungi) were collected in eight commercial Japanese quince plantations in Latvia during the vegetation periods of 2017 and 2018 and two by two in Lithuania and Estonia in 2018. When necessary, collected samples were stored in humidity chambers until macroscopic structures (for example, mycelium, sclerotium, etc.) of fungi appeared on the damage point. A piece of fungus structure or damaged area of quince part was placed on a Petri dish containing potato dextrose agar (hereinafter – PDA) with streptomycin (100 ppm L^{-1}). Fragments of leaves with damages associated with fungal diseases were placed on the PDA directly. Fungi were incubated at 20 °C in the dark. Sample purification was done until a pure culture of fungal isolate was obtained and stored at 5 °C on PDA.

The identification of isolated fungi was performed based on the morphological characteristics of the isolates grown on PDA and using a microscope. Isolates of *Botrytis cinerea* were separated from all obtained isolates for further morphological studies. Isolates were sorted in groups by common morphological features. Representative isolates from each group were selected for the identification by molecular methods.

DNA extraction was done using E.Z.N.A.® HP Fungal DNA Kit (Omega Bio-tek, USA), following the manufacturer's instructions. ITS fragment was amplified using primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR products were sent for sequencing to Latvian Biomedical Research and Study Centre (BMC). Fungal samples were identified using the NCBI BLAST® database.

Morphological characterization of *Botrytis cinerea* isolates

Mycelial pieces (length of square edge approx. 5 mm) from isolates identified as *B. cinerea* were placed on a Petri dish (9 cm diameter) filled with PDA with addition of streptomycin (as described above); each isolate in three replicates for inter-comparison. Samples were grown in dark at the temperature of 20 °C for three weeks.

Morphological traits of *B. cinerea* were recorded 3, 7, 10 days, and three weeks after incubation. Mycelium type, colour, margin and size (diameter in cm), media (reverse side of plate) colour, sclerotia amount (pcs. on plate), diameter (mm), arrangement, colour, development stage and visibility were recorded at each observation.

Classification of *B. cinerea* isolates into distinct morphological types was performed depending on the last assessment after three weeks of incubation of isolates. The number of sclerotia on the plate filled with PDA (hereinafter – plate) was recorded. The classification of sclerotia-forming isolates depending on sclerotia amount on plate was performed, and isolates were separated into five classes: up to 10 sclerotia, 11 to 30, 31 to 50, 51 to 100, and more than 100 sclerotia on plate.

To evaluate the absence or presence of sporulation and sporulation rate on an isolate three weeks after incubation, a microscopic analysis was done using a microscope with 40× magnification (Table 1).

Mycelium type and sclerotia arrangement were described following the methodology previously used by Tanovic et al. (2014) with some modifications. The arrangement of sclerotia on the plate was divided into classes. No scattered arrangement was observed as that in the above-mentioned methodology. Instead of the four types of mycelium used in the mentioned methodology, five types were separated (Table 1).

Table 1. Separating of *B. cinerea* isolates depending on mycelium type, arrangement of sclerotia, and sporulation rate

Mycelium type		Arrangement of sclerotia		Sporulation rate	
designation	description	designation	description*	designation	description
M1	short, aerial	S1	at the edge	no	absence of sporulation
M2	short, tight	S2	in circle around centre	weak	less than 20 spores in field of view
M3	medium, aerial	S3	in circle around outer area	medium	20 to 50 spores in field of view
M4	mycelial masses	S4	irregularly	abundant	more than 50 spores in field of view
M5	thick, woolly				

*Description of basic arrangement of sclerotia. Combinations of characteristics hereinafter were marked, for example, as S1;2 if sclerotia were arranged at the edge and in a circle in one plate etc.

Statistical processing of data

To determine the dependence among qualitative variables, two-way contingence tables were used at a significance level of $\alpha = 0.05$. Programs 'R' (version 3.5.2.), 'R Studio' and 'Excel 2016' were used for data processing.

RESULTS AND DISCUSSION

Symptoms of damages caused by *B. cinerea* on quince

B. cinerea was isolated from fruits with varied symptoms of the disease. Most often, damage of *B. cinerea* appeared as rot on the fruit. The first grey mould symptoms can be recognized as brown sunken spots, sometimes with showy red halo on fruits at all growth stages. During the development of the disease, all fruit or part of it turned evenly brown or, in some cases, with alternating darker and lighter bands. It is observed that the infection of grey mould on quince fruits most often starts from stem side of fruit, sometimes from calix or other parts of quince fruit. In several cases, infection started at the point of contact between the healthy fully developed and rotted fruit. It was found that fruitlets not fallen in the first or second fruit drop and rotted, is a source of grey mould infection during the latest fruit development stages (Jakobija & Bankina, 2018). In moist conditions, grey mycelium covered the rotted surface of fruit. Similar symptoms of grey mould on fruits have been described and identified by Norin & Rumpunen (2003). The causal agent of grey mould was also isolated from wintered rotted fruits (hereinafter – mummies). Mummies from which *B. cinerea* was isolated were dense and brown, in some cases with a brown or pale rustle paper-like surface.

Taking into consideration the fact that *B. cinerea* was found within isolates obtained from damages on dead shoots of quince, it can be concluded that the pathogen can initiate the dieback on quince shoots, as described by Norin & Rumpunen (2003). The bark at the damage point cracked, became dry and separated from wood, and cambium turned dark brown. Most often, infection started from the fruit branch where wintered mummy was hanging.

The causal agent of grey mould was also isolated from the leaves of quince. Most often, *B. cinerea* was isolated from dark brown spots with concentric rings or with a cream-coloured centre and could reach a size of up to 2 mm. Spots were round or irregular, in some cases, central vein of leaf restricted an enlargement of them, located over all leaf e.g. edge. During disease development, spots merged and induced drying of damaged area or yellowing of leaf and premature leaf fall. In a few cases, *B. cinerea* was isolated from small, not more than 1 mm in diameter, red spots with a pale centre.

However, in general, the symptoms of diseases on the leaves caused by *Botrytis cinerea* are not typical and can be easily confused with those of other diseases; therefore, isolation in pure culture is necessary. Information in the literature about isolation of *B. cinerea* from the leaves of *Chaenomeles* spp. in other countries has not been found so far.

Morphological characterization of *B. cinerea* isolates

Entirely 286 fungal isolates were obtained during the study, of which 39 were *B. cinerea*, which were used for the evaluation of the morphological variability.

Three days after incubation on PDA media, the first morphological differences among isolates were detected. Mycelium differed in colour from colourless, white and cream-colored to light grey and grey with entire or wavy margins. Reverse side of plates was cream-colored or white in most cases, and colourless or grey in a few cases. The diameter of colonies varied from 2 to 9 (full plate) cm among isolates. Our data are in contradiction with other investigations; whereas in the investigations by Tanovic et al. (2014), on *B. cinerea* isolates obtained from raspberry at the beginning of incubation, all isolates showed white mycelium with an entire edge. Visible differences appeared only after six days of incubation in similar conditions.

Seven days after incubation, the white colour of mycelium changed to grey, some of isolates stayed cream-colored, but the reverse side of plates, white in previous observation, in most of cases became cream-colored. Mycelium, in most cases, filled the plate. Changes in the formation of mycelium type continued till the last assessment after three weeks of incubation.

Sclerotia-forming isolates were detected on 87.2% of all investigated *B. cinerea* isolates. Investigations of Tanovic et al. (2014) conducted in the same conditions on *B. cinerea* isolates from strawberries showed similar results, i.e., sclerotial-type isolates were observed on 81.5% of all studied isolates. Also, Kumari et al. (2014) reported that the presence or absence of sclerotia formation depends on *B. cinerea* isolate.

The first signs of sclerotia appeared as white beginnings on mycelium – after seven days of incubation, on 61.8% of isolates forming sclerotia. On 32.4% of sclerotia-forming isolates, the first visible signs of sclerotia appeared after ten days of incubation and on 5.9% – after three weeks of incubation.

Fully formed sclerotia were black, 1 to 5 mm in diameter depending on an isolate. The time to full formation of sclerotia fluctuated from 10 to more than 21 days depending on an isolate. These results conform to the findings of Mehra et al. (2019), where sclerotia appeared up to 20 days of the incubation of isolates obtained from capsicum. Different results were obtained in the research of Rasiukevičiūtė et al. (2017) where fully developed sclerotia were detected on *Botrytis cinerea* isolates from apple and strawberry already after 6 to 7 days after incubation on PDA at 20 °C in the dark. This is a reason to consider that the development of *B. cinerea* isolates obtained from Japanese quince is different compared to the development of isolates obtained from other fruits, as it was in the case with apples and strawberries. Also Rasiukevičiūtė et al. (2017) claimed that *B. cinerea* isolates obtained from different plants had variable phenotypic characteristics.

Classification of *B. cinerea* sclerotia-forming isolates depending on sclerotium amount on plate

The number of sclerotia on the plates varied from 5 to 134. Also, Tanović et al. (2009) reported that the amount of sclerotia on plates significantly differed.

Most frequently, sclerotia-forming isolates with 31 to 50 and 51 to 100 sclerotia on plate were detected – proportionally in 35% of cases for both classes. Isolates with less than 10 sclerotia on plate were found in 12% of cases, and with 11 to 30 and with more than 100 sclerotia on plate were found in 9% of cases for both classes. Also, the amount of sclerotia on plate fluctuated among the isolates obtained from different parts of Japanese quince (Fig. 1).

It was established that the amount of sclerotia on plate was significantly dependent on the part of quince from which an isolate was obtained ($p = 0.001$). The largest amount of sclerotia were found on isolates obtained from the fruits of Japanese quince, and the lowest amount was found on isolates obtained from leaves and shoots. In the light of this fact, it can be assumed that infected fruits are the main source for surviving of *B. cinerea* with sclerotia and can be an important reason for primary infection at the beginning of vegetation of Japanese quince, compared to infected leaves and shoots.

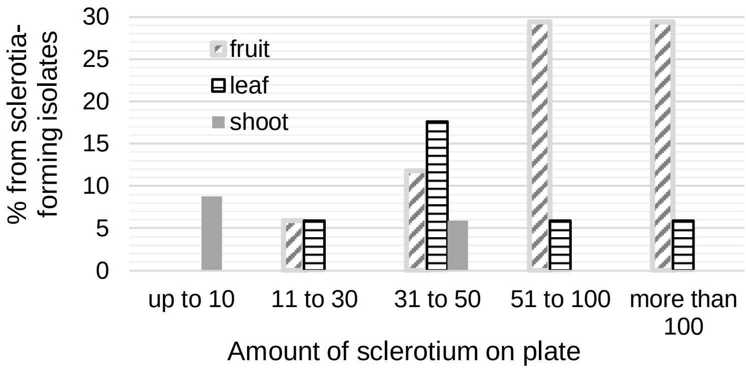


Figure 1. Proportion of sclerotia amount on sclerotia-forming isolates of *B.cinerea* obtained from different parts of Japanese quince.

Classification of *B. cinerea* isolates depending on sporulation

Sporulation was observed on 74.4% of *B. cinerea* isolates after three weeks of incubation. Tanović et al. (2009) obtained different results, where sporulation was observed only on 6.4% of *B. cinerea* isolates obtained from soft fruits in Serbia.

Most frequently (46.2% of cases), abundant sporulation was observed. Medium and weak sporulation was detected in 10.3% and 17.9% of cases respectively (Table 3), whereas in similar conditions, most frequently a medium sporulation was detected by Mehra et al. (2019).

Sporulation rate did not significantly depend on the amount of sclerotium on plate ($p = 0.728$) or on the type of mycelium ($p = 0.289$).

Table 3. Proportion of sporulation rate among *B. cinerea* isolates obtained from different parts of Japanese quince

Sporulation rate	Proportion (%) among isolates obtained from:		
	fruit	leaf	shoot
Abundant	20.5	17.9	7.7
Medium	5.1	5.1	0.0
Weak	10.3	5.1	2.6
No	15.4	5.1	5.1

Sporulation rate fluctuated among the isolates obtained from different parts of quince (Table 3) but did not significantly depend on the part of quince from which an isolate was obtained ($p = 0.898$).

Classification of *B. cinerea* isolates depending on mycelium type and sclerotium arrangement

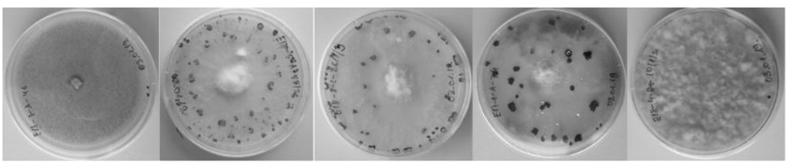


Figure 2. Mycelium types on *B. cinerea* isolates obtained from Japanese quince (left to right): M1 – short, aerial; M2 – short, tight; M3 – medium, aerial; M4 – mycelial masses; M5 – thick, woolly.

Five mycelium types (Fig. 2) were recorded on the isolates of *B. cinerea*. Mycelium type M2 was recorded in 26%, M3 – in 23%, M4 – in 20%, M1 – in 18%, and M5 – in 13% of cases. However, the overall proportion among different types of mycelium was equal. Three mycelium types – aerial, short, and cottony (woolly) – were distinguished among investigated isolates of *B. cinerea* obtained from blackberries by Isaza et al. (2019). This was similar to the findings of Martínez et al. (2008), who studied isolates from ornamental plants.

The research results demonstrated that the type of mycelium did not significantly depend on the part of quince from which an isolate was obtained ($p = 0.153$).

Six types or combinations of *B. cinerea* sclerotium arrangement were observed (Fig. 3).

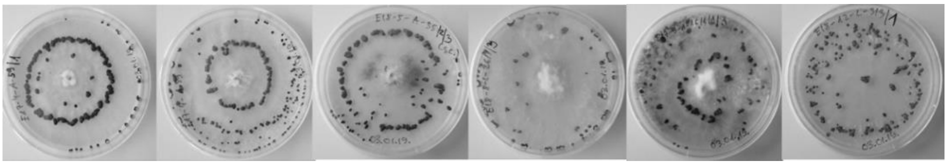


Figure 3. Sclerotium arrangement on *B.cinerea* isolates obtained from Japanese quince (left to right): S1;2 – at the edge, and in circle around the centre; S1;2;4 – at the edge, in circle around the centre, and irregularly; S1;3;4 – at the edge, in circle around outer area, and irregularly; S1;4 – at the edge, and irregularly; S2;4 – in circle around the centre, and irregularly; S4 – irregularly.

Irregular arrangement of sclerotia dominated (detected in 53% of cases) among all sclerotia-forming isolates. Similar results were obtained in the studies of Tanovic et al. (2014) and Kumari et al. (2014) where an irregular placement of sclerotia was found on 45% to 65% and on 58% (respectively) of *B. cinerea* isolates. The arrangement of other isolates was formed in combinations of different types.

During the study, it was found that sclerotium arrangement did not significantly depend on the part of quince from which an isolate was obtained ($p = 0.904$). Also, sclerotium arrangement did not depend on the type of mycelium ($p = 0.689$).

CONCLUSIONS

1. Sclerotium amount significantly depended on the type of mycelium, and sclerotium arrangement did not significantly depend on the part of quince from which an isolate was obtained.

2. The highest amount of sclerotia was formed on isolates of *B. cinerea* obtained from fruits compared to isolates from shoots and leaves. This suggests that infected fruits could be the main source for pathogen surviving and can continuously influence the primary spread of grey mould at the beginning of vegetation period.

3. Among the isolates of B. cinerea after three weeks of incubation at 20 °C in the dark, abundant sporulation dominated; however, a considerable part (26%) of isolates did not sporulate under those conditions.

4. Five types of mycelium and six types or combinations of sclerotium arrangement were recorded on the isolates of *B. cinerea*, which is an evidence for a high morphological variability of B. cinerea.

5. All mentioned data are a justification to conduct further investigations about the traits of B. cinerea in Japanese quince. The results obtained in this research can be used as a visual and informative material for further studies of *B. cinerea*.

ACKNOWLEDGEMENTS. The research was financed by the ERDF project 'Environment-friendly cultivation of emerging commercial fruit crop Japanese quince (*Chaenomeles japonica*) and waste-free methods of its processing', Nr.1.1.1.1/16/A/094.

REFERENCES

Banaś, A. & Korus, A. 2016. Health-promoting qualities of Japanese quince fruit *(Chaenomeles japonica). Medycyna Rodzinna* **2**, 83–85.

Baranowska-Bosiacka, I., Bosiacka, B., Rast, J., Gutowska, I., Wolska, J., Rębacz-Maron, E. … Chlubek, D. 2017. Macro- and microelement content and other properties of *Chaenomeles japonica* L. fruit and protective effects of its aqueous extract on hepatocyte metabolism. *Biological Trace Element Research* **178**(2), 327–337.

Beever, R.E. & Weeds, P.L. 2007. Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. In Elad, Y., Williamson, B., Tudzynski, P., Delen, N. (ed.): *Botrytis: Biology, Pathology and Control*. 29–52.

Chang, S.-W., Kim, S., Yi, E.-S. & Kim, J.-W. 2001. Occurrence of gray mold caused by *Botrytis cinerea* on *Cryptotaenia japonica* in Korea. *Mycobiology* **29**(4), 227–229.

Elad, Y. 1997. Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. *Biological Reviews* **72**(3), 381–422.

Elad, Y., Pertot, I., Prado, A.M.C. & Stewart, A. 2016. Plant hosts of *Botrytis* spp. In *Botrytis*– the fungus, the pathogen and its management in agricultural systems. *Springer, Cham.* 413–486.

Elmer, P.A.G. & Michailides, T.J. 2007. Epidemiology of *Botrytis cinerea* in orchard and vine crops. In Elad, Y.,Williamson, B., Tudzynski, P., Delen, N. (ed.): *Botrytis: Biology, Pathology and Control*, 243–272.

Fedulova, J.A., Kuklina, A.G. & Kashtanova, O.A. 2017. The study of pathogenic mycoflora and entomofauna on the cultivars of chaenomeles (*Chaenomeles* Lindl.) in the Tambov region. (Изучение патогенной микофлоры и энтомофауны на культиварах хеномелеса (*Chaenomeles* lindl.) в Тамбовской области.). *Вестник Мичуринского Государственного Аграрного Университета*. **2**, 25–30. (in Russian)

Grigaliūnaitė, B., Žilinskaitė, S. & Radaitienė, D. 2012. Phytosanitary conditions of *Chaenomeles japonica* in Vilnius University Botanical garden. *Optimization of Ornamental and Garden Plant Assortment, Technologies and Environment: Scientific Articles.* **3**(8), 25–29. (in Lithuanian, summary English).

Hawker, L.E. & Hendy, R.J. 1963. An Electron-Microscope Study of Germination of Conidia of *Botrytis cinerea*. *Journal of General Microbiology* **33**(1), 43–46.

Hennebert, G.L. 1973. *Botrytis* and *Botrytis*-like genera. *Persoonia*. **7**(2), 183–204.

Isaza, L., Zuluaga, Y.P. & Marulanda, M.L. 2019. Morphological, pathogenic and genetic diversity of *Botrytis cinerea* Pers. In Blackberry cultivations in Colombia. *Revista Brasileira de Fruticultura* **41**(6), e-490.

Jakobija, I. & Bankina, B. 2018. Incidence of fruit rot on Japanese quince *(Chaenomeles japonica)* in Latvia. *Research for Rural Development 2018*. **2**, 83–89.

Jarvis, W.R. 1962. The dispersal of spores of *Botrytis cinerea* fr. in a raspberry plantation. *Transactions of the British Mycological Society* **45**(4), 549–559.

Kaufmane, E., Skrivele, M., Rubauskis, E., Strautiņa, S., Ikase, L., Lacis, G., … Priekule, I. 2013. Development of fruit science in Latvia. *Proceedings of the Latvian Academy of Sciences, Section B*. **67**(2), 71–83.

Kumari, S., Tayal, P., Sharma, E. & Kapoor, R. 2014. Analyses of genetic and pathogenic variability among *Botrytis cinerea* isolates. *Microbiological Research* **169**(11), 862–872.

Latvijas lauksaimniecība 2018. Retrieved from https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/01/33/19/Gadazinojums.pdf

Martínez, J. A., Valdés, R., Vicente, M. J. & Bañón, S. 2008. Phenotypical differences among *B. cinerea* isolates from ornamental plants. *Communications in Agricultural and Applied Biological Sciences* **73**(2), 121–129.

Mehra, A.K., Malannavar, A.B. & Banyal, D.K. 2019. Factors affecting the growth of *Botrytis cinerea* and development of capsicum gray mould under protected cultivation. *Plant Disease Research* **34**(1), 76–78.

Mierina, I., Serzane, R., Strele, M., Moskaluka, J., Seglina, D. & Jure, M. 2011. Extracts of Japanese quince seeds - potential source of antioxidants. In E.Straumite (ed.): *Conference Proceedings of the 6th Baltic Conference on Food Science and Technology FOODBALT-2011. Innovations for Food Science and Production.* Latvia University of Agriculture, Riga, pp. 98–103.

Mirzaei, S., Mohammadi Goltapeh, E., Shams-Bakhsh, M., Safaie, N. & Chaichi, M. 2009. Genetic and phenotypic diversity among *Botrytis cinerea* isolates in Iran. *Journal of Phytopathology* **157**(7–8), 474–482.

Nahorska, A., Dzwoniarska, M. & Thiem, B. 2014). Fruits of Japanese quince *(Chaenomeles japonica* (Thunb.) Lindl. ex Spach) as a source of bioactive compounds. *Postępy Fitoterapii* **4**, 239–246. (in Polish, English abstr.).

Norin, I. & Rumpunen, K. 2003. Pathogens on Japanese quince *(Chaenomeles japonica)* plants. In K. Rumpunen (ed.): *Japanese quince - potential fruit crop for Northern Europe*. Department of Crop Science, Swedish University of Agricultural Sciences, pp. 37–54.

Phipps, J.B., Robertson, K.R., Smith, P.G. & Rohrer, J.R. 1990. A checklist of the subfamily *Maloideae (Rosaceae). Canadian Journal of Botany* **68**(10), 2209–2269.

Rasiukevičiūtė, N., Moročko-Bičevska, I. & Sasnauskas, A. 2017. Characterisation of growth variability and mycelial compatibility of *Botrytis Cinerea* isolates originated from apple and strawberry in Lithuania. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences* **71**(3), 217–224.

Rumpunen, K. 2002. *Chaenomeles*: Potential New Fruit Crop for Northern Europe. In J. Whipkey & A. Janick (eds.): *Trends in new crops and new uses.* ASHS Press, Alexandria, VA, pp. 385–392.

Tanović, B., Delibašić, G., Milivojević, J. & Nikolić, M. 2009. Characterization of *Botrytis cinerea* isolates from small fruits and grapevine in Serbia. *Archives of Biological Sciences* **61**(3), 419–429.

Tanovic, B., Hrustic, J., Mihajlovic, M., Grahovac, M. & Delibasic, G. 2014. *Botrytis cinerea* in raspberry in Serbia I: Morphological and molecular characterization. *Pesticidi i Fitomedicina* **29**(4), 237–247.

Weber, C. 1964. The genus *Chaenomeles (Rosaceae). Journal of the Arnold Arboretum* **45**(2), 161–205.

Williamson, B., Tudzynski, B., Tudzynski, P. & Van Kan, J. A. L. 2007. *Botrytis cinerea*: The cause of grey mould disease. *Molecular Plant Pathology* **8**(5), 561–580.

Xu, M., Li, H., Zhang, Z., Zou, Q. 2009. Biological characteristics of gray mold on tomato. *Guizhou Agricultural Sciences* **3**, 68–71.

Zhou, Y.J., Zhang, J., Wang, X.D., Yang, L., Jiang, D.H., Li, G.Q., … Zhuang, W.Y. 2014. Morphological and phylogenetic identification of *Botrytis sinoviticola*, a novel cryptic species causing gray mold disease of table grapes (*Vitis vinifera*) in China. *Mycologia* 1**06**(1), 43–56.

Agronomy Research **18**(1), 137–147, 2020
https://doi.org/10.15159/AR.20.088

Float system and crucial points of the method for seedling production and crop cultivation with or without organic fertilization

P. Kanatas

Agricultural Cooperative of Mesolonghi-Nafpaktia, GR30200 Mesolonghi, Greece
Correspondence: pakanatas@gmail.com

Abstract. Float system is a less-intensive and low-cost technology that enables efficient control of the nutrition of the growing plants. The findings of experiments carried out under float system in various crops with or without organic fertilization need to be reviewed. The importance of float system for growing plants without inorganic fertilization and chemical pesticide use needs also to be pointed out. Float system can enhance root development of growing plants and control the height of produced transplants. This can result in increased yields and improved transplant quality, not only in tobacco transplant production systems but also in the case of vegetable crops such as tomato or lettuce. In the case of crops like tobacco and for the success of float system alkalinity and oxygen content of the water in the nutrient solution and selection of the growing media are crucial. Regarding vegetable crops, the combination of float system and organic fertilization can enhance root development in tomato as compared to the combination and lettuce resulting in high-quality products. Several aromatic and medicinal plants, including basil and spearmint, provide high yields and good product quality in floating systems especially along with organic fertilization. Conclusively, float system is a major method for producing high quality products or transplants. Further investigation is needed regarding the combined effects of organic fertilization and float system on more crops and under different climatic conditions.

Key words: Float system, organic fertilization, root growth, yield, agronomy.

INTRODUCTION

The term 'soilless culture' generally refers to any method of growing plants outside the soil (Savvas, 2003; Gruda et al., 2016). The main reason to deep on new systems avoiding soil use is due unfortunately to several soilborne pests and diseases posing as a major threat to productivity of important crops for human nutrition. Root diseases caused by soil fungi and nematodes occur with great frequency on field where major crops such as vegetables are cultivated (Sherf & MacNab, 1986). In general, all economically important crops can suffer from several diseases that can decrease not only crop yield but also the quality of the harvested products (Agrios, 1988). Given that the use of the soil fumigant to control pest and diseases, methyl bromide has been phased out in industrialized countries, research has been carried out in order to develop alternative crop production strategies that are not dependent on its use as soil sterilizer (Webster et al., 2001; Gilreath et al., 2004; Rosskopf et al., 2005).

Soilless agriculture practices can be a real and efficient alternative. They produce relatively health crops, satisfactory root growth; and consequently, increased yield and product quality can be attained by adopting a low cost and more environmentally-friendly crop production strategy (Gruda et al., 2018; Glatkova & Pacanoski, 2019). The term 'hydroponics' is frequently used as a synonym to 'soilless culture'. However, some scientists are referred to hydroponics as a group of soilless culture systems in which no chemically active growing media are used (Adams, 2002). Water culture systems with nutrient solution as root environment and cultivation on porous growing media are included in hydroponics. Regarding water culture systems, float system, the nutrient film technique (NFT) and the aeroponic system are more suitable to intense greenhouse production systems (Van Os et al., 2008). Float system has been stated as the least risky out of the three systems mentioned above. Increased buffering capacity is the trademark of floating system due to the high ratio of available nutrients per plant that is normally observed in the root zone (Brechner & Both, 1996). This growing system is commonly constituted by polystyrene trays whose surface is cut appropriately so as cells of suitable size are formed. The distance between the cells in the surface of the polystyrene tray is preassigned and the roots of the cultivated plants pass through the cells while the above-ground part of the plants remains above the level of the tray (Savvas, 2016). The trays are floated due to their low specific gravity in polystyrene tanks filled with nutrient solution to a depth of 15–20 cm at least (Resh, 1995) whereas in some cases it can reach the level of 80–100 cm in order to eliminate changes in the chemical composition of the nutrient solution. Increased depth of nutrient solution results in increased buffering capacity of the nutrient solution to the fluctuations of temperature and this trait is well desirable especially in warm regions and climates where high temperatures are observed in great frequency (Bouzo & Kuchen, 2012). Furthermore, the inner surface of polystyrene tanks must be veneered with a material that cannot be penetrated from water in order to avoid any contact between tank surface and the nutrient solution (Savvas, 2016). Regarding the dimensions of the polystyrene tanks, they can vary among different levels. For example, Jensen & Collins (1985) used large tanks of 70 m length, 4 m width and 0.3 m height in contrast with Resh (1997) who suggested the use of narrow tanks of 60 cm width.

The aim of this paper is to review the findings of experiments conducted under float system in various crops with or without organic fertilization and point out the importance of float system as a promising water culture system for several crops.

FLOAT SYSTEM METHOD FOR VARIOUS CROPS

Plant nursery management is considered to be one of the most labor-consuming and not enough mechanized fields of agricultural area. Therefore, the development and introduction of new progressive technologies of cultivation of seedlings is urgent in order to increase quality and productivity per area, as previously described by Klymenko et al. (2010) and Carrasco et al. (2003). The adoption of float system can result in higher yield, for leaf vegetables crops such as lettuce (*Lactuca sativa* L.), corn salad (*Valerianella locusta* (L.) Betcke) or spinach (*Spinacia oleracea* L.) (Nicola et al., 2002; Van Os et al., 2002). The adoption of floating system has been stated as more suitable for growing tobacco (*Nicotiana tabacum* L.) transplants in greenhouses as compared to the case of horticultural crops (Biernbaum, 1992; Bilalis et al., 2008; 2009). In tobacco transplant production, float system technology has been widely studied (Jialai, 1995; Chunlei et al., 1997; Leal, 2001; Jiye & Haiping, 2004; Bu et al., 2008). Moreover, float system is a common strategy adopted regarding either aromatic plants or tomato (*Lycopersicon esculentum* Mill.) transplants production (Wyatt, 1998; Incrocci et al., 2001; Saha et al., 2016). However, other scientists indicate that in special cases it can also be an alternative cultural practice of producing horticultural crops such as radish (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin) because it is less labor intensive and is not harmful for the environment (Salerno et al., 2004).

Tobacco. The most common and recently developed strategy to develop tobacco transplants is the adoption of float system technology. In this system, Styrofoam trays filled with soilless media are floated on a large volume of nutrient solution (Maksymowicz & Palmer, 1993). Two different systems are the most dominant, the direct-seeded and the plug-and-transfer system (Smith et al., 1993). Water quality can have a major impact on the growth of seedlings. Key aspects determining the success of float system in tobacco transplant production are quality, alkalinity and oxygen content of the water in the nutrient solution (Pearce & Palmer, 1997; Reed, 2009). There is evidence that tobacco transplants in the float system can tolerate alkalinity up to 244 mg HCO^{3-} L^{-1}. However, potentially damaging levels of nitrate-N in the nutrient solution occur at high HCO^{3-} concentrations in the nutrient solution while the addition of Ca^{2+} can increase both the Ca and Mg concentrations in shoots, but decrease the concentrations of P and Fe (Pearce et al., 1999). Emphasis must be also given on the careful selection of the growing media. Typical tobacco media consist primarily of peat combined with vermiculite and perlite in various proportions (Fisher & Vann, 2017). In Chile, it was found that substrate selection in the float system did not affect significantly neither the fresh and nor the dry weight of the produced transplants. The same result was recorded for the parameters of stem length, stem diameter and following establishment of the finished transplants in the soil (Carrasco et al., 2000). Moreover, the effect of media selection on the physical properties of the media blends should be evaluated. According to Masaka et al. (2007), small and medium size pine bark particles the recorded porosity values were up to the level of 70% while for the no-pine bark particles the lower porosity were recorded.

Fertilizers commonly provide nitrogen from various combinations of nitrate, ammonium, and urea sources. Tobacco seedlings can use nitrogen in the nitrate and ammonium forms, but urea must be converted to ammonium before the nitrogen can be used by the plant (Fisher & Vann, 2017). Fertilizers containing urea affect negatively tobacco transplants grown in the float system. This can be justified by the findings of Pearce et al. (1998) who noted that no urea-N can be regained from the media solutions. Furthermore, the same authors reported that ammoniacal-N is decreased very slowly in the float water, but quickly nitrified in the media solution while Nitrite-N was accumulated from 60 to 80 mg L^{-1} at 2 to 3 wk after seeding, in float beds where 80% urea-N was applied. The sufficient levels of phosphorus (P) content in tobacco seedlings tissues have not been established. However, Miner and Tucker (1990) indicated that for mature plants, the sufficiency level varies from 2.0 to 5.0 mg g^{-1}. In float system it can be assessed that tissue P content over the level of 4 mg g^{-1} does not inhibit tobacco transplants growth and also that P deficit can put a negative effect on the produced transplants growth and quality. In the study of Masaka et al. (2008) deficiency of copper

(Cu), N, potassium (K), and P resulted in the creation of thin stems whereas the largest reduction in leaf emergence was recorded in sand media subjected to P and K deficiencies where a 64% reduction in leaf count was recorded. A boron deficiency causes bud distortion and death and has been observed in several float systems. In most cases, the water and the fertilizer did not contain any boron. The best solution to this situation is to choose a fertilizer such as a 20-10-20 with a guaranteed micronutrient charge if the water analysis indicates no boron (Fisher & Vann, 2017). Root and shoot weights, stem length, and flowering date of both transplant sources can be promoted by the transplant water fertilizer, but the effects generally are more consistent for transplants produced in the float greenhouse than for those produced in conventional nurseries (Fisher et al., 2001). Regarding the effects of applying organic fertilizers in float systems, it is well established that lower pH and electrical conductivity values of the nutrient solution are strongly related to organic float systems not only in tobacco transplant production but also in the cases of tomato and lettuce (Bilalis et al., 2009). Bilalis et al. (2010) reported that the production of oriental and flue-cured tobacco transplants with over 80% water content was attributed to the application of inorganic fertilizers in the nutrient solution. In the same study, the use of organic fertilizers resulted in greater total nicotine content and N concentration in the leaves of the transplants for both the tobacco types. In recent years, many growers have contracted to grow tobacco organically (Fisher & Vann, 2017). Regardless of it is about an organic or conventional tobacco float tray system, the appropriate distance among the cells in the tray surface is dependent on the needs and the targets of each crop grower. For instance, higher density trays are characterized by increased endurance in comparison to low density trays, but on the contrary their use increases the cost of seedlings production. However, inexpensive low density trays are more preferred by those who wish to sell well-developed plants and either face difficulties in getting trays returned or are concerned about disease issues often related to returned trays (Pearce et al., 2008). The results of Mundell et al. (2012) showed that no significant differences were detected in green-biomass yield among tobacco transplants grown in a tray with 595 cells and transplants grown in float trays with either 288 or 338 cells. The results of the study mentioned above showed that although plant maturation may not be promoted, the use of trays with a large number of cells offer an attractive option for float system by an economical aspect. Moreover, uniform germination and seedling emergence is of great importance in float system. Seeding should begin 50 to 55 days before the anticipated transplanting date using only high-quality, pelleted seeds. New trays should also be wet before filling because dry trays float higher than old trays and because it is difficult to keep the medium from falling through the hole in the bottom of the tray (Fisher & Vann, 2017). Regarding seed trays uniformity, it was indicated by Hartley et al. (2001) that even a 3 to 5 days delay in seeding 25% of the cells can decrease significantly the number of usable transplants produced. There is evidence that seeding date and N- or P-fertilizers application may not significantly affect early growth of roots or shoots in the field, but severe clipping one day before transplanting generally reduced shoot growth and delayed flowering of greenhouse transplants. Proper clipping is an important practice that can increase the number of usable transplants and improve transplant hardiness, stem-length uniformity, and stem diameter. Properly clipped plants with uniform stem lengths are needed to transplant seedlings at the proper depth, and excessive foliage disturbs the timing mechanism. Clipping can also be used to delay transplanting when field conditions are unfavorable (Fisher et al., 2001). There is also concern about the fact that although float system technology cannot be affected by soil pathogens, hazardous fungi such as Oomycete *Pythium myriotylum* (Drechs.) and Basidiomycete *Rhizoctonia solani* (Kühn) have been isolated from diseased plants in tobacco float systems (Anderson et al., 1997; Gutierrez et al., 1997). Except pathogens, attention should be also paid for the control of insects that can cause damage to the transplants and chemical control poses as a very effective strategy against their infestation. For instance, *Agrotis ipsilon*, *Faustinus cubae* and *Myzus persicae* were efficiently controlled by the application of a mixture of imidacloprid plus cyfluthrin in a conventional tobacco float system (Link et al., 2000).

Vegetable crops. Float systems are a successful strategy for the production of leaf vegetables with a short cultivation period such as lettuce, spinach and rocket (Van Os et al., 2002; Nicola et al., 2007) and are also promising for the cultivation of aromatic plants and herbs (Frantz & Welbaum, 1998; Miceli et al., 2003). Given that conventional tomato and lettuce transplant production is labor intensive (Thomas, 1993), the adoption of floating system poses as a low-cost attractive prospective as it eliminates the need for additional irrigation. Regarding vegetable crops, Bilalis et al. (2009) have reported that the combination of float system and organic fertilization resulted in 9–14% greater value of root surface in tomato as compared to the combination of float system along with the application of inorganic fertilizers. Even greater differences have been recorded in lettuce where root surface development was by 19–32% higher in the float system with use of organic fertilization (Bilalis et al., 2009). The positive effects of organic fertilizers application on tomato seedlings quality and growing potential in float systems have also been observed by Özer (2018). Similar were the results of a study carried out in melon (*Cucurbita pepo* L.) where under shade conditions, more efficient root development was observed for the transplants which were produced in the float system with organic fertilization in comparison to the corresponding development in the float system with inorganic fertilization. In tomato, brushing along with delayed phosphorus fertilization provided efficient height control and improved seedlings quality (Rideout & Overstreet, 2003).

For lettuce and tomato for processing, various mixtures of nutrient solutions can be utilized in a float system while it was also found that in the case of brassicas cell volume is another important parameter to consider in order to reduce the cost of float systems for vegetables. In the case of cauliflower, cabbage and broccoli it is possible to utilize a 20 cc/cell (Carrasco et al., 2003). Regarding *Brassica* spp., there is evidence that broccoli *(Brassica oleracea* Botrytis Group) and cabbage (*Brassica capitata* Group) transplants can be efficiently grown in tobacco transplant float systems (Niedziela & Gumbi, 1993). Float systems for the production of leaf vegetables have been increasing, but adequate nutrient solution concentrations are not yet available (Alberici et al., 2008). Regardless of their different composition, nutrient solutions contain high levels of dissolved nutrients with higher values than those generally observed in the natural soil environment (Marschner, 1995). The composition of the nutrient solution is an important aspect affecting the success of float systems and is dependent on the needs of each cultivated crop. For example, different solutions are used for lettuce (Coronel et al., 2009), lamb's lettuce (Gonnella et al., 2004) or rocket (Nicola et al., 2003). The role of electrical conductivity and oxygen content in the nutrient solution needs also to be evaluated when float system is applied in vegetable crops. In a tomato float system, it was noted that root length and branching of plants was promoted when plants were grown in areas of the trunk where EC value was increased (Tataranni et al., 2013). The interference of nutrient solution with organic or inorganic fertilization or chemical elements needs to be further investigated. Malorgio et al. (2009) pointed out that the addition of 0.5 and 1 mg Se L^{-1} in the nutrient solution of the float system leaded to higher leaf biomass per unit area both for lettuce and chicory (*Cichorium intybus* L.), especially during spring period, whereas both ethylene production and phenylalanine ammonia lyase activity were decreased due to Selenium addition. This comes in agreement with the findings of another study carried out in tomato whose conclusion was that the addition of Se in a nutrient solution can be an effective strategy for producing tomatoes with beneficial properties for public nutrition and health (Pezzarossa et al., 2014). The production of perennial vegetable crops in float system has also been studied. It was documented that for artichoke (*Cynara cardunculus* L. subsp. *scolymus* (L.) Hegi) and cultivated cardoon (*C. cardunculus* L. var. *altilis* DC), increased salinity on the nutrient solution reduced the ratio of leaves per plant by approximately 6–19% and leaf dry biomass by 17–28 %. However, improved quality of leaves was attributed to increased salinity in the nutrient solution (Rouphael et al., 2012). This is in full agreement with previous findings of other researchers who showed that an increase in nutrient solution electrical conductivity, through the use of floating system, can improve fresh-cut lettuce characteristics (Scuderi et al., 2011).

Aromatic and Medicinal Plants. Float systems are also promising for the cultivation of aromatic plants and herbs (Thomas, 1993; Miceli et al., 2003). The positive effects of adopting float system instead of soil cultivation on the yields of basil (*Ocimum basilicum* L.) and arugula (*Eruca vesicaria* (L.) Cav.) have been well demonstrated by Incrocci et al. (2001). *E. sativa* showed also good adaptability to floating system and its yield was slightly influenced by different EC levels (D’anna et al., 2003). There is also evidence that in rocket crop under float system, the use of a biostimulant resulted in yield and chlorophyll content regardless of the dose of standard or reduced nutrient solution was used (Vernieri et al., 2005). This finding indicates that the use of biostimulants along with float system technology can reduce the dependence of greenhouse production on the use of inorganic fertilizers. The combined effects of float system and organic fertilization has been well demonstrated by Akoumianaki-Ioannidou et al. (2015) who noticed that dry weight of shoots and leaves as fresh and dry weight of roots of basil seedlings were increased under float system than in seed beds, whereas in spearmint (*Mentha spicata* L.) there was also seed germination ability was favored by the adoption of float system. In addition, float system along with organic fertilization resulted in increased leaf area per plant in basil, whereas this combination did not favor several growth attributes of spearmint.

The results of Kiferle et al. (2013) suggested that the standard N concentration used in hydroponic culture could be significantly reduced, with important implications from the environmental aspect. This conclusion was justified by the facts that root dry weight of basil plants was significantly increased at 0.5 mol m^{-3} NO^{-3} and also that plants grown with 5.0 mol m^{-3} NO^{-3} contained more rosmarinic acid in root and leaf tissues than those grown with higher NO^{-3} concentration. According to Miceli et al. (2003), basil plants developed well in a floating system. There is evidence that several herbs, including basil and spearmint, provide high yields and good product quality when cultivated in soilless systems and in particular in floating systems (Thomas, 1993; Treadwell et al., 2011). Another finding was that keeping plants in the nutrient solution until the harvest, resulted in increased content of NO^{-3}. In the experiment of Saha et al. (2016) submerged basil seedlings were held in polyethylene tanks and were developed in a float raft system. The findings of this study revealed that aquaponic basil plants were higher than hydroponic basil plants and produced 56% more fresh weight but no significant differences were detected regarding either plant quality or concentration of leaf nutrient contents.

CONCLUSION

In summary, float system can certainly be a satisfactory alternative of the cultivation in the soil. It is considered to be a less-intensive and low-cost technology that enables the farmer to control efficiently the nutrition of the growing plants. Furthermore, diseases caused by soil pathogens are reduced when float system technology is adopted. Float system can significantly increase root development of growing plants and control the height of produced transplants. Increased yields and improved transplant quality (and consequently lower transplanting stress) can be achieved through float system technology for various crops like tobacco, vegetables, aromatic and medicinal crops. In all cases, it is crucial to mind about issues such as water quality, oxygen content and salinity, the substance of the growing media and the concentrations of nutrients in the nutrient solution. Float system along with organic fertilization can optimize float system technology and result in higher yields and excellent quality of produced transplants for tobacco, vegetables, aromatic and medicinal crops.

REFERENCES

Adams, P. 2002. Nutritional control in hydroponics. In: *Hydroponic Production of Vegetables and Ornamentals*, Savvas, D. & Passam, H.C. (Eds.)., Embryo Publications, Athens, ISBN: 9608002125, pp. 211–261.

Agrios, G.N. 1988. Plant Pathology. 3rd Edn., Academic Press, New York, ISBN: 9780323139694, 803 pp.

Akoumianaki-Ioannidou, A., Rasouli, M., Podaropoulou, L., Karapanos, I. & Bilalis, D. 2015. Effects of cultivation system and fertilization on seedling production of *Ocimum basilicum* L. and *Mentha spicata* L. *Notulae Botanicae Horti Agrobotanici* **43**, 131–137.

Alberici, A., Quattrini, E., Penati, M., Martinetti, L., Marino Gallina, P. & Ferrante, A. 2008. Effect of the reduction of nutrient solution concentration on leafy vegetables quality grown in floating system. *Acta Horticulturae* **801**, 1167–1176.

Anderson, M.G., Fortnum, B.A. & Martin, S.B. 1997. First report of *Pythium myriotylum* in a tobacco seedling float system in South Carolina. *Plant Diseases* **81**, 227–227.

Biernbaum, J.A. 1992. Root-zone management of greenhouse container-grown crops to control water and fertilizer use. *HortTechnology* **2**, 127–132.

Bilalis, D., Karkanis, A., Triantafyllidis, V., Ladavos, A., Bizos, G., Patsiali, S., Efthimiadou, A. & Papatheohari, Y. 2010. Effects of organic and inorganic fertilization on growth, yield and nicotine content of flue-cured and oriental tobacco (*Nicotiana tabacum* L.) seedlings grown in organic and conventional float system. *Journal of Food, Agriculture & Environment* **8**, 585–589.

Bilalis, D., Kanatas, P. & Konstantas, A. 2008. Effects of shading on root and shoot development of melon (*Cucurbita pepo*) transplants in conventional and organic float system nurseries. In: *Proceedings of the Second Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR)*, 18–20 June, 2008, Modena, Italy, pp.628–630.

Bilalis, D., Kanatas, P., Patsiali, S., Konstantas, A. & Akoumianakis, K. 2009. Comparison between conventional and organic floating systems for lettuce and tomato (*Lactuca sativa* and *Lycopersicon esculentum*) seedling production. *Journal of Food, Agriculture & Environment* **7**, 623–628.

Bouzo, C.A. & Kuchen, M.G. 2012. Effect of temperature on melon development rate. *Agronomy Research* **10**, 283–294.

Brechner, M. & Both, A.J. 1996. Hydroponic Lettuce Handbook. Cornell Controlled Environment Agriculture. http://cea.cals.cornell.edu/attachments/Cornell%20CEA%20Lettuce%20Handbook%20.pdf Accessed 09.10.17.

Bu, Y., Tang, B., Geng, S., Li, Q. & Zhou, R. 2008. Technology development and procedure of tobacco seedling production in sand floating system. *Chinese Tobacco Science* **1**.

Carrasco, G., Rebolledo, P. & Valverde, P. 2000. Floating system: an alternative for producing tobacco transplants in Chile. *Acta Horticulturae* **517**, 241–246.

Carrasco, G., Martínez, A.C., Márquez, O., Osorio, D., Urrestarazu, M. & Salas, M.C. 2003. Vegetable seedlings frown in a float system. *Acta Horticulturae* **614**, 241–245.

Chunlei, Y., Yongxiong, H., Aifei, C.G., Zhengbin, L. & Xinghe, Y. 1997. Study on the technique of tobacco seedling production with the direct-seeded float system. *Acta Tabacaria Sinica* **2**.

Coronel, G., Chang, M. & Rodríguez-Delfín, A. 2009. Nitrate reductase activity and chlorophyll content in lettuce plants grown hydroponically and organically. *Acta Horticulturae* **483**, 137–144.

D'anna, F., Miceli, A. & Vetrano, F. 2003. First results of floating system cultivation of *Eruca sativa* L. *Acta Horticulturae* **609**, 361–364.

Fisher, L.R., Peedin, G.F. & Smith, W.D. 2001. Factors affecting early field growth of flue-cured tobacco transplants produced in the float system. *Tobacco Science* **45**, 35–43.

Fisher, L.R. & Vann, M.C. 2017. Producing healthy transplants in a float system. North Carolina State University: Flue-cured tobacco production guide 2017. North Carolina State University, pp. 44–57.

Frantz, J.M. & Welbaum, G.E. 1998. Producing horticultural crops using hydroponic tobacco transplant systems. *HortTechnology* **8**, 392–395.

Gilreath, J.P., Noling, J.W. & Santos, B.M. 2004. Methyl bromide alternatives for bell pepper (*Capsicum annuum*) and cucumber (*Cucumis sativus*) rotations. *Crop Protection* **23**, 347–351.

Glatkova, G. & Pacanoski, Z. 2019. Evaluating the effects of application modes and soil types on the herbicide efficacy and crop yield of pendimethalin and clomazone on transplanted pepper (*Capsicum annuum* L.). *Agronomy Research* **17**, 430–437.

Gonnella, M., Conversa, G., Santamaria, P. & Serio, F. 2004. Production and nitrate content in lamb's lettuce grown in floating system. *Acta Horticulturae* **644**, 61–68.

Gruda, N., Gianquinto, G., Tüzel, Y. & Savvas, D. 2016. Culture Soil-less. In *Encyclopedia of Soil Sciences*, 3rd Edn., Lal, R. (Ed.)., CRC Press, Florida, USA., ISBN: 9781315161860, pp. 533–537.

Gruda, N., Savvas, D., Colla, G. & Rouphael, Y. 2018. Impacts of genetic material and current technologies on product quality of selected greenhouse vegetables - A review. *European Journal of Horticultural Science* **83**, 319–328.

Gutierrez, W.A., Shew, H.D. & Melton, T.A. 1997. Sources of inoculum and management for Rhizoctonia solani damping-off on tobacco transplants under greenhouse conditions. *Plant Diseases* **81**, 604–606.

Hartley, M.D., Smith, W.D., Spears, J.F., Fisher, L.R. & Schultheis, J.R. 2001. Effect of uniformity of seedling emergence on the percentage of usable transplants produced in the greenhouse float system. *Tobacco Science* **45**, 1–5.

Incrocci, L., Lorenzini, O., Malorgio, F., Tognoni, F. & Pardossi, A. 2001. Growth and quality of two herbs (*Eruca vesicaria* (L.) Cav. and *Ocimum basilicum* L.) g rown in soil and floating system using irrigation water with different NaCl concentrations. *Italus Hortus* **8**, 92–97.

Jensen, M.H. & Collins, W.L. 1985. Hydroponic vegetable production. *Horticultural Reviews* **7**, 483–557.

Jialai, X. 1995. Effect of fertilization, cell size and clipping on the greenhouse production of flue-cured tobacco seedling in the float system. *Acta Tabacaria Sinica* **1**.

Jiye, Q. & Haiping, Z. 2004. Seeding production with float system of flue-cured tobacco. *J. Lis. Teach. College* **2**.

Kiferle, C., Maggini, R. & Pardossi, A. 2013. Influence of nitrogen nutrition on growth and accumulation of rosmarinic acid in sweet basil (*Ocimum basilicum* L.) grown in hydroponic culture. *Australian Journal of Crop Science* **7**, 321–327.

Klymenko, O., Klymenko, M., Kartyzhova, L. & Alechenkova, Z. 2010. Cultivation of sweet cherry (*Cerasus avium* (L.) Moench) seedlings with using of phosphate-mobilizing microorganisms. *Agronomy Research* **8**, 633–636.

Leal, R.S. 2001. The use of Confidor® S in the float, a new tobacco seedling production system in the south of Brazil. *Pflanzenschutz-Nachr. Bayer* **54**, 337–352.

Link, D., Weber, L.F. & Leal, R.S. 2000. Control of the black cutworm, the tobacco stemborer and the green peach aphid with insecticides sprayed on tobacco seedlings produced by float system. *Revista Agricultura (Piracicaba)* **75**, 175–186.

Maksymowicz, B. & Palmer, G. 1993. Selecting a tobacco transplant production system. *Kentucky Cooperative Extension Service*. AGR 155.

Malorgio, F., Diaz, K.E., Ferrante, A., Mensuali-Sodi, A. & Pezzarossa, B. 2009. Effects of selenium addition on minimally processed leafy vegetables grown in a floating system. *Journal of Science of Food & Agriculture* **89**, 2243–2251.

Marschner, H. 1995. *Mineral nutrition of higher plants*. 2nd Edn., Academic Press, London, 889 pp.

Masaka, J., Muguti, P. & Chivandi, E. 2008. Nutrient deficiency effects on the biometric characteristics of tobacco seedlings in float tray systems. *Journal of Plant Nutrition* **31**, 797–808.

Masaka, J., Musundire, R. & Gondongwe, L. 2007. The effect of float seedling growth media combination and particle size distribution on germination and biometric characteristics of tobacco seedlings (*Nicotiana tabacum*). *International Journal of Agricultural Research* **2**, 459–467.

Miceli, A., Moncada, A., Vetrano, F. & D'Anna, F. 2003. First results on yield and quality response of basil (*Ocimum basilicum* L.) grown in a floating system. *Acta Horticulturae* **609**, 377–381.

Miner, G.S. & Tucker, M.R. 1990. Plant analysis as an aid in fertilizing tobacco. In: *Soil Testing and Plant Analysis*, Walsh, L.M. & Beaton, J.D. (Eds.)., Soil Science Society of America Inc., Wisconcin, USA., ISBN: 0891187936, pp, 645–657.

Mundell, R., Chambers, O., O'Daniel, J.P. & Davies, H.M. 2012. Evaluation of float trays with high cell numbers on stand counts and yields in a close-grown tobacco production system. *Tobacco Science* **49**, 4–7.

Nicola, S., Hoeberechts, J. & Fontana, E. 2002. Rocket (*Eruca sativa* Mill.) and corn salad (*Valerianella olitoria* L.) production and shelf-life of two vegetables grown in a soilless culture system. *Acta Horticulturae* **633**, 509–516.

Nicola, S., Hoeberechts, J. & Fontana, E. 2007. Ebb-and-flow and floating systems to grow leafy vegetables: A review for rocket, corn salad, garden cress and purslane. *Acta Horticulturae* **747**, 585–593.

Nicola, S., Hoeberechts, J., Fontana, E. & Saglietti, D. 2003. Cultural technique influences on post-harvest quality of rocket (*Eruca sativa* Mill.). *Acta Horticulturae* **604**, 685–690.

Niedziela, C.E. & Gumbi, P. 1993. Effect of cell size on the growth of broccoli and cabbage transplants in the tobacco float system. *HortScience* **28**, 530.

Özer, H. 2018. The effects of different seedling production systems on quality of tomato plantlets. *Acta Scientarum Polonorum-Hortorum Cultus* **17**, 15–21.

Pearce, B. & Palmer, G. 1997. Nitrogen source effects on the growth and development of Burley tobacco transplants in the float system. *Agronomy Notes* **30**(6), 1–10.

Pearce, R.C., Li, Y. & Bush, L.P. 1999. Calcium and bicarbonate effects on the growth and nutrient uptake of burley tobacco seedlings: Float system. *Journal of Plant Nutrition* **22**, 1079–1090.

Pearce, R.C., Zhan, Y. & Coyne, M.S. 1998. Nitrogen transformations in the tobacco float tobacco system. *Tobacco Science* **42**, 82–88.

Pearce, B., Palmer, G., Bailey, A., Seebold, K. & Townsend, L. 2008. Management of tobacco float systems. In: *Kentucky & Tennessee Tobacco Production Guide ID-160*, Seebold, K. & Pearce, B. (Eds.)., University of Kentucky, Kentucky, USA., pp. 11–22.

Pezzarossa, B., Rosellini, I., Borghesi, E., Tonutti, P. & Malorgio, F. 2014. Effects of Se-enrichment on yield, fruit composition and ripening of tomato (*Solanum lycopersicum*) plants grown in hydroponics. *Scientia Horticulturae* **165**, 106–110.

Reed, T.D. 2009. Float greenhouse tobacco transplant production guide. *Virginia Cooperative Extension Service*. **436**(051), 1–10.

Resh, H.M. 1995. *Hydroponic Food Production*. 5th Edn., Woodbridge Press Publishing Company, 527 pp.

Rideout, J.W. & Overstreet, L.F. 2003. Phosphorus rate in combination with cultural practices reduces excessive growth of tomato seedlings in the float system. *HortScience* **38**, 524–528.

Rosskopf, E.N., Chellemi, D.O., Kokalis-Burelle, N. & Church, G.T. 2005. Alternatives to methyl bromide: A Florida perspective. *Plant Health Progress* **6**, 19.

Rouphael, Y., Cardarelli, M., Lucini, L., Rea, E. & Colla, G. 2012. Nutrient solution concentration affects growth, mineral composition, phenolic acids, and flavonoids in leaves of artichoke and cardoon. *HortScience* **47**, 1424–1429.

Saha, S., Monroe, A. & Day, M.R. 2016. Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems. *Annals in Agricultural Science* **61**, 181–186.

Salerno, A., Pierandrei, F., Rea, E., Colla, G., Rouphael, Y. & Saccardo, F. 2004. Floating system cultivation of radish (*Raphanus sativus* L.): production and quality. *Acta Horticulturae* **697**, 87–92.

Savvas, D. 2003. Hydroponics: A modern technology supporting the application of integrated crop management in greenhouse. *Journal of Food, Agriculture & Environment* **1**, 80–86.

Savvas, D. 2016. *General Horticulture*. Pedio Publications, Athens, ISBN: 9789605467821, pp. 303–305.

Scuderi, D., Restuccia, C., Chisari, M., Barbagallo, R.N., Caggia, C. & Giuffrida, F. 2011. Salinity of nutrient solution influences the shelf-life of fresh-cut lettuce grown in floating system. *Postharvest Biology & Technology* **59**, 132–137.

Sherf, A.F. & MacNab, A.A. 1986. *Vegetable Diseases and Their Control*. Wiley, New York, USA., ISBN: 0471058602, 728 pp.

Smith, W.D., Peedin, G.F., Yelverton, F.H. & Campbell, C.R. 1993. *Producing tobacco transplants in greenhouses. Greenhouse systems*. North Carolina State University, College of Agriculture & Life Sciences, 12 pp.

Tataranni, G., Sofo, A., Casucci, C. & Scopa, A. 2013. Different root growth patterns of tomato seedlings grown hydroponically under an electric field. *Plant Root* **7**, 28–32.

Thomas, B.M. 1993. Overview of the speeding, incorporated, transplant industry operation. *HortTechnology* **3**, 406–408.

Treadwell, D.D., Hochmuth, G.J., Hochmuth, R.C., Simonne, E.H. & Sargent, S.A. 2011. Organic fertilization programs for greenhouse fresh-cut basil and spearmint in a soilless media trough system. *HortTechnology* **21**, 162–169.20

Van Os, E., Gieling, T.H. & Ruijs, M.N.A. 2002. Equipment for hydroponic installations. In: *Hydroponic production of vegetables and ornamentals*, Savvas, D. & Passam, H.C. (Eds.)., Embryo Publications, Athens, ISBN: 9608002125, pp. 103–141.

Van Os, E., Gieling, T.H. & Lieth, H.H. 2008. Technical equipment in soilless production systems. In: *Soilless Culture Theory and Practice*, 2nd Edn., Raviv, M. & Lieth, J.H. (Eds.), Elsevier Publications, London, UK., ISBN: 9780444529756, pp. 147–207.

Vernieri, P., Borghesi, E., Ferrante, A. & Magnani, G. 2005. Application of biostimulants in floating system for improving rocket quality. *Journal of Food, Agriculture & Environment* **3**, 86.

Webster, T.M., Csinos, A.S., Johnson, A.W., Dowler, C.C., Sumner, D.R. & Feryd, R.L. 2001. Methyl bromide alternatives in a bell pepper–squash rotation. *Crop Protection* **20**, 605–614.

Wyatt, J.E. 1998. Tomato transplant production using the float system and cupric hydroxide. *HortTechnology* **8**, 366–369.

Agronomy Research **18**(1), 148–162, 2020
https://doi.org/10.15159/AR.20.014

Combined application of microbial preparation, mineral fertilizer and bioadhesive in production of leek

V. Karpenko[1,*], G. Slobodyanyk[1], O. Ulianych[1], S. Schetyna[1], I. Mostoviak[1] and V. Voitsekhovskyi[2]

[1]Uman National University of Horticulture, Faculty of Horticulture, Ecology and Plants Protection, Department of Biology, Department of Vegetable Growing, Department of Plant Protection and Quarantine, Instytutska street 1, UA20300 Uman, Ukraine
[2]National University of Bioresources and Nature Management, Agrobiological Faculty Department of technologies of storage, processing and standardization of planting products by. prof. B.V. Lesika, Heroiv Oborony street 13, UA03041 Kyiv, Ukraine
[*]Correspondence: unuh1844@gmail.com

Abstract. The research deals with additional fertilizing of leek cultivars Goliath and Tango with bacterial preparation Organic-balance and mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me in combination with adhesive agent of a natural origin Liposam. Field research was performed on the experimental plots of the Department of Vegetable Growing of Uman National University of Horticulture. The research focused on microbiological processes and formation of productivity in the leek crops depending on the combination of preparations.
It has been established that the number of bacteria *Azotobacter* in the rhizosphere of leek increased 2.8 times after a four-time fertilizing with bacterial preparation Organic-balance and bioadhesive Liposam during vegetation. The maximum number of bacteria, including *Azotobacter*, in the rhizosphere of leek, was recorded after a four-time fertilization with DripFert $N_{20}P_{20}K_{20}$ + Me in combination with Organic-balance and Liposam. The share of influence of additional fertilization on the microbiota of the rhizosphere made up 77–97%.
Leek cultivar Tango produced a larger assimilative leaf surface. Depending on the fertilizing the maximum leaf surface and photosynthetic potential of leek cultivars Tango and Goliath were recorded under combination of Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam. The yielding capacity of variety Goliath was better than that one of the variety Tango regardless of fertilizing. To make the growing technology of leek more environmental friendly it is advisable to introduce in the growing technology bacterial preparation Organic-balance together with Liposam. Utilization of these preparations improves crop yield, which makes up 0.6–0.7 t ha^{-1} for variety Goliath and 1.1–2.7 t ha^{-1} for variety Tango correspondingly. The investigated leek varieties had the maximum yields under combined application of Organic-balance + Liposam with fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me, which is by 18–24% higher than provided by plants grown without fertilization.

Key words: leek growing, additional fertilization, microbial preparation, mineral fertilizer, bioadhesive, rhizosphere microbiota, assimilative leaf surface, photosynthetic potential, yield.

INTRODUCTION

Traditional vegetable growing technologies are aimed in obtaining the maximum productivity due to the intensive application of mineral fertilizers and pesticides, which inflicts considerable damage to the environment, leads to the deterioration of the biological state, physical and chemical properties of soil (Martyniuk et al., 2001). Alternative technologies of vegetable growing with the exclusion or limited utilization of chemical substances are safer, though they have a satisfactory economic efficiency (Brumfield et al., 2000; Bulluck et al., 2002). At present, research institutions are working at the introduction of preparations of natural origin, which are consistent with natural biodiversity and increase the productivity of plants (Melero et al., 2006; Sosnowski et al., 2014; Leggo, 2017).

Among such preparations, special attention is paid to the forms, that include microorganisms and products of their metabolism (Golubkina et al., 2018). Colonization of plants rhizosphere with microbiota stimulates growth and development of plants (Grant et al., 2005; Saxena et al., 2005) and promotes the adaptation to biotic and abiotic stresses (Yang et al., 2009; Grover et al., 2011). Because of unstable climatic situation (frequent droughts, increasing temperature, late spring or early autumn frosts) the relevance of using microbial preparations in agriculture is gaining in importance since other strategies of adaptation and effective resource management are money-consuming and short-term. At present bacteria of the genera *Azotobacter chroococcum* and *Bacillus subtilis* are the most wide-spread in the composition of microbial preparations. They participate in important processes of plant growth stimulation: *Azotobacter* is able to fix 60–80 kg ha^{-1} of nitrogen (Kennedy et al., 2004); *Bacillus subtilis* has fungicide properties (Kloepper, 2004).

Moreover, microbial preparations increase the availability of mineral compounds of calcium, phosphorus and iron for cultivated plants (Marra et al., 2012; Sosnowski et al., 2014; Kozhemyakov et al., 2015). Being used at the background of chemical substances they activate antioxidant processes aimed at detoxification of metabolic by-products, induced by the influence of xenobiotics (Karpenko & Prytulyak, 2014).

Under combined utilization of microbial preparations and mineral fertilizers the application rates of the latter can be reduced (Kahiluoto & Vestberg, 1998). Comparing the efficiency of using Humate and bacterial fertilizer Biostar (75% of shredded straw, 24% of mineral complex N : P : K at the ratio of 4 : 4: 4 and 1% of microorganisms *Bacillus sp.* – 5×10^6 CFU in 1 g) the preparation Biostar has the greatest influence on the yield capacity of leek. Under its use, the mass of leek increased 3.1 times compared to untreated plants (Golubkina et al., 2010).

Because of a long vegetation period high content of nutrients within a root system zone is especially urgent for leek. Thus the growing technology implies the application of up to 400 kg ha^{-1} nitrogenous fertilizers. Along with that, complex application of mineral fertilizers and biological preparations promotes a valid increase in yield capacity by 55–86%, even on the soils that have higher and high provision with mobile forms of phosphorus and exchangeable potassium (Syubayeva & Titova, 2015).

With the reference to above mentioned, it is important to develop such elements of leek growing technologies, that would combine the application of microbial preparations and mineral fertilizers and provide high productivity of a plant under the minimal negative influence on the agrocenosis and environment. It was this pertinent issue that determined the aim and the tasks of our research.

MATERIALS AND METHODS

The experimental part of the research was performed in the fields (experimental plots) and in the laboratories of the Department of Vegetable Growing of Uman National University of Horticulture in 2016–2018. The soil on the experimental plots is a podzolic heavy clay black soil with 1.9% of humus content with pH value – 6.3, nitrogen content in the arable top soil layer 103 mg kg^{-1}; mobile forms of phosphorus and potassium make up 122 and 135 mg kg^{-1}. The climate of the region where the experiment was conducted is temperate continental with unstable moistening.

Sixty-day seedlings of leek varieties Goliath and Tango were planted in the experimental plots in the second decade of April, the crops were planted according to the scheme 70×15 cm. The aim of the experiment was to study the efficiency of root additional fertilizing of leek with mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me and bacterial preparation Organic-balance, applied separately and together with bioadhesive Liposam.

Mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me (DripFert™, Turkey, 'Sabera' private limited company', a representative office in Ukraine) is a water soluble fertilizer without chlorine, including balanced complex of microelements: B – 0.03%; Fe – 0.04; Mn – 0.03; Zn – 0.04; Cu – 0.06% on chelate base of EDTA (ethylene diamine tetraacetic acid).

Bacterial preparation Organic-balance (BTU-Center (Ukrainian Biotechnology), Ukraine) is a concentrate of viable and inactivated microorganisms of different taxonomic groups and their active metabolites, namely: cells of bacteria *Bacillus subtilis 221* – 40 ± 10%, *Azotobacter* – 30 ± 10%, *Paenibacillus polymyxa* – 10 ± 5%, *Enterococcus* – 10 ± 5%, *Lactobacillus* – 10 ± 5%, titre $1×10^8 – 1×10^9$ CFU in cm^3 macro- and microelements, biologically active metabolites of bacteria.

Liposam (BTU-Center, Ukraine) is a composition of biopolymers of natural origin, applied as a biological adhesive agent for crop protection agents and fertilizers. It provides a close contact with a treated surface, creates a flexible protective film preserving moisture and applied solutions in the root system.

Variety Goliath (Rijk Zwaan, Holland) is a medium-early leek variety with a vegetation period of 150 days. According to morphological features it belongs to autumn variety and is not frost-resistant. Plants have 80 cm long leaves, pseudo-stems reach 30 cm. This variety is resistant to diseases.

Tango (Moravo seed, Czech Republic) is a mid-season ripening leek variety. According to its morphological features and frost resistance, it belongs to the winter variety. Plants are of medium height, grayish-green leaves are wide. The pseudo-stem has the height of 12 cm and is 5 cm in diameter. The taste is semi-spicy. The variety is recommended for long winter storage.

Two-factor experiment included leek varieties (factor A) Goliath and Tango (control) and bacterial preparation and mineral fertilizer applied separately or together with bioadhesive (factor B): Organic-balance (0.5 L ha^{-1}); Organic-balance (0.5 L ha^{-1}) + Liposam (0.5 L ha^{-1}); DripFert $N_{20}P_{20}K_{20}$ + Me (50 kg ha^{-1}); DripFert $N_{20}P_{20}K_{20}$ +

Me (50 kg ha^{-1}) + Liposam (0.5 L ha^{-1}); Organic-balance (0.5 L ha^{-1}) + DripFert $N_{20}P_{20}K_{20}$ + Me (50 kg ha^{-1}); Organic-balance (0.5 L ha^{-1}) + DripFert $N_{20}P_{20}K_{20}$ + Me (50 kg ha^{-1}) + Liposam (0.5 L ha^{-1}); 7) without fertilization (control).

Preparations were introduced into the soil as water solutions at the rate of 20 m^3 ha^{-1} per watering rate. Four additional fertilizations were performed during leek vegetation: the first fertilization was achieved three weeks after seedlings planting and the other three with 30 days interval. The experiment had four replicates. The allocation of repetitions and variants was multilevel. The plants were hilled up twice in July for the etiolation of pseudo-stem. The harvest was gathered and recorded at the end of the 1st decade of October.

The number of rhizospheric bacteria in the experiment was determined according to the method presented by Zvyagintsev et al. (1991). On the 25th day after the 1st and the 4thfertilizers supplementation the samples were taken from a root zone, from which soil suspension was prepared and sowed on meat-and-peptone agar. Microbiological inoculation was performed four times. The number of bacteria was calculated according to the formula $K = a \times P/m$, where: K – the number of colony-forming units (CFU), units in 1 g of absolutely dry soil; a – the number of microorganism colonies, that were grown on nutritious medium, units; P – dilution of soil extract; m – the mass of absolutely dry soil in 1g of wet soil, g.

The number of nitrogen fixing bacteria *Azotobacter* was recorded on the nutrition medium Ezhbi according to the method of growing colonies around soil clods and were expressed in percentage to the total number of soil clods, that were placed in Petri dish (Hrycajenkoet al., 2003).

Leaf surface area was recorded and photosynthetic potential was calculated during the vegetation period of leek. The leaf surface area (cm^2) of leek was calculated according to the formula: $S = D \times L / 2$ (where D – the length of a leaf, cm, L – the width of a leaf, cm) with further re-calculation in m^2 per 1 ha (Bondarenko & Yakovenko, 2001). Photosynthetic potential (*PhP*), $m^2 \times$ days ha^{-1} was determined according to the Nichiporovich (1972) formula: $PhP = L_1 + L_2 / 200 \times T$, where L_1 – the leaf surface area on the 20th May; L_2 – leaf surface area on the 10h of October, thousand m^2 ha^{-1}; T – the duration of inter-phase period, days. We determined the share of the commercial produce relevant to the collected yield of the pseudo-stem with a diameter of more than 1.5 cm, the harvest was weighed and calculated in t per ha.

The validity of the research and significance of the differences between the mean values of the variables examined were evaluated according to the results of dispersion and correlation analysis of mathematical statistics (Ehrmantraut et al., 2000).

RESULTS AND DISCUSSION

Soil microbiocenosis considerably influences the interaction of introduced bacteria and plants (Stamenov et al., 2012). Under field conditions, the positive effect of bacteria on improving the accessibility of nutrients for plants is not always achieved since the vital activity of microbiota depends on the growing technology and properties of plants (Jarak et al., 2012). Therefore, to determine the regularities of functioning microbial cenosis is an important criterion to evaluate the expediency of any elements of technology, because the fertility of soil and yield capacity of plants depends on the rearrangement of some ecological populations of microorganisms (Avrova, et al., 2005;

Sherstoboyeva et al., 2007). In this respect, special attention should be paid to nitrogen-fixing bacteria *Azotobacter*, that can enrich soil with nitrogen as well as synthesize growth-stimulating compounds and antagonistic compounds to the pathogenic microbiota (McSpadden-Gardener, 2004).

The data of the conducted microbiological analysis of the soil showed that application of the investigated preparations in the technology of growing leek influences considerably the growth of rhizospheric bacteria (Table 1).

Table 1. The number of microbiota in the rhizosphere of leek (mean over three years)

Additional fertilizing (B)	Bacteria, 10^6 CFU in 1 g of soil			
	Variety (Factor A)			
	Goliath	Tango (control)	*Mean value by factor B*	± compared to control
I period of recording (at the beginning of June)[1]				
Without fertilization (comparison variant, control)	1.33	1.92	*1.63*	-
Organic-balance	2.44	2.41	*2.43*	0.80
Organic-balance + Liposam	3.05	4.07	*3.56*	1.94
DripFert $N_{20}P_{20}K_{20}$ + Me	1.82	2.63	*2.23*	0.60
DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	2.15	2.91	*2.53*	0.90
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	3.17	3.76	*3.47*	1.84
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	5.31	4.84	*5.08*	3.45
Mean value by factor A	*2.75*	*3.22*	-	*0.47*
LSD_{05} – 0.25; LSD_{05A} – 0.1; LSD_{05B} – 0.18; Factor influence, % – A – 14; B – 82; AB – 4				
II period of recording (at the beginning of September)[2]				
Without fertilization (comparison variant, control)	3.54	1.68	*2.61*	-
Organic-balance	5.19	4.28	*4.74*	2,13
Organic-balance +Liposam	6.57	6.24	*6.41*	3.80
DripFert $N_{20}P_{20}K_{20}$ + Me	3.15	2.56	*2.86*	0.25
DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	3.87	2.50	*3.19*	0.58
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	5.84	4.69	*5.27*	2.66
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	7.09	7.35	*7.22*	4.61
Mean value by factor A	*5.03*	*4.19*	-	*0.84*
LSD_{05} – 0.33; LSD_{05A} – 0.13; LSD_{05B} – 0.23; Factor influence, % – A – 20; B – 77; AB – 3				

[1]after 25 days since the first additional fertilizing; [2]after 25 days since the fourth additional fertilizing.

Thus, 25 days after the first fertilizing of Goliath and Tango varieties the number of bacteria exceeded 1.4 times the value typical for control. Under joint application of DripFert $N_{20}P_{20}K_{20}$ + Me, Liposam and Organic-balance it increased 1.5–1.7 and 2.0–2.4 times respectively. Contrary to Goliath variety, after the first application of preparation Organic-balance in the plots with Tango variety there was less amount of bacteria compared to the variant with mineral fertilizing. On the plots with Goliath variety after 25 days since the application of Organic-balance the number of bacteria exceeded the variant DripFert $N_{20}P_{20}K_{20}$ + Me by 34%, the variant without fertilizer by 83%. Under combined application of Organic-balance with Liposam the number of bacteria after the first fertilizing was 1.3 and 1.7 times higher compared to the option without bioadhesive on the plots with varieties Goliath and Tango respectively. After the fourth combined application of Organic-balance + Liposam the number of bacteria exceeded the option without bioadhesive 1.3 times (for variety Goliath) and 1.5 times (for variety Tango).

On average during the experiment, the greatest number of bacteria was recorded after the first fertilization with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam application where their number exceeded the options without fertilization 2.5 times (Tango) and 4.0 times (Goliath).

The number of bacteria increased during vegetation in the rhizosphere, except the control variant and DripFert $N_{20}P_{20}K_{20}$ + Me. On the plots with Goliath variety without fertilization at the beginning of September (the second period of recording) the number of bacteria in the rhizosphere of leek was 2.7 times higher compared to the data at the beginning of June (the first period of recording). After the fourth fertilization DripFert $N_{20}P_{20}K_{20}$ + Me on the plots with Goliath variety the total amount of bacteria was lower than without fertilization. However, on average according to factor B after the fourth fertilization DripFert $N_{20}P_{20}K_{20}$ + Me the number of bacteria exceeded the control by 0.25×10^6 CFU, which is significant according to the LSD_{05B} in space of soil.

Under the application of mineral fertilizer together with Liposam the higher amount of bacteria was recorded compared to the variant without bioadhesive. In the option with DripFert $N_{20}P_{20}K_{20}$ + Me together with Organic-balance at the beginning of September the number of bacteria on the plots with Tango and Goliath varieties was correspondingly 3.0 and 2.30×10^6 CFU in 1 g of soil higher than in the plots without fertilization.

Regardless of the variety after the fourth combined application of Organic balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam in the rhizosphere of, leek the number of bacteria was the highest and according to factor B, it exceeded on average the indicators of the plots without fertilizing 2.8 times.

As to nitrogen-fixing microorganisms of the genus *Azotobacter*, their number in the variant with applying only DripFert $N_{20}P_{20}K_{20}$ + Me was by 4–5% lower in the leek rhizosphere of both varieties in September (after the fourth fertilizing) compared to the indexes at the beginning of June (after the first fertilizing) (Table 2).

On average, according to factor B the number of *Azotobacter* bacteria in the option DripFert $N_{20}P_{20}K_{20}$ + Me had no significant difference compared to control. Application of Organic-balance together with mineral fertilizer increased the number of *Azotobacter* bacteria by 5–7% after the first additional fertilization and by 16–18% after the fourth fertilization compared to the variant with the application of only DripFert $N_{20}P_{20}K_{20}$ + Me. In the rhizosphere of Goliath variety, grown without additional fertilization, the number of *Azotobacter* bacteria was the lowest at the beginning of September.

In the variants with a separate application of Organic-balance and with bioadhesive, the number of *Azotobacter* bacteria was higher with maximum indexes in the variant Organic-balance + Liposam after the fourth fertilization. On average, according to factor B after the fourth fertilization with Organic-balance and with Organic-balance + Liposam the number of *Azotobacter* bacteria was 2.5 and 2.8 lower respectively.

In the variants of combined application of Organic balance + Liposam + DripFert $N_{20}P_{20}K_{20}$ + Me the share of bacteria of the genus *Azotobacter* was maximum and at the beginning of June and September it was higher by 13–14% and by 30–31% compared to the plots without additional fertilization within the variety. Similar phenomenon was recorded by other researchers (Colo et al., 2014): the total number of bacteria and the number of nitrogen fixing bacteria in the leek crops inoculated with *Azotobacter* *chroococcum* and *Bacillus subtilis* was higher. According to Tawaraya et al. (1995), leek roots secrete amino acids, sugars and organic acids, creating in this way favorable conditions for the development of different groups of microorganisms, and introduction of microbiological fertilizers into soil changes not only their number, but also their species composition. It is obvious that the increase of the total number of microbiota and *Azotobacter* bacteria in different variants of application of Organic-balance is the result of the introduction of bioagents of this preparation into the rhizosphere of plants. The increase of bacteria number in the soil under their induction causes the activation of microbiological transformations favorable for plants provision with nutrients (Jarak et al., 2006; Khadiga et al., 2015).

Table 2. The number of *Azotobacter* bacteria in the rhizosphere of leek, % (mean over three years)

Additional fertilizing (B)	*Azotobacter*, %			
	Variety (factor A)			
	Goliath	Tango (control)	*Mean value by factor B*	± compared to control
I period of recording (at the beginning of June)				
Without fertilization (comparison variant, control)	17	13	*15.0*	-
Organic-balance	27	26	*26.5*	10.5
Organic-balance + Liposam	33	35	*34.0*	19.0
DripFert $N_{20}P_{20}K_{20}$+Me	20	16	*18.0*	3.0
DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	22	19	*20.5*	5.5
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me	25	23	*24.0*	9.0
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	30	37	*33.5*	18.5
Mean value by factor A	*25.0*	*24.1*	-	0.9
LSD_{05} – 7; LSD_{05A} – 3; LSD_{05B} – 5; Factor influence, % – A – 8; B – 90; AB – 2				
II period of recording (at the beginning of September)				
Without fertilization (comparison variant, control)	14	17	*15.5*	-
Organic-balance	35	38	*36.5*	21.0
Organic-balance + Liposam	41	47	*44.0*	28.5
DripFert $N_{20}P_{20}K_{20}$+Me	16	11	*13.5*	-2.0
DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	27	15	*21.0*	5.5
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me	32	29	*30.5*	15.0
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	44	48	*46.0*	30.5
Mean value by factor A	*30.0*	*29.2*	-	0.80
LSD_{05} – 8; LSD_{05A} – 3; LSD_{05B} – 6; Factor influence, % – A – 1; B – 97 ; AB – 2				

According to statistical analysis the share of factor B influence (additional fertilizing with mineral fertilizer and biological preparation) on the rhizosphere microbiota of leek made up 77–97%, and influence of the variety (factor A) and interaction of factors (AB) was not significant and made up 1–20% and 2–4% respectively. During the periods of recording, there was a strong correlation in the rhizosphere of leek between the total amount of bacteria and the share of *Azotobacter* – $r = 0.73 \pm 0.11$ (at the beginning of June) and $r = 0.92 \pm 0.04$ (at the beginning of September) (Fig. 1).

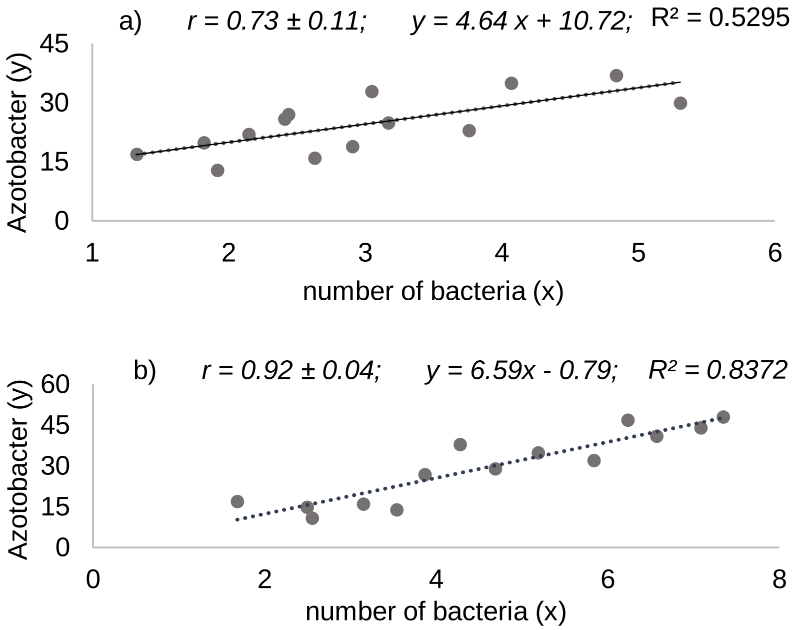


Figure 1. Correlation dependence between the total numbers of bacteria (x), 10^6 CFU in 1 g of soil and the share *Azotobacter* (y), % in the rhizosphere of leek after the first (a) and the fourth (b) additional fertilizing.

Prediction of *Azotobacter* content depending on the total number of bacteria can be calculated using regression equation $y = 4.64x + 10.72$ and $y = 6.59x – 0.79$ for the investigated periods. At the same time, in the experiments of Hajnal-Jafari et al. (2012) the introduction of *Azotobacter chroococcum* did not affect its amount in the soil, therefore correlations between the amount of *Azotobacter* and yield of corn seeds was not found. However, understanding the processes of colonization of rhizosphere by microorganisms is of great importance for the prediction of interaction of microbiota with plants towards increasing their productivity (Stephane et al., 2010).

When mineral fertilizers are applied, the increase of the efficiency of preparations containing associative nitrogen-fixing microbiota can be explained by the fact that nitrogen from the fertilizer stimulates the plant growth at the background of relatively low activity of nitrogen-fixing bacteria at the initial period of vegetation. After a while fertilized plants with rather developed root system and high level of metabolism provide optimal conditions for the activity of rhizosphere microbiota (Syubayeva & Titova, 2015), as the result, a plant gets additional amount of metabolic by-products of diazotrophs (nutrients and growth stimulators).

According to the data of leaf surface area of leek it was found that additional fertilization has a positive effect on the formation of indexes of assimilation activity. Application of Organic-balance alone or its application in the mixture with Liposam promoted the formation of leaf surface during the harvesting period of Goliath and Tango varieties by 3.18–4.21 thousand m^2 ha^{-1} and 0.84–2.25 thousand m^2 ha^{-1} more than plants that were not treated with the preparation (Fig. 2).

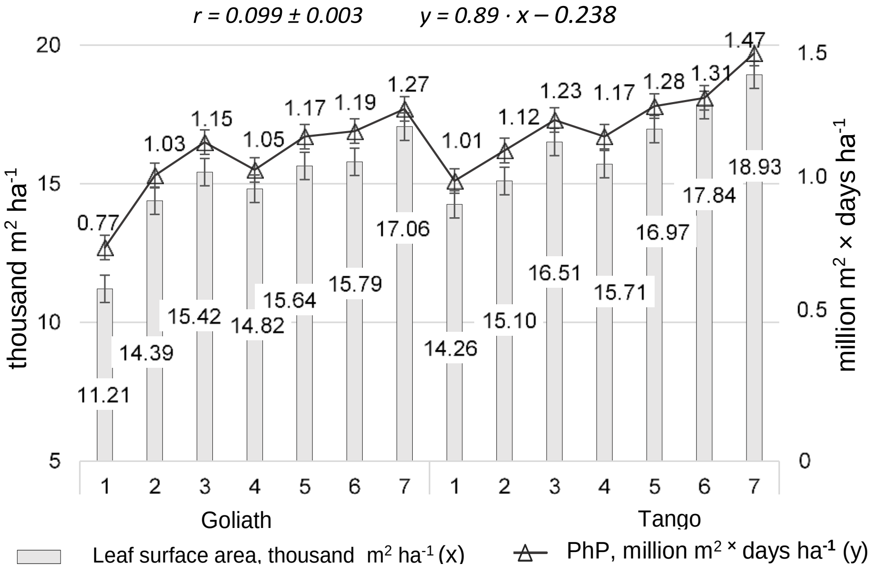


Figure 2. Indexes of assimilation activity of leek, average over 2016–2018:

1) without fertilization (comparison variant - Goliath, control-Tango);
2) Organic-balance;
3) Organic-balance + Liposam;
4) DripFert $N_{20}P_{20}K_{20}$+Me;
5) DripFert $N_{20}P_{20}K_{20}$+Me + Liposam;
6) Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me;
7) Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam

Results of statistical analysis

Leaf area (as of 10.X) (thousand m^2 ha^{-1})					
The year of research					
2016		2017		2018	
$LSD_{0.5}$	*CV*[1], %	*$LSD_{0.5}$*	*CV*[1], %	*$LSD_{0.5}$*	*CV*[1], %
1.72	*11*	*0.95*	*15*	*1.41*	*15*

[1] *– coefficient of variation.*

After additional fertilizing with DripFert $N_{20}P_{20}K_{20}$ + Me on the 10th of October the average increase of leaf surface of Goliath variety made up 3.61 thousand m^2 ha^{-1}, and of Tango variety made up 1.45 thousand m^2 ha^{-1} compared to the variants without fertilization. Fertilization with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me and DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam turned out to be more efficient than fertilizing with DripFert $N_{20}P_{20}K_{20}$ + Me alone, the photosynthetic potential was higher by 0.11–0.14 m^2 × days ha^{-1}. For both varieties the maximum indexes of assimilative leaf surface were recorded when there was a combined application of Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam and they were by 52% (Goliath) and 33% (Tango) higher than in the options without fertilization.

Obtained indexes of the photosynthetic potential of leek indicate correlation dependence with leaf surface area: $r = 0.992 \pm 0.004$. On average over the period from 20th May to 10th October Tango variety had the highest values of photosynthetic potential in the variants Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me and Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam: 1.3–1.5 times higher than in control. The photosynthetic potential of Goliath variety under similar variants of growing was lower than that one of Tango variety. According to dispersion analysis, the power of influence on the assimilative surface of factor A (variety) was 48%, factor B (fertilizing) – 47%. The level of indexes variation was average and made up 11–15%.

The obtained data of assimilation activity of leek depending on additional fertilization with biological or mineral fertilizer were in good agreement with the results of other researchers (Kloepper, 2004; Hosam et al., 2013), who indicated that inoculation with *Pseudomonas* isolates had a stimulating effect on the plant growth. In the research of Khadiga et al. (2015) the maximum leaf surface and dry matter of leek were formed under combined application of *B. subtilis + Ps. fluorescens + Yeast*. According to the data in Abou & Yousry (2012), after inoculation with *Glomus intraradices* biometric parameters of leek were by 25–28% higher compared to the variants without inoculation.

Mazur et al. (2019) reported that the preparation containing *Rhizohumin* bacteria in combination with plant growth regulator Emistym C stimulated the development of leaf apparatus and promoted the increase of the photosynthetic potential of lupine in compared to control by 8.1 m^2 ha^{-1} (27%). The increase of biological indexes of plants under the treatment of microorganisms is considered to reflect the growing activity of rhizosphere microbiota (Mantelin & Touraine, 2004; Tilak et al., 2006).

The prospects of the investigated methods of growing leek under the application of additional fertilizing are evaluated according to the yield capacity and marketability of etiolated pseudo-stem. According to the data of Syubayeva & Titova (2015), the introduction of biological preparation Azophobakterin-AF into soil provides the maximum and valid yield increase of leek at the background of mineral additional fertilizing. Application of *Azotobacter* bacteria and other beneficial bacteria is recommended for the increase of leek, pepper, tomatoes, cucumber, wheat, corn and other crops yield (Kumar et al., 2001; Hajnal-Jafari et al., 2012; Colo et al., 2014). After the inoculation of sugar beet seeds with *Azotobacter chroococcum* the increase of yield of root crops made up 23% (Mrkovački et al., 2016). According to the data of Geel et al. (2006), in the variant without the application of mineral fertilizers leek yield reached 39–45 t ha^{-1}, and after the application of N_{120} it was higher by 3.0–5.5 t ha^{-1}. The maximum yield of dry weight of leek was achieved in the variant when split fertilizing with mineral nitrogen was applied (Savic, 2012).

According to the obtained results regardless of fertilizing the yielding capacity of Tango variety was lower in comparison with Goliath variety (Table 3). In the option without fertilization mean difference was 7.7 t ha^{-1} over three years. Fertilization of Tango variety with DripFert $N_{20}P_{20}K_{20}$+Me alone provided mean yield increase by 7%, whereas fertilizing together with Liposam or Organic-balance – by 14–16%.

In the option where Tango variety was fertilized with Organic-balance the yield was by 1.1 t ha^{-1} higher, or by 80% compared to control. Combined application of bacterial preparation with bioadhesive was more effective, the average yield capacity of Tango variety was by 0.7 t ha^{-1} higher than without Liposam and by 13% higher than in control.

The yield capacity of Tango variety in 2016 was lower than in control when Organic-balance and Organic balance + Liposam were applied, though with insignificant smaller difference (by 0.2–0.5 t ha^{-1}) according to the statistical analysis (LSD_{05B} – *1.4* t ha^{-1}). The yield capacity of Tango variety in 2018 was insignificantly higher than control in the variant with DripFert $N_{20}P_{20}K_{20}$ + Me. Throughout the experiment period the yield capacity of Tango variety was significantly higher under the application of Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam by 2.1–4.6 t ha^{-1} according to the LSD_{05B} – *2.0–2.1* t ha^{-1}. On average over three years complex fertilization of Tango variety with mineral fertilizer and bacterial preparation together with Liposam provided the yield which was by 22% higher than in control.

During the experimental period a considerable increase of yield within Goliath variety was achieved in the variants with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam, and Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me. Goliath variety had the highest yield capacity in 2018 under combined fertilizing with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam with 3.7 t ha^{-1} yield increase compared comparison to variant and 1.9 times higher than in control. Average increase of yield of Goliath variety over three years under fertilizing with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam made up 18% compared to the comparison variant. Under the application of DripFert $N_{20}P_{20}K_{20}$ + Me the yield of Goliath variety was by 0.5–1.0 t ha^{-1} lower compared to additional fertilization with mineral fertilizer together with Liposam or Organic-balance. Yield increase of this variety was lower under the application of Organic balance alone and made up 0.6 t ha^{-1}.

In a whole the yielding capacity of leek was lower under the application of Organic balance separately or together with bioadhesive compared to the variants where it was applied with DripFert $N_{20}P_{20}K_{20}$ + Me. The maximum yield increase was achieved in the variant where Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam were applied: by 18% (Goliath) and by 24% (Tango).

Table 3. The yield of leek pseudo-stem of and marketability of output, 2016–2018

Variety (A)	Additional fertilizing (B)	Yield (t ha^{-1})				Marke-tability (% from total plant mass)
		Years of research			Average over three years	
		2016	2017	2018		
Goliath	Without fertilization (comparison variant)	19.9	20.4	24.6	21.6	62
	Organic-balance	19.2	21.0	26.3	22.2	58
	Organic-balance + Liposam	21.7	21.7	27.0	23.5	60
	DripFert $N_{20}P_{20}K_{20}$ + Me	21.8	22.2	25.7	23.2	55
	DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	22.3	22.5	26.2	23.7	51
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	22.0	23.2	27.4	24.2	57
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	24.1	23.8	28.3	25.4	64
Tango	Without fertilization (control)	12.7	14.2	15.0	13.9	49
	Organic-balance	12.1	15.8	17.1	15.0	61
	Organic-balance + Liposam	12.5	16.2	18.4	15.7	56
	DripFert $N_{20}P_{20}K_{20}$ + Me	13.0	15.0	16.4	14.8	50
	DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	1.34	16.8	17.3	15.8	56
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	13.6	17.0	17.6	16.1	55
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	14.8	17.6	19.6	17.3	54
LSD_{05A}		*0.8*	*0.9*	*0.8*		–
LSD_{05B}		*1.4*	*1.5*	*1.5*		
LSD_{05}		*2.0*	*2.1*	*2.1*		
CV[1], %		*26*	*18*	*22*		

[1] *– coefficient of variation.*

The share of commercial product referred to the harvested yield was determined (Table 3). On average over three years Tango variety had the lowest share of commercial yield in control and under fertilizing with DripFert $N_{20}P_{20}K_{20}$ + Me. The higher yielding capacity of Goliath variety was caused by the higher mass of pseudo-stem than that of Tango variety. Thus, the marketability of Goliath variety without fertilizing was 13% higher than control. Fertilizing of Goliath variety with DripFert $N_{20}P_{20}K_{20}$ + Me did not improve marketabilitys, which was by 7% lower than variant without fertilization. The best marketability of Goliath variety was achieved after combined application of DripFert $N_{20}P_{20}K_{20}$ + Me, Organic-balance and Liposam.

Obtained results as to the efficiency of the application of microbiological preparations are in good agreement with the data of Colo et al. (2014): the mass of leek and output of standard products were significantly higher in the variants with *Azotobacter chroococcum, Bacillus subtilis* treatment. The yielding capacity of leek after the treatment of seeds with *Azotobacter chroococcum*, *Bacillus subtilis and Pseudomonas fluorescens* increased by 6.7–17.1 t ha^{-1} compared to control. The treatment of seeds with a mixture of these strains turned out to be less effective.

In the experiments of Choudhary & Paliwal (2017) the highest yield of broccoli was also achieved under the application of mineral fertilizers together with biological preparations that contain *Azosprillum* and worm compost (2.5 times higher than control). In general, the application of microbial preparations and mineral fertilizers increases the yielding capacity of vegetables and creates the conditions for their effective environmental friendly growth.

It has been established that the application of biological bacterial preparation Organic-balance with Liposam promotes the formation of the optimal microbial cenosis in the rhizosphere of leek at the background of mineral fertilization with DripFert $N_{20}P_{20}K_{20}$ + Me. It proved by the statistically valid increase of the total number of microbiota, including *Azotobacter* bacteria. After a four-time application of Organic-balance + Liposam or together with DripFert $N_{20}P_{20}K_{20}$ + Me the total number of bacteria increased 2.5–2.8 times, the total number of *Azotobacter* bacteria increased 2.8–3.0 times in the rhizosphere of leek compared to the option without fertilization.

The leaf surface area and photosynthetic potential of leek were determined by variety properties as well as by the use of additional mineral fertilization and biological bacterial preparation. Over the period from the 10^{th} of May to the 10^{th} of October the photosynthetic potential of Tango variety was higher than that of Goliath variety, its maximum indexes were recorded in the options with fertilization Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me and Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam, on average the excess made up 18–46% compared to control.

The yield capacity of the investigated varieties of leek increased by 6–7% at the background of mineral fertilization DripFert $N_{20}P_{20}K_{20}$ + Me alone. Under the application of the bacterial preparation Organic-balance separately and together with Liposam the yield increase of Goliath variety was 3–9% and 8–13% of Tango variety compared to the options without fertilization. Application of Liposam or Organic-balance increased the efficiency of mineral fertilizer. Fertilization with DripFert $N_{20}P_{20}K_{20}$ + Me together with Organic-balance provided higher yield capacity of investigated leek varieties by 12–16%. The highest level of yield capacity was recorded under combined fertilization of leek with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam – yield increase was 18% (Goliath) and 24% (Tango).

CONCLUSIONS

To summarize, it can be stated that in order to increase ecological safety of growing technology of leek it is reasonable to apply additional fertilization with biological preparations Organic-balance + Liposam throughout vegetation that promotes the increase in the number of soil microbiota, including nitrogen-fixing *Azotobacter* bacteria, and provides the increase of yield capacity and marketability of a pseudo-stem.

These preparations demonstrate the best efficiency when they are combined with mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me (a four-time additional fertilization). Regardless of fertilization the highest yield capacity and marketability of a pseudo-stem was manifested by leek variety Goliath.

REFERENCES

Abou, El.S. & Yousry, M. 2012. Response of onion and leek plants during the early growth stage to mycorrhizal inoculation on a phosphorus deficient calcareous soil. *Australian Journal of Basic and Applied Sciences* **6**(7), 594–602.

Avrova, N.P., Vorobiev, N.I. & Rysev, M.N. 2005. Microbiological evaluation of the efficiency of use in crop rotations of plant residues and mineral fertilizers. *Reports of the Russian Academy of Sciences* **3**, 19–21 (in Russia).

Bondarenko, G.L. & Yakovenko, K.I. 2001. *Methodology of experimental work in vegetable and melon.* Kharkiv, Fundamentals, 235 pp. (in Ukraine).

Brumfield, R.G., Rimal, A. & Reiners, S. 2000. Comparative cost analyses of conventional, integrated crop management and organic methods. *HortTechnology* **10**, 785–793.

Bulluck, L.R., Brosius, M., Evanylo, G.K. & Ristaino, J.B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology* **19**, 147–160.

Choudhary, S. & Paliwal, R. 2017. Effect of bio-organics and mineral nutrients on yield, quality and economics of sprouting broccoli (*Brassica oleracea var. italica*). *International Journal of Current Microbiology and Applied Sciences* **6**(12), 742–749.

Colo, J., Hajnal-Jafari, T.I., Durić, S., Stamenov, D. & Hamidović, S. 2014. Plant growth promotion rhizobacteria in onion production. *Polish Journal of Microbiology* **63**(1), 83–88.

Ehrmantraut, E., Shevchenko, I. & Nenyn, P. 2000. *Mathematical analysis and interpretation of research. Coll. Science Works Institute for Sugar Beet UAAS* **2**, 189–205 (in Ukraine).

Geel, W.C.A., Radersma, S., Meurs, E.J.J. & Grashoff, C. 2006. Comparison of split nitrogen application strategies in leek (*Allium porrum*) to Reduce n fertilization on sandy soils in the Netherlands. *Acta Horticulturae* **700**, 241–245.

Golubkina, N.A., Zamana, S.P., Tareeva, M.M., Mukhortov, V.Yu. & Pivovarov, V.F. 2010. Comparative assessment of the influence of humates and bacterial fertilizer Biostar on the accumulation of selenium, zinc and copper with the use of organic fertilizers. *Agricultural Biology* **3**, 41–45 (in Russian).

Golubkina, N.A., Seredin T.M., Antoshkina M.S., Kosheleva O.V., Teliban G.C. & Caruso G., 2018. Yield, quality, antioxidants and elemental composition of new leek cultivars under greenhouse organic or conventional system. *Horticulturae* **4**(4), 39.

Grant, C., Bittman, S., Montreal, M., Plenchette, C. & Morel, C. 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science* **85**, 3–14.

Grover, M., Ali, Sk.Z., Sandhya, V., Rasul, A. & Venkateswarlu, B. 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology & Biotechnology* **27**, 1231–1240.

Hajnal-Jafari, T., Latković, D., Đurić, S., Mrkovački, N. & Najdenovska, O. 2012. The use of *Azotobacter* in organic maize production. *Research Journal of Agricultural Science* **44**(2), 28–32.

Hosam, E.A.F., Hamuda, B. & Patkó, I. 2013. PGPR strains selection to improve plant productivity and soil quality agricultural. *International Scientific Practical Conference. 'Food, Technologies & Health'*. Academy Food Research & Development Institute. Plovdiv, Bulgaria, pp. 44–50 (in Bulgaria).

Hrycajenko, Z.M., Hrycajenko, A.O., Karpenko, V.P. 2003. Methods of biological and agrochemical investigations of plants and soil. Nichlava, Kyiv, 320 pp. (in Ukrainian).

Jarak, M., Đukić, D. & Čolo, J. 2006. The effect of bacterization and liming in the production of red clover. *Grassland Science in Europe* **11**, 291–293.

Jarak, M., Mrkovački, N., Bjelić, D., Jošić, D., Hajnal-Jafari, T. & Stamenov, D. 2012. Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. *African Journal of Microbiology Research* **6**, 5683–5690.

Kahiluoto, H. & Vestberg, M. 1998. The effect of arbuscular mycorrhiza on biomass production and phosphorus uptake from sparingly soluble sources by leek (*Allium porrum L.*) in finish field soils. *Biological Agriculture & Horticulture: An International Journal for Sustainable Production Systems* **16**(1), 65–85.

Karpenko, V. & Prytulyak, R. 2014. Physiological changes in plants of spring barley under the influence of biologically active substances. *Bulletin of Uman National University of Horticulture* **4**, 60–65 (in Ukraine).

Kennedy, I.R., Choudhury, A.I.M.A. & Kecskes, M.L. 2004. Non Symbiotic bacterial diazotrophs in crop-farming systems: can their potential or plant growth promotion be better exploited? *Soil Biology & Biochemistry* **36**, 1229–1244.

Khadiga, I. M. El-Gabry, Sanaa S.H. Sarabana & Amal W. Abou El- Khair. 2015. Effect of some microbial activators on onion plantlets growth quality. *Middle East Journal of Agriculture Research* **4**, 932–937.

Kloepper, J.W., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus sp. Phytopathology* **94**, 1259–1266.

Kozhemyakov, A.P., Laktionov, Yu.V., Popova, T.A., Orlova, A.G., Vashlya, O. B., Agafonov, E.V., Guzhvin, S.A., Churakov, A.A. & Yakovleva, M.T. 2015. Agrotechnical bases for creation of improved forms of microbial biopreparats for agriculture. *Agricultural Biology* **50**(3), 369–376 (in Russian).

Kumar, V., Behl, R.K. & Narula, N. 2001. Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under green house conditions. *Microbiological Research* **156**, 87–93.

Leggo, P.J. 2017. The future of organic farming in the light of the development of new biological fertilizers. *Agrotechnology* **6**(4), 6–4.

Mantelin, S. & Touraine, B. 2004. Plant growth promoting bacteria and nitrate availability impacts on root development and nitrate uptake. *Journal of Experimental Botany* **55**, 27–34.

Marra, L. M., Sousa, F.S., Oliveira, S.M., A.Ferreira, P.A. & Soares, B.L. 2012. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. *Plant and Soil* **357**, 289–307.

Martyniuk, S., Gajda, A. & Kuś, J. 2001. Microbiological and biochemical properties of soils under cereals grown in the ecological, conventional and integrated system. *Acta Agrophysica* **52**, 185–192.

Mazur, V.A., Pantsyreva, H.V., Mazur, K.V. & Didur, I.M. 2019. Influence of the assimilation apparatus and productivity of white lupine plants. *Agronomy Research* **17**(1), 206–219. https://doi.org/10.15159/AR.19.024

McSpadden-Gardener, B.B. 2004. The nature and application of biocontrol microbes: *Bacillis spp*. ecology of *Bacillus* and *Paenibacillus spp.* in agricultural systems. *Phytopathology* **94**, 661–664.

Melero, S., Porras, R., Herencia, J.F. & Madejon, E. 2006. Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil and Tillage Research* **90**(1–2), 162–170.

Mrkovački, N.B., Bjelić, D.Đ., Maksimović, L.L., Ćurčić, Ž.P., Ćirić, M.Z. & Živanov, M.S. 2016. The effect of inoculation with *Azotobacter chroococcum* on microorganisms in rhizosphere and sugar beet yield in organic farming. *Matica Srpska Journal of Natural Sciences* **130**, 45–52 (in Serbia).

Nichiporovich, A.A. 1972. *Theoretical foundations of photosynthetic productivity*. Moscow, Nauka, 527 pp. (in Russia).

Savic, D. 2012. Leek hybrid yield potential and relation on adaptation to light interception under nitrogen supply. *Genetika* **44**(3), 549–559.

Saxena, A.K., Lata Shende, R. & Pandey, A.K. 2005. Culturing of plant growth promoting rhizobacteria. In Saxena, A.K.: *Basic research applications of mycorrhizae*. *The IX International Symposium*. Pvt Ltd, New Delhi, pp. 453–474.

Sherstoboyeva, O.V., Shusteruk, T.Z. & Demyanyuk, O.S. 2007. Biological monitoring of soils as a component of ecological monitoring of agroecosystems. *Agroecological journal* **3**, 45–49 (in Ukraine).

Sosnowski, J., Jankowski, K., Vidican, R., Wiśniewska-Kadżajan, B., Malinowska, E. & Kaczorek, A. 2014. Number of microorganisms in the soil cultivated with legume-grass mixtures supplied with phytohormones and nitrogen. *Notulae Scientia Biologicae* **6**(4), 498–504.

Stamenov, D., Jarak, M., Đurić, S., Milošev, D. & Hajnal-Jafari, T. 2012. Plant growth promoting rhizobacteria in the production of English ryegrass. *Plant, Soil and Environment* **58**(10), 477–480.

Stephane, C., Christophe, C. & Angela, S. 2010. Plant growth promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology & Biochemistry* **42**, 669–678.

Syubayeva, A. O. & Titova, V.I. 2015. The influence of the joint application of mineral fertilizers and bio-fertilizers Azophobakterin-AF on yield, taste and the content of macronutrients in green cultures. *Agrarian Science of Euro-North-East* **2**(45), 50–55 (in Russian).

Tawaraya, K., Watanabe, S., Yoshida, E. & Wagatsum, T. 1995. Effect of onion (*Allium cepa*) root exudates on the hyphal growth of Gigaspora margarita. *Mycorrhiza* **6**, 57–59.

Tilak, K.V.B.R., Ranganayaki, N. & Manoharachari, C. 2006. Synergistic effects of plant growth promoting *Rhizobacteria* and *Rhizobium* on nodulation and nitrogen fixation by pigeon pea (*Cajanus cajan*). *European Journal of Soil Science* **57**, 67–71.

Venkateswarlu, B. & Shanker, A.K. 2009. Climate change and agriculture: adaptation and mitigation strategies. *Indian Journal of Agronomy* **54**, 226–230 (in Indian).

Yang, J., Kloepper, J.W. & Ryu, C.M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* **14**, 1–4.

Zvyagintsev, D.G., Aliyeva, I.V., Babaeva, I.P., Byzov, B.A., Guzev, V.S., Dobrovolskaya, T.G., Zenova, G.M., Kozhevin, P.A., Kurakov, A.V., Lysak, L.V., Marfenina, O.E., Mirchink, T.G., Polyanskaya, L., M., Panikov, N.S., Skvortsova, I.N., Stepanov, A.L. & Umarov, M.M. 1991. *Methods of soil microbiology and biochemistry*. Moscow, 1991. 304 pp. (in Russian).

Agronomy Research **18**(1), 163–176, 2020
https://doi.org/10.15159/AR.20.006

Effect of different pesticides combined with Melafen on grain yield and quality of winter wheat

I.Yu. Kuznetsov*, R.R. Alimgafarov, B.G. Akhiyarov, F.F. Safin and A.R. Nafikova

University Federal state budgetary educational institution of higher education 'Bashkir state agrarian university', Department of plant growing, plant breeding and biotechnology, 50 anniversary of October St., 34, RU450001 Ufa, Russia
*Correspondence: kuznetsov_i1@rambler.ru

Abstract. The use and search for new pesticides ensuring high and stable yields is one of the topical issues in winter wheat growing. The aim of the study was to develop theoretical foundations and farming practices for highly productive winter wheat through the use of pesticides of different groups in the southern forest-steppe of the Republic of Bashkortostan. An experiment (2016–2019) with 4 main blocks was conducted to determine the optimal combination of pesticides in cultivation of winter wheat. The pesticides were used at the tillering ((ZGS) 25) and heading stages ((ZGS) 59) of wheat growth. The experiment was replicated 4 times. The study results show that pesticides used to treat winter wheat increased grain yield and improved grain quality characteristics. The best results were reached in the block where treatment included Melafen plant growth promoter combined with the insecticide, herbicide and fungicide at different stages of winter wheat growth. The pesticides used in the experiment confirmed their efficiency. On the whole, the block of variants that used seed treatment produced a yield of 3.33–6.37 t ha^{-1}. The new plant growth promoter Melafen worked well in the experiments on winter wheat, especially in combination with pesticides in different variations. It produced the highest grain yield (6.36–7.41 t ha^{-1}). All experiment variants demonstrated positive economic efficiency. The study results may be useful in developing winter wheat cultivation practices aimed at increasing yields and improving grain quality.

Key words: winter wheat, herbicide, insecticide, fungicide, plant growth promoter, Melafen.

INTRODUCTION

Crop farming produces more than 50% of all agricultural products in Russia. Grain farms take the leading position in crop farming. Grain crops have occupied 57.7–58.1% of all cultivated areas of the country over the past ten years. The Russian Federal State Statistics Service reported that as of 2019 the average annual wheat yield was 1.77–3.12 t ha^{-1} for 2008–2018 (Federal State Statistics Service, 2019). The low grain yields are due to the fact that the applied farming practices were not adapted for use in specific soil and climatic conditions of farms. The choice of the integrated pest, weed and disease management is particularly important (Sandukhadze, 2016).

Winter wheat is one of the most important, most valuable and high-yielding grain crops (Litke et al., 2019). Wheat grain contains 11–20% protein, 63–74% starch, about

2% fat and the same amount of fibre and ash. The most important indices characterizing the quality of wheat are protein and gluten values (Litke et al., 2018). The protein content determines the use of wheat. For instance, grain with 14–15% of protein is required for breadmaking and grain with 17–18% of protein is required for producing pasta (Posypanov et al., 2006).

The current market conditions as well as various ways of wheat use and the need to meet the country's requirements are key factors for increasing grain yield and quality and developing innovative technologies that ensure environmentally safe products and better production efficiency (Pomortsev, 2019). Pesticides are widely used in European crop production to stabilize yields and mitigate losses from weeds, diseases and insects. Substantial crop yield and associated economic losses can occur if weeds are not adequately controlled (Pacanoski & Mehmeti, 2019). While all farmers use herbicides and fungicides in winter wheat, the dairy farmers use significantly fewer insecticides and plant growth regulators in winter wheat. Farmers cultivating more than 150 ha had higher pesticide intensity than farmers with small farms, which was observed for both sandy and clay soil farmers (Jørgensen et al., 2019).

The use of plant growth promoters is a promising and rapidly growing area in farming development (Masterov et al., 2017). Plant growth promoters improve the efficiency of using the genetic potential of crops and high soil fertility (Kargin et al., 2011). Synthetic growth promoters as counterparts of natural phytohormones are of particular interest. The substances have a specific range of physiological activity that reduces many properties of phytohormones on the one hand and enhances the protective effect on the other hand (Kochmin & Bogomazov, 2016). Currently, there are about 40 winter wheat growth promoters recommended for use in the Russian Federation. Among them are Agrostimulin, Vitazim, Cherkaz, Obereg, Symbionta, Vympel, AgroStimul, Agropon C, Albit, Novosil, Alfastim and others (List of pesticides and agrochemicals approved for use on the territory of the Russian Federation: reference edition, 2019).

A lot of scientists from different regions of the Russian Federation are engaged in studies of plant growth promoters. The studies consider the plant growth promoters as substances that increase yield and productivity of wheat and improve grain quality (Lapa, 1998; Ismagilov et al., 2018). One of the studies focused on a synthetic plant growth promoter 'Moddus'. The study was conducted in the forest-steppe of the Middle Volga by Kochim & Bogomazov (2016). They pointed out that the use of the plant growth promoter in spraying of winter wheat crops increased in the leaf surface area by 2.9–5.6 thousand square metres per hectarecompared to the control. The plant growth promoter Moddus increased the 1,000 kernel weight by 0.41–2.50 g. It reduced the height of crops, strengthened the stem and thus increased the crop resistance to lodging. The plant growth promoter Moddus increased the winter wheat yield depending on the treatment time and rate by 5.5–8.6% compared to the control practice. The crop treatment increased the grain bulk density by 14–16 g on average. Profitability rose from 86 to 124% (Kochmin, 2015).

Gruznova et al. (2018) revealed that plant growth regulators had the potential to reduce the effect of heavy metals on winter wheat crops. They studied the influence of a natural plant growth regulator Ribav–Extra and ions of heavy metals (HM) Pb2+, Cu2+, Zn2+ and Ni2+ on the physiological and biochemical indices of the winter wheat (*Triticum aestivum* L.) cultivar 'Mironovskaya 808'. The wheat plants, whose seeds had been treated with Ribav-Extra, were heavier metal-resistant than the untreated ones (Gruznova et al., 2018).

Zhang et al. (2019) found that a plant growth regulator had a positive effect on winter wheat effectiveness. Plant growth regulators also increased the grain filling rate during the 14 days after anthesis, thereby increasing grain weight. The grain number per spike, 1,000-kernel weight, and yield per plant after harvest were also significantly enhanced ($p < 0.05$). Thus, spraying plant growth regulators at the booting stage relieved the adverse effects on physiological activity in wheat flag leaves caused by chilling stress, and 6-BA and SA were particularly effective (Zhang et al., 2019).

Korshunov et al. (2015) studied the effect of synthetic growth promoters (Bigus, Carvitol) on winter wheat. The study was conducted on the farm 'Barsuk T.L.' in Pavlovsky district of the Northern zone of Krasnodar Krai in 2011–2013. Treatment of seeds with the study promoters increased the density of standing plants by 4–8% compared to the control; it enhanced the energy of germination and germination rates. The treatment increased the energy of germination and germination rates and improved productive plant stand. Bigus and Carvitol growth promoters increased this plant density by 50 and 64 number of stems m^{-2} or by 10.1 and 13.0%. The study growth promoters increased the yield by 0.201–0.451 t ha^{-1} (4.51–10.0%) compared to the control. The study found that protein and gluten values rose, grain bulk density and vitreous aspects improved as well (Korshunov et al., 2015).

It should be borne in mind that most of the currently used plant growth promoters are classified as chemical compounds and pollute the environment. They are toxic substances that have a cumulative effect, in some cases they produce a mutagenic effect and may lead to cancer. In this regard, environmentally safe practices of growing agricultural crops prefer growth promoters of the plant origin (Kuznetsov et al., 2019). Also, particular attention should be paid to the application methods and crop treatment mechanisms (Gabitov et al., 2018).

Ecosil is a plant growth promoter of natural origin. It activates metabolic processes, immunity, resistance to diseases and adverse environmental conditions, increases productivity and improves product quality. Ecosil is designed for pre-sowing treatment of seeds and sowing material, as well as for spraying of cultivated and ornamental plants during the growing season. It is recommended for use on 28 cultivars. Masterov et al. (2017) studied the influence of the product on winter wheat. The study was conducted on the educational and experimental crop rotation plot of the Arable Farming Department on the territory of 'Experimental fields of Bashkir State Agricultural Academy' in 2009–2014. The plant growth promoter Ecosil increased grain yield by 0.20–0.24 t ha^{-1}, these were the average data for the study years.

Ishkov (2017), studied treatment of winter wheat with plant-based growth promoters Narciss and Stabilan. The study was conducted on dark gray forest soils of the experimental field, the Soil Science, Arable Farming and Crop growing Department at Kursk State Agricultural Academy in 2013–2014. The study results demonstrated that pre-sowing seed treatment improved wheat grain quality. The study revealed an increase of 5.1–5.4% in gluten with Stabilan, and an increase of 6.7–7.0% in gluten with Narciss. The combined use of Vincent, SC (2.0 L t^{-1}) + Narciss, aq.sol. (1 L t^{-1}) increased the productive tillering capacity by 24.5–25.0%, the number of kernels per head by 30.9–31.2%, the kernel weight taken from one head by 0.31 – 0.36 g compared with the control. Combination of Vincent, SC (2.0 L t^{-1}) + Narciss, aq.sol. (L t^{-1}) produced a higher yield of winter wheat. The yield gain was 0.95 t ha^{-1}, the figure exceeded the control variant by 19.5–19.8%. The variant where the fungicide Lamador (0.2 L t^{-1}) and the plant growth promoter Stabilan, aq.sol. (2.0 L ha^{-1}) were applied at the pre-sowing stage showed the best technological quality indices at the stage of tillering and stem extension. Gluten value reached 26.3%, exceeding the control variant by 2.5–2.8% (Ishkov, 2017).

Soft red winter wheat (SRWW) is an important crop in the mid-Atlantic (Kleczewski& Whaley, 2018). The use of growth regulators such as trinexapac-ethyl (TE) applied with nitrogen applications at pseudo-stem erection [Zadocks (ZGS) 30] has gained more interest in the region as a means to potentially reduce lodging and improve yields. Fungicide use has also increased, with many growers applying fungicides at ZGS 30 and again at ZGS 37 or 60 (Zadoks et al., 1974). These results indicate that TE can be used safely in SRWW production when applied alone or when tank mixed with a fungicide; however, the effect on plant height reduction and potential impact on lodging may be reduced in situations where fungicides are used (Kleczewski & Whaley, 2018).

Synthesis of new preparations with a wide range of biological activity is of great interest. A synthetic plant growth promoter Melafen is a product developed at Institute of Organic and Physical Chemistry named after A.E. Arbuzov (Kazan research centre of the Russian Academy of Sciences. The product is melamine salt of bis (oxymethyl) phosphinic acid. Melafen belongs to nanotechnological preparations. The concentration of the active substance in the solution when exposed to seeds varies between 10–9 – 10–8%. Melafen regulates energy metabolism during seed germination, creating a favorable energy balance, increases the biological usefulness of seeds. Processing of winter wheat seeds with a solution of the drug 'Melafen' in the optimal concentration, strengthens the shaping processes, rational redistribution of accumulated assimilates into economically valuable organs and, thus, increases the yield and quality of grain (Fattakhov et al., 2014).

The review of studies on application of various growth promoters in winter wheat arouses great interest for the issue. In this regard there was a need for a comprehensive study on combined treatment of crops with insecticides, fungicides and herbicides. The aim of the study was thus to develop theoretical foundations and farming practices for highly productive winter wheat through the use of pesticides of different groups in the southern forest-steppe of the Republic of Bashkortostan. The experiment used pesticides for various purposes: Imidor-insecticide system action to combat a wide range of pests on potatoes, cucumbers, tomatoes, sugar beets, cereals, pastures. Active substance Imidacloprid, 200 g L^{-1}, water-soluble concentrate. The chemical class is neonicotinoids. Polaris is a fungicidal mordant intended for pre-sowing treatment of grain seeds. Active substance - (100 g L^{-1} prochlorase + 25 g L^{-1} limazalil + 15 g L^{-1} tebuconazol), microemulsion. Chemical class-imidazoles + triazoles. The title Duo is a systemic fungicide designed to combat a wide range of diseases on grain crops. The active substance is propiconazol + tebuconazol. Preparation form: concentrated colloidal solution. Garnet is a post-emergence herbicide of systemic action for control of dicotyledonous weeds, including those resistant to 2,4-D and MSRA, in crops of grain crops. Water-dispersible granules containing 750 g kg^{-1} tribenuron-metil. Studies conducted by Rebouh et al. (2019) and Khaibullin et al. (2018) support the chosen direction of our research.

In accordance with this, the research was aimed at solving a number of problems, including: - determining the optimal use of pesticides for the production of high-quality grain; - determining the quality indicators of winter wheat grain and commodity class; - determining the economic efficiency of winter wheat crops.

MATERIALS AND METHODS

We conducted field experiments on the experimental field of the Department of Crop Growing, Plant Selection and Biotechnology (Bashkir State Agrarian University) in Ufa district of the Republic of Bashkortostan in 2016–2019. The experiments focused on determining the effect of seed treatment on productivity and quality of winter wheat grain. Soil characteristics were leached Chernozem of heavy loamy granulometric composition. Leached chernozems are characterized by a dark color of the humus horizon, its considerable stretch, lumpy-grained structure with nut-like separations in the sub-arable layer.The capacity of the humus horizon is 45–50 cm, the humus content is 9.7%, the pH of the salt extract is 5.6, the sum of the absorbed bases is 56 mg - equivalent at 100 g, the degree of saturation of the bases-89.2%. The conventional agricultural machinery typically used for the area was used in the experiment. The fallow land was used in the experiment.

Object of the study. The object of research was soft winter wheat (Volzhskaya K variety).

The experiment used a recognized winter wheat variety Volzhskaya K, recommended for the southern forest-steppe zone of the Republic of Bashkortostan. Brief description of the variety used. Originator: OOO NPTs [LLC Research Production Centre] 'Selektsiya'. Breeding record: The variety was bred by individual selection from the population obtained from crossing VSGI winter wheat variety with Kinelskaya 4 variety (1983). The variety entered the State Register of Varieties [2004] for the North - West (2), Central (3), Volga - Vyatka (4), Central Black Earth (5), Middle Volga (7), Ural (9) and Far East (10) regions. The variety is medium-ripening. The genus is erythrospermum. The wheat has good baking qualities; the variety is high-value wheat. The overall baking score is 4.1 points. Variety is of intensive type, winter-hardy (3.7–4.0 points), and drought-resistant (4.1 points). The wheat is susceptible to brown rust (16%). It is highly susceptible to Fusariummold (39%) and Fusarium head blight. The wheat has medium resistance to mildew (14%).

The seeding rate was 5.0 million pieces of seeds per hectare. The sowing depth was 4–6 cm. The territory of the experimental field belongs to a relatively warm, medium-moist area. Climatic conditions could be characterized as continental with dry air and a high level of solar energy. The area features sharp changes in air temperature (Table 1).

The variants were put in an order so that the plots were arranged one after another in one line. The experiment was replicated 4 times. The length of the plot was 3 meters, the width was 1.6 m, the distance between variants was 40 cm, and the protective strip was 2 m. The total area of a variant was 20.8 m^2 and the registration area was 1 m^2. The area under experiment was 800 m^2. Before sowing the seeds were treated according to the experiment pattern. Melafen was applied at of 10 ml t^{-1}, Klen-PSB-0.1seed treater was used to apply Polaris fungicidal chemical at 1.5 L t^{-1}. Cz-3.6 drill was used to seed the crops. SCCS-6A rollers attached to DT-75M tractor were used after the sowing. The following seed treatment was done under the experiment pattern: Granat systemic herbicide at 25 g ha^{-1}, Imidor insecticide at 60 mm ha^{-1}, Titul Duo fungicide at 250 mm ha^{-1}, Melafen growth promoter at 5 mm ha^{-1} and Polaris fungicidal chemical (100 g L^{-1} prochlorase + 25 g L^{-1} imazalil + 15 g L^{-1} tebuconazole). The first H+I+M treatment was carried out at the tillering stage, the second I+F+M treatment was at the heading stage. Doses of applied pesticides are generally recommended for winter wheat crop in the Russian Federation (List of pesticides and agrochemicals allowed for use in the territory of the Russian Federation, 2019).

Southern forest-steppe belongs to the area with insufficient humidity. The total effective temperature is 2,110–2,290 °C. Annual rainfall is 475–570 mm. The distribution of precipitation is extremely uneven. The hydrothermal coefficient is 1.10–1.24. The down-coming active photosynthetic radiation ranges from 1,920 to 2,880 kcal ha^{-1}. The capacity of the humus horizon was 43–48 cm, the entire moisture content in the meter layer of the soil reached 311–347 mm. The arable layer contained the average 8.2–9.1% of humus, total nitrogen of 0.48%, phosphorus of 0.18%, potassium of 0.67%.

Table 1. The experiment pattern

Seq. no.	Variant	Planted area	Tillering stage	Heading stage
	Control	C1	-	-
		C2	H+I	-
		C3	-	I+F
		C4	H+I	I+F
	Seed Treatment	ST1	-	-
		ST2	H+I	-
		ST3	-	I+F
		ST4	H+I	I+F
		ST5	H+I+M	-
		ST6	H+I+M	I+F
		ST7	H+I+M	I+F+M
		ST8	H+I	I+F+M
		ST9	-	I+F+M
	Melafen	M1	-	-
		M2	H+I	-
		M3	-	I+F
		M4	H+I	I+F
		M5	H+I+M	-
		M6	H+I+M	I+F
		M7	H+I+M	I+F+M
		M8	H+I	I+F+M
		M9	-	I+F+M
	Seed Treatment + Melafen	ST+M1	-	-
		ST+M2	H+I	-
		ST+M3	-	I+F
		ST+M4	H+I	I+F
		ST+M5	H+I+M	-
		ST+M6	H+I+M	I+F
		ST+M7	H+I+M	I+F+M
		ST+M8	H+I	I+F+M
		ST+M9	-	I+F+M

Note: ST stands for seed treatment, M for Melafen, H for herbicide, I for insecticide, F for fungicide, C for Control.

The following observations were done based on the conventional methods.

6. Phenological observations of wheat growth stages and determining interstage periods were taken every 10 days.

7. The linear plant growth was studied every 10 days from the shooting stage till crop harvesting by measuring the height of the plants: measurement was done at ten plot points of two discontinuous replicated variants and the average value was found.

8. The number of plants was counted on the fixed sites with an area of 0.25 m^2 in 2-fold repetition The plant stand was assessed in the period when the shoot formed completely and before harvesting.

9. Weeding of the planted area was assessed using quantitative and weight method at wheat growth stages.

10. Leaf surface was determined based on the stages of wheat growth. Leaf area was found using the contour method.

11. Yield formula for winter wheat. We determined the number of productive stems, plant height, head length, number of spikelets per head, number of kernels per height, grain weight per head.

12. The chemical composition of grain and grain vitreousness were analysed in the laboratory of the Bashkir State Agrarian University (the National Standard GOST 54478-2011 method for determining the quantity and quality of gluten in grain, the National Standard GOST 10987-76 method for assessing grain vitreousness.

13. Grain bulk density was measured under the national standard GOST 10840-2017. A litre grain-unit scale was used to measure the grain bulk density: a 2 kg sample of cleaned grain taken from the mean sample was weighed. Each sample was measured twice and the average value with an accuracy of 1 g was obtained.

14. Grain class was determined under GOST 9353-90 for wheat, storage and shipment requirements. We conducted our study in accordance with the procedural guidelines developed by Dospekhov et al. (1987), the state commission for testing agricultural crop varieties (Fedyn, 1983) and the Methodology instructions developed by the Russian Academy of Agricultural Sciences (1997). Statistical methods (dispersion, regression and correlation analyses) were used to analyse experimental results. STATISTICA 5.0 software package for Windows was employed (Rushninsky, 1971).

RESULTS AND DISCUSSION

Our study revealed that the winter wheat stand density tended to decrease as the crop matured. In the tube exit phase (ZGS 40) the index ranged from 615 stems m^{-2} in variant 1 (Control) to 917 stems m^{-2} in variant 14 (Melafen). By the milk maturity stage (ZGS 75) variant 5(ST) and variant 1 (C) showed the lowest indices of 284 stems m^{-2} and 396 stems m^{-2}, respectively, they lagged behind the variant with the highest index by 576 stems m^{-2} and 464 stems m^{-2}, respectively. Variant 14 (M) showed the best result of 860 stems m^{-2}. The high performance of variants with growth stimulant treatment is confirmed by the results of Jørgensen L.N., Kudsk P. & Ørum J.E. (2019).

At the complete maturity stage the plant stand density varied from 260 stems m^{-2} in variant 5 (ST) to 820 stems m^{-2} in variant 14 (M). Among the variants that used pre-sowing treatment variant 14 (M) had the highest index of stand density, the index was 820 stems m^{-2} and the value was 2.8 times higher than variant 1 (Control). Application of herbicide+insecticide at the tillering stage resulted in that variant 15 treated with Melafen reached the value of 756 stems m^{-2}, thus demonstrating the optimal index with the treatment pattern. Variant 24 (ST+M) had the lowest index of 564 stems m^{-2}.

The analysis of the correlation dependence of the density of standing on the pesticides used in the experiment and their combination according to the experimental scheme revealed a number of relationships. Variant (Control) has a dependence above the average (r = 0.693–0.701), variant (Seed Treatment) - above the average (r = 0.685–0.691), variant (Melafen) has a high dependence (r = 0.825–0.854), variant (Seed Treatment + Melafen) - above average and high (r = 0.781–0.829).

The study showed that in 2018 winter wheat had different standing height depending on the growth stage and treatment type. At the booting stage the indices varied from 39.9 cm (ST+M) to 58.2 cm (C). At the heading stage variant 20 (M) and variant 31 (ST+M) showed the highest indices of 92.6 cm and 91.9 cm, respectively. At the milk maturity stage variants where pre-sowing ST+M treatment was done demonstrated the optimal results: variant 26 had the height of 100.0 cm and variant 23 had the height of 98.1 cm. The lowest indices were found in variant 3 (Control) at 79.9 cm and variant 10 (ST) at 80.8 cm. At the complete maturity stage, the indices varied depending on the treatment from 110 cm in variant 19 (M), to 84 cm in variant 3 (Control). Variant 19 (M) showed the best index of winter wheat height at 110 cm treated with H+I+M at the tillering stage and with I+F at the heading stage. Seed treatment produced the best results when the crop was H+I+M treated at the tillering stage (variant 9) and I+F+M treated at the heading stage (variant 13). ST+M treatment reached the greatest results when the crop was H+I treated at the tillering stage and I+F treated at the heading stage (variant 26).

Our research shows a positive effect of the growth stimulant Melafen on the growth processes of winter wheat. However, in the studies of Korshunov et al. (2015), the use of the growth stimulator Modus led to a decrease in the height of winter wheat plants, which we explain not by rational redistribution of accumulated assimilates, since the regulators have different chemical composition. The behavior of the growth regulator can be influenced by the weather factor, as our previous research shows.

The issue of grain quality is one of the major issues in grain production as the quality has a direct effect on the cost of grain. To improve wheat grain quality is particularly relevant as the grain protein and gluten values tend to drop over the past few years. The grain quality in winter wheat was determined by the year conditions at the waxy maturity stage. For instance, low temperatures and high precipitation in 2018 resulted in a longer ripening period. This had a negative effect both on the grain bulk density due to feeble and shriveled grain and the gluten weight ratio and quality (Table 2).

Table 2. Quality indices of winter wheat grain (Scientific training centre at Bashkir State Agrarian University, 2016–2019)

Seq. no.	Crude gluten weight ratio, %	Gluten quality group	Grain bulk density, g L^{-1}	Vitreousness, %	Class
1	28.04	Group 2	671	98	3
2	29.72	Group 2	746	97	3
3	30.84	Group 3	717	90	No class
4	32.28	Group 2	749	97	3
5	31.60	Group 2	708	96	3
6	28.80	Group 2	740	98	3
7	28.56	Group 2	749	97	3
8	27.64	Group 2	751	98	3
9	28.32	Group 2	752	95	3
10	29.36	Group 2	748	95	3
11	26.92	Group 2	758	99	3
12	29.60	Group 2	754	98	3
13	30.80	Group 2	752	96	3
14	28.12	Group 2	734	96	3
15	29.52	Group 2	750	97	3
16	28.84	Group 2	767	97	3
17	28.24	Group 2	754	97	3
18	29.00	Group 2	754	97	3
19	29.00	Group 2	770	97	3
20	29.84	Group 2	765	97	3
21	29.60	Group 2	759	94	3
22	28.80	Group 2	772	97	3
23	31.00	Group 2	757	99	3
24	28.96	Group 2	741	97	3
25	28.84	Group 2	762	97	3
26	30.44	Group 2	763	96	3
27	28.12	Group 2	756	96	3
28	29.56	Group 2	752	97	3
29	28.52	Group 2	761	97	3
30	29.24	Group 2	761	97	3
31	29.00	Group 2	763	96	3

Note: red colour stands for class 1, blue colour for class 1–2 and yellow colour for class 3.

Variant 4 had the highest gluten weight ratio index of 32.28%, the index is characteristic of the first quality group; the rest belonged to the second quality group. All variants except variant 8 and variant 11 had the indices of strong wheat; variants 8 and 9 had the indices of high-value wheat. All of the treated variants except for variant 3 belonged to wheat class 3 based on quality indices such as gluten weight ratio, grain bulk density and vitreousness. This is due to the fact that based on the gluten weight ratio the variants belong to the second group, i.e. wheat class 3, though most of the variants suit wheat class 1 or 2 based on grain bulk density and vitrousness indices.

Table 3. Economic efficiency of winter wheat grain (Scientific training centre at Bashkir State Agrarian University, 2016–2019)

Seq no.	Variant	Yield, t ha^{-1}	Gross output value, rouples per hectare	Production costs per 1 hectare	Notional net return from 1 hectare, roubles	The cost price of 1 centner (100 kg) of grain, rubles	Profitability, %
	C1	4.54	39,952.0	27,165.0	12,787.0	598.4	47.0
	C2	5.00	44,000.0	27,635.0	16,365.0	552.7	59.2
	C3	4.21	37,048.0	27,576.0	9,472.5	655.0	34.3
	C4	4.85	42,680.0	26,922.0	15,758.0	555.1	58.5
	ST1	3.33	29,304.0	27,328.0	1,975.6	820.7	7.2
	ST2	4.78	42,064.0	27,913.0	14,151.0	584.0	50.7
	ST3	5.15	45,320.0	27,990.0	17,330.0	543.5	61.9
	ST4	6.15	54,120.0	28,521.0	25,599.0	463.8	89.7
	ST5	6.01	52,888.0	28,271.0	24,617.0	470.4	87.0
	ST6	5.92	52,096.0	28,696.0	23,400.0	487.7	81.5
	ST7	6.26	55,088.0	28,931.0	26,157.0	462.2	90.4
	ST8	6.37	56,056.0	28,743.0	27,313.0	451.2	95.0
	ST9	6.21	54,648.0	28,313.0	26,335.0	455.9	93.0
	M1	6.36	55,968.0	27,572.0	28,396.0	433.5	102.9
	M2	6.54	57,552.0	28,014.0	29,538.0	428.3	105.4
	M3	7.00	61,600.0	28,089.0	33,511.0	401.3	119.3
	M4	7.19	63,272.0	28,519.0	34,754.0	396.6	121.8
	M5	6.67	58,696.0	28,223.0	30,473.0	423.1	107.9
	M6	7.15	62,920.0	28,723.0	34,197.0	401.7	119.0
	M7	7.41	65,208.0	28,958.0	36,250.0	390.8	125.1
	M8	7.32	64,416.0	28,747.0	35,669.0	392.7	124.0
	M9	7.09	62,392.0	28,298.0	34,094.0	399.1	120.4
	ST+M1	6.31	55,528.0	27,881.0	27,647.0	441.9	99.1
	ST+M2	6.32	55,616.0	28,295.0	27,321.0	447.7	96.5
	ST+M3	6.87	60,456.0	28,383.0	32,073.0	413.1	113.0
	ST+M4	6.36	55,968.0	28,738.0	27,230.0	451.9	94.7
	ST+M5	6.26	55,088.0	28,483.0	26,605.0	455.0	93.4
	ST+M6	6.39	56,232.0	28,938.0	27,294.0	452.9	94.3
	ST+M7	6.10	53,680.0	29,115.0	24,566.0	477.3	84.3
	ST+M8	5.89	51,832.0	28,879.0	22,953.0	490.3	79.4
	ST+M9	6.41	56,408.0	28,536.0	27,872.0	445.2	97.6

Economic efficiency of cultivating winter wheat varieties was measured and a positive effect was marked in all variants. Table 3 shows that the gross output value was determined by the yield obtained in the experiment. The control variant had 4.21–4.85 t ha^{-1}or 37.0–44.0 thousand roubles per hectare. Variant 2 (Control) with H+I treatment at the tillering stage showed the best efficiency. Seed treatment with Polaris chemical increased grain yield to 4.78–6.37 t ha^{-1} when crops were treated with pesticides during the growing season; the chemical reduced the yield to 3.33 t ha^{-1} in variant 5 compared to the control index of 4.54 t ha^{-1}. As a result, the gross output value amounted to 29.3–56.0 thousand roubles in this block. Variant 12 with ST +HI (tillering stage)+IFM (heading stage) reached the highest gross output value. There was a decrease of 0.09 t ha^{-1} in efficiency in the block with Melafen applied at the tillering stage.

Treatment of winter wheat seeds with the ST chemical combined with the plant growth promoter resulted in an increase of 39.9–41.6% in crop yields up to 5.89–6.87 t ha^{-1}. Variant 25 with ST+M (pre-sowing stage)+IF (heading stage) had the top indices in this block. There was a decrease in grain yields with Melafen applied during the growing season in variants 27, 29 and 30, an increase of 0.08 and 0.1 t ha^{-1} was revealed in variants 28 and 31. The results of the research are consistent with the results obtained by Korshunov et al. (2015). In their experiments, the use of growth regulators increased the yield by 0.20–0.45 ha^{-1} (4.51–10.0%) in relation to the control. Also increased indicators of protein and gluten in the grain, its nature and vitreous.

The block where seeds were treated with Melafen showed the highest crop yields. The grain yield was 6.36–7.41 t ha^{-1}, the index exceeded the control by 51.4–52.7%. Two leading variants were found in the block: variant 20 M (pre-sowing stage)+HIM (tillering stage)+IFM (heading stage) had the grain yield of 7.41 t ha^{-1} and variant 21 M (pre-sowing stage)+HI (tillering stage)+IFM (heading stage) had the grain yield of 7.32 t ha^{-1}. Variant 20 and variant 21 had also the highest gross output values of 65.2 and 64.4 thousand roubles per hectare, respectively.

Production costs exceeded 26 thousand roubles in all variants and ranged from 26.9 to 29.1 thousand roubles per hectare. Variant 29 ST (pre-sowing stage)+HIM (tillering stage)+HIM (heading stage) had the highest production costs. Production costs had an effect on the cost price of 1 centner of grain. The cost price of grain ranged within 390.8–820.7 roubles per centner. Variant 5 in the block where seeds were treated with Polaris chemical had the highest cost price of 820.7 roubles per centner compared to the control of 552.7–655.0 roubles per centner. The block where seeds were treated with Melafen plant growth promoter had the lowest cost price of 390.8–433.5 roubles per centner. Variant 20 had the optimal index in the experiment.

In terms of the efficiency of cultivating winter wheat it should be noted that all variants had a positive outcome as notional net return from 1 hectare amounted to 1.9–36.2 thousand roubles. The control block had the index of notional net return at 1.9–27.3 thousand roubles, the index was 28.3–36.2 thousand roubles in the block with Melafen application, the figure was 22.9–32.0 thousand roubles in the block with ST+M treatment. Variant 20 and variant 21 had the highest notional net return.

Profitability is the key indicator in assessing the economic efficiency. In the experiment indices were 7.2–125.4%. The control block had profitability indices of 34.2–59.2%. The indices were 7.23–95.0% when seeds were treated with Polaris fungicide, the figures ranged within 102.9–125.7% in the block where seeds were treated with Melafen plant growth promoter; seed treatment combined with Melafen ST+M produced 79.4–113.0%

Variants 16–22 had profitability of 107.9–125.1% demonstrating thereby stable high efficiency. Variant 2 M (pre-sowing stage) +HIM (tillering stage)+IFM (heading stage) showed the best economic efficiency.

Today, more than ever before application of pesticides in winter wheat is an urgent issue. The use of plant growth promoters is increasingly supported by scientists and manufacturers (Peng et al., 2019). At the same time, other areas of pesticide use in winter wheat are being tested (Moss et al., 2019).

In this regard, experience of Iranian researchers Shourbalal et al. (2019) may be of interest The important results of this research work are: 1) sowing winter wheat as spring wheat (vernalization not required) resulted in optimum yield amounts by priming and spraying techniques using gibberellins, kinetin and 6-benzyl adenine. This is of significance, with respect to the issue of global warming. 2) Shortening vernalization in winter wheat, which is especially important under arid and semi-arid conditions, as the plant is subjected to different stresses including drought. 3) Improving wheat grain quality by the increased rate of protein and gluten. It is possible to plant winter wheat under arid and semi-arid conditions using gibberellins, kinetin and benzyl adenine. Such a method results in alleviating the adverse effects of global warming on wheat production. It is also possible to plant winter wheat under different stresses including drought and cold by controlling the vernalization process (Sayed Shourbalal et al., 2019).

So plant growth promoters have the capacity both to speed up plant growth and slow it down. Kumar et al. (2019) conducted the experiment with retardants and had impressive findings. This study was conducted during winter season of 2016–17 and 2017–18 at Rajasthan Agricultural Research Institute, Durgapura, and Jaipur to evaluate the effect of nutrient management and growth retardants on wheat (*Triticum aestivum* L.). Results revealed that the application of 150% RDF+FYM led to higher plant height and dry matter accumulation of wheat at all the growth stages (60 days after sowing (DAS), 90 DAS and at harvest) except at 30 DAS (where it was maximum with 150% RDF). The grain, straw and biomass yields of wheat due to different growth retardant treatments were found statistically at par. The application of 150% RDF+ FYM increased the gross return by 50.74, 13.10 and 3.83% and mean net returns by 60.56, 12.96 and 3.13% over control, 100% RDF and 150% RDF, respectively (Kumar et al., 2019).

The plant growth promoter was further tested on various crops in different regions of the Russian Federation (Ulyanovsk, Kurgan, Ryazan regions, Krasnodar territory) and in Bulgaria for a number of years. The tests were conducted on winter rye, winter and spring wheat and barley, peas, fodder crops (Sudanese grass, fodder millet, spring rape), etc. The studies found that treatment of seeds with Melafen plant growth promoter increased yields from 10.0 to 34.0%. It was also found that under the influence of Melafen there was a more intense absorption of elements of mineral nutrition, it significantly increased the resistance of winter crops to stresses in the autumn and winter period and improved the quality of crop products. Melafen provided a relatively stable increase in crop yields and accelerated ripening of the crop. The plant growth promoter enhanced the tillering stage of winter wheat. Longer wheat heads and larger number of kernels per head resulted in the increased grain weight per plant. The experiments reported an increase of 12.0–12.8% in yields (Fattakhov et al., 2014).

Our experiments confirmed that application of the plant growth promoter applied for winter wheat was efficient. Melafen plant growth promoter combined with pesticides in different variations produced the maximum grain yield of 6.36–7.41 t ha^{-1} while the control index was 4.21–5.00 t ha^{-1}. The experiment also confirmed the improved gluten quality and higher bulk density in grain due to the plant growth promoter. In contrast to previous experiments, we studied various options for the use of pesticides in combination with the growth promoter. We observed a negative effect of wheat seed treatment and a better effect that the seed treatment had when combined with Melafen plant growth promoter. The study proved that maximum efficiency of crops was achieved when the plant growth promoter was applied at all treatment stages Melafen (pre-sowing stage) + (herbicide+insecticide+Melafen (tillering stage)) + (insecticide+fungicide+Melafen (heading stage)).

CONCLUSIONS

The study findings let us conclude that pesticides used in the experiment to increase grain yield of winter wheat and grain quality have confirmed their efficiency. High economic efficiency was characterized by the use of the growth stimulant Melafen. The control variants reached yield indices of 4.21–5.00 t ha^{-1}. Seed treatment with Polaris chemical led to lower yield indices in some variants, the indices were 3.33–6.37 t ha^{-1} in the block with seed treatment. The plant growth promoter Melafen worked well in the experiment on winter wheat, especially in combination with pesticides in different variations. It produced the highest grain yield (6.36–7.41 t ha^{-1}). All experiment variants demonstrated positive economic efficiency. The notional net return from 1 hectare ranged from 1.9 thousand roubles to 36.2 thousand roubles. The control block had notional net return at 1.9–27.3 thousand roubles, the index was 28.3–36.2 thousand roubles in the block with Melafen application, and the figure was 22.9–32.0 thousand roubles in the ST + M block. Variant 20 showed the best economic efficiency in the experiment: Melafen (pre-sowing stage) + (herbicide + insecticide + Melafen (tillering stage)) + (insecticide +fungicide +Melafen (heading stage)).

REFERENCES

Dospekhov, B.A., Vasiliev, I.P. & Tulikov, A.M. 1987. *Hands-on course in agriculture*. 2nd revised edition. Agropromizdat, Moscow, 383 pp.

Fattakhov, S.G., Kuznetsov, V.V. & Zagoskina, F.M. (Eds.) 2014. *Melafen: mechanism of action and scope*. 2nd ed. Printing-Service XXI, Kazan, 408 pp. (in Russian).

Federal State Statistics Service. 2019. *Russian Statistical Yearbook*. Rosstat Publ., Moscow, 511 pp. (in Russian).

Fedyn, M.A. 1983. *Methods of state agricultural crop variety testing*. 3 edition. Kolos, Moscow, 184 pp.

Gabitov, I., Mudarisov, S., Gafurov, I., Ableeva, A., Negovora, A., Davletshin, M., Rakhimov, Z., Khamaletdinov, R., Martynov, V. & Yukhin, G. 2018. Evaluation of the Efficiency of Mechanized Technological Processes of Agricultural Production. *Journal of Engineering and Applied Sciences* **13**, 8338–8345.

Gruznova, K.A., Bashmakov, D.I., Miliauskienė, J., Vaštakaitė, V., Du-Chovskis, P. & Lukatkin, A.S. 2018. The effect of a growth regulator ribav-extra on winter wheat seedlings exposed to heavy metals. *Zemdirbyste* **105**(3), 227–234.

Ishkov, I.V. 2017. Effect of pre-sowing seed treatment with fungicides and growth regulators on the productivity of winter wheat. *Chief Agronomist* **10**, 20–24 (in Russian).

GOST 54478-2011. Seed. Methods for determining the quantity and quality of gluten in wheat - Electronic resource [access Mode] https://standartgost.ru/g/%D0%93%D0%9E%D0%A1%D0%A2_%D0%A0_54478-2011 free.

GOST 10987-76. Seed. Methods for determining vitreous - Electronic resource [access Mode] https://standartgost.ru/g/%D0%93%D0%9E%D0%A1%D0%A2_10987-76 free.

GOST 10840-2017. Seed. Method for determining nature-Electronic resource [access Mode] https://standartgost.ru/g/%D0%93%D0%9E%D0%A1%D0%A2_10840-2017 free.

GOST 9353-2016. Wheat. Technical conditions-Electronic resource [access Mode] https://standartgost.ru/g/%D0%93%D0%9E%D0%A1%D0%A2_9353-2016 free.

Ismagilov, R.R., Gaysina, L.F., Akhiyarova, L.M., Ayupov, D.S., Nurlygayanov, R.B., Akhiyarov, B.G., Abdulvaleev, R.R., Malyutina, K.V., Ismagilov, K.R. & Abdulloev, V.K. 2018. Crop Yields and Baking Qualities of Winter Rye F1 Hybrids Grain in the Forest-Steppe of the Republic of Bashkortostan. *Journal of Engineering and Applied Sciences* **13**, 6487–6493.

Jørgensen, L.N., Kudsk, P. & Ørum, J.E. 2019. Links between pesticide use pattern and crop production in Denmark with special reference to winter wheat. *Crop Protection* **1**, 147–157.

Kargin, V.I., Nemtsev, S.N., Zakharkina, R.A. & Kargin, Yu.I. 2011. Effectiveness of biological products in spring wheat growing. *Reports of the Russian academy of agricultural sciences* **1**, 35–38 (in Russian).

Khaibullin, M.M., Kirillova, G.B., Yusupova, G.M., Kagirov, E.S., Ismagilov, R.Z., Rakhimov, R.R., Sergeev, V.S., Khaziev, F.H., Gaifullin, R.R. & Bagautdinov, F.Y. 2018. Influence of Percentage Fertilizer Systems on Change of Agrochemical Properties of the Arable Layer of Leach Chernozem and on the Crops Productivity of Crop Rotation. *Journal of Engineering and Applied Sciences* **13**, 6527–6532.

Kleczewski, N.M. & Whaley, C. 2018. Assessing the utility of the growth regulator trinexapac-ethyl and fungicides in the mid-Atlantic soft red winter wheat production systems. *Crop Protection* **104**, 60–64.

Kochmin, A.G. & Bogomazov, S.V. 2016. Agricultural methods for increasing the productivity of winter wheat in the forest-steppe zone of the middle Volga region. *Feeding of farm animals and fodder production* **4**, 61–73 (in Russian).

Kochmin, A.G. 2015. Yield and quality of winter wheat grain, depending on the preceding crop and the use of growth regulators 'Moddus'. Innovative technologies in the agricultural sector: theory and practice. *Proceedings of the 3rd All-Russia scientific and practical conference held at Interdisciplinary research and documentation centre 'Penza state Agricultural Academy'*. Penza, pp. 65–69 (in Russian).

Korshunov, A.A., Grabovskaya, T.V. & Lazareva, A.S. 2015. The productivity of winter wheat, depending on growth regulators on various backgrounds of mineral nutrition. *Taurian Bulletin of Agricultural Science* **2**, 50–54 (in Russian).

Kumar, S., Sharma, P.K., Yadav, M.R., Sexena, R., Gupta, K.C., Garg, N.K. & Yadav, H.L. 2019. Impact of nutrient management practices and plant growth regulators on growth, productivity and profitability of wheat (Triticumaestivum). *Indian Journal of Agricultural Sciences* **89**(4), 604–609.

Kuznetsov, I. Yu., Povarnitsyna, A.V. Akhmetzyanov, M.R. & Vafin, I.Kh. 2019. The effectiveness of the use of Melafen growth stimulator in the treatment of winter wheat seeds with 'Polaris' chemical. *Bulletin of Kazan State Agrarian University***14** (2/53), 15–19.

Lapa, V.V. 1998. Application of multi-component fertilizer ECO-FLASH in agricultural crop growing. *NTI and the market* **1**, 11–12 (in Russian).

List of pesticides and agrochemicals approved for use on the territory of the Russian Federation: reference edition. 2019. Mozhaisk Printing and Publishing, Mozhaisk, 792 pp. (in Russian).
Litke, L., Gaile, Z. & Ruža, A. 2019. Effect of nitrogen rate and forecrop on nitrogen use efficiency in winter wheat (Triticum aestivum). *Agronomy Research* **17**(2), 582–592. doi.org/10.15159/AR.19.040
Litke, L., Gaile, Z. & Ruža, A. 2018. Effect of nitrogen fertilization on winter wheat yield and yield quality. *Agronomy Research* **16**(2), 500–509. http://dx.doi.org/10.15159/ar.18.064
Masterov, A.S., Karaulny, D.V. & Plevko, E.A. 2017. Ecosil in the fertilizersystem of agricultural crops. *Chief Agronomist* **10**, 14–18 (in Russian).
Methodology instructions for conducting field experiments on grain crops. 1997. Russian Academy of Agricultural Sciences, Moscow, 156 pp.
Moss, J.W., Tubbs, R.S., Gray, T.L., Smith, N.B. & Johnson, J.W. 2019. Assessment of Double-Crop and Relay-Intercropping Systems of Peanut with Soft Red Winter Wheat and Residual Herbicides. *Crop, Forage and Turfgrass Management* **3**(1), cftm2016.10.0069.
Pacanoski, Z & Mehmeti A. 2019. Pre-emergence grass weed control in winter wheat (Triticum aestivum L.) with soil applied premixed herbicides influenced by precipitation. *Agronomy Research* **17**(6), 2386–2398. https://doi.org/10.15159/ar.19.198
Peng, J., Yang, X., Luo, M.-N. &Liu, X.-P. 2019. Effects of spraying a blended plant growth regulator on the tillering, growth, and the cold-resistance of different wheat varieties. *Agricultural Research in the Arid Areas* **37**(1), 137–143.
Posypanov, G.S., Dolgodvorov V.E., Zherukov, B.H. etc. M. 2006. *Crop production*. Kolos, Sprinting, 612 pp. (in Russian).
Pomortsev, O.A., Pomortseva, A.A. & Trofimtsev, Y.I. 2019(June). Cyclic Organization of Geological Environment: Permafrost Zone of Yakutia. I*n IOP Conference Series: Earth and Environmental Science* **272(**2), 022059.
Rebouh, N.Y., Polityko, P.M., Pakina, E., Plushikov, V.G., Norezzine, A., Gadzhikurbanov, A., Vvedenskiy, V., Duksi, F. & Iguer-Ouada, M. 2019. Impact of three integrated crop protection treatments on the varieties of winter wheat (Triticumaestivum L.) in the Moscow area, Russia. *Research on Crops* **20**(1), 16–168.
Rushninsky, L.Z. 1971. *Mathematical processing of experimental results*. Nauka, Moscow, 192 pp.
Sandukhadze, B.I. 2016. Development and results of winter wheat growing in the central Non-Chernozem region. *Advances in science and technology of the agro-industrial complex* **9**, 15–18 (in Russian).
Sayed Shourbalal, S.K., Soleymani, A. & Javanmard, H.R. 2019. Shortening vernalization in winter wheat (Triticumaestivum L.) using plant growth regulators and cold stratification. *Journal of Cleaner Production* **219**, 443–450.
Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. Decimal code for the growth stages of crops. *Weed Research* **14**(6), 415–421.
Zhang, W., Huang, Z., Xu, K., Liu, L., Zeng, Y., Ma, S. & Fan, Y. 2019. The effect of plant growth regulators on recovery of wheat physiological and yield-related characteristics at booting stage following chilling stress. *ActaPhysiologiaePlantarum* **41**(8), 133.

Agronomy Research **18**(1), 177–182, 2020
https://doi.org/10.15159/AR.20.016

Agroecological prospects of using corn hybrids for biogas production

V.A. Mazur[1], H.V. Pantsyreva[1,*], K.V. Mazur[1], R.O. Myalkovsky[2] and O.O. Alekseev[1]

[1]Vinnytsia National Agrarian University, 3, Soniachna Str., UA21008 Vinnytsia, Ukraine
[2]State Agrarian and Engineering University, 13, Shevchenko Str., UA32300 Kamianets-Podilskyi, Ukraine
[*]Correspondence: pantsyreva@vsau.vin.ua

Abstract. Ukraine is an agricultural country with great agricultural potential for biogas production, which is the key to fertile soils and favorable climatic conditions for energy crops, including corn. The article analyzes the experience of using biogas in Ukraine and the world, its mechanism of production. The leader in biogas production in the world is the European Union in general and Germany in particular. The total number of biogas plants in Europe exceeds 11 thousand, of which 7.2 thousand in Germany. Installed biogas, which is released in the process of complex fermentation of organic waste, consists of a mixture of gases: methane – 55–75%, carbon dioxide – 23–33%, hydrogen sulfide – 7%. An important sector of renewable energy sources in biogas production is presented and the prospects for its use are determined. The energy dependence of our country on the volumes of imported natural gas is analyzed. The main aspects of biogas production are explored using renewable energy sources that are inexhaustible in our crane and the phased operation of the biogas plant is investigated. The real advantages of the need for biogas production and use in our country are outlined. Problems aimed at the development of alternative energy have been proved in order to detect environmental pollution. It has been established that in Ukraine the use of corn silage to improve the efficiency of biogas production at existing biogas stations has not been used so far. The problems of increasing the yield of corn plants have been proved not only by breeding and genetic methods, but also by cultivation technology.

Key words: biogas, corn, renewable energy, the intensification of production, pollution.

INTRODUCTION

Total EU biogas production in 2017 was 10.9 million tonnes (equivalent to 13.5 billion cubic meters of natural gas), of which 6.7 million tonnes was produced in Germany, with an annual increase of 31.3%. One of the important sectors of renewable energy in the world is the production and use of biogas. Ukraine is an agricultural country with great agricultural potential for the production of alternative energy sources, which is the key to fertile soils and favorable climatic conditions for growing energy crops (Kaletnik, 2010; Klimchuk, 2017; Bezvikonnyi, 2018). However, our country still remains energy dependent. The irreversible depletion of the world's energy reserves, the rising cost of energy, and the problems of environmental pollution are forcing most developed countries to formulate their energy strategies for the development of alternative energy.

Experimental research has shown that scientists' views on the feasibility of producing biofuels such as biodiesel and bioethanol are controversial. This is all due to the high cost of technology. Given this, it is extremely important to produce biogas based on corn hybrids (Kolchinskij, 2008; Kaletnik, 2010).

One of the promising areas of energy conservation in agricultural enterprises is the production of biogas as a type of renewable energy sources, which is supplied by agriculture for raw materials (Alimov et al., 1994). After all, the production of biogas and high-yield crops, including corn, is ensured on the basis of the use of plant material obtained as a result of cultivation. It is the diversification of energy sources based on the principles of sustainable development that is a global trend and an urgent need in Ukraine. One of the important sectors of renewable energy. According to the International Energy Agency, by 2030, the share of electricity generated from alternative sources will double compared to today, accounting for about 16% of total production (Wilkie et al., 2000).

The common practice of intensifying and increasing gross biogas production is the use of co-occurring methane fermentation of various raw materials, including those specifically grown for biogas production. In Ukraine, the use of corn silage to improve the efficiency of biogas production at existing biogas stations has not yet been used. There is also limited scientific and practical data on the basis of which it is possible to justify the technological modes of operation of the biogas plant, depending on the proportion of corn silage in the mixture with manure waste (Thran, 2010; Westerholm et al., 2012; Klimchuk, 2017).

MATERIALS AND METHODS

In the process of research used conventional methods. Dry organic matter (Table 2) content was determined by weighting of the initial biomass samples, drying in dry matter weights Shimazu at 105 °C and then placed for ashing in oven (‘Nabertherm’ type) at 550 °C. All the components were carefully mixed together and filled in bioreactors. All bioreactors were placed into heated thermostat SNOL in the same time before starting of anaerobic digestion. Gas released from each bioreactor was collected in storage bag positioned outside of the thermostat container. Gas volumes were measured using flow meter (Ritter drum-type gas meter). The composition of gases, including oxygen, carbon dioxide, methane, and hydrogen sulphide was measured help by gas analyser (model Pronova SSM). The substrate pH value was measured before and after finishing off the AD process, using a pH meter (model TDS-986) with accessories. Scales (Certus CBA-300-0) was used for weighting of the total weight of substrates before and after the AD process. Fermented cattle manure (from 120 L bioreactor working in continuous mode) was used as the inoculum. Batch mode AD process was ongoing at temperature 38 ± 0.5 °C. Biogas released was collected in gas bags for further measurements of gas volume and elemental composition. Biogas and methane volumes and gases composition were measured during AD process at regular time intervals. The AD process was provided until biogas emission ceases. Obtained experimental data were processed using appropriate statistical methods. All data was recorded in the experiment log and on the computer. All bioreactors were connected to calibrated gas storage bags and taps, placed in an oven and set at a working temperature of 38 ± 0.5 °C. The amount and composition of the released gas was measured daily. Bioreactors were also shaken daily by mixing the substrate to wet and reduce the floating layer. The fermentation took place in a single filling (batch) mode and lasted until the biogas was released (25 days). The material for the research were hybrids of corn Bogatyr, Emilio, Dialog.

RESULTS AND DISCUSSION

Biogas production is efficient and attractive to investors technology, but Ukraine is at the beginning stage of introducing renewable energy sources, and the scientific and technical problems of biogas production and use are not quite formal (Rakotojaona, 2013; Mazur et al., 2018; Pantsyreva, 2018; Guo et al., 2020). Therefore, the study, analysis and borrowing of the experience of biogas production in the world and its implementation at agronomic enterprises of Ukraine is of particular relevance.

Biogas, which is released in the process of complex fermentation of organic waste, consists of a mixture of gases: methane – 55–75%, carbon dioxide – 23–33%, hydrogen sulfide – 7% (Angelidaki et al., 2009; Mazur & Pantsyreva, 2017; Pantsyreva, 2019b; Vdovenko et al., 2018a). One of the main trends in crop and livestock production is the development of complex technologies using methane digestion processes for biomass utilization, for biogas production (Noorollahi, 2015; Vdovenko et al., 2018b; Alavijeh et al., 2020).

Silage corn is by far the most important crop for use in biogas plants. Corn is also called the C4 plant because of its high dry mass yield (Wellinger et al., 2013). The technology needed to process this culture is usually always available in the business or well-known and inexpensive. Corn is easy to silo and even when used cleanly does not cause disturbances in the operation of biogas plants (Sosnowski et al., 2018; Mazur et al., 2019; Ovcharuk, 2019; Pantsyreva, 2019a).

Today there are already special varieties for use in biogas installations. These varieties generally produce more biomass yield. The best time for harvesting is its readiness for silage, digestion and weather. As a rule, corn at harvest should have a dry matter content of 28–35% and be in a state between milky ripeness and fitness for flour.

The main indicator of the effectiveness of growing any crop is its yield. The problems of increasing the yield of maize plants are solved not only by breeding methods, but also by cultivation technology. For the 2015–2017 years, the highest yields (Table 1) were recorded in the areas of the middle-aged Emilio hybrid (12.57 t ha^{-1}).

Table 1. Agrotechnical characteristics of corn hybrids (average for 2015–2017)

Hybrid	Group ripeness	Yield, t ha^{-1}	Length of the cob, cm	Diameter of the cob, cm
Bogatyr	medium early	11.01	17.1	4.5
Emilio	medium	12.57	18.5	4.7
Dialog	medium	11.77	18.3	5.0

LSD0.5 t ha^{-1}: A-0.05, B-0.6, C-0.07, AB-0.12, AC-0.11; BC-0.14, ABC-0.09;
2015 LSD0.5 t ha^{-1}: A-0.04; B-0.05, C-0.05, AB-0.05, AC-0.06, BC-0.07,ABC-0.1;
2016 LSD0.5 t ha^{-1}: A-0.05, B-0.06, C-0.05, AB-0.08, AC-0.07, BC-0.08, ABC-0.1;
2017 LSD0.5 t ha^{-1}: A-0.06, B-0.07, C-0.05, AB-0.07, AC-0.08, BC-0.9, ABC-0.13.

The study found that the highest quantitative and linear rates were recorded in the middle-matured group of corn hybrids. Thus, the cob and its diameter averaged 18.4 cm and 4.9 cm, respectively.

As the main indicators used to evaluate the properties of each individual experimental parallel, certain agro-technical and physical-chemical parameters of maize properties intended for biogas production were taken. In particular, the yield and linear parameters of the cob, as well as the individual technological properties of biomass (dry matter, ash, N, P, K, C, hydrogen and oxygen). These physicochemical indicators are the minimum necessary to determine the prospects of using corn for biogas production (Table 2).

Table 2. Physicochemical characteristics of corn hybrids (average for 2015–2017)

Hybrid	Dry matter,%	Ash,%	N, %	P,%	K, %	C, %	H_2, %	O_2, %
Bogatyr	28.5	4.6	1.00	0.30	0.80	39.8	4.3	36.5
Emilio	29.9	5.8	1.62	0.54	1.56	40.3	5.0	41.4
Dialog	28.9	4.9	1.33	0.44	1.01	39.9	4.8	37.0

Thus, the highest indicators of dry matter and ash content were recorded in the areas of the middle-aged hybrid Emilio, which were respectively 29.9% and 5.8 %. Somewhat lower rates are recorded in the hybrid Dialog, which is a genetically determined trait.

In the process of methane fermentation, the ratio C: N is constant changes because the carbohydrate is released with biogas constantly and the nitrogen is stored in the bioreactor and is only released when the sludge is unloaded (Fig. 1).

Corn silage is characterized by low NH4 content and high C: N ratio. This result can be explained by the fact that mixing cattle manure and corn silage will optimize the components of the mixture in terms of both ammoniacal nitrogen and C: N.

If the ratio C: N in the litter is large, then there will be a shortage of nitrogen serve as a limiting factor for methane fermentation. If the ratio is low, then there is a large amount of ammonia that is toxic to the methanogens. In the process of methane fermentation, the ratio C: N is constantly changing because the carbohydrate is released with biogas constantly, and nitrogen is stored in the bioreactor and is only released when the sludge is unloaded.

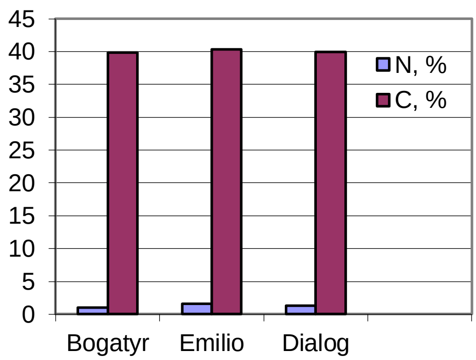


Figure 1. Value content (%) of C and N corn hybrids (average for 2015–2017).

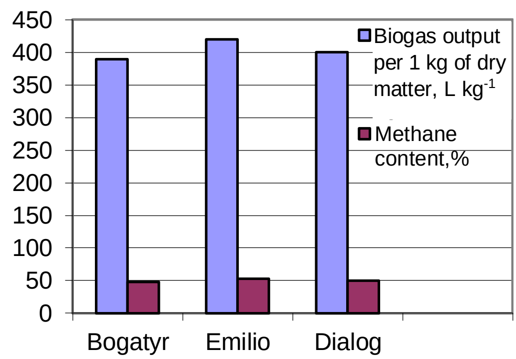


Figure 2. Biogas yield from corn hybrids and methane content (average for 2015–2017).

According to the results of the research, from the corn silage, a certain amount of biogas with different methane content can be obtained during processing (Fig. 2)

In Ukraine, biogas production from agro-industrial raw materials can be estimated at 1.6 million tonnes of conventional fuel. Considering the current technological possibilities of using green mass as raw material for biogas production, the potential of biogas fuel can be considered to be substantially high.

Therefore, biogas production is an efficient and attractive technology due to the presence of significant raw material potential, favorable natural and climatic conditions and low cost of this type of energy. However, Ukraine is at the initial stage of introducing renewable energy sources, and the scientific, technical and economic problems of biogas production and use are insufficiently studied. Thus, the study of foreign experience on these problems and its introduction in agricultural enterprises of Ukraine is of particular relevance.

CONCLUSION

Agrarian and industrial complex of Ukraine, producing large amounts of organic waste, has the resources for biogas production, capable of replacing 1.5 billion cubic meters of gas per year. With the extensive use of corn silage, this potential can be increased to 18 billion cubic meters in terms of natural gas.

The amount of biogas produced depends on the physical and chemical properties of the raw material. To predict the production of biogas, the need to develop new methods, to analyze the potential of biogas production from crop residues and manure. The model should take into account the biogas potential of agricultural crop statistics.

REFERENCES

Alavijeh, R.S., Karimi, K. & van den Berg, C. 2020. An integrated and optimized process for cleaner production of ethanol and biodiesel from corn stover by Mucor indicus. *Journal of Cleaner Production* **249**, pp. 399–408.

Alimov, D.M. 1994. *Production technology of crop production*. K: Higher School, 287 pp.

Angelidaki I., Alves M., Bolzonella D., Borzacconi L., Campos J.L., Guwy A.J., Kalyuzhnyi S., Janicek P. & van Lier J.B. 2009. Defining the biomethane potential: proposed protocol for batch assays. *Water Science & Technology*, WST **59.5**, pp. 1–8.

Bezvikonnyi, P.V. 2018. Effectiveness of combined application of fungicides and foliar fertalization of microfertilizers on beet crops. *Taurian Scientific Bulletin.* Ed. **100**, 9–14.

Guo, H., Zhang, M., Dong Z., Wang Q., Xia D., Lv J. & Yu, H. 2020. The mechanisms of biogenic methane metabolism by synergistic biodegradation of coal and corn straw. *Bioresource Technology* **298**(February), 122577.

Kaletnik, G.M. 2010. Biofuels. *Food, Energy and Environmental Security of Ukraine*: Monograph. K: High-tech Press,. 516 pp.

Klimchuk, O.V. 2017. Economic and technological processes of efficient regional biogas production in Ukraine. *Economy*. *Finances*. *Management*: topical issues of science and practice. No. **2**, pp.104–117.

Kolchinskij, J.L. 2008. Problems of development of bio-energetics in the Russian Federation. *Agronomy Research* **6**(Special issue), 221–227.

Mazur, V.A. & Pantsyreva, H.V. 2017. Influence of technological methods of cultivation on the yield and quality of white lupine grain in the conditions of the right-bank forest-steppe of Ukraine. *Agriculture and forestry*. Vinnytsia, VSAU, No. **7**. T 1, pp. 27–36.

Mazur, V.A., Pantsyreva, H.V., Mazur, K.V. & Didur, I.M. 2019. Influence of the assimilation apparatus and productivity of white lupine plants. *Agronomy research* **17**(1), 206–219. https://doi.org/10.15159/AR.19.024

Mazur, V.A., Didur, I.M., Pantsyreva, H.V. & Telekalo, N.V. 2018. Energy-economic efficiency of grain-crop cultures in the conditions of the right-bank Forest-Steppe of Ukraine. *Ukrainian J. Ecol.* **8**(4), 26–33.

Ovcharuk, V.I., Mulyarchuk O.I., Myalkovsky R.O., Bezvikonnyi, P.V., Kravchenko, V.S. & Klymoych, N.M. 2019. Parameters of beet plants. *Bulletin of the Uman National University of Horticulture*. No. **1**, pp. 70–75.

Noorollahi, Y., Kheirrouz M., Asl, H.F., Yousefi, H. & Hajinezhad, A. 2015. Biogas production potential from livestock manure in Iran. *Renewable and Sustainable Energy Reviews* **50**, 748–754. doi: https://doi.org/10.1016/j.rser.2015.04.190

Pantsyreva, H.V. 2019a. Morphological and ecological-biological evaluation of the decorative species of the genus Lupinus L.. *Ukrainian Journal of Ecology*, **9**(3), 74–77.

Pantsyreva, H.V. 2019b. Technological aspects of biogas production from organic raw materials. *Bulletin of KhNTUSG them*. P. Vasilenko. Kharkiv, pp. 276–290.

Pantsyreva, H.V. 2018. Research on varietal resources of herbaceous species of Paeonia L. in Ukraine. *Scientific Bulletin of the NLTU of Ukraine* **28**(8), pp. 74–78. https://doi.org/10.15421/40280815

Rakotojaona, L. 2013. Domestic biogas development in developing countries. INIS-FR--16-0051 Enea Consult. 33, 1–25.

Sosnowski, J., Matsyura, A., Jankowski, K. & Przytuła, M. 2018. Cell wall fractions in the biomass of Dactylis glomerata and Festuca pratensis. *Ukrainian Journal of Ecology* **7**(1), 66–70.

Thran, D. 2010. *Methodenhandbuch Energetische Biomassenutzung*, (Methods Manual Energetic use of biomass), Leipzig, 93 pp. (in German)

Vdovenko, S.A., Prokopchuk, V.M., Palamarchuk, I.I. & Pantsyreva, H.V. 2018a. Effectiveness of the application of soil milling in the growing of the squash (Cucurbita pepo var. giraumontia) in the right-benk forest stepp of Ukraine. *Ukrainian J. Ecol.*, **8**(4), 1–5.

Vdovenko, S.A., Pantsyreva, G.V., Palamarchuk, I.I. & Lytvyniuk, H.V. 2018b. Symbiotic potential of snap beans (Phaseolus vulgaris L.) depending on biological products in agrocoenosis of the right-bank forest-steppe of Ukraine. *Ukrainian J. Ecol.* **8**(3), 270–274.

Wellinger, A., Murphy, J. & Baxter, D. 2013. *The biogas handbook*. Woodhead Publishing, 512 pp.

Westerholm, M., Hansson, M. & Schnürer, A. 2012. Improved biogas production from whole stillage by co-digestion with cattle manure. Swedish Institute of Agricultural and Environmental Engineering, Science Direct. *Bioresour Technol.* **114**(Jun), pp. 314–319.

Wilkie, A.C., Riedesel, K.J. & Owens, J.M. 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass and Bioenergy* **19**, 63–102.

Agronomy Research **18**(1), 183–193, 2020
https://doi.org/10.15159/AR.20.008

Grain yield response of facultative and winter triticale for late autumn sowing in different weather conditions

B. Mazurenko*, S. Kalenska, L. Honchar and N. Novytska

National University of Life and Environmental Science, Department of Plant Science, Heroiv Oborony street 15, UA03041 Kyiv, Ukraine
*Correspondence: mazurenko.bohdan@nubip.edu.ua

Abstract. Climate change is affecting the growing conditions of winter cereals. Peculiarities of organogenesis and their impact in grain yield of facultative triticale depend on different nitrogen fertilization can help to avoid adverse effects of unfavorable conditions. Field experiment was conducted in zone of the Right-Bank Forest-Steppe of Ukraine. The experiment included 2 late autumns sowing periods and fertilization system with few variants of nitrogen fertilization applied in spring. Features of organogenesis of two winter varieties and facultative triticale Pidzimok kharkivskiy were determined by apical meristem microscopy from emergence till heading. Was established process of apical meristem differentiation in facultative triticale has non-linear relation between temperature and number of spikelets. The efficiency of apical meristem differentiation reaches its maximum at 12 °C. Grain yield of triticale varieties depend on studied factors but main impact had weather conditions. Grain yield of facultative triticale significantly exceeds winter varieties and had a lesser difference between sowing period than winter cultivars. Crops in the first sowing period were more productive than in the second. Facultative triticale has great productivity potential in late autumn sowing and can realize it in various conditions. Reduced yields in late sowing are lower than in winter cultivars.

Key words: apical meristem, nitrogen fertilization, organogenesis, sowing period.

INTRODUCTION

Sustainable grain production depends on growing technologies. Sowing time is the key to successful wintering and ensures efficient use of resources. Autumn climatic conditions do not allow sowing in optimal terms in recent years. Cereals germinate later and vegetative period in the fall is shrinking. Winter varieties accumulate less sugars what reduce their winter hardiness. On the other hand, winter hardiness depends on conditions before winter and may difference in same variety.

Low temperature tolerance (LT) depends on daylight length and exposure of period (Limin & Fowler, 2006). Late autumn is preceded by shortening day length that positive affected on LT-tolerance genetic potential. Triticale and wheat get half of their cold resistance in 2 weeks and full in 6–7 weeks. Expression of VRN-A1 allele is a major cause of delayed plant development on early vegetative stages in the autumn. Late autumn sowing can be effective when cereals wintering in BBCH 05–09.

VRN-1 gene expression occurs in plant after germination at low temperature (Humphreys et al., 2006). Increase in temperature could result in a failed or insufficient vernalization of winter wheat. This imperfection is manifested in varieties with high vernalization requirement and sensitivity to day length. Spring and facultative triticale have not such disadvantages.

Productivity of winter crops decreases when sowing is delayed (Yildirim et al., 2013). The main reasons of low efficiency are decrease in the number of productive stems and grains in the ear. The weight of grain in spike depends on the grain size and its filling in the later stages of ripening. This allows to increase yields, when the number of spikes per m^2 and grains in the spike has been determined.

The experience of spring triticale growing (Grocholski et al., 2007) with late autumn sowing showed that their winter hardiness is sufficient by the typical winter conditions with snow cover.

Facultative crops in late autumn sowing have many advantages because their development may occur by winter or spring types depending on environmental conditions. Triticale seeds can sprout at 1–3 °C.

Germinated seeds stay viable at low temperature and they vernalization are continuously. When vegetation continues for a long time the plants do not switch to ‘double ridge’ stage in the autumn. They increase in root system mass and accumulate solute carbohydrates. Grain and biomass yield of facultative triticale had a less dependence on sowing periods than in winter varieties.

MATERIALS AND METHODS

Field experiments were conducted in 2016–2019 (3 vegetative seasons for winter cereals). Triticale was cultivated in the Right-bank Forrest-Steppe of Ukraine (49°46´ N, 30°44´ E). The soil is typical low-humic black, the arable layer of which is characterized by the following agrochemical and agro-physical indices: humus content – 4.31–4.63%; pH – 7.2, easily hydrolyzed nitrogen (according to Kornfeld) – 152.3–167.0 mg; P_2O_5 in acetic acid extract (according to Chirikov) – 109.0–142.0 mg; exchangeable potassium (according to Chirikov) – 127.0–132.0 mg per 1 kg of soil.

Climate conditions

Meteorological indicators were obtained from the meteorological station #33466(Mironivka). The calculation of monthly average temperature and summary of precipitations was carried out during vegetative period (October–June).

Establishment of weather condition deviation was conducted by equation:

$$K_{sdw} = \frac{X_i - \bar{X}}{S} \tag{1}$$

where K_{sdw} – coefficient of weather conditions deviation; X_i – multi-annual average parameter; $\bar{X}$ – average/summary parameter in year; S – standard deviation in observed period.

$|K_{sdw}|$ < 1 – typical condition, $|K_{sdw}|$ from 1 to 2 – conditions differences from typical, > 2 – rarely or abnormally condition.

Result of weather condition analysis and their typicality by the e.g. (1) are showed in Table 1.

Table 1. Monthly average temperature[1] (°C) and precipitations during triticale vegetation in 2016–2019

Month	Temperature, °C				
	2016/2017	2017/2018	2018/2019	Average	Average multi-annual
X	6.7	8.6	10.5[a]	8.6	7.9
XI	1.4	3.5	0.1[a]	1.7	2.0
XII	-1.8	2.2[b]	-1.9	-0.5	-2.5
I	-5.3	-3.0[b]	-4.9	-4.4	-5.6
II	-2.6	-3.6	0.4[b]	-2.0	-4.5
III	6.1[a]	-1.8	4.7[a]	3.0	0.4
IV	10.5[a]	13.3[b]	10.4[a]	11.4	8.6
V	15.4	18.4[b]	17.3[a]	17.0	15
VI	20.6[a]	20.2[a]	22.6[b]	21.1	18
Average per season	5.7	6.4	6.6	6.2	4.4
	Precipitations, mm				
X	118.9[a]	115[a]	36.6	90.2	27
XI	45.9	86.2[a]	32.2	54.8	39
XII	46.2	161.1[b]	108.3[a]	105.2	44
I	39.8	104.3[b]	53.6	65.9	34
II	48.5[a]	54.1[b]	37	46.5	32
III	19.2	114.3[a]	44.4	59.3	33
IV	55.7	23.1	68.1	49	45
V	38.0	42.1	66.5[a]	48.9	44
VI	27.7	162.6[a]	156.9[a]	115.7	77
Sum per season	439.9	862.8	603.6	635.4	375.0

[1]Data from meteorological station #33466 (Mironivka); [a] – conditions differences from typical; [b] – rarely or abnormally conditions.

Spring vegetation was restored in 3rd decade of March in 2017 and 2019, but 2018 (restoration on 2nd decade of April) had a significant difference from them. Temperature conditions in this month had deviations from ordinary conditions. They were more warm than multi-annual parameter. This month has been typical by precipitations in both years, but their quantity was increased in 2018. Temperature conditions of April are important because there is a transition from vegetative to generative development. The temperature conditions were not typical and exceeded multi-annual parameter by 1.8 °C in 2017 and 2019. Weather conditions in 2018 were rarely and the average monthly temperature exceeded the multi-annual by 4.7 °C.

Sampling and methods

Features of facultative triticale development and affect of factors on grain yield were obvserved in the field experiment by 3-factorial scheme (Table 2). The experiment was established in 4 replications. The size of elementary plots was 32 m^2 (25.2 m^2 to harvesting). Each year, 36 kg P ha^{-1} (superphosphate, 18% P) and 72 kg K ha^{-1} (potassium chloride, 60% K) were applied before sowing on all plots. Nitrogen fertilizer applied on subplots depending of research programme. Tillage system included only one plowing after preceding crop harvesting (soybean). Was conducted cultivation on sowing depth (2–4 cm) before sowing. Triticale was sown with 15-cm inter-row spacing with rate 450 grains per square meter. Weren't applied pesticides during research. Plots were harvested in July. Yield per hectare was calculated to 14% moisture.

Table 2. Scheme of field experiment

Variety Factor A	Fertilization system Factor B	Code FS[2]	Sowing date Factor C
A1. Pidzimok kharkivskiy	B1. $P_{36}K_{72}$	N_0	C1. 2nd decade
A2. Amur	B2. $P_{36}K_{72}$ + $N_{25(11-13)}$[1]	N_{25}	of October
A3. Obrii Myronivs`kyi	B3. $P_{36}K_{72}$ + $N_{25(11-13)}$ + $N_{55(23)}$	N_{80}	C2. 3rd decade
	B4. $P_{36}K_{72}$ + $N_{25(11-13)}$ + $N_{55(25)}$ + $N_{20(49)}$	N_{100}	of October

[1]BBCH scale; [2]Code FS(fertilizing system) uses in text.

All researched varieties have good winter and cold resistance (Table 3). They are able to form high grain yield in diffirent conditions (drought and salt resistance).

Table 3. Variety characteristic

Variety	Type	Thousand kernel weight, g	Protein content, %	Grain yield potential, t ha^{-1}	Freezing injurious temperature, °C
Pidzimok kharkivskiy	facultative	40–45	13–16	9.2	-16.5
Amur	winter	50–60	13–15	9.6	-18.5
Obrii Myronivs`kyi	winter	45–50	12–15	9.7	-18.0

The date of organogenesis stage beginning was the moment when 10% of plants entered in the stage. Were analyzed 10 plants from each variant to establish organogenesis stage by apical meristem microscopy. Organogenesis stages was established by Kuperman scale (Kuperman, 1969). For establishing average number of spikelets on spike was analyzed 30 plants in ripening stage, sampled from each experimental plot.

Statistical analysis

Relation between parameter was determined by linear and polylinear correlation. Evaluation of regression coefficient significance was derived by means of *t-test*, adopting zero hypothesis and 0.05 confidence level. *ANOVA* (confident interval Fisher`s LSD) and cluster analysis were conducted by Statistica 13.3.

RESULTS AND DISCUSSION

Influence of weather conditions on spike elements formation

Peculiarity of triticale organogenesis in non-traditional sowing dates is that it germinates in autumn and spike organs are formed in the spring. Restoration of spring vegetation occurs at daily temperature more 5 °C. At initial stages, development of plants is most affected by temperature regime. The date of spring restoration and temperature regime is a key factor, influencing on apical meristem differentiation. Average monthly air temperatures over the years was increased in compared to multi-annual average parameter. This influenced on apical meristem differentiation, especially in 2018. The linear size of the apical meristem increased more rapidly in 2018 than in other years, but the number of double ridges did not increased (Fig. 1).

Development of apical meristem in crops of the second period was slower but under abnormal conditions was accelerated in compare with 1st sowing date (C1). Differentiation occurred differently depending on variety and temperature from germination to double ridge formation. The highest duration of double ridge formation and more favorable temperature conditions in variants with first sowing period. This stage occurred at higher average daily temperatures in crops of 2nd sowing date (C2) that affected on differentiation and potential spike structure. Differentiation in facultative triticale at this stage was similar to the winter variety Amur.

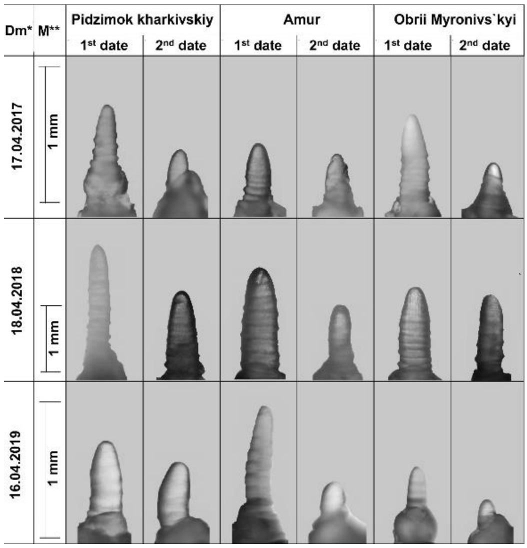


Figure 1. Apical meristem development (main shoot) in BBCH 25, *Dm – date of microscopy, m** – measurement.

Decrease in some phases of development duration was observed in crops during the late autumn sowing (Hall et al., 2013). Reduction in duration of double ridge is occurs in decrease of spikelets and grains number, but an increase in weight of thousand kernels. Self-regulatory ability of the plant increases flow of dry matter to the grain, which in turn realizes photosynthetic potential in grain yield.

The main difference in the process of apical meristem differentiation between winter and facultative triticale was relationship between formation of double ridges and average daily temperature during this process (Fig. 2). Double ridges develop to spikelets in future.

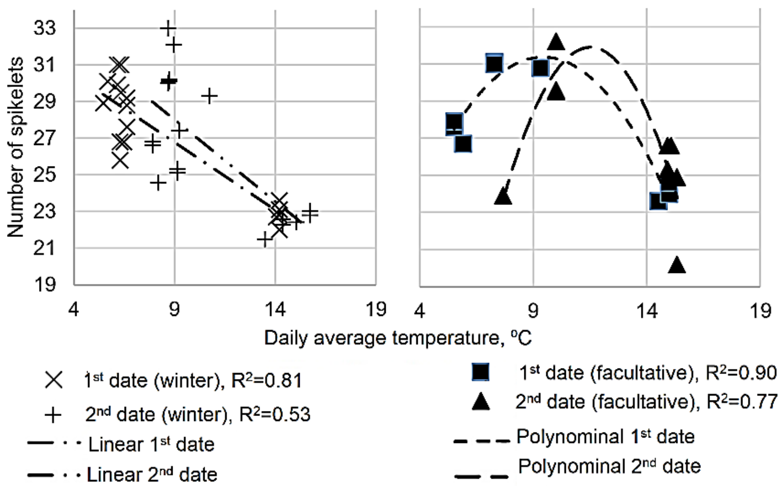


Figure 2. Correlation between spikelets number (at the ripening phase) and the average daily temperature during double ridge formation.

The total number of spikelets after apical meristem differentiation depended on the average daily temperature in winter and facultative triticale. The nature of this relationship was different. In winter varieties with average daily temperature increasing was a linear decrease in the number of spikelets. But a nonlinear relation was observed in facultative triticale for both sowing periods. This relationship was described by a quadratic function (inverted parabola) with focus in the range 9–12 °C. After spring vegetation restoration, increasing of average daily temperature to the focus point caused increase in number of spikelets per spike. If temperature during differentiation is more than 12 °C the total number of spikelets in the spike decreases.

Impact of weather condition on productive shoots number per m^2 and grain yield

Main factor affecting the yield of triticale is productive shoots number per m^2 (Fig. 3). Gustavo et al. (2013) founds the main impact on grain yield has a number of grains per m^2, what correlate with number of shoots per m^2. Quantity of grain and weight of thousand kernels reflect on weather conditions and nutrition. Shoots density depends on these factors too. Precipitations affect the number of shoots with enough nutrients. If autumn vegetation is shorter than ordinary condition there is a decrease in the number of spikes per m^2, the number of grains per spike is similar, but thousand kernels and grain weight per spike can increase (Santiveri et al., 2004; Slafer et al., 2014; Wenda-Piesik et al., 2016).

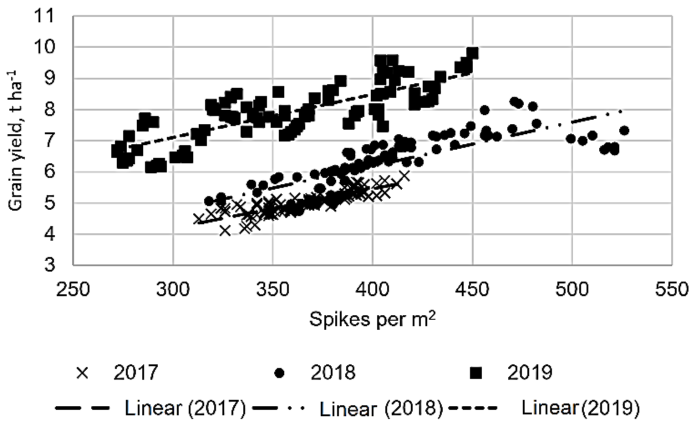


Figure 3. Regression analysis between grain yield and density per m^2 for 3 triticale varieties in every year of research.

Relation between grain yield and number of productive shoots per m^2 had a similar point but variate depend on year conditions (Table 4).

Table 4. Regression between grain yield and number of shoots per m^2

Year	Regression equation	R^2	R
2017	y = 0.0127x + 0.3922	0.74	0.86
2018	y = 0.0142x + 0.5173	0.61	0.78
2019	y = 0.0139x + 2.9403	0.64	0.80

Grain yield deviation from the regression equation was the smallest in 2017. Correlation coefficients between grain yield and number of grains per m^2 was 0.86. High accuracy of the model is due to the low variation of spike grain weight, thousand kernels weight and number of grains in different varieties in 2017. May and June were more warm than multi-annual condition (has a deviation from ordinary condition), but precipitations were lower (in normal range) what impacted on filling the grain in ripening phase. Precipitations before anthesis has a significant effect on formation processes but high temperatures after anthesis can limit the yield (Kalenska et al., 2018).

Weather conditions were similar in May and June on two next years. Temperature condition was rarely by average monthly parameter. They exceeded the multi-annual by 3.4 °C and impacted on triticale development from jointing (stem elongation) to anthesis. Temperature conditions had a deviation in June, but intensive rainfalls reduced the negative effects of high temperatures in this period. Decrease in yield compared to 2019 (similar to June) due to the number of spikelet's formed in double ridge. High temperature in period apical meristem differentiation decreased formation processes.

The higher yield triticale formed in 2019 than in two previously years. Main reason is increased rainfalls from spring restoration to ripening which impacted on efficiency of dry matter synthesis and grain filling. Correlation between grain yield and number spikes per m^2 was like in the previous year. More favorable conditions in double ridge formation period and enough precipitation in anthesis-ripening allowed to form higher yield due to the bigger mass of grain from the spike.

Increasing rainfalls leads to increased grain yield. Difference in grain yield between varieties was reduced at high precipitations rates (Linina & Ruza, 2018).

Response to fertilization system and sowing dates varied by variety depend on the weather condition during vegetation. Variety Pidzimok kharkivskyi (Table 5) had a low difference between variants in 2016/17 vegetation period but some variants of second sowing period decreased in grain yield when nitrogen dose was increased. Increase in the nitrogen dose leads to increased yields on all variants of first sowing date in 2017/18. Significant difference in sowings of 2nd sowing date was observed at variant N_{80} in that year. There was no significant difference between N_{80} and N_{100} variants for different sowing dates, but facultative triticale of 1st sowing date had a bigger grain yield than 2nd.

Table 5. Grain yield (t ha^{-1}) of winter and facultative triticale Pidzimok kharkivskyi

Sowing date	Fertilizing system	Year		
		2016/17	2017/18	2018/19
C1	B1	5.31 ± 0.11^{a}	6.42 ± 0.10	8.25 ±0.03^{a}
	B2	5.22 ± 0.04^{ab}	7.15 ± 0.10^{b}	8.51 ± 0.02^{ab}
	B3	5.35 ± 0.11^{abc}	7.31 ± 0.10^{bc}	8.75 ± 0.11^{bc}
	B4	5.54 ± 0.03^{cd}	8.14 ± 0.06	9.51 ± 0.10^{d}
C2	B1	5.51 ± 0.07^{acdg}	6.67 ± 0.03^{d}	7.72 ± 0.13^{e}
	B2	5.70 ± 0.08^{dg}	6.83 ± 0.11^{de}	7.81 ± 0.09^{e}
	B3	5.26 ± 0.04^{abce}	7.15 ± 0.07^{bc}	8.61 ± 0.12^{bc}
	B4	5.30 ± 0.02^{abce}	6.75 ± 0.03^{de}	9.24 ± 0.13^{d}
Average		5.40 ± 0.04	7.05 ± 0.09	8.56 ± 0.011

Means in columns with the different letter are highly significantly different according to the Fisher's test ($P \leq 0.05$) Value with ± represent the standard errors. Without letter has significant difference from other variants.

Nitrogen fertilization increased the yield in variety Amur (Table 6) every year, but the lower impact was observed in 2016/17. Dose of nitrogen had a significant difference on triticale of first sowing period, but in crops of second period almost did not change the yield level. Situation in 2017/18 was changed because first fertilization by 25 kg ha^{-1} N increased yield more than the next fertilization. Level of yield had insignificant difference in the adjacent variants of nitrogen fertilization. In 2018/19 the situation was similar to the previous year, but nitrogen fertilization significantly increased yield only by high doses of nitrogen (N_{80} and N_{100}) compare with N_0 and N_{25} variants of first sowing period. There was no significant difference between the variants of nitrogen fertilization, but they had a significant difference compare to variant without fertilizing. Application of nitrogen fertilizers may be a determining factor for productivity by the short autumn vegetation, but grain yield has not a significant difference because of the nitrogen dose (Petunenko et al., 2016).

Table 6. Grain yield (t ha^{-1}) of winter variety Amur

Sowing period	Fertilizing system	Year		
		2016/17	2017/18	2018/19
C1	B1	4.61 ± 0.01^{a}	6.00 ± 0.06^{a}	7.59 ± 0.12^{a}
	B2	4.88 ± 0.03	6.80 ± 0.13^{b}	7.54 ± 0.09^{ab}
	B3	5.02 ± 0.03	6.99 ± 0.13^{bc}	8.03 ± 0.12^{c}
	B4	5.12 ± 0.02	7.13 ± 0.14^{c}	9.24 ± 0.13
C2	B1	4.22 ± 0.04	5.17 ± 0.06	7.77 ± 0.02^{abc}
	B2	4.64 ± 0.02^{ab}	5.70 ± 0.07	8.26 ± 0.11^{cd}
	B3	4.74 ± 0.04^{c}	6.17 ± 0.05^{ab}	8.04 ± 0.04^{cd}
	B4	4.71 ± 0.02^{bc}	6.20 ± 0.10^{ab}	8.38 ± 0.06^{d}
Average		4.74 ± 0.05	6.27 ± 0.12	8.10 ± 0.10

Means in columns with the different letter are highly significantly different according to the Fisher's test ($P \leq 0.05$) Value with ± represent the standard errors. Without letter has significant difference from other variants.

Table 7. Grain yield (t ha^{-1}) of winter variety Obrii Myronivs`kyi

Sowing period	Fertilizing system	Year		
		2016/17	2017/18	2018/19
C1	B1	4.65 ± 0.06^{a}	5.60 ± 0.08	6.54 ± 0.04^{a}
	B2	4.87 ± 0.03^{b}	6.04 ± 0.05	7.20 ± 0.07^{b}
	B3	4.87 ± 0.13^{b}	6.36 ± 0.09^{a}	7.28 ± 0.07^{b}
	B4	5.03 ± 0.07^{bc}	6.52 ± 0.09^{a}	7.82 ± 0.05
C2	B1	4.99 ± 0.02^{bcd}	4.88 ± 0.07^{b}	6.20 ± 0.02
	B2	5.05 ± 0.05^{abcde}	4.96 ± 0.07^{bc}	6.45 ± 0.09^{a}
	B3	4.83 ± 0.06^{bd}	4.93 ± 0.07^{bc}	6.83 ± 0.11
	B4	5.06 ± 0.02^{cde}	5.24 ± 0.02	7.60 ± 0.05
Average		4.92 ± 0.03	5.57 ± 0.11	6.99 ± 0.10

Means in columns with the different letter are highly significantly different according to the Fisher's test ($P \leq 0.05$) Value with ± represent the standard errors. Without letter has significant difference from other variants.

Variety Obrii Myronivs`kyi (Table 7) had not significant difference in grain yield between many variants of first sowing period but yield was changing unsystematic depending of nitrogen dose in 2016/17. There was a significant decrease in yield at second sowing period compare to the first in 2017/18. Nitrogen fertilizers significantly increased the yield in N_{25} and N_{80} variants of second sowing period but had not difference between N_{80} and N_{100}. Only variant N_{100} had a significant difference compare to other sowings of second sowing period in that year. Fertilization significantly increased the yield of crops first and second sowing period in 2018/19 but yield of the second sowing period was lower. Gibson et al. (2007) showed that some varieties had not significant difference in grain yield depend on nitrogen dose, but the number of productive shoots was increased and, accordingly, grain mass per spike was decreased.

Grain yield varied in different years depending on precipitations. Precipitations contributed to the tillers formation, but this process depended on available nitrogen. Estrada-Campuzano et al. (2012) in their studies indicated that grain mass from the main shoot under different nitrogen doses has not significant impact, but nitrogen fertilizers increased grain yield from the tiller. The main role of additional nitrogen nutrition was manifested in increase of thousand kernels weight at the tillers and increase in the grains number. The application of high rates of nitrogen fertilizers has lesser impact on grain yield. Efficiency of high nitrogen norm depends on weather condition more than low one (Litke et al., 2018).

Peculiarities of the varieties in relation to the sowing periods and fertilization system in interaction of 'grain yield – the number of productive shoots' were identified with using cluster analysis (Fig. 4).

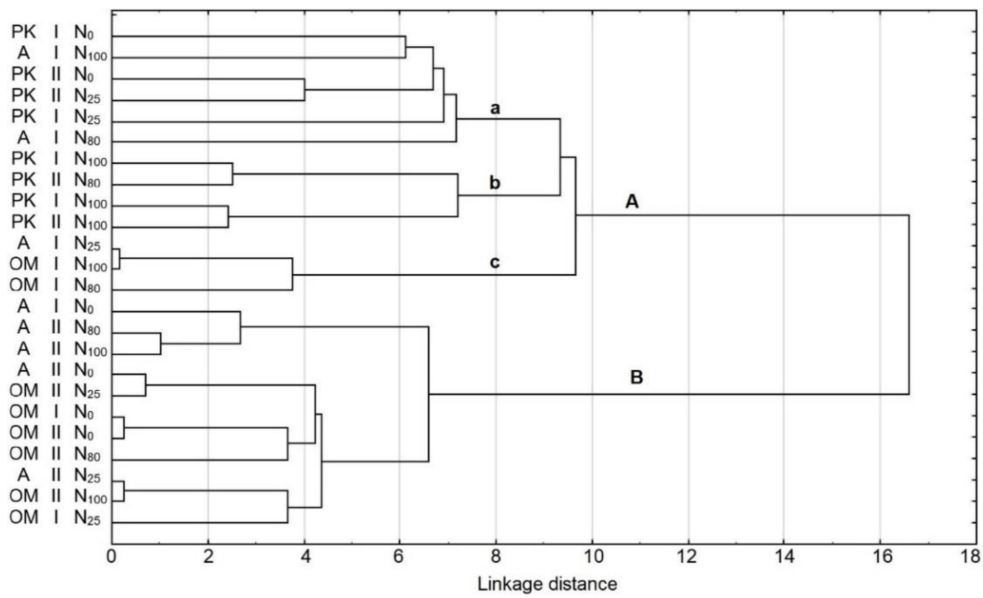


Figure 4. Cluster analysis of studied variants by relation 'grain yield – productive shoots per m^2'; PK – Pidzimok kharkivskyi; A – Amur; OM – Obrii mironivs'kyi; I – 2nd decade of October; II – 3rd decade of October; N_0, N_{25}, N_{80}, N_{100} – nitrogen fertilizing (B1 – B4).

All variants are grouped into two groups at the 10tn linkage distance: high performance (**A**) and low performance (**B**). High-performance crops divided on 3 groups on 8th linkage distance.

First group (**a**) include variants of Pidzimok kharkivskiy with application 25 kg ha^{-1} N and without nitrogen fertilizers by the two sowing dates. Variety Amur of first sowing date with application 80 and 100 kg ha^{-1} N was included too. The unifying feature of this group was the high number of productive shoots and relative yield level, which did not reach the maximum.

Second group (**b**) included variety Pidzimok kharkivskyi with high level of nitrogen nutrition. On 3rd linkage distance this group divided on 2 lesser in depend on dose of nitrogen. Sowing date has not effect on this group.

Third group (**c**) had intermedium level of yield and number of productive shoots. There were included winter varieties Amur and Obrii Myronivs`kyi of first sowing period with high level of nitrogen fertilizing.

Large group of low-performance variants (**B**) include Obrii Myronivs`kyi with low nitrogen doses and Amur by 2nd sowing date. These variants characterized by lowest number of productive shoots and accordingly yield level. Grain yield may vary insignificantly because of the different nitrogen doses (Lestingi et al., 2010) in different condition, but can change chemical content of grain.

CONCLUSIONS

Weather conditions significantly affected the process of triticale organogenesis at the early stages and yield. Increasing of nitrogen fertilization doses leads to linear increase in yield due to the increase of productive shoots number. The sowing period affected the yield of winter triticale much greater extent than facultative variety. Facultative triticale has a greater yield potential than variety and can realize it in different weather condition.

ACKNOWLEDGEMENTS. We thank the Plant Production Institute nd. a. V. Ya. Yuryev of NAAS for providing the seeds of facultative triticale (Pidzimoc kharkivskiy) for the experiments, and V.M. Remeslo Myronivka Instutute of Wheat for providing the seeds of winter triticale (varieties Amur and Obrii Myronivs`kyi).

REFERENCES

Estrada-Campuzano, G., Slafer, G.A. & Miralles, D.J. 2012. Differences in yield, biomass and their components between triticale and wheat grown under contrasting water and nitrogen environments. *Field Crops Research* **128**, 167–179.

Gibson, L.R., Nance, C.D. & Karlen, D.L. 2007. Winter Triticale Response to Nitrogen Fertilization when Grown after Corn or Soybean. *Agronomy Journal* **99**(1), 49–58.

Grocholski, J., Sowinski, J., Kulczycki, G. & Wardega, S. 2007. The Effect of Sowing Date of Facultative Wheat Varieties Cultivated on Silt-loam Soil on Yield and Plant Morphology. *Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wrocławiu-Rolnictwo* **91**(560), 7–12.

Gustavo, A.S., Savin, R. & Sadras, V.O. 2013. Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crop Res*. **157**, 71–83.

Hall, R.M. 2013. Influence of sowing date (autumn vs. spring) on crop development, yield and structure of wheat and triticale. W: S. Dušková (red.). *Current Trends in Agronomy for Sustainable Agriculture, Brno*, pp. 110–120.

Humphreys, M.W., Yadav, R.S., Cairns, A.J., Turner, L.B., Humphreys, J. & Skøt, L. 2006. A changing climate for grassland research. *New Phytologist* **169**(1), 9–26.

Kalenska, S., Yeremenko, O., Novitska, N., Yunyk, A., Honchar, L., Cherniy, V. & Rigenko, A. 2018. Enrichment of field crops biodiversity in conditions of climate changing. *Ukrainian Journal of Ecology* **9**(1), 19–24.

Kuperman, F.M. 1969. Physiology of development, growing and organogenesis of wheat. *In Physiology of Agricultural Crops* **4**, 7–203 (in Russian).
Lestingi, A., Bovera, F., De Giorgio, D., Ventrella, D. & Tateo, A. 2010. Effects of tillage and nitrogen fertilisation on triticale grain yield, chemical composition and nutritive value. *Journal of the Science of Food and Agriculture* **90**(14), 2440–2446.
Limin, A.E. & Fowler, D.B. 2006. Low-temperature tolerance and genetic potential in wheat (*Triticum aestivum* L.): response to photoperiod, vernalization, and plant development. *Planta* **224**(2), 360–366.
Linina, A. & Ruza, A. 2018. The influence of cultivar, weather conditions and nitrogen fertilizer on winter wheat grain yield. *Agronomy Research* **16**(1), 147–156. https://doi.org/10.15159/AR.18.034
Litke, L., Gaile, Z. & Ruža, A. 2018. Effect of nitrogen fertilization on winter wheat yield and yield quality. *Agronomy Research* **16**(2), 500–509. https://doi.org/10.15159/AR.18.064
Petunenko, I., Kalenska, S. & Liebhard, P. 2016. Formation of yield and quality of winter wheat varieties depending of nitrogen nutrition in semiarid climate. *Science Journal NUBiP Ukraine. Series 'Agronomics'* **235**, 9–24 (in Ukrainian).
Santiveri, F., Royo, C. & Romagosa, I. 2004. Growth and yield responses of spring and winter triticale cultivated under Mediterranean conditions. *European Journal of Agronomy* **20**(3), 281–292.
Slafer, A.G., Savin, R. & Sadras, V.O. 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crop Res.* **157**, 71–83.
Wenda-Piesik, A., Holková, L., Solařová, E. & Pokorný, R. 2016. Attributes of wheat cultivars for late autumn sowing in genes expression and field estimates. *European Journal of Agronomy* **75**, 42–49.
Yildirim, M., Koc, M., Akinci, C. & Barutcular, C. 2013. Variations in morphological and physiological traits of bread wheat diallel crosses under timely and late sowing conditions. *Field Crops Res.* **140**, 9–17.

Agronomy Research **18**(1), 194–205, 2020
https://doi.org/10.15159/AR.20.009

Use of pyrophyllite to reduce heavy metals mobility in a soil environment

S. Murtić[1,*], H. Čivić[2], E. Sijahović[2], I. Koleška[3], J. Jurković[4] and M. Tvica[5]

[1]University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Plant Physiology, Zmaja od Bosne 8, BA71000 Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Plant Nutrition, Zmaja od Bosne 8, BA71000 Sarajevo, Bosnia and Herzegovina
[3]University of Banja Luka, Faculty of Agriculture, Department of Plant Physiology and Nutrition, Bulevar vojvode Petra Bojovića 1A, BA78000 Banja Luka, Bosnia and Herzegovina
[4]University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Chemistry, Zmaja od Bosne 8, BA71000 Sarajevo, Bosnia and Herzegovina
[5]University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Pedology, Zmaja od Bosne 8, BA71000 Sarajevo, Bosnia and Herzegovina
[*]Correspondence: murticsenad@hotmail.com

Abstract. This study revealed the effects of pyrophyllite ore materials on heavy metals mobility in soil plots located near the steel mill in Zenica (Bosnia and Herzegovina). The experiment was set up in a randomized block design with four pyrophyllite treatment rates i.e. 0, 200, 400 and 600 kg ha^{-1} in three replications. Analyses of the heavy metals (Cu, Zn, Mn, Ni, Cr, Pb, Cd) in soil and plant samples were performed using atomic absorption spectrophotometry. Pyrophyllite addition in soil was found to reduce the availability of all tested heavy metals in the studied soil. The pyrophyllite addition at a rate of 200 kg ha^{-1} reduced Mn, Cu and Zn available forms in soil by 11.1, 20.4 and 11.2%, respectively, compared with control. The pyrophyllite addition at higher rates i.e. 400 and 600 kg ha^{-1} had an even higher impact on the decrease in Mn and Zn mobility in studied soil in comparison with 200 kg ha^{-1}. Additionally, these pyrophyllite rates have the ability to reduce Ni mobility in studied soil. The study also found a positive effect of all pyrophyllite treatments to reduce heavy metals accumulation in the leaves of potato grown on the studied soil. In sum, the results of this study indicate that pyrophyllite treatment could be an effective technique for improving the environmental quality of soils and alleviating the hazards of heavy metals to plants. However, further studies are necessary to confirm or denied this hypothesis.

Key words: accumulation, clay minerals, soil-plant system.

INTRODUCTION

Aluminosilicate clay minerals such us zeolite, pyrophyllite and bentonite have been widely studied regarding their suitability to be used in retention of heavy metal ions and thus for the protection, improvement and also remediation of soils polluted by heavy metals (Chaves et al., 2015; Lee et al., 2019). These minerals, based on a threedimensional aluminosilicate framework with numerous channels and cavities, have high retention properties which make them very useful in decreasing bioavailability of heavy metals in soils (Jemeljanova et al., 2019). Most scientists have noted that the ability of aluminosilicate minerals to reduce the mobility and thus availability of heavy metals in soils for plants is the result of their high cation exchange capacity, high surface area, and pore volume enabling the entry and retention of heavy metals in their inter layers (Wang & Li, 2011; Uddin, 2017; Xu et al., 2017).

The use of aluminosilicate minerals as heavy metals retention materials has advantages upon many other remediation techniques in terms of their non-toxic nature, low-cost, and high efficiency (Sharma et al., 2018). Among aluminosilicates, one of the least used materials in the soil remediation is pyrophyllite, which is a result of its relatively lower presence in nature, but also insufficient research on its use as a remediation material.

Pyrophyllite belongs to the class of aluminosilicates with form of the 2:1 layer i.e., the pyrophyllite structure consists of an octahedral Al-O layer sandwiched between two opposing tetrahedral Si-O layers. The bonding between these sandwiches is weak resulting with pyrophyllite's softness (Mohs hardness is between 1 and 1.5). Furthermore, the pyrophyllite has high density (2.7 and 2.9 g cm^{-3}), a relatively high cation exchange capacity (between 50 and 70 meq 100 g^{-1}) and a pH ranging from neutral to slightly alkaline. It occurs in all shades of colour, particularly white, grey-white, and greenish pink, depending upon the presence of coloured minerals in pyrophyllite ore (Churakov, 2006).

Over the past several decades, the steel industry in Zenica has been associated with emission of large quantities of harmful substances, affecting negatively the air quality, as well as the quality of the soils in the Zenica area, more precisely their use value. Among harmful substances, heavy metals are of great concern due to its adverse effects on human health (Felix-Henningsen et al., 2010; Imeri et al., 2016). However, the presence of heavy metals in soils does not necessarily predict adverse effects on plants and consequently human health if they do not occur in a quantity above permissible limits established by legislation and in forms that is easily absorbed by plants (Kulokas et al., 2019). The normal range of Cu, Zn, Mn, Ni, Cr, Pb and Cd in leaves of plants are 5–20 mg kg^{-1}, 20–100 mg kg^{-1}, 15–150 mg kg^{-1}, 0.02–50 mg kg^{-1}, 0.1–1 mg kg^{-1}, 0.5–30 mg kg^{-1} and 0.1–2.4 mg kg^{-1}, respectively (Chaney, 1989; Kastori et al., 1997).

The main objective of this study was to examine the ability of pyrophyllite ore materials from Parsovići – Konjic to decrease the availability of heavy metals in soils located near the steel mill in Zenica. An additional goal of this study was to evaluate pyrophyllite efficiency in reducing heavy metals accumulation in leaves of potato grown on these soils. Potato is food crops that are mostly grown in the study area and therefore is selected as the subject of this study.

MATERIALS AND METHODS

Materials

The pyrophyllite ore materials from the deposits 'Parsovići–Konjic' (Bosnia and Herzegovina) were used in this study. The particle size of the pyrophyllite materials used in the experiment was smaller than 500 µm.

The median total SiO_2, Al_2O_3, K, Ca, Mg, Cu, Ni, Zn, Co, Mn, Pb and Cr of pyrophyllite ore materials used in this study were 67.6%, 19.1%, 0.3%, 6.7%, 0.1%, 1.4 mg kg^{-1}, 2.7 mg kg^{-1}, 25.7 mg kg^{-1}, 0.4 mg kg^{-1}, 93.1 mg kg^{-1}, 8.0 mg kg^{-1} and 0.8 mg kg^{-1}, respectively. The results mentioned above were obtained from the Laboratory at the Faculty of Agriculture and Food Sciences University of Sarajevo.

Study area

Three agricultural soil plots in Gradište, north-western suburb of Zenica (44°22'5" N, 17°89'85" E) were chosen as a study area (Fig. 1). The area has a temperate oceanic climate (cool summers and cool but not cold winters), with an average annual mean temperature and rainfall of 11.3 °C and 992 mm, respectively. It typically lacks a dry season as rainfall is dispersed evenly throughout the year.

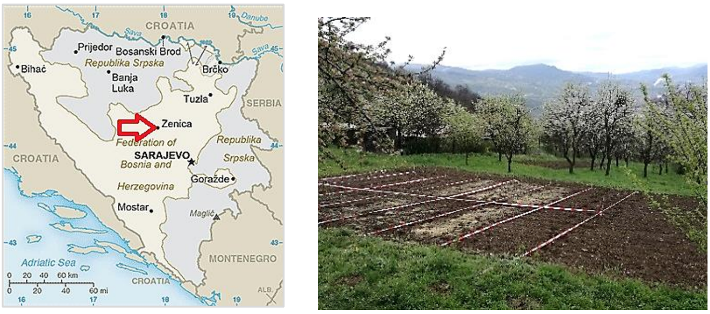


Figure 1. Location map of the study site.

The investigated soil plots had approximately 1,000 m^2 in area and were located at a very close distance from each other (up to 500 m). These soils were chosen for investigation because it is located near the steel mill, but also because the populations in this area are fully or partially engaged in agriculture. Accordingly, the results of this study could provide specific data regarding the adverse impacts of heavy metals emission from steel factory on food crops production in the investigated area. All investigated soils were classified as Eutric Cambisol based on the Word Reference Base for Soil Resources (FAO, 2014). Slightly acid to neutral reaction, medium texture, good physical properties, moderate organic matter content and base saturation of more than 50% is a typical characteristic of this type of soil.

Soil samples from investigated soil plots were collected once in March 2019 from 0–30 cm depth, using clean steel shovel. Five soil samples from each experimental plot were randomly gathered and mixed properly to obtain a composite soil sample. Thereafter, composite soil samples were placed in plastic bags and brought to the laboratory.

The soil samples were air–dried, crushed and ground using porcelain mortar and pestle, passed through a 2 mm and 1 mm sieve and then stored in paper bags until chemical analysis. Soil with highest concentrations of heavy metals (Cu, Zn, Mn, Ni, Cr, Pb, Cd) was used for conducting the experiment.

Experimental design

The experimental soil area was divided into twelve equal plots. Each plot had area 20 m^2 and the size of each unit plot was 5×4 m. The experiment was set up in a randomized block design with four pyrophyllite treatment in three replications. Distances between two plots were 1 m and the blocks were 2 m apart. Experiment treatments were as follows:

1. T_1 – soil without pyrophyllite i.e. control treatment
2. T_2 – soil with pyrophyllite at rate of 200 kg ha^{-1}
3. T_3 – soil with pyrophyllite at rate of 400 kg ha^{-1}
4. T_4 – soil with pyrophyllite at rate of 600 kg ha^{-1}.

Recommended pyrophyllite ore rate was recalculated based on experimental plot area (20 m^2). Pyrophyllite ore materials in all experimental plots were applied seven days before planting potato. All other agrotechnical measures needed for optimum potato growth (pest control measures, irrigation) were performed identically on all experimental plots until the time of potato technological maturity.

Concentrations of plant-available forms of heavy metals in soils and heavy metal concentrations in potato leaves were determined at the end of experiment i.e. potato technological maturity stage. All samples of soils and potato leaves in the experiment area were collected at the same time.

Soil analysis

Before performing experiment, the following soil chemical properties were analysed: soil reaction (pH), organic matter (OM), available forms of phosphorus (available P) and potassium (available K), and total forms of heavy metals (Cu, Zn, Mn, Ni, Cr, Pb, Cd). Available forms of heavy metals in soil samples were determined at the end of experiment.

Soil pH was measured in H_2O and 1 M KCl in a 1:2.5 soil: solution ratio with a Mettler Toledo 320 pH meter. OM was measured by chromic acid digestion method (ISO 14235, 1998) and available forms of phosphorus and potassium by Egnér–Riehm method (Egnér et al., 1960).

Total heavy metals in soil samples were extracted by mineralizing 1 g of dry weight sample with 21 mL aqua regia (HNO_3: HCl 1:3) for 16 h at ambient temperature. Then, the flask solution was heated on hotplate under reflux for 2 h at 180 °C, cooled down to room temperature, filtered through quantitative filter paper into 100 mL flasks and diluted to the mark with deionized water (ISO 11466, 1995).

Plant-available forms of heavy metals in soil samples were extracted by EDTA solution (0.01 M ethylenediaminetetraacetic acid (EDTA) and 1 M $(NH_4)_2CO_3$, adjusted to pH 8.6) as follows: 10 g of air-dried soil was transferred into 100 mL plastic bottle, and then 20 mL EDTA solution was added. The bottle solution was shaken for 30 min at 180 rpm in an orbital shaker and thereafter the extract was filtered through quantitative filter paper into 25 mL flask and diluted to the mark with deionized water (Trierweiler & Lindsay, 1969).

Total and available heavy metal concentrations in soil samples were determined by atomic absorption spectrometry (ISO 11047, 1998) and their content expressed as mg per kg dry weight.

Plant sampling and analysis

Healthy green potato leaves without signs of parasites or disease from each experimental plot were carefully collected at the stage of potato technological maturity in quantity of approximately 200 g. The leaf samples were air-dried and separately powdered with a stainless-steel mill and stored in paper bags until analysis.

Total heavy metals in leaf samples were extracted by mineralizing 1 g of dry weight sample with 14 mL HNO_3 + $HClO_4$ mixture (2.5:1 v/v) for 4 h at ambient temperature. Thereafter, the flask solution was heated on a hotplate for 30 min, cooled down to room temperature, filtered through quantitative filter paper into 50 mL flasks and diluted to the mark with deionized water (Lisjak et al., 2009). Heavy metal concentrations in leaf samples were also determined by atomic absorption spectrometry (ISO 11047, 1998) and their content expressed as mg per g dry weight.

Statistical analysis

All measurements were done in triplicates and the results were presented as mean ± standard deviation. The collected data were analysed statistically using Microsoft Excel 2013 software program, and the significant differences between the variants were determined using *Least Significant Differences test* at 0.05 level of probability ($P \leq 0.05$).

RESULTS

Basic chemical properties of the studied soils

The results of the soil chemical analysis are presented in Table 1.

Chemical analyses showed that the sampled soils from studied were slight acidic to neutral with moderate levels of organic matter and high content of available forms of phosphorus (P_2O_5) and potassium (K_2O). Soil 1 had highest concentrations of heavy metals (Cu, Zn, Mn, Cr, Pb, Cd) and therefore this soil was selected for conducting the experiment.

Table 1. Chemical analysis of soil sample

Parameter	Unit	Soil 1	Soil 2	Soil 3
pH H_2O	pH unit	7.4	7.3	7.4
pH KCl	pH unit	6.7	6.6	6.8
organic matter	%	2.81	2.23	2.48
P_2O_5	mg 100 g^{-1}	20.84	8.08	3.22
K_2O	mg 100 g^{-1}	60.9	30.3	34.9
Cu	mg kg^{-1}	47.1	41.2	40.2
Zn	mg kg^{-1}	53.9	56.7	50.0
Mn	mg kg^{-1}	1,411.9	1,322.1	1,400.3
Ni	mg kg^{-1}	120.2	160.0	155.9
Cr	mg kg^{-1}	31.1	18.0	20.3
Pb	mg kg^{-1}	69.5	54.1	55.0
Cd	mg kg^{-1}	0.25	0.12	0.13

Available forms of heavy metals in the soil after pyrophyllite treatment

The concentrations of available forms of Cu, Zn, Mn, Pb, Ni, Cr and Cd in soil plots (mg kg^{-1} dry weight), depending on the pyrophyllite treatment, are presented in Table 2 and 3. These results are also presented by a histogram 1 and 2 for easer visualization (Fig. 2).

The presented data illustrates the concentrations of available forms of heavy metals in soil after pyrophyllite treatment. The addition of pyrophyllite reduced the availability of all tested heavy metals in the studied soil compared to control treatments but the magnitude of the effect was not the same for all treatments. However, the statistical analysis does not confirm the significant effect of added pyrophyllite on the reduction of Cr availability in soils.

Table 2. Concentrations of available forms of Cu, Zn, Mn and Pb in the studied soil

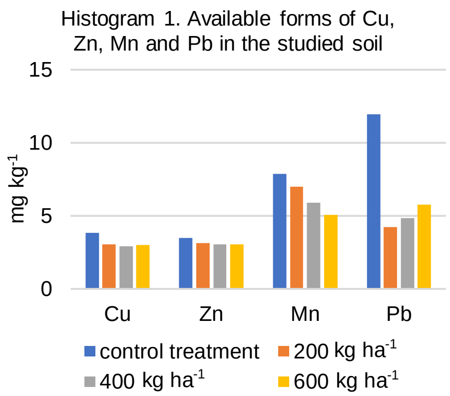
Treatment[1]	Cu	Zn	Mn	Pb
T_1	3.83 ± 0.24[a]	3.49 ± 0.15[a]	7.87 ± 0.25[a]	11.95 ± 1.61[a]
T_2	3.05 ± 0.50[b]	3.10 ± 0.14[b]	7.00 ± 0.26[b]	4.20 ± 0.44[b]
T_3	2.89 ± 0.24[b]	3.02 ± 0.08[b]	5.89 ± 1.35[c]	4.85 ± 1.42[b]
T_4	3.08 ± 0.36[b]	3.01 ± 0.40[b]	5.07 ± 0.78[d]	5.73 ± 2.91[b]
$Lsd_{0.05}$	0.451	0.203	0.713	1.851

Values marked with different letters in the same column indicate significantly differences. [1]Experimental treatment: T_1 – control treatment (without pyrophyllite); T_2 – pyrophyllite at rate of 200 kg ha^{-1}; T_3 – pyrophyllite at rate of 400 kg ha^{-1}; T_4 – pyrophyllite at rate of 600 kg ha^{-1}.

Table 3. Concentrations of available forms of Ni, Cr and Cd in the studied soil

Treatment[1]	Ni	Cr	Cd
T_1	0.37 ± 0.03[a]	0.107 ± 0.02	0.091 ± 0.02[a]
T_2	0.26 ± 0.04[b]	0.106 ± 0.02	0.050 ± 0.01[b]
T_3	0.25 ± 0.05[b]	0.105 ± 0.01	0.048 ± 0.01[b]
T_4	0.24 ± 0.03[b]	0.105 ± 0.03	0.046 ± 0.01[b]
$Lsd_{0.05}$	0.056	-	0.017

Values marked with different letters in the same column indicate significantly differences. [1]Experimental treatment: T_1 – control treatment (without pyrophyllite); T_2 – pyrophyllite at rate of 200 kg ha^{-1}; T_3 – pyrophyllite at rate of 400 kg ha^{-1}; T_4 – pyrophyllite at rate of 600 kg ha^{-1}.



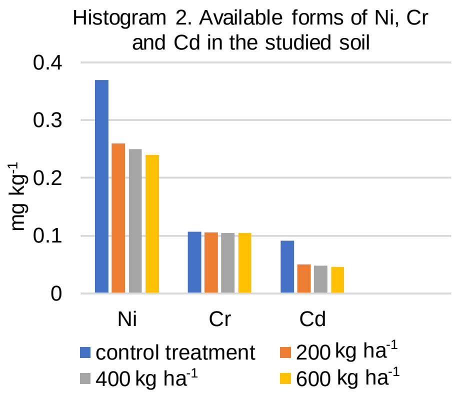


Figure 2. The concentrations of available forms of heavy metals in soil depending on the pyrophyllite treatment.

Heavy metal concentrations in leaves of potato after pyrophyllite treatment

The heavy metal concentrations (Cu, Zn, Mn, Pb, Ni, Cr and Cd) in potato leaves (mg kg^{-1} dry weight), depending on the pyrophyllite treatment, are presented in Table 4 and 5. These results are also presented by a histogram 3 and 4 for easer visualization (Fig. 3).

The presented data have shown that pyrophyllite treatment, regardless of the applied rates, significantly reduced accumulation of heavy metals (Cu, Zn, Mn, Pb, Ni and Cr) in potato leaves as compared to control treatment. The study also found a positive effect of pyrophyllite treatment to reduce Cd accumulation in potato leaves, but these findings did not reach statistical significance.

Table 4. Concentrations of Cu, Zn, Mn and Pb in potato leaves

Treatment[1]	Cu	Zn	Mn	Pb
T_1	19.4 ± 5.6^{a}	44.5 ± 7.2^{a}	177.9 ± 69.2^{a}	18.0 ± 1.7^{a}
T_2	12.5 ± 1.0^{b}	30.0 ± 4.6^{b}	78.3 ± 26.1^{b}	5.4 ± 2.9^{b}
T_3	12.0 ± 2.9^{b}	28.1 ± 8.3^{bc}	71.5 ± 10.7^{b}	5.8 ± 1.8^{b}
T_4	11.2 ± 0.7^{b}	24.6 ± 1.7^{c}	63.7 ± 11.1^{b}	3.8 ± 2.6^{b}
$Lsd_{0.05}$	2.94	5.24	35.34	2.14

Values marked with different letters in the same column indicate significantly differences. [1]Experimental treatment: T_1 – control treatment (without pyrophyllite); T_2 – pyrophyllite at rate of 200 kg ha^{-1}; T_3 – pyrophyllite at rate of 400 kg ha^{-1}; T_4 – pyrophyllite at rate of 600 kg ha^{-1}.

Table 5. Concentrations of Ni, Cr and Cd in potato leaves

Treatment[1]	Ni	Cr	Cd
T_1	10.6 ± 4.7^{a}	1.81 ± 0.32^{a}	0.24 ± 0.1
T_2	3.0 ± 2.1^{b}	0.93 ± 0.36^{b}	0.24 ± 0.1
T_3	2.5 ± 1.2^{b}	0.91 ± 0.61^{b}	0.23 ± 0.1
T_4	2.1 ± 1.7^{b}	0.85 ± 0.24^{b}	0.20 ± 0.2
$Lsd_{0.05}$	2.50	0.35	–

Values marked with different letters in the same column indicate significantly differences. [1]Experimental treatment: T_1 – control treatment (without pyrophyllite); T_2 – pyrophyllite at rate of 200 kg ha^{-1}; T_3 – pyrophyllite at rate of 400 kg ha^{-1}; T_4 – pyrophyllite at rate of 600 kg ha^{-1}.

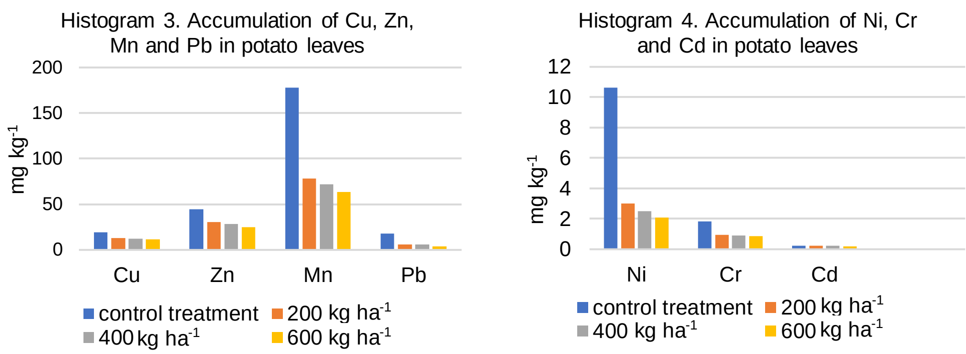


Figure 3. Accumulation of heavy metals in soil depending on the pyrophyllite treatment.

DISCUSSION

This paper is an attempt to describe and evaluate the efficiency of pyrophyllite ore material to decrease the mobility of heavy metals in soil and thus their availability for plants. The results of this study demonstrate the high potential of pyrophyllite to immobilize heavy metals in studied soil, regardless of the amount of applied material. These findings are generally in line with previous studies (Kim et al., 2013; Park et al., 2017; Rath et al., 2017).

In the present study, the addition of pyrophyllite was especially successful in reducing Pb and Cd mobility in soil. The addition of pyrophyllite at rate of 600 kg ha^{-1} reduced Pb and Cd available forms in soil by 52.1 and 49%, respectively, compared with control treatment. The effect of pyrophyllite at rate of 200 and 400 kg ha^{-1} on the decrease Pb and Cd mobility in soil was also highly significant. These results are in agreement with results obtained by Singh et al. (2016).

Considering the soils polluted by Pb and Cd may pose hazards to food crops and consequently human health (Tchounwou et al., 2012; Alle et al., 2016; Borgulat et al., 2018; Luo et al., 2019), the results mentioned above provide important scientific information in an effort to improve soil protection and quality by applying pyrophyllite. Our opinion is pyrophyllite has the potential to be included in the inventory of efficient soil remediation techniques, especially because the use of pyrophyllite for soil remediation is simple to operate, cost effective and very reliable.

Osacky et al. (2015) reported that the ability of pyrophyllite and other clay minerals to reduce the mobility of heavy metals in soils is the result of their potential to form complexes with heavy metals in their inter layers or on surface areas. The pyrophyllite primarily binds heavy metals in the space between the layers, due to their large pore volume (Ismadji et al., 2015). The efficiency of pyrophyllite to immobilize heavy metals in soil also depends on their dosage, surface area as well as soil physical and chemical properties.

Furthermore, the speciation or chemical form of the element play an important role in in the fixation of heavy metals on the pyrophyllite surface. Namely, heavy metals often have different levels of mobility depending on the specific metal oxidation state (Violante et al., 2010). For example, Cr (III) is, in general, much less toxic and mobile in soils than Cr (VI), and therefore reactions that reduce Cr (VI) to Cr (III) are of great importance for soil remediation.

Although numerous studies confirm that pyrophyllite has high efficiency in removing heavy metals from the soil and water (Prasad & Saxena, 2008; Chawla et al., 2018; Panda et al., 2018), the heavy metal binding mechanisms on pyrophyllite are not completely clear. Scheidegger et al. (1996) have attempted to explain the binding mechanisms between Ni and pyrophyllite surfaces. They reported pH is the primary factor that controls Ni binding on pyrophyllite. The study found that Ni sorption on pyrophyllite, in the lower pH region (i.e., $pH < 7$), increased with decreasing ionic strength. Contrary, Ni sorption on pyrophyllite, in the higher pH region ($pH > 7$ with high Ca^{2+} level), was slower. These results can be explained by the fact that Ni with other ions (primarily Ca^{2+}) compete for the same free sites on the pyrophyllite adsorptive complex. Similar results were reported by Gou et al. (2018).

The results of this study also showed that pyrophyllite addition at a rate of 400 and 600 kg ha^{-1} in soil has the ability to reduce Ni mobility in studied soil (neutral to weakly basic), indicating the pyrophyllite possesses some mechanisms for Ni retention even in the higher pH region. Zhao et al. (2017) noted the inner-sphere complexes between Ni and pyrophyllite surface areas (with no intervening water molecules) were dominant mechanisms for Ni retention.

In the present study, pyrophyllite also demonstrated the high potential to immobilize Mn, Cu and Zn in studied soil. Namely, the pyrophyllite addition at a rate of 200 kg ha^{-1} reduced Mn, Cu and Zn available forms in soil by 11.1, 20.4 and 11.2%, respectively, compared with control treatment (without pyrophyllite). The pyrophyllite addition at higher rates i.e. 400 and 600 kg ha^{-1} had an even higher impact on the decrease in Mn and Zn mobility in studied soil in comparison with 200 kg ha^{-1} pyrophyllite rate. These findings indicate that pyrophyllite ore material used in this research is potentially useful additive to bind Mn and Zn ions in soils and that their mobility in soil decreases with increasing pyrophyllite rates.

However, a better understanding of the pyrophyllite sorption mechanism could make a significant contribution to improving pyrophyllite efficiency to reduce heavy metals mobility in soils. An interesting data related to pyrophyllite is the fact that pyrophyllite has the ability to easily disperse in water, enabling a higher area of pyrophyllite exposure in the soil and thus its activity (El Gaidoumi et al., 2019), and finally pyrophyllite could increase soil pH, thus resulting in less heavy metal mobility (Newton & Sposito, 2015). All the above-mentioned scientific data are undoubtedly associated with its sorption mechanism.

As shown in Table 3, all pyrophyllite treatments were also significantly reduced the accumulation of heavy metals (Cu, Zn, Mn, Ni, Cr, Pb and Cr) in the leaves of potato grown on the studied soil. In addition, the pyrophyllite treatments reduced Cd accumulation in potato leaves, but these effects were not statistically significant. The results also showed that 600 kg ha^{-1} pyrophyllite rate had the highest effect on the reduction of heavy metals accumulation in potato leaves. This treatment reduced the accumulation of Cu, Zn, Mn, Ni, Cr, Pb and Cr in the potato leaves by 42.1, 44.5, 64.2, 80.0, 52.8, 78.9 and 17.8%, respectively, compared to control treatment. The effect of 200 and 400 kg ha^{-1} pyrophyllite rate was also significant but less pronounced.

Generally, the results of this study demonstrated that the addition of pyrophyllite in tested soil reduced heavy metals mobility, thus resulting with low accumulation of heavy metals in potato leaves. One exception to this general rule was the behaviour of Cr in soil- plant system. Namely, the pyrophyllite treatments did not significantly reduce the concentration of Cr available forms in studied soil at the end of experiment, but significantly reduced its accumulation in the leaves of potato grown in the same soil plots. This inconsistent result could be potentially attributed to Cr speciation. As is well known, the Cr mobility in soil can change drastically depending on change in soil pH, redox potential, microbial activity, content of organic matter, content and type of clay minerals etc. Accordingly, changes in Cr chemical forms (speciation) are possible in a relatively short-term, and thus its bioavailability (Huang et al., 2018). We assume that Cr mobility in soil in the initial stages of potato growth were higher, resulting in higher Cr uptake by the potato. However, further studies are necessary to confirm or denied this hypothesis as well as other hypotheses presented in this study.

CONCLUSIONS

Pyrophyllite addition in soil was found to reduce the availability of tested heavy metals in the studied soil, indicating that pyrophyllite treatment could be an effective technique to stabilize soils polluted by heavy metals. Under experimental conditions of the study, the pyrophyllite addition at a rate of 200 kg ha^{-1} reduced Mn, Cu and Zn available forms in soil by 11.1, 20.4 and 11.2%, respectively, compared with control. The pyrophyllite addition at higher rates i.e. 400 and 600 kg ha^{-1} had an even higher impact on the decrease in Mn and Zn mobility in studied soil in comparison with 200 kg ha^{-1}. Additionally, the accumulation of all tested heavy metals in leaves of potato grown on soil plots treated with pyrophyllite was found to be lower than those in non-treated plots, indicating that pyrophyllite can also alleviate the risk for plants associated with heavy metals.

ACKNOWLEDGEMENTS. This study was financially supported by the Environmental Fund of the Federation of Bosnia and Herzegovina.

REFERENCES

Alle, V., Kondratovics, U., Osvalde, A. & Vikmane, M. 2016. Differences in cadmium accumulation and induced changes in root anatomical structures in plants used for food. *Agronomy Research* **14**(S2), 1249–1260.

Borgulat, J., Mętrak, M., Staszewski, T., Wiłkomirski, B. & Suska-Malawska, M. 2018. Heavy Metals Accumulation in Soil and Plants of Polish Peat Bogs. *Polish Journal of Environmental Studies* **27**(2), 537–544.

Chaney, R.L. 1989. Toxic element accumulation in soils and crops: Protecting soil fertility and agricultural foodchains. In Bar-Yosef, B., Barrow, N.J. & Goldshmid, J. (ed.): *Inorganic contaminants in the Vadose Zone*. Springer, Berlin, pp. 140–158.

Chaves, L.H.G, Tito, G.A. & Chaves, B. 2015. Bentonite Application in the Remediation of Zinc Contamination Soil. *Chemical Engineering Transactions* **58**, 745–750.

Chawla, A., Prasad, M., Goswami, R., Ranshore, S., Kulshreshtha, A. & Sinha, A.S.K. 2016. Kinetic model for sorption of divalent heavy metal ions on low cost minerals. *Korean Journal of Chemical Engineering* **33**(2), 649–656.

Churakov, S.V. 2006. Ab Initio Study of Sorption on Pyrophyllite: Structure and Acidity of the Edge Sites. *The Journal of Physical Chemistry B* **110**(9), 4135–4146.

Egnér, H., Riehm, H. & Domingo, W.R. 1960. Studies on the chemical soil analysis as a basis for the assessment of the nutrient status of soils. II. Chemical extraction methods for determination of phosphorus and potassium. *Kungliga Lantbrukshögskolans Annaler* **26**, 199–215 (in German).

El Gaidoumi, A., Doña-Rodríguez, J.M. & Melián, E.P. 2019. Mesoporous pyrophyllite-titania nanocomposites: synthesis and activity in phenol photocatalytic degradation. *Research on Chemical Intermediates* **45**(2), 333–353.

Felix-Henningsen, P., Urushadze, T., Steffens, D., Kalandadze, B. & Narimanidze, E. 2010. Uptake of heavy metals by food crops from highly-polluted Chernozem-like soils in an irrigation district south of Tbilisi, eastern Georgia. *Agronomy Research* **8**(1), 781–795.

FAO. 2014. 'International soil classification system for naming soils and creating legends for soil maps.' Word Soils Resources Reports, Rome, Italy, 2014. Available at: http://www.fao.org/3/i3794en/I3794en.pdf

Gou, W., Siebecker, M.G., Wang, Z. & Li, W. 2018. Competitive sorption of Ni and Zn at the aluminum oxide/water interface: an XAFS study. *Geochemical Transactions* **19**(1), 9.

Huang, W., Jiao, J., Ru, M., Bai, Z., Yuan, H., Bao, Z. & Liang, Z. 2018. Localization and Speciation of Chromium in Coptis chinensis Franch. using Synchrotron Radiation X-ray Technology and Laser Ablation ICP-MS. *Scientific Reports* **8**(1), 8603.

Imeri, R., Kullaj, E., Duhani, E. & Millaku, L. 2016. Impact of rootstock on heavy metal bioaccumulation in apple plant grown near an industrial source in Obiliq, Kosovo. *Agronomy research* **17**(1), 100–110.

Ismadji, S., Soetaredjo, F.E. & Ayucitra, A. 2015. *Clay Materials for Environmental Remediation*. SpringerBriefs in Green Chemistry for Sustainability. Springer, UK, 113 pp.

ISO 11047. 1998. 'Determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc - Flame and electrothermal atomic absorption spectrometric methods.' International Organization for Standardization, Geneva, Switzerland. Available at: https://www.iso.org/standard/24010.html

ISO 11466. 1995. 'Extraction of trace elements soluble in aqua regia.' International Organization for Standardization, Geneva, Switzerland. Available at: https://www.iso.org/standard/19418.html

ISO 14235. 1998. 'Soil quality – Determination of organic carbon in soil by sulfochromic oxidation.' International Organization for Standardization, Geneva, Switzerland. Available at: https://www.iso.org/standard/23140.html

Jemeljanova, M., Ozola, R. & Klavins, M. 2019. Physical-chemical properties and possible applications of clay minerals and humic acid composite materials. *Agronomy research* **17**(S1), 1023–1032. doi.org/10.15159/AR.19.019

Kastori, R., Petrović, N. & Arsenijević-Maksimović, I. 1997. Heavy metals and plants. In: Kastori, R. (ed): *Heavy Metals in the Environment*, pp. 195–257 (in Serbian).

Kim, J., Lee, C., Park, J., Kang, J., Choi, N. & Kim, S. 2013. Use of pyrophyllite clay for fluoride removal from aqueous solution. *Desalination and Water Treatment* **51**(16), 3408–3416.

Kulokas, M., Zaleskas, V., Pedišius, N., Praspaliauskas, M. & Buinevičius, K. 2019. Properties of biofuel fly ash and capabilities of its use for agricultural needs. *Agronomy research* **17**(2), 540–550. doi.org/10.15159/ar.19.051

Lee, D.S., Lim, S.S., Park, H.J., Yang, H.I., Park, S.I., Kwak, J.H. & Choi, W.J. 2019. Fly ash and zeolite decrease metal uptake but do not improve rice growth in paddy soils contaminated with Cu and Zn. *Environment International* **129**, 551–564.

Lisjak, M., Spoljarevic, M., Agic, D. & Andric, L. 2009. *Practical Plant Physiology*. Faculty of Agriculture, Osijek, 25 pp. (in Croatian).

Luo, K., Liu, H., Zhao, Z., Long, J., Li, J., Jiang, C. & Rao, C. 2019. Spatial Distribution and Migration of Cadmium in Contaminated Soils Associated with a Geochemical Anomaly: A Case Study in Southwestern China. *Polish Journal of Environmental Studies* **28**(5), 3799–3807.

Newton, A.G. & Sposito, G. 2015. Molecular Dynamics Simulations of Pyrophyllite Edge Surfaces: Structure, Surface Energies, and Solvent Accessibility. *Clays and Clay Minerals* **63**(4), 277–289.

Osacky, M., Geramian, M., Ivey, D.G., Liu, Q. & Etsell, T.H. 2015. Influence of Nonswelling Clay Minerals (Illite, Kaolinite, and Chlorite) on Nonaqueous Solvent Extraction of Bitumen. *Energy Fuels* **29**(7), 4150–4159.

Panda, L., Rath, S.S., Rao, D.S., Nayak, B.B., Das, B. & Misra, P.K. 2018. Thorough understanding of the kinetics and mechanism of heavy metal adsorption onto a pyrophyllite mine waste based geopolymer. *Journal of Molecular Liquids* **263**, 428–441.

Park, J., Kang, J. & Kim, S. 2017. Comparative Analysis of Bacteriophages and Bacteria Removal in Soils and Pyrophyllite-Amended Soils: Column Experiments. *Water, Air & Soil Pollution* **228**(3), 103.

Prasad, M. & Saxena, S. 2008. Attenuation of divalent toxic metal ions using natural sericitic pyrophyllite. *Journal of Environmental Management* **88**(4), 1273–1279.

Rath, S.S., Singh, S., Rao, D.S., Nayak, B.B. & Mishra, B.K. 2017. Adsorption of heavy metals on a complex Al-Si-O bearing mineral system: Insights from theory and experiments. *Separation and Purification Technology* **186**, 28–38.

Scheidegger, A.M., Sparks, D.L. & Fendorf, M. 1996. Mechanisms of Nickel Sorption on Pyrophyllite: Macroscopic and Microscopic Approaches. *Soil Science Society of America Journal* **60**(6), 1763–1772.

Sharma, S., Tiwari, S., Hasan, A., Saxena, V. & Pandey, L.M. 2018. Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils. *3 Biotech.* **8**(4), 216.

Singh, S., Jena, S.K. & Das, B. 2016. Application of pyrophyllite mine waste for the removal of cadmium and lead ions from aqueous solutions. *Desalination and Water Treatment* **57**(19), 8952–8966.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. & Sutton, D.J. 2012. Heavy metal toxicity and the environment. *EXS* **101**, 133–164.

Trierweiler, J.E. & Lindsay, W.L. 1969. EDTA-ammonium carbonate soil test for zinc. *Soil Science Society of America Proceedings* **39**, 49–54.
Uddin, M.K. 2017. A review on the adsorption of heavy metals by clay minerals, with special focus on the past decade. *Chemical Engineering Journal* **308**, 438–462.
Violante, A., Cozzolino, V., Perelomov, L., Caporale, A.G. & Pigna, M. 2010. Mobility and bioavailability of heavy metals and metalloids in soil environments. *Journal of Soil Science and Plant Nutrition* **10**(3), 268–292.
Wang, X.L. & Li, Y. 2011. Measurement of Cu and Zn adsorption onto surficial sediment components: New evidence for less importance of clay minerals. *Journal of Hazardous Materials* **189**(3), 719.
Xu, Y., Liang, X., Xu, Y., Qin, X., Huang, Q., Wang, L. & Sun, Y. 2017. Remediation of heavy metal-polluted agricultural soils using clay minerals: A review. *Pedosphere* **27**(2), 193–204.
Zhao, X., Qiang, S., Wu, H., Yang, Y., Shao, D., Fang, L., Liang, J., Li, P. & Fan, Q. 2017. Exploring the Sorption Mechanism of Ni (II) on Illite: Batch Sorption, Modelling, EXAFS and Extraction Investigations. *Scientific Reports* **7**, 8495.

Agronomy Research **18**(1), 206–215, 2020
https://doi.org/10.15159/AR.20.074

Aphid complex associated with potato in agro-climatic conditions of Kosovo

F. Musa[1], D. Krasniqi[2] and S. Musa[1,*]

[1]University of Prishtina, Faculty of Agriculture and Veterinary, Plant Protection Department, Boulevard Bill Clinton p.n. Prishtina, XK10000 Prishtina, Republic of Kosovo
[2]Phytosanitary Corporate Sara&Meti sh.p.k. Prishtina. Analytical, Food and Agriculture Laboratory, Street Kamer Loshi No.76. XK10000 Prishtina, Republic of Kosovo
[*]Correspondence: sarandamusa1@gmail.com

Abstract. Field surveys for aphids infesting potato crops in three most important localities for potato production in Kosovo (Podujevë, Prishtinë and Vushtrri) were conducted in vegetation seasons of 2018–2019. With the purpose to monitor and confirm the aphid composition in potatoes, aphid leaf count was used as a method which is applied worldwide. Sampling for aphids from the leaves of potato plants was undertaken during cropping season, every 10 days, where 100 leaves were taken at random and checked in triplicates, from each field. During of this experimental work, the following aphid species were registered *Myzus persicae*, *Macrosiphum euphorbiae*, *Aphis nasturtii* and *Aulacorthum solani*. From the total number of aphids recorded (4,210 individuals), the following percentage belongs to the different aphids: *M. persicae* (57.24%), *A. nasturtii* (25.72%), *A. solani* (7.77%), *M. euphorbiae* (5.68%), and other none identified aphids (3.59%). Relying on the results of this research we have confirmed to exist statistical significant differences with regard to the number of aphid's species according to the localities and aphids that infests the potatoes. With the goal to manage and control aphids spread infields with potatoes, it is extremely useful to evaluate and to see the possibility for an Integrated Management against these pests at farmer level.

Key words: aphids, ANOVA, *Myzus persicae*, potato crop.

INTRODUCTION

The potato is a vegetable of the family *Solanaceae*, together with tomato, eggplant, pepper, tobacco, etc. (Hooker, 1986), and its origin is South America. According to Forghani et al. (2018) potato was seen as one among the best vegetables, which is consumed by human beings in daily regime.

Potatoes are produced commercially in all provinces in Kosovo. The yield and dry matter of tubers of the potatoes is affected by environmental conditions as well their changes (Eremeev et al., 2003).

During the vegetation season, potato is being attacked by several harmful organisms that cause considerable damages to this crop. The major pests of potato are aphids, whiteflies, potato tuber moth, white grubs, Colorado beetle, nematodes, leafhoppers and thrips (Hiiesaar et al., 2006; Kruus, 2012; Kumara et al., 2017). Among the insect pests, aphids play a major role by transmitting a number of viral diseases causing qualitative and quantitative losses (Sigvald, 1990; Woodford, 1992).

Potato is seriously affected by different aphids among which *M. persicae* is extremely important. Potato production not just in Kosovo but worldwide is affected and at risk by damages caused from *M. persicae* as a very harmful insect. The direct damage is feeding on the plants and the indirect damages caused to potato planting materials as a vector of several viruses (Lapointe et al., 1987; Trivedi et al., 2002; Musa et al., 2004). *M. persicae* is known to transmit over 100 viruses in different plants (Raman, 1985).

All aphids that are known so far possess piercing-sucking type of the mouthparts. Some aphid's possess wings and can fly, while the others are wingless and cannot fly. Aphids, during their feeding activities, may cause a considerable damage on plants, but they are of even greater economic significant since they are very efficient to transmit viruses and play the role as a vector of viruses to the plants (Close & Lamb, 1961; DiFonzo et al., 1997; Davis et al., 2007; Vučetić et al., 2013; Avila et al., 2014). In this aspect the alatae (winged forms) are more important than the apterae (wingless forms). According to Woodford (1992) there are above 30 species of alate aphids transmit potato virus Y, whereas *M. persicae* is extremely efficient vector of viruses namely potato leafroll. Regarding potato seed production, in Netherlands is reported that viruses are mainly transmitted by aphids such as *M. persicae* and *A. nasturtii* (Struik & Wiersema, 1999).

The most present and abundant species in potato fields are *M. persicae, A. solani*, *M. euphorbiae*, but there are other aphids as well.

The aphidofauna in different countries of the world has been reported to be very ample and different. According to (Stufkens & Teulon, 2001) in New Zealand the aphid fauna of potato crops has been dominated by three species, *M. persicae*, *A. solani*, and *M. euphorbiae,* whereas in Iran around 14 species of aphids have been recorded and identified as harmful pests on potato fields such as *M. persicae, Aphis gossypii, Aphis craccivora, Therioaphis trifolii, Acyrthosiphon pisum* and *M. euphorbiae* (Rezvani, 2001; Rezvani, 2010). Some authors (De Bokx & Piron, 1990) reported that during their surveys from 1983–1987 in Netherlands 122 aphid species were confirmed in potato fields. On the other side Muthomi et al. (2009) reported that the most abundant species in water pan traps were *M. euphorbiae* as well *M. persicae*.

Darwish (2018) showed that potato cultivars tested had significant variation regarding their susceptibility against infestation by aphids as well other sucking insects.

Regarding to these pests, nymphs and adults feed on young and soft parts of the plants. They insert stylet in soft tissues of the potatoes and feed with plant juices. This feeding causes distortion mainly the leaves and tubers. Aphids secrete also substances that are sticky and are called 'honeydew'. This sweet substance attracts ants and sooty fungi which can discolor the plant making them undesirable for their sale.

The purpose of our work has been to investigate the most spread aphids and relative abundance of these pests in potatoes cultivated in three main important production regions of Kosovo.

MATERIALS AND METHODS

Localities

Field surveys for aphids infesting potato crops in three most important localities for potato production in Kosovo (Podujevë, Prishtinë and Vushtrri) were undertaken in the 2018–2019 growing season. Podujevë is situated at 42°55' latitude, 21°12' longitude, and at the altitude of 620 m above sea level. Prishtina is situated at 42°40' latitude, 21°90' longitude, and at the altitude of 597 m above sea level, whereas Vushtrri has the following geographical parameters: latitude 42°46', longitude 21°04' and altitude of 500 m.

Potato cultivar

The potato cultivar Agria from Netherlands was included in the experiment in three respective localities were the surveys was undertaken. To evaluate and verify the aphids in potatoes, aphid leaf count was used as a method which is widely applied in different countries in the world (Raman, 1985; Sigvald, 1990).

Method of sampling

With the aim to investigate and identify aphid species, from the leaves of potato crop, aphid sampling was performed every 10 days, during the entire vegetation season. In this case 100 leaves were checked in three replications, from potatoes of each experimental plot. The results obtained and shown below in Table 2 are the average numbers for potato aphids recorded in both years of this research. Every sample consisted of a look on the leaves of a potato crop by walking through the field with potato in a W pattern, as well sampling the edges of the paddock. Potato leaves of all positions were examined and all aphids found were collected.

Laboratory work

The samples were provided with a label that contained all relevant data, the date of sampling, locality, and number of the field and were brought to the Crop Protection Laboratory which is a part of the Faculty of Agriculture to identify and describe the aphid species. In the laboratory, winged and wingless aphids were differentiated (immature forms were not distinguished), whereas aphids the immature forms were identified by taking into account their proximity and similarity to the adults they were collected with. Aphids sampled from the leaves were kept and preserved in 80% alcohol and later on were prepared for future work as slide mounted. After that plant aphids were identified to species level with the use of a stereo binocular microscope using keys (Teulon, 1999; Teulon et al., 1999; Blackman & Eastop, 1984). For precise and proper identification of the aphids there were taken in consideration mainly the following morphological aspects: the shape, the size as well as the segments number in antennae, distribution of the veins in the wings, shape of cauda and cornicles, number and length of hair on the legs and cauda, etc.

Statistical analysis

The results obtained were processed statistically using ANOVA two-ways and LSD of 1% and 5%, with MSTA-C software from the University of Michigan, while data processing was done through Microsoft Office 2007.

Meteorological data

Taking into the account that temperature and rainfalls have great impact on aphid appearance and distribution, we decided to collect meteorological data from the metrological stations based in Podujevë, Prishtinë and Vushtrri (Table 1).

Table 1. Meteorological data during vegetation period (2018–2019)

Month	Podujevë		Prishtinë		Vushtrri	
	Temperature (ºC)	Precipitate (mm)	Temperature (ºC)	Precipitate (mm)	Temperature (ºC)	Precipitate (mm)
April	13.2	45.6	14.3	52.7	12.8	62.4
May	15.9	69.3	16.1	60.4	15.0	71.5
June	19.1	59.5	16.8	55.3	16.2	58.7
July	23.4	49.2	23.2	64.5	22.7	67.1
August	21.7	41.7	22.5	39.2	20.5	35.6
September	17.8	44.3	15.8	54.8	16.2	63.2
October	16.5	57.1	13.5	62.6	13.3	60.8
Average	18.2	52.4	17.5	55.6	16.7	59.9

From the Table 1, the values represents the average temperatures during the two growing seasons (2018–2019) and are as follows: 18.2 °C recorded in Podujevë, 17.5 °C in Prishtinë and 16.7 °C in Vushtrri.

As it is shown in the Table 1 the average of rainfall ranged from 52.4 mm in Podujevë, to 55.6 mm in Prishtinë and 59.9 mm in Vushtrri.

RESULTS AND DISCUSSIONS

During this experimental work, regarding to the appearance and aphid distribution in potatoes there were identified four aphid species: mostly *M. persicae* as well *A. nasturtii*, but to some extent also *M. euphorbiae* as well *A. solani* (Table 2).

Table 2. Distribution of the aphids in potato crop, growing season 2018–2019

Locality	Aphid species	Frequency of aphid sampling												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
Podujevë	*M. persicae*	34	47	39	29	46	51	38	29	40	33	27	21	434
	A. nasturtii	11	25	21	15	19	14	10	8	23	16	9	14	185
	A. solani	7	15	9	7	6	9	11	3	4	10	7	5	93
	M. euphorbiae	3	9	14	9	5	7	13	19	8	7	5	2	101
	Other	3	7	6	3	4	1	5	2	2	4	3	5	45
Prishtinë	*M. persicae*	26	39	57	40	36	49	55	43	38	26	18	31	458
	A. nasturtii	9	14	34	16	12	10	9	15	9	14	21	9	172
	A. solani	2	6	12	5	9	4	11	3	8	2	9	2	73
	M. euphorbiae	6	4	8	5	3	9	6	4	7	5	2	6	65
	Other	8	3	1	3	2	2	7	5	2	2	2	4	41
Vushtrri	*M. persicae*	59	82	68	109	137	244	183	122	97	213	108	96	1,518
	A. nasturtii	32	43	41	35	52	146	98	45	32	127	54	21	726
	A. solani	17	28	25	9	16	22	14	3	9	12	4	2	161
	M. euphorbiae	10	16	13	5	7	5	2	1	6	4	3	1	73
	Other	6	9	5	6	2	4	4	3	5	11	2	8	65

Note: These values are the average of aphids from two years of investigations (2018–2019).

Based on the result we have obtained (the average number of aphids during two years of investigation per each sampling date) we can see that the number and distribution of different aphids was very heterogenic (Table 2).

During of these surveys it was confirmed that the time and frequency of appearance of different aphids throughout the monitoring season was different, depending to localities and climatic conditions, so the highest or maximum number recorded of these pests, almost of all aphids was confirm in May (Table 2). Even the other group of aphids, which we did not manage to determine precisely, but were recorded during these surveys, was present in different periods of time in different localities and has had various frequencies throughout the vegetation period, causing considerable damages to the potato crop.

Of the total number of plant aphids found, the largest numbers were wingless in relation to winged forms (Table 3). The relationship between wingless and wingless forms also varied. The smallest ratio was found in other species of plant aphids in the locality Vushtrri (2.42) and the highest in *M. persicae* in Podujevo (32.38) while the average ration regardless of the aphid species was 8.44. Winged forms, though smaller in numbers, are more dangerous, especially in potato seed production, due to the transmission of virus diseases. The winged aphids fly very well but are also helped by the wind and quickly are spread over long distances thus spreading viral diseases, so winged forms of plant aphids are largely responsible to spread the viruses. According to various authors worldwide aphids as vectors of potato viruses transmit them in two agronomically relevant ways: either persistently or none persistently. According to Struik & Wiersema (1999), not all aphids can transmit potato viruses. On the other hand, some viruses can be transmitted by many different aphids, whereas other viruses require specific vectors.

Table 3. Aphid ratio records between alata and aptera

Locality	Aphid species	Aphids		Ratio aptera/ alata
		Aptera	Alata	
Podujevë	*M. persicae*	421	13	32.38
	A. nasturtii	157	28	5.61
	A. solani	72	21	3.43
	M. euphorbiae	85	16	5.31
	Other	38	7	5.43
Prishtinë	*M. persicae*	413	45	9.18
	A. nasturtii	145	27	5.37
	A. solani	54	19	2.84
	M. euphorbiae	49	16	3.06
	Other	37	4	9.25
Vushtrri	*M. persicae*	1,392	126	11.05
	A. nasturtii	659	67	9.84
	A. solani	140	21	6.67
	M. euphorbiae	56	17	3.29
	Other	46	19	2.42
Total		3,764	446	8.44

There are also large differences among aphids species with regard to the efficiency with which they transmit viruses. A highly efficient species is *M. persicae*, while species such as *A. nasturtii* is 2 times less efficient than M. persicae or *M. euphorbiae* is 10 times less efficient than *M. persicae*, and hence are much less threatening (Struik & Wiersema, 1999).

From the total numer of aphids collected (4,210 individuals) in Vushtrri were recorded 2,543 individuals (60.40%). The number of plant aphids in the Podujevë and Prishtina sites was approximately similar, whith 859 individuals (20.38%) and 809 (19.22%) individuals recorded (Fig. 1).

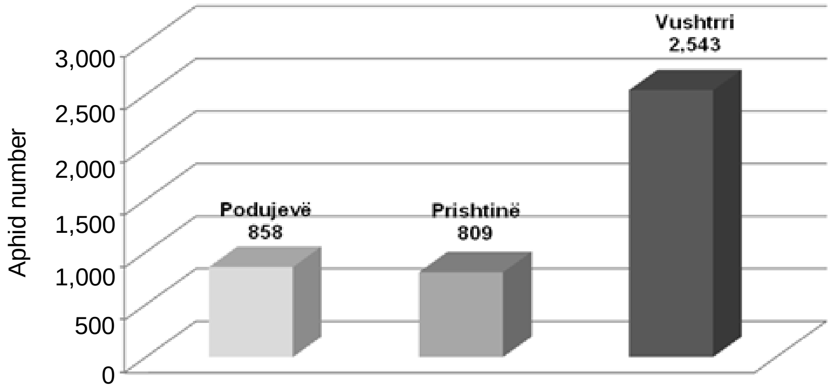


Figure 1. Number of aphid species during vegetation season 2018–2019.

The highest number of aphids present in the locality of Vushtrri according to our opinion is because of several factors, (a) potato mono-cropping pattern, where traditionally in this locality the potato crop is grown in monoculture, year by year in the same field, with consequent favorable conditions to the over-wintering of almost all aphid species, (b) nitrogen application in high doses, which stimulates plant growth, (c) a mild climate compared to the other localities, (d) low altitude level with higher aphid pressure compared with other sites, and (e) insecticides use with broad spectrum from the farmers which is in this locality very often at high rates that have great impact to predators and other parasites of aphids as well. It was proved by Jansson & Smilowitz, 1986 that population of *M. persicae* tend to increase with increasing levels of nitrogen application, while Barlow (1962) reported the correlation of weather parameters and the level of aphids. Aphid specific predators like syrphids, coccinellids and chrysopids are considered of particular importance to decrease aphid populations. In this respect Zamani et al. (2007) reported also for the importance of two parasitoids *Aphidius matricariae* as well *Aphidius colemani*.

The results obtained, with regard to the aphids in potatoes, in the three localities where this crop was cultivated, revealed that both *M. persicae* as well *A. nasturtii* are most prevalent species among the aphids recorded (Fig. 2). From the total number of aphid species collected, as an average of two years of survey, from the leaves of potato plants (4210 aphids) *M. persicae* participated with 2,410 individuals (57.24%), *A. nasturtii* with 1,083 individuals (25.72%), *A. solani* with 327 individuals (7.77%) and *M. euphorbiae* with 239 individuals (5.68%). Other aphid species, presented as other participated with 151 individuals (3.59%). Our results are approximately in line with the results of the other authors who confirmed the highest presence of these aphids in potato crop (Miln, 1978; Berlandier, 1997; Stufkens & Teulon, 2001).

During of these surveys it was shown that *M. persicae* tends to colonize the lower senescing leaves of potato plants, whereas *M. euphorbiae* and *A. solani* are found mainly on new leaves of the potato plants. Regardless the species of plant aphids present or plant part where they were concentrated, the damage was considerable. During their feedings these pests with their stylet or piercing mouth apparatus extracted nutrients from the affected parts of the plants, especially from the leaves, resulting in increased plant deformations, twisting and plant stagnation. These attacks of plant tissues from these pests finally leaded to a decrease the quality as well the yield of potato plants. Concerning the plant aphids that where present and the damages caused to potato plants, there is a tremendous work worldwide by various authors and the results of our research are approximately similar to the data reported by these researchers (Musa et al., 2004; Vučetić et al., 2013; Avila et al., 2014).

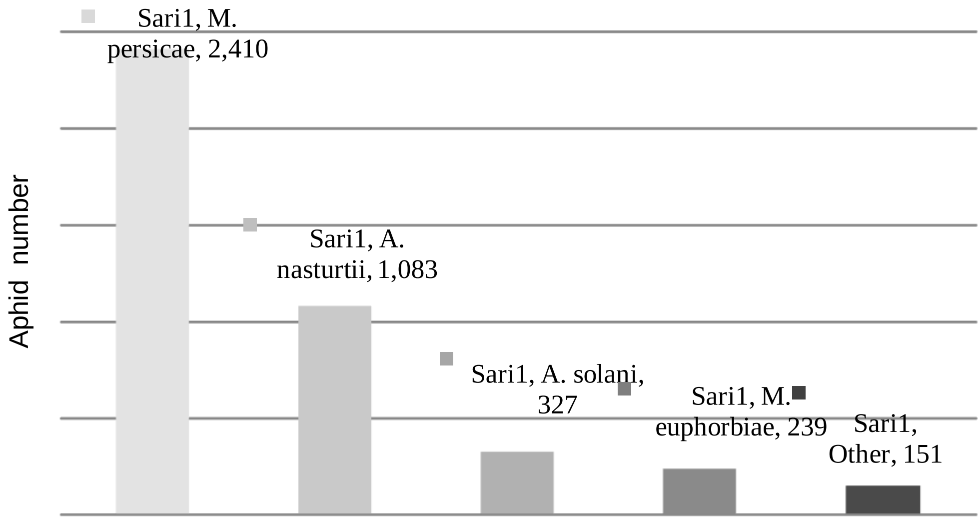


Figure 2. Aphid species composition in potato crop in three localities.

From the table of analysis of variances and LSD (Table 4), it is shown that significant differences were confirmed between three localities where the potato was cultivated (Podujevë, Prishtinë and Vushtrri).

Table 4. Comparison of aphid frequency in potato crop, ANOVA

Localities (A)	Aphid species (B)					Average (A)
	M. persicae	*A. nasturtii*	*A. solani*	*M. euphorbia*	Other	
Podujevë	36.17	15.42	7.75	8.42	3.75	14.30*
Prishtinë	38.17	14.33	6.08	5.41**	3.42	13.48**
Vushtrri	126.50**	60.50	13.42	6.08	5.42	42.38**
Average (B)	66.94**	30.08**	9.08 Ns	6.64 Ns	4.19**	Interaction A × B**
Factor		A	B	B × A		A × B
LSD	0.01	12.8795	10.3128	20.8130		17.8623
	0.05	9.4757	7.8345	13.5698		15.5404

The highest number of plant aphids, regardless aphid species, as an average of two years, was recorded in the locality Vushtrri (42.38 aphids), whereas the lowest number of aphids was recorded in the locality Prishtinë (13.48 aphids). The differences observed in terms of the number of aphids in Vushtrri compared to the other two localities were proved to be highly significant, while no statistically significant differences were found between the two other localities (Podujevë and Prishtinë). In this regard we can state that potato crop cultivated in the locality of Vushtrri has been the most affected by plant aphids whereas potato cultivated in other two localities has been less affected by aphids.

Statistically highly significant differences similarly were shown regarding to aphid species recorded (Factor B). In fact, regarding the aphids the highest number was recorded with *M. persicae* (66.94 aphids), while the lowest number of individuals was confirmed to *M. euphorbiae* (6.64). The differences, concerning to aphid numbers, recorded between *M. persica*e to *A. nasturtii*, also the differences between these two aphids and other species recorded during these researches, are statistically highly significant (Table 4), whereas between *A. solani* and *M. euphorbiae* these differences statistically were confirm to be no significant.

With respect to interaction of factors A × B (locality × aphid species), there were recorded statistically significant differences at different level, concerning the aphid numbers in the leaves of potatoes. In this respect, for example, the highest number of aphids was recorded for *M. persica* in Vushtrri (126.5 aphids) whereas the lowest one for *M. euphorbiae* in the locality of Prishtinë (5.41 aphids).

CONCLUSIONS

Based on these researches conducted during two years of experimental work with regard to aphid distribution in potato crop cultivated in agro climatic condition of Kosovo, the following conclusions might be drawn: Plant aphids are very common in potato crop cultivated in different agro-climatic condition in Kosovo. The results obtained showed that *M. persicae* as well *A. nasturtii* were the most prevalent aphid species found in potato crop. Regarding the other two species, *A. solani* also *M. euphorbiae* they were registered in potato crop as well. During of these surveys we have found that *M. persicae* tends to colonize the lower senescing leaves of potato plants, whereas *M. euphorbiae* and *A. solani* are found mainly on new leaves of the potato plants. Aphids, during their feedings, may cause a considerable damage on plants, but they are of even greater economic significant since they are very efficient to transmit viruses and have the role as a vector of viruses to the plants. The time as well the frequency of certain types of aphids in potato was different throughout the vegetation season, causing considerable damage to this crop. Based on the ANOVA and testing with LSD, highly significant statistical differences were shown among the localities included in the experiment regarding aphids recorded there. The highest number of aphids was recorded in Vushtrri and the smallest number in Prishtina and Podujevë. Significant differences also were shown to exist as well regarding the other aphids. The highest number of plant aphids, almost to all species, was confirm at the beginning of summer (June-July), when necessarily measures should be applied to prevent potato damages that might be caused by aphids. With the aim to get rid such a pest from potato crop, it would be extremely useful to investigate the opportunity of an Integrated strategy for Pest Management at farmer level.

ACKNOWLEDGEMENTS. We would like to thank the experts and technicians at Faculty of Agriculture and Veterinary, from the Prishtina University for their help and assistance in the laboratory and field work. We utilize the opportunity to thank the farmers who permitted us to establish and run the experiments in their fields.

REFERENCES

Avila, A.L., Vera, M.A., Ortego, J., Willink, E., Ploper, L.D. & Conci, V.C. 2014. Aphid (Hemiptera: Aphididae) diversity in potato production areas in Tucumán, Argentina. *Florida Entomologist* **97**(4), 1284–1289.

Barlow, C.A. 1962. The influence of temperature on the growth of experimental populations of *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) (Aphididae). *Canadian Journal of Zoology* **40**(2), 146–156.

Berlandier, F.A. 1997. Distribution of aphids (Hemiptera: Aphididae) in potato growing areas of Southwestern Australia. *Australian Journal of Entomology* **36**, 365–375.

Blackman, R.L. & Eastop, V.F. 1984. *Aphids on the world's crops: Identification and information guide.* John Wiley Sons, London. p. 476.

Close, R. & Lamb, K.P. 1961. Trapping study of some winged aphid vectors of plant virus diseases in Canterbury, New Zealand. *New Zealand Journal of Agricultural Research* **4**(5–6), 606–618.

Darwish, A.A.E. 2018. Variations in the Susceptibility of some Potato (*Solanum tuberosum* L.) cultivars to Infestation with certain piercing sucking insect pests. *Journal of Plant Protection and Pathology, Mansoura Univ.*, **9**(12), 849–853.

Davis, J.A., Radcliffe, E.B. & Ragsdale, D.W. 2007. Resistance to green peach aphid, *Myzus persicae* (Sulzer), and potato aphid, *Macrosiphum euphorbiae* (Thomas), in potato cultivars. *American Journal of Potato Research* **84**, 259–269.

De Bokx, J.A. & Piron, P.G.M. 1990. Relative efficiency of a number of aphids species in the transmission of potato virus Y^N in the Netherlands. *Net. J. Pl. Path*. **96**, 237–246.

DiFonzo, C.D., Ragsdale, D.W., Radcliffe, E.B., Gudmestad, N.C. & Secor, G.A. 1997. Seasonal abundance of aphid vectors of potato virus Y in the Red River Valley of Minnesota and North Dakota. *Journal of Economic Entomology*. **90**(3), 824–831.

Eremeev, V., Jõudu, J., Lõhmus, A., Lääniste, P. & Makke, A. 2003. The effect of pre–planting treatment of seed tubers on potato yield formation. *Agronomy Research* **1**(2), 115–122.

Forghani, S.H., Hassani, F., Ahadiyat, A. & Rezvani, A. 2018. Comparison of important aphids population on seed potato farms. *Journal of Entomology and Zoology Studies* **6**(1), 410–416.

Hiiesaar, K., Metspalu, L., Joudu, J. & Jogar, K. 2006. Over–wintering of the Colorado potato beetle (Leptinotarsa decemlineata Say) in field conditions and factors affecting its population density in Estonia. *Agronomy Research* **4**(1), 21–30.

Hooker, W.J. 1986. *Compendium of potato diseases.* St. Paul, Minnesota 55121, USA, 125 pp.

Jansson, R.K. & Smilowitz, Z. 1986. Influence of Nitrogen on population parameters of potato insects: abundance, population growth, and within-plant distribution of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). *Environmental Entomology* **15**, 49–55.

Kruus, E. 2012. Impact of trade on distribution of potato rot nematode (*Ditylenchus destructor*) and other plant nematodes. *Agronomy Research* **10**(1–2), 319–328.

Kumara, B.B., Kalleshwaraswamy, C.M., Shahid, A., Kadian, M.S. & Venkataravanappa, V. 2017. Species composition and population dynamics of aphids influencing potato virus Y (PVY) incidence in Karnataka. *Journal of Entomology and Zoology Studies* **5**(6), 1242–1246.

Lapointe, S.L., Tingey, W.M. & Zitter, T.A. 1987. Potato virus Y transmission reduced in an aphid-resistant potato species. *Phytopathology* **7**(6), 819–822.

Miln, A.J. 1978. Potato aphids in the Manawatu. *Proceedings of the 31st New Zealand Weed and Pest Control Conference:* pp. 79–84.

Musa, F.M., Carli, C., Susuri, L.R. & Pireva, I.M. 2004. Monitoring of *Myzus persicae* (Sulzer) in potato fields in Kosovo. *Acta agriculturae slovenica* **83**(2), 379–385.

Muthomi, J.W., Nyaga, J.N., Olubayo, F.M., Nderitu, J.H., Kabira, J.N., Kiretai, S.M., Aura, J.A. & Wakahiu, M. 2009. Incidence of Aphid-Transmitted Viruses in Farmer-Based Seed Potato Production in Kenya. *Asian Journal of Plant Sciences* **8**, 166–171.

Raman, K.V. 1985. *Transmission of potato viruses by aphids*. International Potato Center, Lima. Peru. Technical information bulletin 2.

Rezvani, A. 2001. *Key to the aphids (Homoptera: Aphidinea) in Iran.* 1th Ed, Agricultural Education press, Karaj, Iran. 10–15.

Rezvani, A. 2010. *Aphids (Homoptera: Aphidoidea) of herbaceous plants in Iran.* 1th Ed, Mehr Gol Press, Teheran, Iran, 564 pp.

Sigvald, R. 1990. Aphids on Potato Foliage in Sweden and their Importance as Vectors of Potato Virus Y , *Acta Agriculturae Scandinavica* **40**(1), 53–58.

Struik, P.C. & Wiersema, S.G. 1999. *Seed potato technology*. Wageningen Pers, Wageningen. The Netherlands, 383 pp.

Stufkens, M.A.W. & Teulon, D.A.J. 2001. Aphid species on potato crops in Canterbury. *New Zealand Plant Protection* **54**, 235–239.

Teulon, D.A.J. 1999. *Illustrated multiple–entry key for winged aphids in New Zealand*. Crop & Food Research Report No. 614. Crop & Food Research, Lincoln, New Zealand.

Teulon, D.A.J., Eastop, V.F., Stufkens, M.A.W. & Harcourt, S.J. 1999. *Illustrated multiple entry key for apterous aphids of economic importance in New Zealand.* Cropinfo Confidential Report No.612. Crop & Food Research, Lincoln, New Zealand, p. 140.

Trivedi, T.P., Paul Khurana, S.M., Puri, S.N., Bhar, L.M., Mehta, S.C., Jain, R.C., Singh, G., Chaudhari, S.M., Mohasin, M., Dhandapani, A. & Chona, N. 2002. Development of forewarning system of potato aphid (Myzus persicae) on potato (Solanum tuberosum) in India. *Indian Journal of Agricultural Sciences* **72**(6), 341–345.

Vučetić, A., Vukov, T., Jovičić, I. & Petrović-Obradović, O. 2013. Monitoring of aphid flight activities in seed potato crops in Serbia. *ZooKeys*. (**319**), 333–346.

Woodford, J.A.T. 1992. Virus transmission by aphids in potato crops. *Netherlands Journal of Plant Pathology* **98**, 47–54.

Zamani, A.A., Talebi, A., Fathipour, Y. & Baniameri, V. 2007. Effect of temperature on life history of Aphidius colemani and Aphidius matricariae (Hymenoptera: Braconidae), two parasitoids of Aphis gossypii and Myzus persicae (Homoptera: Aphididae). *Environmental Entomology* **36**, 263–271.

Agronomy Research **18**(1), 216–227, 2020
https://doi.org/10.15159/AR.20.038

Analysis of the selective value of promising *Melissa officinalis* L. subsp. *altissima* (Smith.) Arcang variety

N.V. Nevkrytaya[1], V.S. Pashtetsky[1], I.A. Novikov[1], N.N. Petrishina[1], A.V. Mishnev[1] and A.I. Repetskaya[2]

[1]Research Institute of Agriculture of Crimea (RIAC), 150 Kievskaya street, 295493 Simferopol, Republic of Crimea
[2]V.I. Vernadsky Crimean Federal University, Prospekt Vernadskogo 4, 295007 Simferopol, Republic of Crimea
[*]Correspondence: nevkritaya@mail.ru

Abstract. The aim of this research was to study a promising MD 1–17 *Melissa officinalis L. subsp. altissima* (Smith.) Arcang. variety sample obtained in the collection of the gene pool of the Research Institute of Agriculture of Crimea (RIAC) in comparison with Krymchanka (selected in the RIAC) and Lada (selected in the All-Russian research Institute of medicinal and aromatic plants(VILAR) varieties of lemon balm *M. officinalis* L. subsp. *officinalis*. In 2017–2019, in the Department of Essential Oil and Medicinal Plants of the RIAC,a competitive variety trial of lemon balm varieties was conducted in accordance with the methodological recommendations for the selection of essential-oil plants. A territory where this study was conducted belongs to one of the five agroclimatic regions – the upper piedmont, warm, not enough humid; to the northern subarea with moderately mild winters. Weather conditions during the years of competitive variety trial varied significantlya fact that allowed assessing the adaptability of studied variety samples and forecasting the nature of productivity potential realization in different growing conditions. As a result, it was found that MD 1–17 variety sample significantly exceeds other varieties in terms of yield of fresh raw materials, on average, by 62.2 and 77.4%, and in yield of air-dried raw materials, on average, by 32.2 and 52.2%, respectively. In terms of obtaining essential oil from air-dried raw materials, this variety sample exceeds the best in this parameter Crimean variety Krymchanka by 56.3%. Basic components of its essential oil are caryophyllene (25.3–35.9%) and germacrene D (17.7–31.2%) with almost complete absence or insignificant amount of citral (0.1–7.3%); the proportion of latter in essential oils of Krymchanka and Lada varieties can reach 36.6% or more. Novelty of this study includes the creation of the first variety of a new promising essential oil plant –*M. altissima*. Raw materials of this variety and products of its processing may be of interest for different ways of use, including the perfumery and cosmetics industry, for food purposes as a component of tea compositions, etc.

Key words: essential oil plants, *Melissa officinalis*, *Melissa altissima*, competitive variety trial, variety, variety sample, essential oil, productivity parameters, morphological and biological parameters.

INTRODUCTION

Melissa officinalis L .is among the promising, high demanded plants due to the essential oil contained in its raw materials and other valuable extractive substances that determine its use in perfumes, cosmetics, pharmaceuticals, food industries, and medicine Voytkevich, 1999; Moradkhani et al., 2010; Alekseeva, 2011; Papoti et al., 2019). Developing of source breeding material is carried out both by classic methods, and modern biotechnological ones (Egorova & Stavtseva, 2016; Yakimova & Egorova, 2019). Source material for breeding work includes collection samples. In the collection of essential-oil and medicinal plants of the Institute, along with the registered varieties of lemon balm *Melissa officinalis* L. subsp. *officinalis,* there is a sample of *Melissa altissima* - *M. officinalis* L. subsp. *altissima* (Smith.) Arcang. A preliminary study of the collection sample showed that *M. altissima*is characterized by a higher yield of green mass, has a medicinal component composition of essential oils that differs from *M. officinalis*, and may be of interest both for the perfumery and cosmetics industry and for using as a component of tea compositions for food purposes. *M. altissima* significant antipsoriatic in vivo activity open considerable therapeutic capabilities against this severe autoimmune disease (Ulianych et al., 2019).

In selective breeding studies of high-potential species (subspecies) of plants that were previously not used for cropping, a comparative test of breeding samples with varieties of related species (subspecies) is often carried out (Dimas et al., 2020).

The aim of this research is to study morphological and biological parameters and productivity parameters of promising MD 1–17*M. M.officinalis* subsp. *altissima* variety sample in comparison with Krymchanka (selected in the Research Institute of Agriculture of Crimea (RIAC)) and Lada (All-Russian research Institute of medicinal and aromatic plants (VILAR)) varieties of M. *officinalis* subsp. *officinalis*. Selection trials are carried out in the Department of Essential Oils and Medicinal Plants of the (RIAC), aimed at creating highly productive varieties of a number of essential-oil plants.

MATERIALS AND METHODS

The study was conducted in 2017–2019 in the Department of Essential Oils and Medicinal Plants of the RIAC. The experimental site was located in the eastern part of Crimea (village Krymskaya Roza, Belogorsky district). The climate of this region is moderately continental. This area belongs to one of the five agroclimatic regions –upper piedmont, warm, not enough humid; to the northern subarea with moderately mild winters (Savchuk, 2006). According to long-term meteorological observations, average annual air temperature in the village Krymskaya Roza is 10 °C. Period with positive air temperature lasts for 292 days a year. Average temperature of the warmest month, July, is +21 °C, that of the coldest, January, is –0.8 °C. Temperature maximum in summer up to 40 °C and minimum in winter down to – 30–35 °C is possible. Average long-term amount of precipitation is 498 mm, during the growing season – 280 mm. Average annual humidity is 70%. Hydrothermal coefficient is 0.91 what indicates arid weather conditions. Soil at the study site is southern carbonate heavy loamy chernozem (pH – 7.0–7.2, humus content in arable layer – 2.7–3.0%, total nitrogen – 0.12%, phosphorus – 0.10%, potassium – 1.0%).

A nursery for competitive variety trial (CVT) was established on April 05, 2017. Seedlings of tested variety samples were obtained by vegetative propagation (rooting of green cuttings). The length of double row plots was 5 m, space between rows – 0.6 m, plot area – 6 m^2. The number of plants in the plot was 34 (17 plants per row). The experiment was carried out in three replications.

Registration and analysis of basic morphological and biological parameters (plant height and diameter, ratio of fresh and air-dried mass of raw materials, structure of fresh and air-dried raw materials), productivity parameters (productivity of fresh and air-dried raw materials, amount of obtained essential oil) and biochemical parameters (content of essential oil in fresh and air-dried raw materials) was carried out in full blossom phase in accordance with the guidelines for essential-oil plants (Biochemical methods…, 1972; Selection of essential-oil plants, 1977).

Chromatographic analysis of the composition of essential oil was carried out using Crystal 5000.2 gas chromatograph under the following technical conditions: carrier gas –helium A grade; type of detector – flame ionization; capillary column CR-WAXms 30 m×0.32 mm; film thickness in stationary phase – 0.5 μm; detector temperature – 250 °C; evaporator temperature – 230 °C; carrier gas flow rate – 1.9 mL min^{-1}. Starting temperature of the column is 75 °C with an exposure of 1 minute; heating rate 4 °C min^{-1}; final column temperature 220 °C without exposure; analysis duration – 37.3 min; split ratio 1:20. Components were defined by comparing the chromatographic profiles of essential oil (fingerprints method) after preliminary analysis of samples using Agilent Technologies 6890N gas chromatograph/mass spectrometer with Agilent 5973N mass selective detector and Crystal 5000.2 chromatograph under the same chromatographic conditions (Zenkevich et al., 2003; Leontiev et al., 2006).

Statistical processing of the obtained data was performed according to B.A. Dospekhov using software package Microsoft Office Excel 2010 (Dospekhov, 2012).

RESULTS AND DISCUSSION

Weather conditions during the years of competitive variety trial varied significantly. It should be noted that contrasting conditions are very useful for selection trials since they allow evaluating the adaptability of studied variety samples and forecasting the nature of productivity potential realization in different growing conditions. As one can see in Fig. 1, in the years of conducting the competitive trial, during the period from plant growth to blooming phase, temperature conditions of all months, except for April, exceeded long-term average values (norm). Maximum temperature in April-May when vegetal mass was actively growing was noted in 2018. The highest temperatures in June were in 2019.

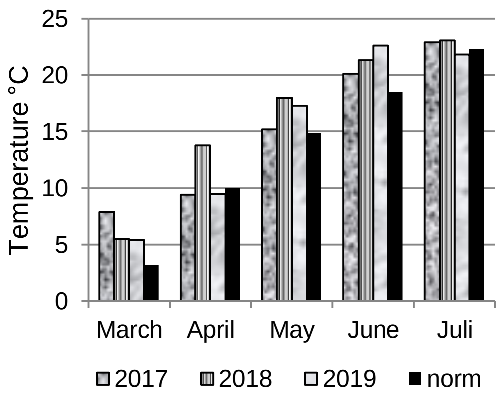


Figure 1. Temperature conditions (°C) of the periods of active vegetation, 2017–2019.

The period of active vegetation during the years of study was the most contrasting by the amount of precipitation (Fig. 2).

The maximum amount of precipitation significantly exceeding long-term average values (norm) of April and May was in 2017. Conditions in 2019 were arider, but in 2018, this period turned out to be extremely hot and arid. The amount of precipitation registered in May 2018 was due to single productive rain (30 mm) in the first decade. The difference in weather conditions led to different periods of time required for the ontogenesis phases.

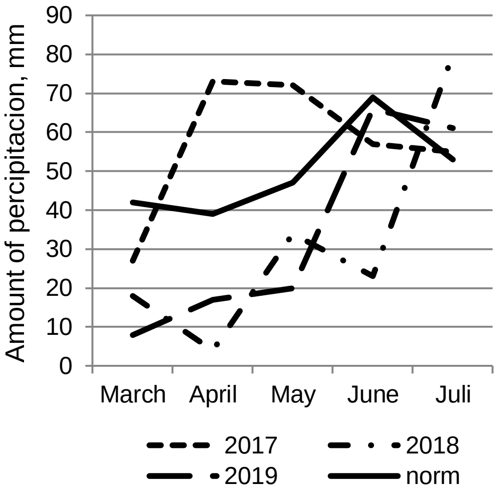


Figure 2. Amount of precipitation (mm) during the period of active vegetation, 2017–2019.

The nursery was founded on April 05, 2017. Measurements were performed during the blossom period, on July 10–11. In 2018, the start of plant growth was noted on March 05. Blooming occurred much earlier than in the previous year due to extreme weather conditions. The measurements were conducted were performed on June 15 (*M. officinalis*) and June 21 (*M. altissima*). In 2019, growing was noted on April 26, and count was performed on July 01.

Analysis of morphological and biological parameters of the plants of studied samples showed that Krymchanka and Lada lemon balm varieties are similar in plant height which is, on average, 49.9 and 55.4 cm, respectively (Table 1). Plants of MD 1–17 sample have a significantly greater height with the average value of 82.0 cm what is 27–32 cm higher than that of plants of the compared varieties.

When drying fresh raw materials of both varieties, its weight decreases, on average, by 2.8 times. The weight of air-dried raw materials of MD 1–17 sample is 3.5 times less than the weight of fresh raw materials.

As a result of analysis of the structure of raw materials, it was found that the weight of fresh raw materials without stems (leaves and inflorescences) in varieties is 69.5% of the total weight, and in MD 1–17 sample it is less, on average, by 11%. The difference between these parameters for air-dried weight is 6.5%.

The loss of more moisture during drying of fresh raw materials, as well as a smaller proportion of inflorescences and leaves in the structure of raw materials in MD 1–17 sample are due to the more massive stem.

M. officinalis subsp. *altissima* plants significantly exceed *M. officinalis* subsp. *officinalis* in height and have a denser leaf blade. Higher quantitative parameters of plants can be associated with different ploidy of subspecies. In a number of literary sources, it is specified that the chromosome set of *M. officinalis* subsp. *altissima* includes 64 chromosomes and that of *M. officinalis* subsp. *officinalis* – 32 chromosomes (Miceli et al., 2006; Kittler et al., 2015). It is known that polyploidy causes an increase in a number of quantitative parameters (plant height, leaf thickness, etc.) (Javadian et al., 2017; Vershinina et al., 2017; Wang et al., 2017; Aqafarini et al., 2019).

Table 1. Characteristics of morphological parameters of lemon balm variety samples. CVT, 2017–2019

Variety sample	Bush height, cm	Bush diameter, cm	Ratio of air-dried weight of raw materials to the weight of fresh raw materials, %	Share of inflorescences and leaves in the total weight of fresh raw materials, %	Share of inflorescences and leaves in the total weight of air-dried raw materials, %
2017					
Krymchanka	46.4	61.9	33.4	78.5	65.3
Lada	47.4	64.4	35.6	78.4	64.9
MD 1–17	70.9	60.7	21.0	66.3	64.9
$LSD_{0.5}$	3.4	3.9			
2018					
Krymchanka	53.0	73.0	37.0	65.7	59.1
Lada	62.3	76.0	37.5	66.6	60.6
MD 1–17	86.7	84.0	34.8	55.5	50.5
$LSD_{0.5}$	7.4	10.1			
2019					
Krymchanka	51.1	62.3	33.9	64.3	62.4
Lada	55.4	60.9	34.6	63.1	61.3
MD 1–17	88.8	75.5	28.8	53.6	51.9
$LSD_{0.5}$	4.3	6.0			
Average for 2017–2019					
Krymchanka	49.9	65.7	34.8	69.5	62.3
Lada	55.4	67.1	35.9	69.4	62.3
MD 1–17	82.0	77.3	28.2	58.5	55.8
$LSD_{0.5}$byfactor A (sample)	3.6	4.4			
$LSD_{0.5}$by factor B (year)	3.6	4.4			

Studying productivity parameters revealed the absence of significant differences between Krymchanka and Lada varieties in terms of yield of both fresh and air-dried raw materials (Table 2).

The lowest yield of raw materials was registered in the 1st year of trial (year of nursery foundation). The total yield of fresh raw materials of MD 1–17 variety sample in 2017 did not significantly differ from that of Lada and exceeded the yield of Krymchanka.

The samples did not differ in the yield of air-dried raw materials.

The productivity of fresh and air-dried raw materials of MD 1–17 variety sample without stems significantly exceeded the yield of varieties only in the third yearwhat is due to a high proportion of stems in the total weight.

It should be noted that the yield of raw materials of both Krymchanka and Lada variety did not differ in the 2nd and 3rd years of vegetation while that of MD 1–17 variety sample was the highest in the 3rd year of vegetation.

In addition to more active growth of vegetativebiomass due to the increased age of plants, different weather conditions during these years had a significant effect on yield. Genotype and year showed the equal effect on the formation of fresh raw materials (proportion of influence was 0.4 and 0.4), and for the air-dried weight of raw material, the influence of weather conditions during a year was more significant compared with this of genotype (proportion of influence was 0.6 and 0.16, respectively).

Table 2. Characteristics of economically valuable parameters of lemon balm varieties during the years of competitive variety trial (CVT)

Variety sample	Parameter	Green crop, t ha^{-1}		Weight content of essential oil, %			Amount of obtained essential oil, kg ha^{-1}	
		fresh raw materials	air-dried raw materials[1]	in fresh raw materials	in absolutely dried raw materials	in air-dried raw materials	from fresh raw materials	from air-dried raw materials
2017								
Krymchanka	total	6.93	2.34	0.046	0.144	0.192	3.15	4.49
Lada	weight	8.32	2.96	0.000	0.000	0.062	0.00	1.23
MD 1–17	of raw	10.48	2.21	0.027	0.082	0.137	2.80	3.01
$LSD_{0.5}$	materials	**2.84**	**0.90**	**0.010**	**0.030**	**0.080**	**2.03**	**1.88**
Krymchanka	weight	5.44	1.52	0.083	0.262	0.392	4.30	5.85
Lada	without	6.36	1.91	0.029	0.092	0.146	1.89	2.83
MD 1–17	stems	6.96	1.43	0.087	0.267	0.291	6.08	4.17
$LSD_{0.5}$		**2.54**	**0.38**	**0.050**	**0.140**	**0.120**	**2.37**	**2.22**
2018								
Krymchanka	total	12.22	4.55	0.046	0.151	0.083	5.73	3.86
Lada	weight	13.56	5.06	0.013	0.041	0.050	1.76	2.53
MD 1–17	of raw	18.33	6.39	0.038	0.121	0.117	6.97	7.45
$LSD_{0.5}$	materials	**2.83**	**1.21**	**0.020**	**0.060**	**0.030**	**3.04**	**2.46**
Krymchanka	weight	8.28	2.67	0.100	0.329	0.200	8.19	5.28
Lada	without	9.06	3.06	0.053	0.179	0.092	4.85	2.82
MD 1–17	stems	10.11	3.28	0.063	0.200	0.258	6.37	8.46
$LSD_{0.5}$		**2.43**	**0.78**	**0.030**	**0.100**	**0.050**	**2.14**	**2.27**
2019								
Krymchanka	total	12.11	3.80	0.027	0.087	0.113	3.16	4.29
Lada	weight	12.33	4.27	0.000	0.000	0.046	0.00	1.96
MD 1–17	of raw	26.67	7.67	0.015	0.047	0.121	3.93	9.24
$LSD_{0.5}$	materials	**2.65**	**0.16**	**0.028**	**0.089**	**0.010**	**3.38**	**1.24**
Krymchanka	weight	7.83	2.37	0.054	0.183	0.162	4.19	3.83
Lada	without	7.78	2.62	0.020	0.070	0.079	1.59	1.82
MD 1–17	stems	14.27	3.98	0.073	0.233	0.242	10.42	9.63
$LSD_{0.5}$		**2.32**	**0.68**	**0.011**	**0.040**	**0.041**	**1.12**	**1.54**

[1]Weight of air-dried raw materials was calculated based on the weight loss during drying of raw materials (%) and the ratio of plant parts in air-dried raw materials (%).

Spring 2019 that was colder in comparison to 2018 hindered the formation of a high yield of raw materials expected for the 3rd year of vegetation. In general, according to the results of three years of competitive variety trial, MD 1–17 *M. officinalis* subsp. *altissima* variety significantly exceeded Krymchanka and Lada *M. officinalis* subsp. *officinalis* varieties inproductivity of fresh and air-dried raw materials (Table 3).

Table 3. Comparative analysis of economically valuable parameters of lemon balm varieties, CVT, 2017–2019

Variety sample (factor A)	Year (factor B)	Green crop, t ha^{-1}		Weight content of essential oil, %			Amount of obtained essential oil, kg ha^{-1}	
		fresh raw materials	air-dried raw materials	in fresh raw materials	in absolutely dry raw materials	air-dried raw materials	fresh raw materials	air-dried raw materials
Total weight of raw materials								
M. officinalis subsp. *officinalis*, Krymchanka variety	2017	6.94	2.34	0.046	0.144	0.192	3.15	4.49
	2018	12.22	4.55	0.046	0.151	0.083	5.73	3.86
	2019	12.11	3.80	0.027	0.087	0.113	3.16	4.29
	average	**10.42**	**3.56**	**0.040**	**0.128**	**0.129**	**4.01**	**4.21**
M. officinalis subsp. *officinalis* Lada variety	2017	8.32	2.96	0.000	0.000	0.062	0.00	1.23
	2018	13.56	5.06	0.013	0.041	0.050	1.76	2.53
	2019	12.33	4.27	0.000	0.000	0.046	0.00	1.96
	average	**11.40**	**4.10**	**0.004**	**0.014**	**0.053**	**0.59**	**2.13**
M. officinalis L.subsp. *altissima* (Sm.) Arcang. MD 1–17 variety sample	2017	10.47	2.21	0.027	0.082	0.137	2.80	3.01
	2018	18.33	6.39	0.038	0.121	0.117	6.97	7.45
	2019	26.67	7.67	0.015	0.047	0.121	3.93	9.24
	average	**18.49**	**5.42**	**0.026**	**0.083**	**0.125**	**4.57**	**6.58**
$LSD_{05\ factor\ A}$		1.17	0.44	0.009	0.030	0.020	1.13	0.89
$LSD_{05\ factor\ B}$		1.17	0.44	0.009	0.030	0.020	1.13	0.89
Weight without stems								
M. officinalis subsp. *officinalis*, Krymchanka variety	2017	5.44	1.52	0.083	0.262	0.392	4.30	5.85
	2018	8.28	2.67	0.100	0.329	0.200	8.19	5.28
	2019	7.83	2.37	0.054	0.183	0.162	4.19	3.83
	average	**7.18**	**2.18**	**0.079**	**0.258**	**0.251**	**5.56**	**4.99**
M. officinalis subsp. *officinalis* Lada variety	2017	6.36	1.91	0.029	0.092	0.146	1.89	2.83
	2018	9.06	3.06	0.053	0.179	0.092	4.85	2.82
	2019	7.79	2.62	0.020	0.070	0.079	1.59	2.07
	average	**7.73**	**2.53**	**0.034**	**0.113**	**0.105**	**2.78**	**2.57**
M. officinalis L.subsp. *altissima* (Sm.) Arcang. MD 1–17 variety sample	2017	6.96	1.43	0.087	0.267	0.291	6.08	4.17
	2018	10.11	3.28	0.063	0.200	0.258	6.37	8.46
	2019	14.27	3.98	0.073	0.233	0.242	10.42	9.63
	average	**10.45**	**2.90**	**0.074**	**0.233**	**0.264**	**7.63**	**7.42**
$LSD_{05\ factor\ A}$		0.99	0.25	0.016	0.047	0.032	0.93	0.88
$LSD_{05\ factor\ B}$		0.99	0.25	0.016	0.047	0.032	0.93	0.88

Comparison of variety samples in the content of essential oil should be correctly performed according to the mass fraction of essential oil in absolutely dry and air-dried raw materials (Tables 2, 3). The lowest value of this parameter for all years of trial was typical for Lada variety. In 2017 and 2019, in the total weight of fresh raw materials of this variety, only traces of essential oil were found, and only in extremely hot and arid year such as 2018, the content of essential oil in absolutely dry raw materials amounted to 0.041%.Regarding the other two varieties, the content of essential oil in the total weight of absolutely dry raw materials was higher in 2017 and 2019 in Krymchanka variety in comparison with MD 1–17 variety sample; in 2018, differences between them were unreliable. The content of essential oil in absolutely dry raw materials (without stems which are dead weight since they actually have no secretory reservoirs) in 2017 was equal in these varieties in 2018, it was higher in Krymchanka variety, in 2019 – in MD 1–17 sample.

Differences between these varieties in terms of the content of essential oil in air-dried raw materials in 2017 and 2019 are insignificant, and in 2018, this parameter was higher in MD 1–17 variety.

In general, according to the results of the competitive variety trial, the content of essential oil in the total weight of absolutely dry raw materials was higher in Krymchanka variety. For all other characteristics, the parameters of Krymchanka and MD 1–17 variety sample had no significant difference (Table 3).

Different intensity of essential oil accumulation in fresh raw materials over these years is associated with differences in weather conditions. Favorable conditions for oil-forming process are high air temperature and minimal rainfall. Summer 2018 would most of best correspond to such requirements. But extreme aridity obviously did not allow accomplishing productivity potential of studied varieties in full.

The content of essential oil in air-dried raw materials, as a rule, did not differ over these years regardless of weather conditions. The basic factor influencing essential oil accumulation is the genotype of the sample (proportion of influence was 0.5–0.6).

The highest amount of essential oil from fresh raw materials for all samples was obtained in 2018 with its extreme weather conditions; it happened due to a combination of increased yield and high content of essential oil in raw materials (Table 2, 3). Lack of precipitation and relatively low temperatures in spring 2019 prevented Krymchanka and Lada varieties from forming a high yield of raw materials. Active growth of vegetal mass compared to the previous year was observed just in MD 1–17 variety sample. Intensive rains during blooming period did not contribute to the accumulation of essential oil, despite high temperatures in June.

In general, according to competitive variety trial, Lada variety was characterized by the lowest amount of obtained essential oil (Table 3). MD 1–17 variety sample significantly exceeds Krymchanka variety in the amount of essential oil during processing of fresh raw materials without stems and air-dried raw materials.

An important parameter that determines the value of essential oil and main directions of its use is the composition and content of basic components. Basic components of lemon balm essential oil, as a rule, include:citral, citronellal, geraniol, citronellol, caryophyllene (Belgin et al., 2009; Sharafzadeh et al., 2011; Mesut Uyanik & Bilal Gurbuz, 2014; Efremov et al., 2015). As the comparison of literature data and obtained information showed, we are talking about *M. officinalis* subsp. *officinalis*. However, the ratio of components in essential oil is very unstable and depends on many factors. This statement applies to all essential-oil plants in general, as evidenced by information from many scientific publications (Alban Ibraliu et al., 2011; Morozov et al., 2015; Shelepova& Khusnetdinova, 2018).

When studying the subspecies *M. officinalis* L. subsp. *altissima,* a number of researchers found that basic components of its essential oil are caryophyllene and germacrene D (Van den Berg et al., 1997; Basta et al., 2005; Božović et al., 2018).

During competitive variety trial, the analysis of component composition of essential oil of lemon balm samples was carried out (Table 4).

Table 4. Comparative analysis of component composition of essential oil of lemon balm varieties. CVT, 2017–2019

Variety sample	Raw materials	Year	Caryo-phyllene	Caryophyllene oxide	Citral			Germacrene D
					neral	geranial	total	
Fresh raw materials								
M. officinalis subsp. *Officinalis* Krymchanka variety	total weight	2017	16.4	1.9	14.1	20.6	34.7	15.4
		2018	14.5	2.5	2.4	4.3	8.7	24.4
		2019	19.4	1.6	5.8	11.3	17.1	23.1
	without stems	2017	17.1	2.2	12.6	20.6	33.2	16.9
		2018	18.4	2.1	7.6	12.7	20.3	17.9
		2019	19.5	1.3	9.0	15.6	24.6	22.1
M. officinalis subsp. *officinalis* Lada variety	total weight	2017	19.4	2.4	7.3	11.0	17.3	19.1
		2018[1]	-	-	-	-	-	-
		2019[1]	-	-	-	-	-	-
	without stems	2017	16.6	1.9	14.5	22.1	36.6	11.8
		2018	18.9	6.1	8.9	14.3	23.2	14.9
		2019	17.8	3.1	2.8	7.7	10.5	26.5
M. officinalis subsp. *altissima* MD 1–17 variety sample	total weight	2017	**35.9**	**1.9**	**0.1**	**0.0**	**0.1**	**17.7**
		2018	**30.9**	**3.6**	**0.6**	**0.3**	**0.9**	**22.6**
		2019	**25.3**	**4.4**	**2.4**	**0.6**	**3.0**	**27.7**
	without stems	2017	**29.9**	**2.1**	**1.9**	**3.6**	**5.5**	**29.5**
		2018	**34.3**	**2.7**	**0.5**	**0.6**	**1.1**	**23.0**
		2019	**26.6**	**1.7**	**2.3**	**5.0**	**7.3**	**31.2**
Air-dried materials								
M. officinalis subsp. *officinalis* Krymchanka variety	total weight	2017	18.2	1.8	14.6	21.8	36.4	16.3
		2018	11.9	17.9	3.1	6.1	9.2	12.3
		2019	14.1	16.5	7.2	14.0	21.2	10.6
	without stems	2017	19.0	1.9	18.6	25.9	44.4	13.7
		2018	15.4	13.0	6.9	12.1	19.0	12.0
		2019	15.4	12.8	10.2	18.4	28.6	9.5
M. officinalis subsp. *officinalis* Lada variety	total weight	2017	22.6	2.2	8.4	13.4	21.8	21.1
		2018	10.7	19.4	3.9	6.9	10.8	11.0
		2019	17.4	12.9	6.1	11.9	18.0	13.3
	without stems	2017	18.7	1.7	13.4	19.2	32.6	17.0
		2018	14.4	13.4	9.4	17.4	26.8	9.6
		2019	16.4	11.4	10.0	18.2	28.2	11.0
M. officinalis subsp. *altissima* MD 1–17 variety sample	total weight	2017	**25.3**	**2.4**	**0.9**	**1.1**	**2.0**	**27.6**
		2018	**29.5**	**9.7**	**1.1**	**2.5**	**3.6**	**19.3**
		2019	**26.5**	**7.0**	**2.0**	**0.1**	**2.1**	**29.9**
	without stems	2017	**25.4**	**1.9**	**1.1**	**1.2**	**2.3**	**21.8**
		2018	**31.4**	**7.3**	**1.1**	**2.8**	**3.9**	**21.8**
		2019	**25.2**	**7.6**	**2.0**	**0.1**	**2.1**	**31.2**

[1]Component composition was not defined due to insufficient or lack of essential oil.

Basic component of the essential oil of both varieties of lemon balm (*M. officinalis* subsp. *officinalis*) is citral (a mixture of isomers – E-geranial and Z-neral). Citral content varied significantly from year to year from 8.8 to 36.6%. Based on the data obtained, it can be assumed that the optimal conditions for the accumulation of citral in essential oil are increased humidity and high air temperature.

In addition to citral, essential oil from fresh raw materials of lemon balm varieties also contains caryophyllene (14.5–19.5%) and germacrene D (11.8–26.5%) in significant quantities. The content of these components turned out to be more stable over the years in both varieties.

Component composition of MD 1–17 *M. officinalis* L. subsp. *altissima* variety sample significantly differed from the composition of essential oils in the compared varieties what is in accordance with published data (Efremov et al., 2015; Aqafarini et al., 2019). Its basic components are caryophyllene (25.3–35.9%) and germacrene D (17.7–31.2%) with almost complete absence or insignificant amount of citral (0.1–7.3%) (Table 4).

A comparative analysis of extractives content in the air-dried raw materials of *M. officinalis* of the studied varieties was carried out in the Department of the Processing and Standardization of Essential Oil Raw Materials of the RIAC. In accordance with the current standard (FS 42-3645-98 'Melissa officinalis herb'), at least 22% of extractives should be contained in lemon balm raw materials.In all three varieties Krymchanka, Lada, and MD 1–17, the content of extractives exceeded this value and amounted to 42.3; 39.0 and 28.1%, respectively.

CONCLUSIONS

As a result of the comperative variety trial of MD 1–17 variety sample of *M. officinalis* L. subsp. *altissima* (Smith.) Arcang in comparison with Lada and Krymchanka varieties of *M. officinalis* L. subsp. *officinalis*, it was revealed that the studied variety sample:

– exceeds varieties in the yield of fresh raw materials, on average, by 62.2 and 77.4%, respectively; by the yield of air-dried raw materials, on average, by 32.2 and 52.2%, respectively;

– considering the amount of essential oil from air-dried raw materials, it significantly exceeds the best Krymchankavariety by 56.3%;

– differs from varieties in the composition of essential oil. Its basic components are caryophyllene (25.3–35.9%) and germacrene D (17.7–31.2%) with a small amount of citral (0.1–7.3%); proportion of the latterin the essential oil of Krymchanka and Lada varieties reached a maximum of 36.6%.

Data obtained allow applying for registration of a new lemon balm variety Tavrida.

REFERENCES

Aqafarini, A., Lotfi, M., Norouzi, M. & Norouzi, G.2019. Induction of tetraploidy in garden cress: morphological and cytological changes. *Plant Cell Tiss Organ Cult*. **137**(3), 627–635. doi: 10.1007/s11240-019-01596-5

Alban Ibraliu, Xuefei Mi, Fetah Elezi. 2011.Variation in essential oils to study the biodiversity in *Satureja montana* L. *Journal of Medicinal Plants Research* **5**(14), 2978–2989.

Alekseeva, A.V. 2011. *Melissa officinalis* as a promising source of import-substituting neurotropic drugs, *Medical Almanac* **1**(14), 233–237 (in Russian).

Basta, A., Tzakou, O. & Couladis, M. 2005. Composition of the leaves essential oil of *Melissa officinalis*L. from Greece. *Flavour Fragr. J.*, **20**, 642–644. doi: 10.1002/ffj.1518

Belgin, C, Arif, I. & Bilal, G. 2009. GC/MS Analysis of Herbage Essential Oil from Lemon Balms (*Melissa officinalis* L.) Grown in Turkey. *Journal of Applied Biological Sciences* **3**(2), 149–152.

Biochemical methods for the analysis of essential-oil plants and essential oils: coll. of scientific materials. 1972. Simferopol: National Research Institute of Essential Oil Plants, 107 pp. (in Russian).

Božović, M., Garzoli, S., Baldisserotto, A., Romagnoli, C., Pepi, F., Cesa, S., Vertuani, S., Manfredini, S. & Ragno, R. 2018. Melissa officinalis L. subsp. altissima (Sibth. & Sm.) Arcang. essential oil: Chemical composition and preliminary antimicrobial investigation of samples obtained at different harvesting periods and by fractionated extractions. *Industrial Crops and Products* **117**, 317–321.

Dimas, D., Tomou, E.-M., Karamani, C., Sfiniadakis, I., Siakavella, K.I., Liakopoulou, A., Hatziantoniou, S., Rallis, M. & Skaltsa, H. 2020. Melissa officinalis ssp. altissima extracts: A therapeutic approach targeting psoriasis in mice. *Journal of Ethnopharmacology* **246,** 112208–112213. doi.org/10.1016/j.jep.2019.112208

Dospekhov, B.A. 2012. *Methods of field experience (with the basics of statistical processing of study results).* Moscow, Kniga po trebovaniyu, 352 pp. (in Russian).

Egorova, N.A. & Stavtseva, I.V. 2016. Using biotechnological methods for developing source breeding material for some essential-oil plants. *Materials of the Kuban State Agrarian University* **2**(59), 122–131 (in Russian).

Efremov, A.A., Zykova, I.D. & Gorbachev, A.E. 2015. Component composition of *Melissa officinalis* oil in Krasnoyarsk localities according to chromatography-mass spectroscopy. Chemistry of Plant Raw *Materials* **1**, 77–81. doi: 14258/jcprm.201501415 (in Russian).

Javadian, N., Karimzadeh, G., Sharifi, M., Moieni, A. & Behmanesh, M. 2017. *In vitro* polyploidy induction: changes in morphology, podophyllotoxin biosynthesis, and expression of the related genes in *Linum album* (*Linaceae*). *Planta*. **245**, 1165–1178. doi: 10.1007/s00425-017-2671-2

Kittler, I., Schrader, O., Kastner, U. & Marthe, F. 2015. Chromosome number and ploidy level of balm (*Melissa officinalis*). *Molecular Cytogenetics*. **8**, 61–66. doi: 10.1186/s13039-015-0166-z

Leontiev, V.N., Shutova, A.G., Kovalenko, N.A., Supichenko, G.N. & Spiridovich, E.V. 2006. Gas chromatographic identification of essential oils. Materials of Belarusian State University Vol. **1**. 1, 261–267 (in Russian).

Mesut, Uyanik & Bilal, Gurbuz. 2014. Chemical Diversity in Essential Oil Compositions of Leaf, Herb and Flower in Lemon Balm (*Melissa officinalis* L.). *Turkish journal of Agricultural and Natural Sciences* **1**(2), 210–214.

Miceli, A., Negro, C. & Tomassi, L. 2006. Essential Oil of *Melissa romana (*Miller) Grown in Southern Apulia (Italy). *Journal of Essential Oil Research* **18**(5), 473–475. doi: 10.1080/10412905.2006.9699144

Moradkhani, H., Sargsyan, E., Bibak, H., Naseri, B., Sadat-Hosseini, M., Fayazi-Barjin, A. & Meftahizade, H. 2010. *Melissa officinalis* L., a valuable medicine plant: A review. *Journal of Medicinal Plants Research* **4**(25), 2753–2759.

Morozov, A.I., Dmitriev, L.B. & Dmitrieva, V.L. 2015. Effect of the cultivation of sod-podzolic soil on the ratio of the components of *Mentha piperita* L. essential oil. *Issues of Biological, Medical and Pharmaceutical Chemistry* **8**, 36–42 (in Russian).

Papoti, V.T., Totomis, N., Atmatzidou, A., Zinoviadou, K., Androulaki, A., Petridis, D. & Ritzoulis, C. 2019. Phytochemical content of *Melissa officinalis* L. Herbal preparations appropriate for consumption. *Processes* **7** (2), 88. doi.org/10.3390/pr7020088

Savchuk, L.P. 2006. *Climate and essential-oil-bearing plants of the foothills of the Crimea*. Simferopol, 76 pp. (in Russian).

Selection of essential-oil plants (guidelines) Ed. by A.I. Arinshtein. 1977. Simferopol: National Research Institute of Essential Oil Plants, 150 pp. (in Russian).

Sharafzadeh, S., Khosh-Khui, M. & Javidnia, K. 2011. Aroma profile of leaf and stem of lemon balm (*Melissa officinalis*L.) Grown under Greenhouse Conditions.*Advances in Environmental Biology* **3**(1), 547–551.

Shelepova, O.V. & Khusnetdinova, T.I. 2018. Effect of using growth regulators on the component composition of the essential oil in tops and fruits of *Anethum graveolens. Chemistry of Plant Raw Materials* **1**, 217–220. doi: 10.14258/jcprm.2018012020 (in Russian).

Ulianych, O., Yatsenko, V., Didenko, I., Vorobiova, N., Kuhnyuk, O., Lazariev, O. & Tretiakova, S. 2019. Agrobiological evaluation of Allium ampeloprasum L. variety samples in comparison with Allium sativum L. cultivars. *Agronomy Research* **17**(4), 1788–1799. doi: 10.15159/AR.19.192

Van den Berg, T., Freundl, E. & Czygan, F.C. 1997. *Melissa officinalis* subsp. *altissima*: characteristics of a possible adulteration of lemon balm. *Pharmazie*. **52**, 802–808.

Vershinina, Z.R., Kuluyev, B.R., Gerashchenkov, G.A., Knyazev, A.V., Chemeris, D.A., Gumerova, G.R., Baymiev, A.Kh. & Chemeris, A.V. 2017. Evolution of genome editing method. *Biomika*. **9**(3), 245–270 (in Russian).

Voytkevich, S.A. 1999. *Essential oils for perfumes and aromatherapy*. Moscow: Food industry. 284 pp. (in Russian).

Wang, L.J, Sheng, M.Y., Wen, P.C. & Du, J.Y. 2017. Morphological, physiological, cytological and phytochemical studies in diploid and colchicine-induced tetraploid plants of *Fagopyrum tataricum* (L.) Gaertn. *Botanical Studies* **58**(1), 2–14. doi: 10.1186/s40529-016-0157-3

Yakimova, O. & Egorova, N. 2019. Induction of micropropagation *in vitro* for *Melissa officinalis* L. *In Vitro Cellular & Developmental Biology-Animal*. **55**(1), 75.

Zenkevich, I.G., Pimenov, A.I., Pozharitskaya, O.N., Shikov, A.N. & Makarov, V.G. 2003. Comparison of chromatographic profiles as a method for defining components of medicinal plant raw materials in complex preparations. *Plant Resources* **39**(3), 143–152 (in Russian).

Agronomy Research **18**(1), 228–240, 2020
https://doi.org/10.15159/AR.20.081

What happens to peat during bog fires? Thermal transformation processes of peat organic matter

V. Ozols, I. Silamikele, L. Kalnina, D. Porshnov, L. Arbidans, J. Krumins and M. Klavins*

University of Latvia, Faculty of Geography and Earth Sciences, Department of Environmental Sciences, Raina blvd. 19, LV 1586 Riga, Latvia
*Correspondence: maris.klavins@lu.lv

Abstract. Bog fires are a serious natural phenomena. Major increase in the number of fires has happened during the last decades due to bog transformation into agricultural lands, accidents and human activities. During bog fires the peat is exposed to high temperatures due to which chemical transformation and even mineralisation of peat can occur. The aim of the study was to analyse the impacts of the bog fires on the bog as an ecosystem, advance the understanding and knowledge of fire impact on peat and humic matter properties and application possibilities. As the material for the study peat samples from burnt sites and thermally treated peat were used. To reveal peat transformation during bog fires, thermogravimetric analysis of peat samples were done, where amounts of bitumens, humic acids and mineral matter were estimated. During bog fires thermal modification of peat properties takes place, resulting in full mineralisation of peat and release of mineral substances. Bog fires lead to development of peat char, bitumens and significant changes in structure and properties of peat humic acids. However, from perspective of application of peat as a substrate and from perspective of impacts on the bog ecosystems, the effects are negligible.

Key words: bog fires, humic substances, peat, recultivation, torrefication.

INTRODUCTION

Bog fires can be considered as serious natural event, considering their significant dimensions (from local scale fires, to regional), significant increase during last decades due to bog transformation to agricultural lands, climate change, accidents and human activities. Bog fires has raised attention at first due to resulting air pollution and adverse impacts on human health. Fires has happened in Europe (Shvidenko et al., 2011), South East Asia (Usup et al., 2004; Hayasaka et al., 2014), South America (Aragão et al., 2018) and elsewhere (Turetsky et al., 2015). Bog fires contribute at the global warming as huge amounts of greenhouse gasses are emitted (Page et al., 2002). While peatlands can burn with open fire, smouldering peatland fires are wildfires with the largest fuel consumption in the world. Peatland fires cause large-scale accumulation of smoke at low altitudes in the atmosphere, which results in the decrease of air quality (Hu et al., 2017). Large smouldering peatland wildfires are very rare events at the local scale, but when they happen, they severely affect peatlands, producing physical, chemical and biological changes to peat (Rein et al., 2008). However not only air pollution is of importance, but the manifestation of fires on bogs and adjacent territories are of equivalent significance. Locally bog fires results in destruction of valuable and unique ecosystems, landscapes, change of hydrological regime in bogs, threats to forest and other ecosystems (Turetsky et al., 2015). All these aspects urge to take actions to protect bogs from accidental fires. However, bog fires changes peat chemical and physical properties. During fires the peat is subjected to high temperature transformation of the peat organic matter, finally to mineralization of peat. It can be supposed that peat after bog fires have significantly different properties than original peat and thus can influence the peat supporting capacity to life on bogs, peat revegetation after bog fires and, of course, can influence the quality of peat if it is mined and used for production of growing media.

It is a very simple question: What happens to peat during bog fires? However, the answer is by far not so easy as very few studies are dedicated to the studies of peat transformation during bog fires. There are studies of soil organic matter transformation during forest fires (González-Pérez et al., 2004; De la Rosa et al., 2012), peat properties during recultivation activities (Rosenburgh et al., 2013) as well as water retention capacity of peat after fires (Thompson & Waddington, 2013). Prolonged heating and the large loss of the peat mass change functional properties of peat humic substances. It is believed that 1 h under the temperature that exceeds 300 °C leads to 90% of mass loss of burnt peat layers and complete peat sterilization (Rein et al., 2008). Specifically impact of bog fires on major group of peat organic substances – humic substances has been studied (Kihara et al., 2015).

Peatland fires in natural peatlands with unaffected groundwater level are extremely rare, while in peatlands with peat excavation sites, deforestation and forest degradation, that all are linked to peatland drainage, they are very common, especially in summer seasons with low rainfall. Moisture content controls peat ignition, dry peat ignites very easily and can burn for months, smouldering underground and re-emerge away from the initial source (Rein et al., 2008). Smouldering peatland fires are more likely to appear in raised bogs than in fens and these fires are highly unpredictable and uncontrollable and thus difficult to extinguish (Svensen et al., 2003). Still the existing studies cover only a minor part of the knowledge needed to understand the impacts of the bog fires on the peat properties.

The aim of the study is to analyse the impacts of the bog fires on example of thermal treatment of peat, on the peat and especially humic matter properties to advance the understanding of the bog fire impacts on the peat properties and bog ecosystem functioning.

MATERIALS AND METHODS

Peat sampling

Peat samples after bog fires were sampled in Saukas bog (Lat: 56,374520; Lon: 25,311041) where recently (July 2019) fire have happened. For further studies upper layer of the peat (further – coked peat) with evident impact of the bog fire (presence of black particles of peat char) were sampled (altogether 24 samples, each 1 kg – dry weight). For further analysis the coked peat was dried (105 °C) and homogenized.

Peat thermal treatment

To study changes of peat properties during bog fires peat from Saukas bog were chosen – typical raised bog as in details described in previous studies (Klavins et al., 2009). Samples were finely ground and homogenised. Each sample was moistened in demineralised water, and excess water was pressed out to reproduce real life wet peat in a bog. Approximately 100 mL of wet peat by volume was densely packed in a separate cast iron capsule and charred in a muffle furnace (Nabertherm B180). Each sample was charred in 4 temperatures – 150 °C; 225 °C; 300 °C and 375 °C for 2 hours with a temperature rise of 5 °C min^{-1}. After charring samples were cooled and packed for further analysis.

Scanning electron microscopy (SEM)

Peat samples from Sauka Bog with low decomposition degree – natural peat and charred at 150 °C, 225 °C, 300 °C and 375 °C for 2 hours as described earlier were used. Each sample was analysed by taking 2 to 5 images with up to 10 points for each sample. For each point chemical data graph was obtained. Electronic microscopy analysis was done using scanning electron microscope Phenom ProX. Images for viewing and morphology evaluation were based on 10 kV mode. Images used for chemical analysis have a 15 kV powerful electron beam and often a point intensity to more accurately transmit the beam, thereby achieving X-ray emission from the material. Energy dispersive spectroscopy (EDS) were done at resolution 132 eV (for manganese K-alpha peak) using ultra-thin silicon nitride (Si_3N_4) drift detector with active surface 25 mm^2. The EDS resolution was 132 eV (for manganese K-alpha peak). Maximum reception of emitted X-rays were 300,000 signals per second.

Characterization of peat

1.00 g of sample (natural, heated or coked peat) was extracted 10 mL of $CHCl_3$. After 24 hours the extract was filtered through paper in pre-weighted Petri dish. After drying in air and at 40 °C the dried lipids on Petri dish were weighed and the difference in weight between an empty, dry Petri dish and dried Petri dish containing waxes and bitumens was calculated by subtracting and expressed in percent.

The pH of peat is measured in an aliquot of sample and a reagent with the mass ratio of 1:5. The solution is prepared from 1-part of peat and 5 parts of a reagent, whether it be a 0.01M $CaCl_2$ or deionized water. 10 g of dry peat is weighed into an Erlenmeyer flask and 50 mL of deionized water is added to it. The sample is then put in an orbital shaker for 1 hour and then filtered through a filter paper and the pH of the sample is measured with a pH meter. Total dissolved solids (TDS) and conductivity (σ) were measured in an aliquot of filtrated sample. They show the combined content of organic and inorganic substances in a liquid.

Extraction of humic substances from peat

Approximately 1 gram of each peat sample was weighed in a 100 mL glass screwcap bottle and 50 mL of 4% NaOH was added to each sample. Samples were placed in an orbital shaker (Biosan PSU-20i) and shaken for 24 hours. After shaking samples were filtered through a filter paper. 1 mL of each sample was transferred to a 100 mL volumetric flask and diluted 100 times with distilled water. Total organic carbon in humic substance solutions were analysed with a total organic carbon analyser

(Shimadzu TOCV-CSN). 20 mL of each charred peat extract was acidified to pH of 2 with concentrated sulfuric acid to precipitate humic acids from fulvic acids. Precipitated humic acids were separated from fulvic acids by filtration. Each fulvic acid solution was diluted 10 times and analysed with a total organic carbon analyser.

Excitation-Emission Matrix Fluorescence Spectroscopy

Humic solutions were prepared at concentrations of 80–90 mg L^{-1}. The excitation-emission matrix spectrum of the prepared samples on an AQUALOG fluorometer was taken using a 1 cm glass cuvette. The laboratory reagent blank was distilled water. The fluorescence signals were normalised to the spectrometer lamp reference intensity, with spectral corrections applied by the instrument software. The emission spectra were scanned from 250 to 600 nm and from 250 to 600 nm in excitation wavelength in 5 nm steps. The spectra obtained were analyzed by PARAFAC, using MATLAB R2014a v. 5.3.0.532 software. Matrices obtained in the excitation-emission matrix fluorescence spectroscopy were corrected and treated with the parallel factor analysis on *MATLAB R2014a v.5.3.0.532* software with *drEEM* toolbox (Krumins, 2018). Data were corrected for the inner filter effects with the subtraction of the blanks, but the fluorescence intensity was converted to Raman units (Lawaetz & Stedmon, 2009; Kothawala et al., 2013). Following the procedure of parallel factor analysis, the excitation-emission matrices of peat humic substances were disassembled into independent components (Murphy et al., 2013).

Thermogravimetrical analysis

TA Instruments – Waters LLC SDT Q600 was used to conduct thermal analysis – thermogravimetry (TG) and differential thermogravimetry (DTG). Sample of peat (5 mg) was heated in a small crucible. Pyrolysis process were ensured in inert atmosphere with nitrogen gas flow of 100 mL min^{-1}. Constant heating rate 20 °C min^{-1} was used during TG process. Samples were heated from room temperature to 105 °C, kept isothermal for 5 minutes to detect amount of moisture and then heated to 900 °C, where samples were held isothermal for 5 minutes in oxygen atmosphere to detect fixed carbon and ash content. Data of weight loss (w%) and derivative weight loss was recorded (w%/°C) during whole TG analysis.

Peat mineralisation

Peat sample was pre-treated in different conditions in a muffle furnace (Nabertherm B180) – dry peat; charred peat at 375 °C; dry peat charred at 900 °C; peat charred at 375 °C one more time at 900 °C. Both fresh peat and ash were macerated in 100 mL of distilled water and shaken in an orbital shaker (BioSan PSU-20i) at 200 rpm for 24 hours. After shaking the samples were filtered through a filter paper. Na; Ca; Mg and K were analysed in the filtered water phase with an ICP-OES (Thermo Scientific iCAP 7000 series). Sulphate ions in filtered water phase were measured turbidimetrically with a spectrophotometer (Hach-Lange DR 2800) at 610 nm wavelength. Peat water extract was titrated with 0.02M $AgNO_3$ using potassium chromate as an indicator to determine chloride ions.

RESULTS AND DISCUSSION

Bog fires and their occurrence in Latvia

Extended periods of dry, hot and windy weather promote increase of frequency of bog fires. It is expected that also in Latvia incidence of fires in territories of bogs will significantly increase both in pristine mires and peat harvesting sites due to climate change, including increase of average air temperature and prolonged dry periods. Peatlands are significantly different in respect to the way fire spreads in peat - it is determined by peat properties (Huang & Rein, 2018).

Most of the bog fire events nowadays are directly connected with human activity (Aragão et al., 2018). Analysis of peat geological sections indicate that even during the Stone Age when human activity was insignificant in the territory of current Latvia periodical burning of peatlands did naturally occur. Before human inhabitation, larger areas of forests and mires were affected by wildfire of natural origin. Gradually human activity has changed the pattern of fire events thus influencing ecological conditions and natural processes (Suveizda, 2016). Impact of bog fires caused by human activity on local ecosystem is much greater than naturally occurring wildfire.

Data from 2011 to 2018 show that there have been more than 40 cases of peatland burnings in Latvia. In most cases territories affected by fire are not significant – up to 1ha - due to the fact that fire is quickly noticed and extinguished before spreading further. However, in hard to access places fire spreads rapidly and affected territories are extensive, for example, in 2018 conflagration in Stikli Mire complex affected 529 ha of both pristine mire and peat harvesting site. More than 21 peatland burnings were reported in 2018.

During this period the fires in peatlands have occurred from February until November. Most of them took place during hot summer months – July and August. Although rare, but occasional repeated ignition and burning of peat occurs, usually during or after smoldering (after longer period of time).

Naturally occurring wildfires in boreal zone emphasizes the need for successful monitoring of conflagration and wildfire and reduce of adverse effects on environment. It can be achieved by research, analysing and understanding combustibility, flammability, fire spreading and burning and peat properties itself. Peat properties are quite different in both in the region of the Baltic States in comparison with other regions, like Ireland, the Netherlands, as well as peat properties are different even in nearby locates sites. Therefore, it is very important to carry out peat studies in details to understand how to protect peatlands from fires and how to use peat more effectively with added value.

Changes of peat properties during bog fires – experimental study

In the thermally treated peat samples and in the samples from naturally burnt sites a number of parameters has been determined, such as amount of water soluble substances, tars and bitumens, trace elements, humic substances and others. Besides to that, the visual appearance of peat was studied using Scanning Electron Microscopy (SEM) and Thermogravimetrical Analysis (TGA).

Obtained SEM images and data of chemical composition revealed in graphs show gradual changes in both peat structure and chemical composition. Results of dry natural and dry charred peat samples heated at 150 °C and 375 °C are shown in Fig. 1.and Fig. 2. and discussed in details.

Both analysed peat samples are represented by low decomposed raised bog type *Sphagnum* peat and in their botanical composition *Sphagnum fuscum* dominates up to 90% among moss remains forming peat. *Sphagnum* leaves have structure with pores resulting soak up water like a sponge. These large, clear, structural, dead cells of leaves have the large water-holding capacity, which may hold 16 to 26 times as much water as their dry weight (Klavins et al., 2009). Particularly these *Sphagnum* leaf structure properties are also preserved as the *Sphagnum* dies and forms peat and determines that natural peat moisture content is 90–96%. As shown in the obtained images, even at 150 °C heating, the structure and pores of the Sphagnum leaves are still well preserved. The pores are still partially retained heated at 375 °C while leaves are torned by heating.

In charred Sphagnum peat, both the structure and pores of the Sphagnum leaves are damaged before heating and there is increasing heating effect observed along the increase of heating temperature. The leaf structure becomes shapeless and the pores are compressed. More expressed changes happened also because of chemical processes in charred sample, while in dry and only heated sample changes are not significant.

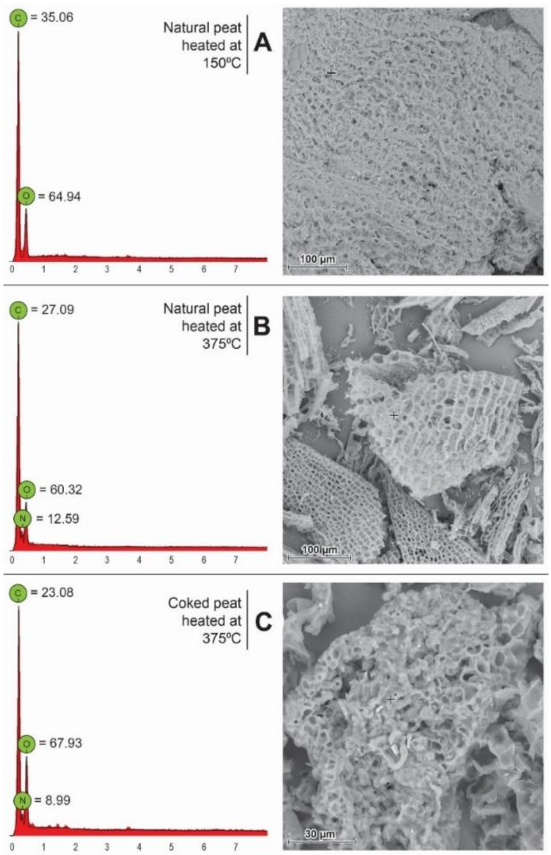


Figure 1. Scanning electron microscopy images of natural peat heated at 150 °C (A); at 375 °C (B) and coked peat, heated at 375 °C as well as elemental composition of corresponding peat samples obtained using energy dispersive spectroscopy (EDS).

Chemical composition of both analysed samples is different. In chemical composition of dried natural peat the main chemical element is oxygen reaching approximately 61–69% and it does not vary significantly with the heating temperatures. From other elements determined predominantly only carbon, the amount of which is fluctuating 27–37%. In the chemical composition of charred peat oxygen varied from 61 to 68%, and decrease of carbon, but there more other elements (Si, K, Al, N, Br) are determined, however in small quantities.

The thermogravimetrical analysis of the natural peat, peat obtained from sites after bog fires provides the information of the processes going on during bog fires – transformation of peat organic matter, torrefication or charring ending up in formation of peat char in presence of limited amounts of oxygen or full mineralization of peat

(Fig. 2). The first stage of peat thermal transformation – removal of constitutionally bound water happening at 110 °C. At somewhat higher temperatures (200–300 °C), the destruction takes place of less condensed components, such as polysaccharides or of a part of humic substances. The stage of destructive drying or torrefication is entered around 200 °C. As it is showed by earlier studies (Klavins & Porshnov 2011; Klavins & Porshnov, 2013), destructive drying significantly changes water uptake properties of peat by increasing its hydrophobicity due to removal of hydrophillic hydroxyl and carboxyl functional groups from the surface of the material.

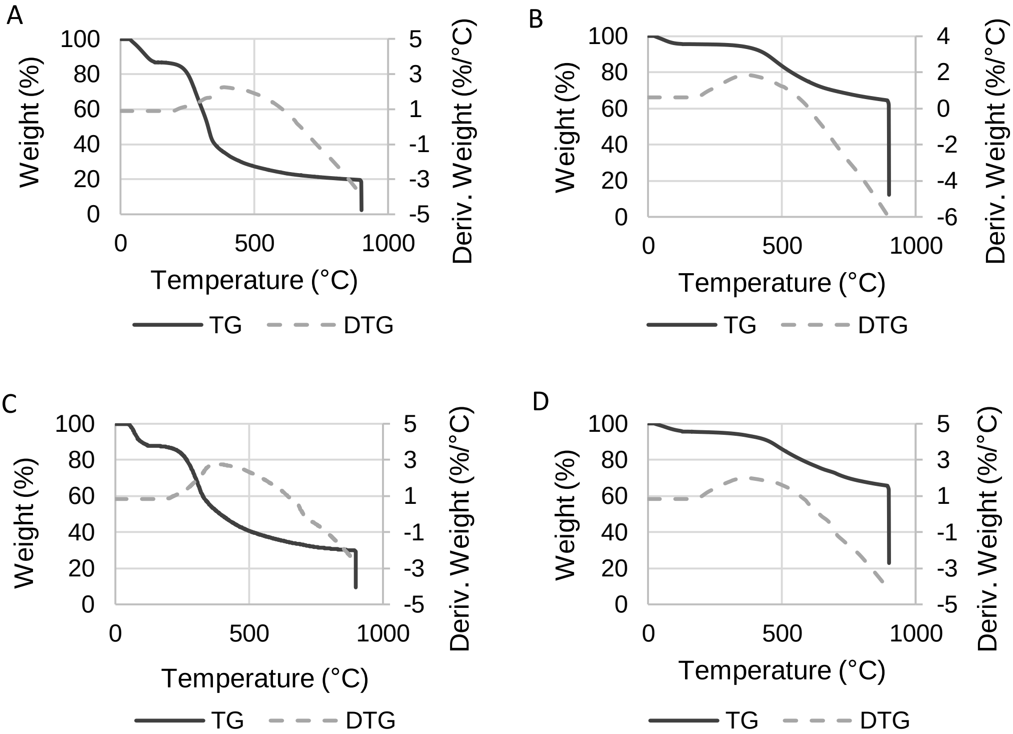


Figure 2. Thermogravimetrical analysis of peat sample decomposition: A – natural peat; B – natural peat torrefied at 375 °C; C – coked peat; D – coked peat torrefied at 375 °C.

As it is showed on Fig. 2. water uptake decreases more than two times after the thermal treatment this fact undoubtedly plays significant role considering biological as well as chemical processes after the fire. Finally, at about 350–500 °C thermal effects are attributed to the pyrolysis of the more condensed materials, such as aromatic compounds of lignin and remaining humic acids (Almendros et al.; 1982). The TGA analysis do not reveal significant differences between natural peat and coked (charred) peat (Fig. 2, A, C) as well as natural peat and coked peat after thermal treatment (torrefication) (Fig. 2, B, D).

Thus, a conclusion can be drawn that despite the visually seen differences in the peat appearance (presence of small carbonaceous particles in coked peat) the actual differences in the major part of the peat mass after peat fires are not so significant. However, if the thermal transformation is deeper such as in case of the torrefication resulting in the total transformation of peat mass in carbonaceous mass, the TGA demonstrates significantly differing the behaviour (Vitazek et al., 2019). Results of experimental thermal treatment of peat obtained from bog fire sites provides understanding of chemical processes taking place during bog fire in the layer of peat. Contrary to the upper layer where complete mineralisation of material take place, processes in the deeper layers differ significantly and must be characterised as pyrolytic decomposition or as torrefication: depending on temperature experienced by material during the fire. The next stage of the thermal decomposition: pyrolysis (decomposition of polymeric chains) takes place at temperatures higher than 350 °C, as we see in Fig. 3, this process involves crosslinking reactions producing significant amounts of fixed carbon.

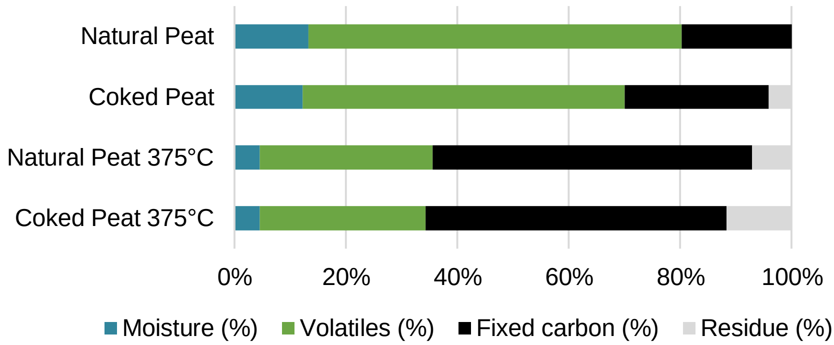


Figure 3. Proximate analysis of peat, coked peat and natural and coked peat after treatment at 375 °C.

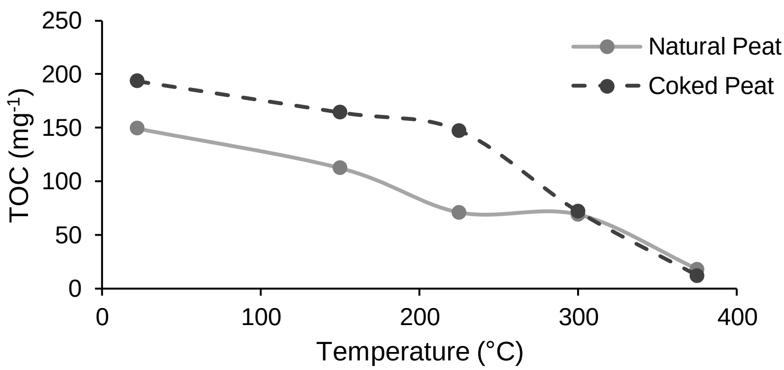


Figure 4. Changes of concentration of humic substances (expressed as total organic carbon concentration) in peat (natural and coked) depending on the temperature of the following thermal treatment.

Thermal treatment of the peat significantly reduces the amount of humic substances available in the peat (Fig. 4). The yields of humic substances in coked peat is significantly lower (~ 25%) than in natural peat and the yield of humic substances depends on the temperature of the thermal treatment of the peat. Such changes can be related to the condensation of peat organic matter and related decrease of solubility. The humic substances in the peat can affect the ability of peat to support development of vegetation on bogs and thus the reduction of the availability of the humic substances on the intensity of the thermal treatment (temperature) shows the impacts of bog fires on the peat properties and following application possibilities.

The thermal treatment of peat results in increase of the pH as well as in the concentrations of dissolved substances (as indicated by the changes of TDS and conductivity of the aqueous extracts of the peat) (Table 1). Also, slight increase in concentrations of inorganic ions, representing the mineral matter of peat can be observed in coked peat in comparison with natural peat as well as in the peat depending on the temperature of the thermal treatment. As the re-vegetation of peatlands after bog fires, depends on availability of nutrients, this finding illustrates the impacts of bog fires on the peatlands.

Table 1. Changes of composition of peat water soluble components and peat humic substances

Peat sample	pH	TDS, ppm	σ, μS cm^{-1}	TOC, mg g^{-1}	W_{HA}, %	$W_{FA, TOC}$, %	W_{lipids}, %
Coked Peat (CP)	4.50	55.9	110.0	193.7	79.1	20.9	1.23
Natural Peat (NP)	4.97	43.8	86.9	149.4	70.2	29.8	1.15
NP 150 °C	5.10	25.6	51.3	112.3	60.8	39.2	1.33
NP 225 °C	5.00	38.3	72.2	70.9	60.2	39.8	1.85
NP 300 °C	5.06	34.6	70.6	68.9	52.8	47.2	2.30
NP 375 °C	6.28	15.2	31.4	17.8	52.4	47.6	1.95
CP 150 °C	4.56	50.9	101.2	164.5	75.0	25.0	1.45
CP 225 °C	4.58	60.4	111.4	147.5	71.1	28.9	2.50
CP 300 °C	5.28	27.6	63.4	71.8	48.7	51.3	3.20
CP 375 °C	5.37	17.0	35.0	12.1	47.9	52.1	2.80

On the other hand also the total amount of humic substances (as indicated by the total organic carbon in peat extracts with O.1 N NaOH) is decreasing depending on the temperature of the thermal treatment of the peat as well as in peat after bog fires in comparison with unaffected peat. The increase of the temperature of the thermic treatment reduces the amounts of humic acids and increases the percentage of fulvic acids (more soluble part of the humic matter pool).

The excitation-emission matrices of peat humic substances correspond to a system with a number of fluorescent components. Peak fluorescence for natural and charred peat humic substances is similar and locates around λ 440 nm of emission wavelength with indication of electron-donating functional groups in the material. Fluorescence at this emission wavelength suggests the presence of complex structural components with high relative degree of aromatic condensation and high relative number of conjugated fluorophores (Enev et al., 2014).

The difference in the fluorescence between natural and charred humic substances emerges in the relative intensity, which in charred peat humic substances is higher, suggesting increase in aromaticity due to prolonged heat impact on peat. It is believed that peat fire induces increased production of benzene, which is least complex aromatic hydrocarbon.

Data suggests that smouldering peatland fire also affect humic substances in the fluorescence excitation region between λ 450–500 nm with the emission of λ 500–575 nm, functionalities at this range due to heat impact seems to collapse. Following the procedure of parallel factor analysis, a two-component model with 99.96% probability was generated and validated by the residual examination and split-half analysis. Simple two-component model reveals severe physical and chemical changes to peat humic substances due to peatland fire, especially a significant decrease in humic substance fluorescence intensity (more than 1,000 arbitrary units) in λ 500–575 nm emission region.

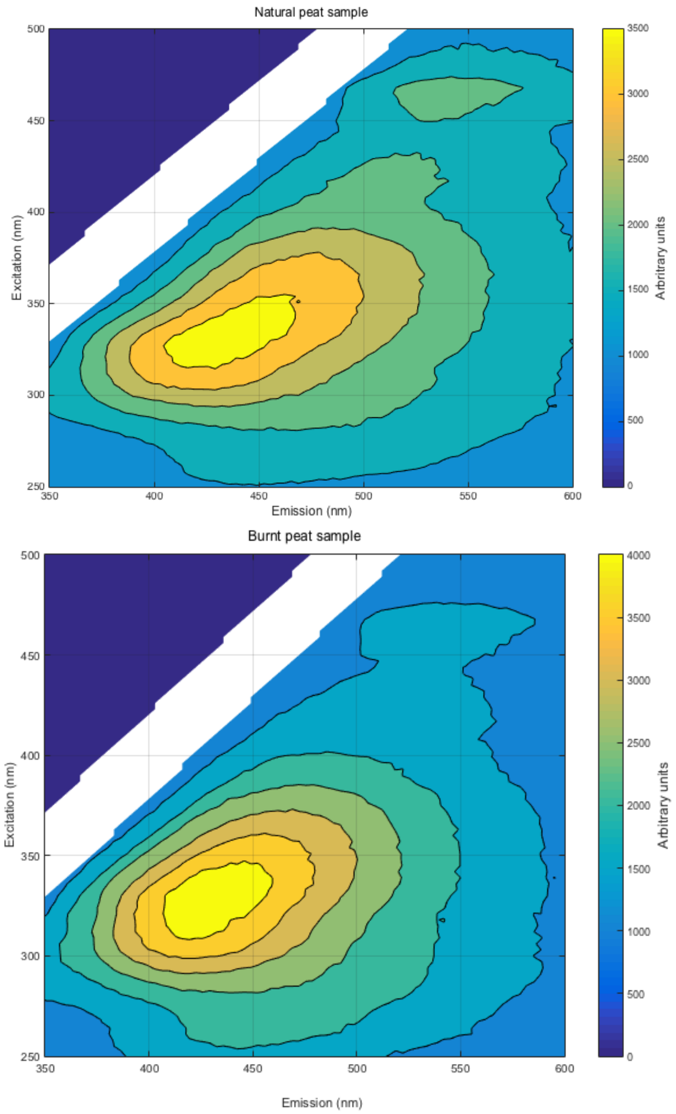


Figure 5. Excitation-emission matrices of *Sphagnum* moss peat humic substances in a) natural settings and b) after a smouldering peatland fire.

Consequences of bog fires on peat properties and possible impacts of recovery of peatlands after bog fires

The bog fires result in significant changes of peat properties. A first the development of peat char (biochar) particles is evident: after peat fires - everyone can observe black char on the surface of bogs. Peat char is much more stable in respect to degradation than peat and can remain in peat layers for thousands of years. Also, SEM figures proves the significant changes in the view of peat particles.

The structure and pores of the *Sphagnum* leaves from the dried natural peat is preserved even after heating, while the structure and pores of the burned peat are significantly deformed and do not change significantly when heated, suggesting that the peat has been exposed to really high temperatures during fire.

Irrespective of the heating temperature, the chemical composition of natural peat mainly is represented by the oxygen (61–69%) and carbon (27–37%), but the presence of other elements is small, however the mineral substances (inorganic ions) after bog fires can leak out from the peat.

The chemical composition of burnt peat is more variable than that of natural peat. Although the main elements are the same as in natural peat - oxygen and carbon - it still contains up to 12% silicon (Si), as well as other chemical elements (Br, Al, K, N) have been determined.

The structure of the charred peat is significantly and irreversibly affected, possibly resulting in loss of peat absorption properties.

CONCLUSIONS

The peat thermal decomposition results in release of mineral substances (at first inorganic ions) from the peat as demonstrates the changes in pH, TDS as well as analysis of water-soluble substances. The ions, such as K^+, Ca^{+2} and others in mineral mater poor peat can be considered as nutrients and they can greatly support burned peatland re-vegetation, however not with the indigenous Sphagnum species, but rather with higher plants.

Another major way of impacts of peat thermal transformation (burning) includes impacts on peat organic matter as demonstrated by changes of peat humic substances. The decrease of humic acid concentration and increase of fulvic acid (lower molecular mass, higher solubility directly can influence the drainage water composition from peatlands – their brownification as well as the increase of mineral substances in the drainage.

From perspective of peat mining – the admixtures of burnt peat will not have significant adverse effects on the plant development or rather even can be considered as beneficial at production of growth substrates. However, from perspective of recovery of native vegetation especially in raised bogs, peat fires can support development of higher vegetation.

Data suggests that due to prolonged heat impact on peat it is characteristic for aromatic and condensed molecules to increase in number, while more labile compounds collapse into smaller, more stable formations. It is believed that during peat fire high amounts of aromatic hydrocarbon is produced, which significantly increases the aromaticity of peat.

Only two major functional compounds can be traced in humic substances of both natural and charred peat, which means that severe physical and chemical changes have been made to peat material and due to prolonged heating, most functionalities have been destroyed.

ACKNOWLEDGEMENTS. This study was funded by the European Regional Development Fund grant number 1.1.1.2/16/I/001 under the post-doctoral research project number 1.1.1.2./VIAA/1/16/008 as well as Latvia Science Council project ‘Properties and structure of peat humic substances and possibilities of their modification’ lzp-2018/1-0009. Additional support was provided by the University of Latvia project ’Studies of impact by peatland burning on the environment and bog recovery intensity’ with partners JSC ’Latvia’s State Forest’, The Nature Conservation Agency and Latvian Peat Association.

REFERENCES

Almendros, G., Polo, A. & Vizcayno, C. 1982. Application of thermal analysis to the study of several Spanish peats. *Journal of Thermal Analysis* **24**, 175–182.

Aragão, L.E., Anderson, L.O., Fonseca, M.G., Rosan, T.M., Vedovato, L.B., Wagner, F.H., Camila, V.J., Silva, C.V.J., Silva, C.H.L., Arai, E., Aguiar, A.P., Barlow, J., Berenguer, E., Deeter, M.N., Domingues Lucas, G., Gatti, L., Gloor, M., Malhi, Y., Marengo, J.A., Miller, J.B., Phillips, O.L., Sassan, S. & Barlow, J. 2018. 21st Century drought-related fires counteract the decline of Amazon deforestation carbon emissions. *Nature communications* **9**(1), 536.

De la Rosa, J.M., Faria, S.R., Varela, M.E., Knicker, H., González-Vila, F.J., González-Pérez, J.A. & Keizer, J. 2012. Characterization of wildfire effects on soil organic matter using analytical pyrolysis. *Geoderma* **191**, 24–30.

Enev, V., Pospisilova, L., Klucakova, M., Liptaj, T. & Doskocil, L. 2014. Spectral characterization of selected natural humic substances. *Soil and Water Research* **9**(1), 9–17. https://doi.org/10.17221/39/2013-SWR

González-Pérez, J.A., González-Vila, F.J., Almendros, G. & Knicker, H. 2004. The effect of fire on soil organic matter–a review. *Environment International* **30**(6), 855–870.

Hayasaka, H., Noguchi, I., Putra, E.I., Yulianti, N. & Vadrevu, K. 2014. Peat-fire-related air pollution in Central Kalimantan, Indonesia. *Environmental Pollution* **195**, 257–266.

Huang, X. & Rein, G. 2019. Upward-and-downward spread of smoldering peat fire. *Proceedings of the Combustion Institute* **37**(3), 4025–4033.

Hu, Y., Fernandez-Anez, N., Smith, T.E.L. & Rein, G. 2017. Review of emissions from smouldering peat fires and their contribution to regional haze episodes. *International Journal of Wildland Fire* **27**(5), 293–312. https://doi.org/10.1071/WF17084

Kihara, Y., Sazawa, K., Kuramitz, H., Kurasaki, M., Saito, T., Hosokawa, T. & Tanaka, S. 2015. Effects of peat fires on the characteristics of humic acid extracted from peat soil in Central Kalimantan, Indonesia. *Environmental Science and Pollution Research* **22**(4), 2384–2395.

Klavins, M. & Porshnov, D. 2011. Approaches for peat modification to improve oil sorption capacity. In: *Proceedings of the 4th WSEAS international conference on Energy and development-environment-biomedicine/World Scientific and Engineering Academy and Society (WSEAS)*, pp. 48–53.

Klavins, M. & Porshnov, D. 2013. Development of a new peat-based oil sorbent using peat pyrolysis. *Environmental Technology* **34**(12), 1577–1582.

Klavins, M., Silamikele, I., Nikodemus, O., Kalnina, L., Kuske, E., Rodinov, V. & Purmalis, O. 2009. Peat properties, major and trace element accumulation in bog peat in Latvia. *Baltica*, **22**(1), 37–49.

Kothawala, D.N., Murphy, K.R., Stedmon, C.A., Weyhenmeyer, G.A. & Tranvik, L.J. 2013. Inner filter correction of dissolved organic matter fluorescence. *Limnology and Oceanography Methods* **11**(12), 616–630. https://doi.org/10.4319/lom.2013.11.616

Krumins, J. 2018. *Parallel factor analysis of peat humic substance 3D fluorescence data. Data treatment and analysis*. University of Latvia: Riga, Latvia, 36 pp.

Lawaetz, A.J. & Stedmon, C.A. 2009. Fluorescence intensity calibration using the Raman scatter peak of water. *Applied Spectroscopy* **63**(8), 936–940. https://doi.org/10.1366/000370209788964548

Murphy, K.R., Stedmon, C.A., Graeber, D. & Bro, R. 2013. Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Analytical Methods* **5**(23), 6541–6882. https://doi.org/10.1039/c3ay41160e

Page, S.E., Siegert, F., Rieley, J.O., Boehm, H.D.V., Jaya, A. & Limin, S. 2002. The amount of carbon released from peat and forest fires in Indonesia during 1997. *Nature* **420**(6911), 61.

Rain, G., Cleaver, N., Ashton, C., Pironi, P. & Torero, J.L. 2008. The severity of smouldering peat fires and damage to the forest soil. *Catena* **74**(3), 304–309. https://doi.org/10.1016/j.catena.2008.05.008

Rosenburgh, A., Alday, J.G., Harris, M.P., Allen, K.A., Connor, L., Blackbird, S.J., Eyre, G. & Marrs, R.H. 2013. Changes in peat chemical properties during post-fire succession on blanket bog moorland. *Geoderma* **211**, 98–106.

Shvidenko, A.Z., Shchepashchenko, D.G., Vaganov, E.A., Sukhinin, A.I., Maksyutov, S.S., McCallum, I. & Lakyda, I.P. 2011. Impact of wildfire in Russia between 1998–2010 on ecosystems and the global carbon budget. In *Doklady Earth Sciences* **441(**2), 1678–1682.

Suveizda, L. 2016. Forest fires in Latvia from 1990 till 2014. In: Management examples of nature: forests. [Meža ugunsgrēki Latvijā laika periodā no 1990. līdz 2014. gadam. Aktuāli savvaļas sugu un biotopu apsaimniekošanas piemēri: meži in Latvian] SIA 'Masterprint', Rīga, 89–94.

Svensen, H., Dysthe, D.K., Bandlien, E.H., Sacko, S., Coulibaly, H. & Planke, S. 2003. Subsurface combustion in Mali: Refutation of the active volcanism hypothesis in West Africa. *Geology* **31**(7), 581–584. https://doi.org/10.1130/0091-7613(2003)031<0581:SCIMRO>2.0.CO;2

Thompson, D.K. & Waddington, J.M. 2013. Peat properties and water retention in boreal forested peatlands subject to wildfire. *Water Resources Research* **49**(6), 3651–3658.

Turetsky, M.R., Benscoter, B., Page, S., Rein, G., Van Der Werf, G.R. & Watts, A. 2015. Global vulnerability of peatlands to fire and carbon loss. *Nature Geoscience* **8(**1), 11.

Usup, A., Hashimoto, Y., Takahashi, H. & HAYASAKA, H. 2004. Combustion and thermal characteristics of peat fire in tropical peatland in Central Kalimantan, Indonesia. *Tropics* **14**(1), 1–19.

Vitázek, I., Ondro, T., Sunitrová, I., Majdan, R. & Šotnar, M. 2019. Thermoanalytical investigation of selected fuel during isothermal heating. *Agronomy Research* **17**(6), 2455–2459 https://doi.org/10.15159/AR.19.216

Agronomy Research **18**(1), 241–255, 2020
https://doi.org/10.15159/AR.20.013

Physiological indicators and yield of the Chinese cabbage cultivated at different soil water tensions

D. Paulus*, I. C. Zorzzi, D. Becker, G.A. Nava, A.L. de Paula and F.M. de Paula

Federal University of Technology - Paraná, Campus Dois Vizinhos, Faculty of Agronomia, Department of Agronomy, Street adress Estrada para Boa Esperança, km 04, postal code 157, Dois Vizinhos, Paraná, Brazil
*Correspondence: dalvapaulus@gmail.com

Abstract. The development and yield of Chinese cabbage is influenced by soil moisture. The objective of this study was to evaluate the physiological indicators, development, and yield of Chinese cabbage (*Brassica rapa subsp. pekinensis* (Lour.) Rupr.) grown at different soil water tension ranges. Two experiments were conducted (2016–2017) in the Olericulture Sector of the Federal University of Technology of Paraná. Two cultivars of the Chinese cabbage, Eikoo and Kinjitsu, and four soil water tension ranges 13–17, 23–27, 33–37, and 43–47 kPa were studied. Eikoo presented higher relative chlorophyll index, photosynthesis, and fresh leaf mass than did Kinjitsu. Physiological indicators transpiration (5.8 mmol H_2O m^{-2} s^{-1}), photosynthesis (14.5 µmol CO_2 m^{-2} s^{-1}), stomatal conductance (0.31 mol H_2O m^{-2} s^{-1}), and WUE (39.4 kg m^{-3}) were higher at 13–17 kPa soil water tension. Soil water tension ranges with high water restrictions reduced the fresh leaf mass of both cultivars. Fresh leaf mass decreased by 236.2 and 191.7 g $plant^{-1}$ in the highest soil water tension range in 2016 and 2017, respectively, when compared with the fresh leaf mass at the 13–17 kPa tension range. The lowest water consumption was observed at the 13–17 kPa tension range. The year 2017 resulted in higher internal CO_2 concentration, transpiration rate, fresh leaf mass, number of irrigations and water consumption compared to the year 2016. Thus, the irrigation regime for the most optimal Chinese cabbage cultivation should maintain the soil water tension range at 13–17 kPa.

Key words: *Brassica rapa*, photosynthesis, soil moisture, tensiometer, yield.

INTRODUCTION

Water shortages are increasingly reported in agricultural systems in the wake of climate change–a phenomenon that will negatively affect the livelihoods of rural communities and food security in urban areas. In the same vein, the agricultural sector is growing at a rapid pace and is responsible for mobilizing large volumes of water (Godfray & Garnett, 2014). Addressing water scarcity requires cultivation strategies to save water as efficient use of irrigation water is becoming increasingly important.

Low water-use efficiency, both under irrigation systems and in the field, calls for the introduction of new approaches to enhance efficient exploitation of water resources. The development of systems that maintain or increase yield while minimizing such negative impacts is critical for the achievement of sustainable production (Godfray &

Garnett, 2014). Strategies for reducing water loss and conserving the water resources available for agricultural production, such as deficit irrigation, which aims to increase water-use efficiency while ensuring effective irrigation by reducing the amount of water used (Zorica & Stikic, 2018), alternative wetting and drying (AWD), which has been demonstrated to decrease water use by 23–33% under rice cultivation (Carrijo et al., 2018), in addition to water reuse and adoption of localized irrigation methods (drip and micro sprinkler), with efficiencies greater than 90%, are some alternative approaches that could enhance water-use efficiency (Zorica & Stikic, 2018).

Proper irrigation requires careful consideration of the amount of water and the timing of its application to the soil based on the stage of plant growth. Improper irrigation, either in the form of excessive or inadequate irrigation, results in crop water stress and reduced yield. Excessive irrigation could also cause the pollution of water sources due to the loss of plant nutrients by leaching, runoff, and soil erosion (Yazgan et al., 2008).

The amount of water to be applied by the irrigation system is dependent on soil moisture (Mantovani et al., 2009). One of the most used methods for indirect determination of soil moisture employs tensiometers, because they are easy to install and maintain allow you to determine soil moisture and calculate the net irrigation depth (Shock & Wang, 2011). However, it is necessary to know beyond the matrix potential of soil water, the soil's capacity to store water in the root zone and the maximum tension for irrigation return at levels that do not cause damage to crop development (Shock & Wang, 2011). Tensiometers have been used widely in the management of vegetable crops to determine irrigation frequency for the recommended soil water tension of 10–20 kPa (Marouelli, 2008).

Brassica rapa is an important fresh and processed vegetable, especially in Asian countries. With the migration of the Asian population to other parts of the world, it has become increasingly popular in countries around the world. Due to its flavour and high nutritional value, such as vitamins, minerals, and fiber, the demand for this vegetable is constantly growing (Acikgoz, 2016). According to Artemyeva & Solovyeva (2006), *Brassica* vegetables also have high amounts of plant secondary metabolites, which have anti-carcinogenic, antioxidant, antibacterial, and antiviral properties, in addition to boosting the immune system, reducing inflammation, and preventing the development of cardiovascular diseases.

However, it is a highly water-demanding leafy vegetable grown mainly in a protected environment and using drip irrigation (Maršalkienė et al., 2014), so soil moisture should be kept close to field capacity throughout the growth cycle (Filgueira, 2008). Scheduling irrigation is essential for maximizing the productivity of the crop under different agronomic management practices (Maršalkienė et al., 2014). Research results are presented for this crop, such as indications of cultivars for regions with warm climate (Seabra et al., 2014), fertilizer use (Pascual et al., 2013), nitrogen level, irrigation frequency and planting date (Maseko et al., 2017). However, there is little research related to irrigation management, informing the appropriate soil water tension range for crops, which does not harm the development, productivity and quality of Chinese cabbage and also advise farmers on critical crop limit and soil moisture parameter to determine the frequency of irrigation.

Physiological aspects of a plant, such as photosynthesis and plant development, are closely related to plant's water status and directly affect its yield (Silva et al., 2015). Understanding the changes in these plant parameters in relation to soil water properties prevailing during the cultivation of Chinese cabbage will help to develop an effective irrigation schedule that will increase its quality and productivity. Therefore, the objective of this study was to evaluate the physiological indicators, development, and productivity of Chinese cabbage under different soil water tension ranges.

The present study was conducted as an important contribution to irrigation management practices and assist in deciding which water management option to practice when producing Chinese cabbage under different water tensions in the soil. We hypothesized that the effects of water stress on soil and cultivar on growth regulation, physiology, and efficiency in the use of Chinese cabbage water are related to soil-plant-atmosphere interactions, in order to obtain high yields. In particular, this information should facilitate the establishment of scientific irrigation systems for Chinese cabbage, help maintain sustainable land use and provide a theoretical basis for the sustainable development of agriculture.

MATERIALS AND METHODS

Characterization of the experimental area

Two experiments were performed, in the months of February to May and in the years 2016 and 2017, at the Experimental Station of the Federal Technological University of Paraná (25°42′S, 53°06′W and 520 m altitude). The experiments were conducted in a greenhouse covered with a 150-µm thick transparent polyethylene under controlled light and temperature conditions. The climate in the region according to Köppen classification is humid subtropical (Cfa), with no defined dry season and the warmest month average temperature of 22 °C (Alvares et al., 2013).

Soil physical and chemical analysis

Polyethylene pots (18 L) were filled with dry soil that was previously crushed and sieved through a 2 mm mesh sieve. The soil was Dystroferric Red Latosol (Embrapa, 2018), collected from the ravine, from the top 0–20 cm layer, located in the Experimental Area of the University with 78.3% clay, and therefore characterised as very clay-rich (USDA, 2017). The soil physical and chemical characteristics measured during the pre-planting stage are listed in Table 1.

Granulometry (clay, silt and sand) analyses were performed at the Laboratory of Physics and Soil Water of the University of Passo Fundo – Rio Grande do Sul – Brazil according to the methodology described by Gee & Bauder (1986). For the analyzes was used as dispersant aqueous sodium hydroxide solution (4 kg m^{-3}) and Boyoucos densimeter for the readings. The first reading was performed 40 seconds after 6 hours of agitation to determine the total sand and the second reading 2 hours later to determine the clay, and the silt fraction was determined by difference. The separation of the sand fraction was performed by washing and sieving in a 0.053 mm mesh sieve. After the samples were oven dried, the sand fractions were separated by very thick (> 1 mm), thick (0.5–1.0 mm), medium (0.25–0.5 mm), thin (0.105–0.25 mm) and very thin (< 0.105 mm).

The analyzes of soil density, particle size, microporosity, macroporosity and total porosity were carried out at the Soil Laboratory of the Federal Technological University of Paraná – Dois Vizinhos, according to the methodology proposed by Embrapa (1997), using six replications in soil each analysis.

Table 1. Physical and chemical characteristics of experimental soil in the 0 to 20 cm depth layer

Measurement	Results
Clay (%)	78.3
Silt (%)	16.7
Sand (%)	5.0
Macroporosity (%)	0.31
Microporosity (%)	0.42
Total porosity (%)	0.73
Particle Density (g cm^{-3})	3.52
Soil density (g cm^{-3})	0.95
pH $CaCl_2$	5.80
Organic matter (%)	1.88
P (mg dm^{-3})	11.78
K^+ ($cmol_c$ dm^{-3})	0.45
Ca^{+2} ($cmol_c$ dm^{-3})	6.10
Mg^{+2} ($cmol_c$ dm^{-3})	3.70
Cu (mg dm^{-3})	4.71
Fe (mg dm^{-3})	45.53
Zn (mg dm^{-3})	12.29
Mn (mg dm^{-3})	188.54
Sum of bases (mg dm^{-3})	10.25
$H^+ + Al^{+3}$ ($cmol_c$ dm^{-3})	3.18
Base saturation (%)	76.32

Notes: Methodologies according to Teixeira et al. (2017), where organic matter by moist digestion; P, K, Cu, Fe, Zn and Mn extracted with Mehlich's –I solution; pH in $CaCl_2$ – 1:2.5; Ca, Mg and Al exchangeable extracted with KCl – 1 mol L^{-1}.

Soil density was determined using the volumetric ring method, where the volume of the ring was calculated, and then the soil and the ring weighed together, stored in a greenhouse at 105 °C until constant mass, and then removed and weighed after cooling. Soil density was calculated using Eq. (1):

$$Density\ (g\ cm^3) = \frac{a}{b} \qquad (1)$$

where a – dry sample weight at 105 °C (g); b – ring volume (cm^3).

Microporosity (Eq. 2) and macroporosity (Eq. 3) were determined using the stress table method, where in samples saturated with known mass are placed under a stress table and regulated using a 60 cm water column. After 24 h, they are removed and weighed, the percentage saturation determined, and then the samples transferred to an oven and dried at 105 °C until a constant mass is achieved.

$$Microporosity = \frac{(a-b)}{c} \qquad (2)$$

where a – mass of sample taken from tension table; b – dry sample weight at 105 °C; c – volume of the ring.

$$Macroporosity = saturation\ percentage - \ microporosity \qquad (3)$$

Total porosity was obtained by Eq. 4.

$$Total\ porsity = microporosity\ +\ macroporosity \qquad (4)$$

To determine particle density, the density (Eq. 1) and total porosity (Eq. 4) of four samples were determined. Particle density was calculated using Eq. (5):

$$Particle\ density\ (g\ cm^3) = \frac{a}{b} \qquad (5)$$

where a – soil density (known mass by volume (g); b – volume occupied by solids (100 – total porosity) (cm^{-3}).

The soil water retention curve was determined from undisturbed samples collected at 0.10 m depth in the field, with six replications (Fig. 1). Volumetric humidity at low tensions points (6 and 10 kPa) was determined using porous plate funnels; the humidity at 1,500 kPa tension was estimated from that at 100 and 300 kPa tensions using Richards chamber and pedotransfer functions (Michelon et al., 2010).

The relationship between stress and volumetric humidity was adjusted using the model proposed by van Genuchten (1980).

The adjustment was conducted in RETC (van Genuchten et al., 1991) with the following parameters at a depth of 0.10 m: residual volumetric humidity ($m^3\ m^{-3}$) = 0.078; volumetric humidity at saturation ($m^3\ m^{-3}$) = 0.43; model tuning parameters: m = 0.359; n = 1.56; α = 0.036, and R^2 = 0.95.

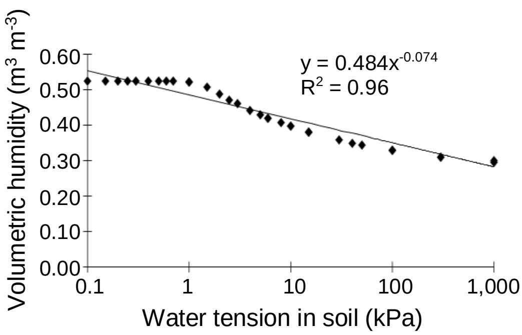


Figure 1. Soil water retention curve 0.10 m depth.

Plant Material and Cultural Practices

Chinese cabbage seedlings from two cultivars, Eikoo and Kinjitsu, were grown in 200-cell polyethylene trays filled with commercial Carolina Soil® substrate. The seeds were bought from the company Horticeres seeds®.

The plants were transplanted 20 days after sowing when they developed between four and six definitive leaves (Segovia et al., 2000) in both experiments.

Mean temperature, relative humidity, and radiation data for the region were obtained from the UTFPR weather station (16 universal time coordinated), located near the experimental unit. Potential evapotranspiration was estimated by the Penman-Monteith method (Allen et al., 1998). The climatic elements used to estimate potential evapotranspiration comprise a set of factors such as maximum and minimum temperature, real vapor pressure, net radiation, and wind speed. The mean temperature and mean evapotranspiration were 17.6 °C and 0.78 mm day^{-1} in the first experiment and 21.8 °C and 1.75 mm day^{-1} in the second experiment, respectively. The temperature conditions were within the optimal range for the culture (Filgueira, 2008).

Pest control such as aphid (*Aphis gossypii* G.), *Diabrotica speciosa* and disease such as erwinia (*Erwinia carotovora*) were performed according to the recommendations by Segovia et al. (2000). Fertilization was calculated based on the soil chemical characteristics and the recommendations by Segovia et al. (2000). Nitrogen fertilization (urea) was conducted in three applications of 40 kg ha^{-1}, at planting, at 15 days, and at 30 days after transplantation. The base fertilization with phosphorus (Monoammonium phosphate) and potassium (Potassium chloride) was applied at 100 kg ha^{-1} P_2O_5 and 40 kg ha^{-1} K_2O, in a single application to the pots.

Irrigation management and water consumption

Soil moisture for each observed tension was determined using the water retention curve according to the depth of the root system (100 mm), the pot area (0.07 m²), and the average moisture value of the range soil water tension (current θ); the replacement volume was calculated so that it reaches field capacity (FC) of 0.397 $m^3\ m^{-3}$, according to Mantovani et al. (2009).

Irrigation management was performed based on the soil water matrix potential, determined by tensiometers installed at a depth of 10 cm in each experimental unit. Tension readings were taken daily in the late afternoon with a digital tensiometer. When the mean tension reached the treatment tension range, irrigation was performed until reaching FC. For soil water tension ranges 13–17, 23–27, 33–37, and 43–47 kPa at the time of irrigation, the application irrigation depths were 3.14, 9.29, 14.57, and 18.86 mm, respectively. Water was distributed evenly along the irrigation slide using a graduated beaker.

The number of irrigations was monitored during the experiments, and their frequency was expressed as the number of days between two irrigations. Total water consumption of the crop was calculated at the end of the experiments for each treatment based on the number of irrigations and the net irrigation depth, and expressed in millimetres (mm).

Water use efficiency (WUE – kg m^{-3}) was calculated according to Eq. (6), proposed by Doorenbos & Kassan (1994):

$$WUE = \frac{Y}{W} \qquad (6)$$

where Y – is the Chinese cabbage yield (kg ha^{-1}); W – amount of water applied (m^3 ha^{-1}).

Treatments and Experimental Design

The experimental design was completely randomized in a 2×4 factorial scheme, with the two cultivars (Eikoo and Kinjitsu) and four soil water tension ranges (13–17, 23–27, 33–37, and 43–47 kPa), with five repetitions. One plant was transplanted per pot and the pots were arranged at 30×60 cm spacing.

Evaluated Parameters

Physiological parameters

The relative chlorophyll index was determined in five plants 70 days after transplantation on two expanded leaves from the middle third of each plant using a portable chlorophyll meter Chlorofilog (Falker®, made in Porto Alegre – Rio Grande do Sul, Brazil).

The net CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gS, mol H_2O m^{-2} s^{-1}), intracellular CO_2 concentration (Ci, μmol CO_2 mol^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and water use efficiency (WUE, %) were evaluated between 9:00 and 11:00 on two fully developed, healthy leaves per plant, one in the lower third and one in the middle third of the plant at 70 days after transplantation. The measurements were conducted using a gas exchange measuring system equipped with LI-6400XT infrared gas analyser (IRGA) (LI-COR, Lincoln, NE, USA), with automatic CO_2 injector and artificial light source. The conditions in the measuring chamber were kept constant at 1,300 μmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) and 400 μmol CO_2 mol^{-1} during the measurements.

Growth and yield parameters

The harvests in both experiments were performed 88 days after sowing, when the cabbage head was firm to the touch (Segovia et al., 2000). Compactness of the head was evaluated using a grade scale proposed by Souza et al. (2013) with the scores from 0 to 5, where 0, plants with total head absence; 1, plants with a head without a defined core;

2, plants with apparent core head and loose peripheral leaves; 3, plants with heads that have a defined core and peripheral leaves in the beginning stage of compaction; 4, plants with heads that have a defined core and compact peripheral leaves, but the leaves can be visually delimited; and 5, plants with compact head without visually delimited peripheral leaves.

Fresh leaf mass was determined on a precision digital scale (0.0001 g).

Statistical Analysis

The data obtained were subjected to the analysis of variance (F test) and the effect of the treatments was evaluated by the Scott-Knott means test ($P > 0.05$). Statistical analyses were performed in SAS Studio (2017).

RESULTS AND DISCUSSION

Physiological parameters

The relative chlorophyll index was higher in Eikoo than in Kinjitsu in 2016 and 2017, and it was the highest under the tension range of 13–17 kPa (Table 2). The year 2016 resulted in higher chlorophyll content. It was 8.9% higher at the tension range of 13–17 kPa than at the 43–47 kPa tension range. As the soil water stress became more negative, the chlorophyll content decreased. The higher soil water stress affected pigment concentration and reduced the photosynthetic capacity. The results are consistent with the findings of other studies. Hanci & Cebeci (2014) observed that water stress decreased the chlorophyll and carotenoid concentrations in onion (*Allium cepa L.*). In addition, the total chlorophyll concentrations in eggplant (*Solanum melongena* L.) at high water stress (40% field capacity) decreased by 55% when compared with the total chlorophyll concentrations in the control (100% field capacity) (Kirnak et al., 2001). Furthermore, decreased or unaltered chlorophyll concentrations have been reported in other species under water stress, depending on the duration and severity of the stress (Pirzad et al., 2011).

Table 2. Chlorophyll relative index of Chinese cabbage cultivars Eikoo and Kinjitsu exposed to different soil water tension ranges in two experiments (2016–2017)

Soil water tension (kPa)	Chlorophyll relative index					
	2016			2017		
	Eikoo	Kinjitsu	Mean	Eikoo	Kinjitsu	Mean
13–17	49.00	44.60	46.80 a*	43.5	36.6	40.05 a
23–27	46.20	43.40	44.80 b	39.5	34.2	37.50 b
33–37	46.40	43.10	44.75 b	40.5	37.4	36.90 b
43–47	45.32	42.00	45.66 b	37.6	37.5	36.50 b
Mean	46.73 A	43.27 B	45.00 A**	40.27 A	36.4 B	38.34 B
C.V. (%)		14.0				12.4

*Means followed by the same uppercase letter in the row and lowercase in the column do not differ statistically by the Scott-Knott test ($p > 0.05$); **Means of years (2016–2017); C.V. Coefficient of variation.

According to Taiz & Zieger (2016), water deficiency is one of the environmental stresses responsible for the reduction of leaf pigments and lowered photosynthesis rate. This effect is associated with a limited capacity for synthesis and greater degradation of total chlorophylls, which stimulated the plants to use alternative energy dissipation routes to avoid photoinhibition and photoxidation under stress conditions.

A differential behaviour in gas exchange was observed between the two cultivars photosynthesis values (net CO_2 assimilation), internal CO_2 concentration (Ci), and transpiration rate (E) were higher in Eikoo in 2016 and 2017 (Table 3). The year 2017 resulted in higher internal CO_2 concentration and transpiration rate. The higher photosynthesis values calculated for Eikoo may be related to the higher relative chlorophyll index, which provided higher photosynthetic efficiency. Chlorophyll directly reflects the overall photosynthesis and assimilates formation that is linked with the overall crop growth and productivity (Ghodke et al., 2018).

The net CO_2 assimilation, internal CO_2 concentration, and transpiration were higher in the soil water tension range of 13–17 kPa than in other ranges, possibly due to the greater water availability at this tension range (Table 3). The greater water availability bestows a higher internal concentration of carbon.

Table 3. Assimilation of liquid CO_2 (A), internal CO_2 concentration (Ci), and transpiration rate (E) of Chinese cabbage cultivars Eikoo and Kinjitsu exposed to different soil water tension ranges in two experiments (2016–2017)

A (µmol CO_2 m^{-2} s^{-1})						
		2016			2017	
Soil water tension (kPa)	Eikoo	Kinjitsu	Mean	Eikoo	Kinjitsu	Mean
13–17	14.5	12.7	13.6 a*	15.8	13.2	14.5 a
23–27	11.9	11.5	11.7 b	12.3	12.0	12.2 b
33–37	11.5	10.0	10.7 b	12.8	10.5	11.6 b
43–47	10.3	9.5	9.9 b	11.2	9.7	10.5 c
Mean	12.3 A	10.9 B	11.6^{ns} **	13.0 A	11.3 B	12.1
C.V. (%)	20.86				19.30	
Ci (µmol CO_2 mol^{-1})						
13–17	262.9	270.3	266.6 a	291.5	285.0	288.3 a
23–27	257.5	250.6	254.0 b	284.7	259.4	272.1 b
33–37	242.0	257.8	249.9 b	274.0	266.6	270.3 b
43–47	230.3	273.2	251.7 b	262.5	258.3	260.4 c
Mean	248.17B	263.0 A	255.6 B**	278.2 A	267.3 B	272.7 A
C.V. (%)	10.6				11.4	
E (µmol H_2O m^{-2} s^{-1})						
13–17	5.5	4.7	5.1 a	6.2	5.4	5.8 a
23–27	4.9	3.0	3.9 b	5.7	3.7	4.7 b
33–37	4.3	3.7	4.0 b	4.4	4.7	4.5 b
43–47	4.0	3.5	3.7 b	4.1	4.4	4.2 b
Mean	4.7 A	3.7 B	4.2 B**	5.1 A	4.5 B	4.8 A
C.V. (%)	20.3				22.8	

*Means followed by same capital letters in the row and lowercase in the column do not differ statistically by Scott-Knott test ($p > 0.05$); **Means of years (2016–2017); C.V. Coefficient of variation.

The highest rate of net assimilation measured at 13–17 kPa tension is linked to the increased amount of internal CO_2 concentration at the time when the plants exhibited the highest stomatal conductance. Thus, water and CO_2 are among the limiting factors of photosynthesis, and the higher diffusive resistance of stomata reduces photosynthesis, mainly by restricting leaf gas conduction. Therefore, water restriction in the soil water tension range of 23–47 kPa may have inhibited photosynthesis due to stomatal conductance for both water and CO_2 flow decreased by closing the stomata (Nemeskéri & Helyes, 2019), which also explains the lower transpiration rate and productivity under these tension ranges.

The increase in the internal CO_2 concentration values is accompanied by increased stomatal conductance. Thus, stomatal limitation would be the main factor linked to photosynthetic performance; i.e. the larger the stomatal opening, the greater the diffusion of CO_2 into the substomatal chamber (Nascimento et al., 2018).

Stomatal conductance (g_s) was significantly higher in Eikoo than in Kinjitsu (Table 4), and it was higher at 13–17 kPa tension than at other soil water tensions. The amount of water available in the soil at this tension range was sufficient to keep stomata open and provide favourable conditions for the plant to perform physiological processes, without negatively affecting stomatal opening. Under conditions of optimum water availability (field capacity), the transpiration rate was generally high, which explains the increase in transpiration and stomatal conductance of Chinese cabbage plants at the tension range of 13–17 kPa. When soil water becomes scarce, the plant lowers its transpiration rate to reduce water loss and preserve the water available in the soil (Silva et al., 2015).

Table 4. Stomatal conductance (gS) of Chinese cabbage cultivars Eikoo and Kinjitsu exposed to different soil water tension ranges in two experiments (2016–2017)

gS (mol H_2O m^{-2} s^{-1})						
Soil water	2016			2017		
tension (kPa)	Eikoo	Kinjitsu	Mean	Eikoo	Kinjitsu	Mean
13–17	0.33	0.25	0.29 a*	0.35	0.27	0.31 a
23–27	0.25	0.20	0.22 b	0.27	0.22	0.24 b
33–37	0.21	0.19	0.20 b	0.25	0.18	0.21 b
43–47	0.23	0.16	0.19 b	0.24	0.17	0.20 b
Mean	0.26 A	0.20 B	0.23 $^{ns\ **}$	0.28 A	0.21 B	0.24
C.V. (%)	15.7			19.3		

^{ns} not significant by Scott-Knott test ($p > 0.05$); *Means followed by same capital letters in the row and lowercase in the column do not differ statistically by Scott-Knott test ($p > 0.05$); ** Means of years (2016–2017); C.V. coefficient of variation.

Growth and yield parameters

Eikoo was characterized by more compact heads, and higher biomass accumulation, compared with Kinjitsu in both experiments (Table 5). The year 2017 resulted in higher fresh leaf mass. Compactness is valued by consumers and is used as an indicator of proper head formation. The treatment with 13–17 kPa water tension range produced cabbage with a preferred head formation, as indicated by compactness value of 4.1, which is considered to denote a head of better quality and consumers favour plants with very firm, whitish leaves (Seabra et al., 2014). Therefore, cultivation of the Chinese cabbage at a soil water tension of 13–17 kPa is an important feature for producers that will help to reduce volume during transport and provide gains in head weight.

The soil water tension range of 13–17 kPa improved head compactness, and fresh mass compared with the other ranges in 2016 and 2017. Tension ranges with lower available soil water resulted in less compact and malformed heads, thus corroborating a previous study which connected water deficit during head formation with shrunken and malformed heads (Beshir, 2017).

Table 5. Head compactness, fresh leaf mass (FLM), and yield of Chinese cabbage cultivars Eikoo and Kinjitsu grown at different soil water tension ranges in two experiments (2016–2017)

Head compactness						
Soil water	2016			2017		
tension (kPa)	Eikoo	Kinjitsu	Mean	Eikoo	Kinjitsu	Mean
13–17	4.1	3.2	3.7 a*	4.4	3.7	4.1 a
23–27	3.6	3.0	3.3 b	3.8	3.1	3.4 b
33–37	3.3	2.8	3.0 b	3.5	2.9	3.2 b
43–47	2.9	2.5	2.7 b	3.0	2.8	2.9 b
Mean	3.5 A	2.9 B	3.2 $^{ns\,**}$	3.7 A	3.1 B	3.4
CV (%)	21.4			20.6		
FLM (g plant^{-1})						
13–17	880.5 Aa	750.1 Ba	815.3	1180.5 Aa	880.3 Ba	1030.40
23–27	760.7 Ab	655.4 Bb	708.0	1025.3 Ab	820.2 Bb	922.75
33–37	690.4 Ab	640.5 Ab	665.5	995.0 Ab	797.8 Bb	896.40
43–47	582.8 Ac	575.4 Ac	579.1	897.0 Ac	780.3 Bb	838.65
Mean	728.6	655.35	691.97 B**	1024.45	819.65	922.05 A
CV (%)	12.8			16.0		

*Means followed by same capital letters in the row and lowercase in the column do not differ statistically by Scott-Knott test ($p > 0.05$); **Means of years (2016–2017); C.V. coefficient of variation.

Higher soil water stress due to water deficit significantly reduced the leaf fresh mass production (Table 5). This pattern may have resulted from the fact that, at lower tensions (up to 13–17 kPa), the number of irrigations (40.5) was greater and the interval between irrigations was less (1.3 days; Table 6), which contributed to higher formation, growth, and compactness of head.

The fresh leaf mass at a 13–17 kPa tension was higher than that reported by Averbeke & Netshithuthuni (2010) and Maseko et al. (2017). This inconsistency in the fresh leaf mass may be due to cultivar type, higher photosynthetic activity, and environmental factors such as temperature and water availability (Maseko et al., 2017).

Tangune et al. (2016) reported that the irrigation of broccoli (*Brassica oleracea* var. italica) crop when soil water tension reached 15 kPa resulted in higher commercial fresh mass (0.76 kg), and mean inflorescence diameter (20.5 cm).

Chinese cabbage and other leafy vegetables require frequent irrigation, at least twice a week, to maintain soil water content close to field capacity. This will provide the optimum conditions for leaf growth, which is a function of cell expansion, a physiological process highly sensitive to water stress (Costa et al., 2007), probably due to its superficial root system, i.e. more than 90% of the roots extend in the top 35 cm soil layer and 20 cm from the stem (Averbeke & Netshithuthuni 2010).

Irrigation management and water consumption

The response of cultivars to the irrigation management was similar in both experiments (Table 6). Soil water tension ranges governed the number and frequency of irrigations and the amount of irrigated water in 2016 and 2017. Increasing tension ranges reduced the number of irrigations and therefore their frequency. However, water consumption increased by 63% and 52% in 2016 and 2017, respectively, when comparing the water consumption at soil water tension of 13–17 kPa with that at 43–47 kPa. The year 2017 resulted in higher number of irrigations and water consumption.

Table 6. Number of irrigations, interval between irrigations, and water consumption of Chinese cabbage cultivars Eikoo and Kinjitsu exposed to different soil water tension ranges in two experiments (2016–2017)

Soil water tension (kPa)	2016 Eikoo	2016 Kinjitsu	2016 Mean	2017 Eikoo	2017 Kinjitsu	2017 Mean
	Number of irrigations					
13–17	25.0	25.0	25.0 a*	41.3	39.8	40.5 a
23–27	15.0	15.0	15.0 b	21.5	21.5	21.5 b
33–37	13.0	11.0	12.0 c	16.3	14.3	15.2 c
43–47	12.0	10.0	11.0 d	16.5	13.5	15.0 c
Mean	16.3	15.3^{ns}	15.8 B**	23.9	22.3^{ns}	23.1 A
C.V. (%)	6.0			15.2		
	Interval between irrigations (dia^{-1})					
13–17	1.9	1.5	1.7 c	1.3	1.3	1.3c
23–27	3.1	3.1	3.1 b	2.6	2.6	2.6b
33–37	3.7	4.3	4.0 a	3.3	3.8	3.6a
43–47	3.5	4.1	3.8 a	3.1	4.0	3.5a
Mean	3.0	3.3^{ns}	**3.2** ^{ns**}	2.89	2.58^{ns}	**2.74**
C.V. (%)	0.75			17.7		
	Water consumption (mm)					
13–17	78.6	78.1	78.3 d	149.6	124.0	136.8 c
23–27	139.3	139.3	139.3 c	199.6	199.9	199.7 b
33–37	204.3	172.6	188.5 b	234.9	205.3	220.1 b
43–47	231.4	192.6	212.0 a	311.1	254.6	282.9 a
Mean	163.4	145.7^{ns}	154.6 B**	223.8	195.7^{ns}	209.8 A
C.V. (%)	6.24			11.4		

^{ns} not significant by Scott-Knott test ($p > 0.05$); Means followed by the same lowercase letter in the column did not differ statistically by the Scott-Knott test ($p > 0.05$); **Means of years (2016–2017); C.V. Coefficient of variation.

With the increase of tension ranges, the number of irrigations was reduced. However, this longer interval between two irrigations caused a water stress to the crop, reducing the rate of photosynthesis and productivity and increasing water consumption.

Kirnak et al. (2017) in studies conducted with chickpea reported that compared to dry treatment without any irrigations, 54.13% decrease was observed in plant water consumption in full irrigation treatment (323 mm). On the other hand, irrigations significantly increased chickpea yields compared to treatment without irrigation.

The average number of irrigations (15.8 and 23.1), the frequency of irrigations (3.2 and 2.7 days), and water consumption (154.6 and 209.8 mm) differed between 2016 and 2017, respectively. These differences may be related to the lower potential evapotranspiration and average temperature observed in 2016 (0.78 mm day^{-1} and 17.6 °C, respectively) when compared with the values in 2017 (1.75 mm day^{-1} and 21.8 °C, respectively).

Similar results were reported by Ünlükara et al. (2017) studied a response of spinach (*Spinacia oleracea* L. Matador) to the salinity of irrigation water in indoor and outdoor greenhouses to reveal different climatic conditions about the salinity tolerance of the plant; they found that the highest water consumption of 36% was observed due to higher indoor temperature, being one of the climatic factors that directly influenced water consumption and salinity tolerance.

For water use efficiency, cultivars responded differently, Eikoo was more efficient in water use compared to Kinjitsu (Table 7). This greater efficiency of 'Eikoo' is the result of higher rates of liquid assimilation, internal CO_2 concentration, and transpiration, which resulted in greater production capacity (biomass accumulation). According to Beshir (2017) the WUE of the crops can be increased by increasing the transpiration rate of the crops to produce greater biomass (CO_2 assimilation) and yield per unit of water used.

Silva et al. (2018a) found that interannual climate variations due to changes in air temperature and relative humidity had significant effects on the growth, productivity and evapotranspiration variables of the coriander (*Coriandrum sativum* L.) cultivated in a tropical environment. The lowest values of yield and WUE in coriander occurred in the summer growing season, due to the higher values inherent to the evapotranspiration of the crop.

The ranges of water tension in the soil significantly influenced the efficiency of water use (Table 7). The WUE was 36.3% higher in the soil water tension range 13–17 kPa, compared to 43–47 kPa. In water tensions in the soil with greater water restriction, water consumption was higher, but productivity was not increasing, resulting in less efficiency in water use. Similar results were reported by Silva et al. (2018b) for lettuce cultivars, found a significant increase in water consumption and a lower WUE in the summer, due to the higher values of the crop evapotranspiration.

Table 7. Water use efficiency of Chinese cabbage cultivars Eikoo and Kinjitsu exposed to different soil water tension ranges in two experiments (2016–2017)

WUE (kg m^{-3})						
Soil water	2016			2017		
tension (kPa)	Eikoo	Kinjitsu	Mean	Eikoo	Kinjitsu	Mean
13–17	44.2	34.6	39.4 a*	46.4	27.1	36.6 a
23–27	25.3	18.1	21.7 b	33.6	18.4	26.0 b
33–37	17.8	16.5	17.2 b	22.3	17.8	20.1 b
43–47	15.1	12.4	13.8 c	15.1	11.5	13.3 c
Mean	25.6 A	20.4 B	23.0^{ns} **	29.4 A	18.7 B	24.1
C.V. (%)	12.4			19.3		

*Means followed by same capital letters in the row and lowercase in the column do not differ statistically by Scott-Knott test ($p > 0.05$); ** Means of years (2016–2017); C.V. Coefficient of variation.

Xiang et al. (2019) observed that the highest productivity and WUE of Chinese cabbage were obtained with an irrigation level of 120% crop evapotranspiration and an irrigation frequency of four days intervals. The results of that study showed that the amount of irrigation had a greater effect on the yield and WUE of Chinese cabbage than the frequency, due to a positive correlation between crop growth and soil moisture.

Thus, the evapotranspiration of the crops is strongly influenced not only by the water content in the soil, but also by the temperature of the air that the plant is submitted (Silva et al., 2018a). Evapotranspiration can be used as an indicator of the evapotranspiration demand by the crop, and therefore, summer-grown crops will likely have higher water consumption.

The frequency of irrigation is directly determined by soil type and weather conditions (Shock & Wang, 2011). The use of tensiometers or other soil moisture monitoring devices contributes to improved productivity and quality of Chinese cabbage as their use ensures a more efficient irrigation and reduces water costs.

In the face of climate change and its impacts on soil water reservoirs, Várallyay (2010) reports on the possibilities for sustainable soil moisture management and the necessary measures for the rational control of water in crop production, such as increasing water use efficiency; reducing evaporation and runoff; thus increasing the water storage capacity and the available soil moisture range, important measures for the rational control of water.

CONCLUSIONS

The research showed that the soil water tension is an important parameter for programming irrigation.

For greater photosynthetic efficiency, productivity, head compactness, water use efficiency, and better development of Eikoo and Kinjitsu cultivars, these crops should be irrigated when the water tension in the soil is in the 13–17 kPa range. At the highest soil water tension ranges, water restriction influenced photosynthesis, head compactness, and yield of the two cultivars negatively.

The lowest irrigation frequency and water consumption were observed under low potential evapotranspiration and mild temperature conditions. The least water consumption was observed at the 13–17 kPa tension range.

The year 2017 resulted in higher internal CO_2 concentration, transpiration rate, fresh leaf mass, number of irrigations and water consumption compared to the year 2016.

The study results help farmers plan irrigation of Chinese cabbage so that it results in productivity and efficient use of water without wasting water.

ACKNOWLEDGEMENTS. We would like to thank to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting scholarship to the researcher Dislaine Becker and Editage (www.editage.com) for English language editing.

REFERENCES

Acikgoz, F.E. 2016. Seasonal variations on quality parameters of pak choi (*Brassica rapa* L. subsp. *chinensis* L.). *Adv Crop Sci Tech*. **4**, 233. doi:10.4172/2329-8863.1000233

Allen, R.G., Pereira, L.S., Raes, D. & Smith, M. 1998. Crop evapotranspiration – guidelines for computing crop water requirements. *Irrigation and Drainage Paper* **56**. 300 pp.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M. & Sparovek, G. 2013. Köppen's climate classification map for Brazil. *Meteorolo. Z.* **22**, 711–728. doi:10.1127/0941-2948/2013/0507.

Artemyeva, A.M. & Solovyeva, A.E. 2006. Quality evaluation of some cultivar types of leafy *Brassica rapa*. *Acta Hortic*. **706**, 121–128.

Averbeke, W.V. & Netshithuthuni, C. 2010. Effect of irrigation scheduling on leaf yield of non-heading Chinese cabbage (Brassica rapa L. subsp. chinensis). S Afr J Plant Soil. 27, 322–327.

Beshir, S. 2017. Review on estimation of crop water requirement, irrigation frequency and water use efficiency of Cabbage production. *Journal of Geoscience and Environment Protection* **5**, 59–69.

Carrijo, D.R., Akbar, N., Reis, A.F.B., Li, C., Gaudin, A.C.M., Parikh, S.J., Green, P.G. & Linquist, B.A. 2018. Impacts of variable soil drying in alternate wetting and drying rice systems on yields, grain arsenic concentration and soil moisture dynamics. *Field Crops Res*. **222**, 101–110. https://doi.org/10.1016/j.fcr.2018.02.026

Costa, J.M., Ortuño, M.F. & Chaves, M.M. 2007. Deficit irrigation as a strategy to save water: physiology and potential application to horticulture. J. Integrative Plant Biol. 49, 1421–1434.

Doorenbos, J. & Kassam, A.H. 1994. *Effect of water on crop yield*. Campina Grande: UFPB. (*FAO, Irrigation and drainage studies* **33**), 306 pp.

Embrapa – Brazilian Agricultural Research Corporation. 1997. *National Soil Research Center*. (2nd ed.). Rio de Janeiro, 212 pp.

Embrapa – Brazilian Agricultural Research Corporation. 2018. *Brazilian system of soil classification* (5nd ed.). Rio de Janeiro, 355 pp.

Filgueira, F.A.R. 2008. *New Olericulture Handbook*: *Modern agrotechnology in the production and marketing of vegetables* (2nd ed.). Viçosa: UFV, 412 pp.

Gee, G.W. & Bauder, J.W. 1986. Particle size analysis. In: Klute, A. (Ed.) *Methods of soil analysis: physical, chemical and mineralogical methods.* Madison: American Society of Agronomy, pp. 383–411.

Godfray, H.C.J. & Garnett, T. 2014. Food security and sustainable intensification. *Phil. Trans. R. Soc. B*. **369**, 2012–2073. http://dx.doi.org/10.1098/rstb.2012.0273

Ghodke, P.H., Andhale, P.S., Gijare, U.M., Thangasamy, A., Khade, Y.P., Mahajan, V. & Singh, M. 2018. Physiological and biochemical responses in onion crop to drought stress. *Int.J.Curr.Microbiol.App.Sci.* **7**(1), 2054–2062.

Hanci, F. & Cebeci, E. 2014. Investigation of proline, chlorophyll and carotenoids changes under drought stress in some onion (*Allium cepa* L.) cultivars. *Turkish Journal of Agricultural and Natural Sciences* **2**, 1499–1504.

Kirnak, H., Kaya, C., Tas, I. & Higgs, D. 2001. The influence of water deficit on vegetative growth, physiology, fruit yield and quality in eggplants. *Bulg. J. Plant Physiol.* **27**, 34–46.

Kirnak, H., Varol, I.S., Irik, H.A. & Ozaktan, H. 2017. Effects of irrigation applied at different growth stages on chickpea yield. *Agronomy Research* **15**(5), 1928–1933. https://doi.org/10.15159/AR.17.046

Mantovani, E.C., Bernardo, S. & Palaretti, L.F. 2009. *Irrigation: principles and methods*. 3. ed. Viçosa: UFV, 355 pp.

Marouelli, W.A. 2008.*Tensiometers for irrigation control in vegetables*. Brasília: Embrapa. 15pp.

Maršalkienė, N., Sliesaravičius, A., Ramaškevičienė, A., Žilėnaitė, L. & Švirmickas, G.J. 2014. Agrobiological potential of some soil crucifers of Lithuania's spontaneous flora. *Zemdirbyste* **101**, 153–160.

Maseko, I., Beletse, Y.G., Nogemane, N., Plooy, C.P., Musimwa, T.R. & Mabhaudhi, T. 2017. Productivity of non–heading Chinese cabbage (*Brassica rapa* subsp. chinensis) under different agronomic management factors. *S Afr J Plant Soil*. **34**, 275–282.

Michelon, C.J., Carlesso, R., Oliverira, Z.B., Knies, A.E., Petry, M.T. & Martins, J.D. 2010. Pedotransference functions to estimate water retention in some soils of Rio Grande do Sul. *Cienc Rural* **40**, 848–853.

Nascimento, J.L., Almeida, A.A.F., Barroso, J.P., Mangabeira, P.A.O., Ahnert, D., Sousa, A.G.R., Silva, J.V.S. & Baligar, V.C. 2018. Physiological, ultrastructural, biochemical and molecular responses of young cocoa plants to the toxicity of Cr (III) in soil. *Ecotoxicology and Environmental Safety* **159**, 272–283.

Nemeskéri, E. & Helyes, L. 2019. Physiological responses of selected vegetable crop species to water stress. *Agronomy*. **9**(447), 1– 19. doi:10.3390/agronomy9080447

Pascual, P.R.L., Jarwar, A.D. & Nitural, P.S. 2013. Fertilizer, fermented activators, and EM® utilization in pechay (*Brassica pekinensis*) production. *Pak. J. Agri., Agril. Eng., Vet. Sci.* **29**, 56–69.

Pirzad, A., Shakiba, M.R., Salmasi, S.Z., Mohammadi, S.A., Darvishzadeh, R. & Samadi, S. 2011. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L. *J. Med. Plant. Res*. **5**(12), 2483–2488.

Seabra, S.J., Pereira, A.S. & Araujo, K.L. 2014. Performance of Chinese cabbage cultivars in Cáceres – MT. *Hortic Bras*. **32**, 504–507.

Segovia, J.F.O., Melem, N.J., Azevedo, J.S. & Lopes, R.P. 2000. *The cultivation of Chinese cabbage in Amapá*. Macapá: Embrapa Amapá, 20 pp.

Shock, C.C. & Wang, F.X. 2011. Soil water tension, a powerful measurement for productivity and stewardship. *HortScience*. **46**(2), 178–185.

Silva, F.G., Dutra, W.F., Dutra, A.F., Oliveira, I.M., Filgueiras, L.M.B. & Melo, A.S. 2015. Gas exchange and chlorophyll fluorescence in eggplant plants under irrigation depths. *Brazilian Journal of Agricultural and Environmental Engineering* **19**(10), 946–952.

Silva, T.G.F., Silva, B.B., Holanda, R. M., Brito, J.I.B., Braga, C.C., Souza, E.P. & Silva, M.T. 2018a. Evapotranspiration, crop coefficient and water use efficiency of coriander grown in tropical environment. *Hortic Bras.* **36**(4), 446–452. https://dx.doi.org/10.1590/s0102-053620180404

Silva, T.G.F., Tavares, A.L., Sousa, I.F., Silva, T.G.F., Holanda, R.M., Souza, E. P., Silva, B.B., Braga, C.C. & Almeida, R.S.R. 2018b. Evapotranspiration, water use efficiency and crop coefficient of three lettuce varieties grown in a tropical region. *Sci. Agrar.* **41**(3), 798–805.

Soil Science Division Staff. 2017. *Soil survey manual*. Ditzler, C., Scheffe, K. & Monger, H.C. (eds.). USDA Handbook 18. Government Printing Office, Washington, D.C., 639 pp.

Souza, A.L., Junior, S.S., Diamante, M.S., Souza, L.H. & Nunes, M.C.M. 2013. Behavior of lettuce cultivars under tropical climate. *Revista Caatinga* **26**, 123–129.

Statistical Analysis System. SAS Studio. 2017. *http://www.sas.com/en_us/software/university–edition.html//*. Acessed 15.2. 2017.

Taiz, L. & Zeiger, E. 2016. *Plant Physiology*. Porto Alegre: Artmed, 888 pp.

Tangune, F.B., Pereira, G.M., Sousa, R.J. & Gatto, R.F. 2016. Production of drip irrigated broccoli under different soil water stresses. *Semin Cienc Agrar*. **37**, 7–16.

Teixeira, P.C., Donagemma, G.K., Fontana, A. & Teixeira, W.G. 2017. *Manual of soil analysis methods.* 2.ed. Rio de Janeiro: CNPS, 573 pp.

Ünlükara, A., Yurtyeri, T. & Cemek, B. 2017. Effects of Irrigation water salinity on evapotranspiration and spinach (*Spinacia oleracea* L. Matador) plant parameters in greenhouse indoor and outdoor conditions. *Agronomy Research* **15**(5), 2183–2194. https://doi.org/10.15159/AR.17.041

Van Guenuchen, M.T. 1980. A closed form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci Soc Am* J. **44**, 892–898.

Van Genutchten, M.T., Leij F.J. & Yates S.S. 1991. *The RETC code for quantifying the hydraulic function of unsaturated soils: version 6.02 V.S.* Riverside: USDA, Salinity Laboratory. Avaiable at: http://www.pc–progress.com/en/Default.aspx?retc–downloads, 20 April 2016.

Várallyay, G. 2010. The impact of climate change on soils and on their water management. *Agronomy Research* **8**, 385–396.

Xiang, Y., Zou, H., Zhang, F., Qiang, S., Wu, Y., Yan, S., Wang, H., Wu, L., Fan, J. & Wang, X. 2019. Effect of irrigation level and irrigation frequency on the growth of mini Chinese cabbage and residual soil nitrate nitrogen. *Sustainability* **11**, 111.

Yazgan, S., Ayas, S., Demirtas, C., Buyukcangaz, H. & Candogan, B.N. 2008. Deficit irrigation effects on lettuce (*Lactuca sativa* var. Olenka) yield in unheated greenhouse condition. *Journal of Food Agronomy and Environment* **6**, 357–361.

Zorica, J. & Stikic, R. 2018. Partial root-zone drying technique: from water saving to the improvement of a fruit quality. Front Sustain Food Syst 1, 1–3. doi: 10.3389/fsufs.2017.00003

Agronomy Research **18**(1), 256–266, 2020
https://doi.org/10.15159/AR.20.010

Influence of fertilizer application on biomass yield and nutritional quality of Mustard Spinach (*Florida)* Broadleaf in South Africa

K.A. Seetseng[1], A.S. Gerrano[1,*], S. Mavengahama[2], H.T. Araya[1] and C.P. Du Plooy[1]

[1]Agricultural Research Council, Vegetable and Ornamental Plants, Private Bag X293, Pretoria 0001, South Africa
[2]Crop Science Department and Food Security and Safety Research Group, Faculty of Natural and Agricultural Sciences, North-West University P/Bag X2046, Mmabatho 2735, South Africa
[*]Correspondence: agerrano@arc.agric.za

Abstract. Mustard Spinach (Florida Broadleaf) is an indigenized leafy vegetable grown in Southern Africa. It is a good source of vitamins and mineral nutrients. An understanding of its response to fertilizer application is important in developing cultural practices for improved yield of the crop. Furthermore, improving fertilizer use efficiency such as nitrogen (N), phosphorus (P) and potassium (K) would result in improving cropping system. Therefore, a field experiment was conducted in 2010 and 2011 winter cropping season to determine the influence of NPK applications and their interactions on biomass yield and nutritional values of Mustard spinach. A randomized complete block design replicated four times were used for the experiment. ANOVA showed significant variation among the treatments. The values for total fresh biomass yield ranged from 252–4,510 and 820–4,982 kg ha^{-1} in 2010 and 2011 cropping seasons, respectively. Omission of P had 4,510 kg ha^{-1} of total fresh biomass yield, while omission of K had 4,506 kg ha^{-1} in the first season. The lowest N content (2.63%) was recorded when nitrogen fertilizer was omitted. Full NPK application increased the content of N and K on the leaf tissues of Mustard Spinach. The highest percentage of dietary fiber was observed when K was omitted. The values recorded for ascorbic acid content varied from 126.94 and 117.42 mg $100g^{-1}$, respectively for both seasons. Iron was more concentrated on the treatments, where K was omitted. Mustard Spinach responded to fertilizer application and the results validated that application of NPK had a beneficial effect on increased production and productivity of the crop tested for small scale farmers.

Key words: mustard spinach, marketable, nutrient omission, dietary fibre, interaction, outperformed, micronutrients.

INTRODUCTION

Africa has a wide ranges of indigenous and indigenized vegetables that are under-utilized and marginalized (Smartt & Haq, 1997) can be utilized for food, nutritional and health security. Mustard Spinach (*Florida Broadleaf*), *Brassica juncea* is an exotic leafy vegetable crop mainly grown in most parts of South Africa for its edible parts (Maboko, 2013). The fresh leaves of Mustard Spinach are mainly eaten in different forms (Grubben & Denton, 2004). In South Africa, it is commonly known by the local name 'Motshaina' specially in the northmost part of the country (Maboko, 2013). Maboko (2013) further reported that the crop is a cool season and fast growing crop that reaches maturity at 35–65 days after transplanting to the field. The crop is a good source of dietary fiber, provitamin A, ascrobic acid, vitamin K, thiamine, riboflavin, vitamin B6, folate and mineral nutrients (Van Wyk, 2005). Mustard spinach is commonly grown to provide essential mineral and organic nutrients to humans for the benefit of health, nutritional and food security.

Agricultural production systems in many developing countries needs to provide enough food coupled with nutrients to meet human needs, therefore cultivation of vegetables and their agronomic practices has to be intensified. Intensified agricultural production includes cultivation practices such as irrigation, fertilizer application as well as nutrient use efficiency to maximise yield potential of the crop. Nitrogen, phosphorus and potassium are key mineral nutrient in the production of agricultural crops as they enhance yield by promoting cell division and expansion in leaves, and root development (Makus, 1992). Leafy vegetables such as cabbage, spinach, lettuce and celery provide humans with many kinds of vitamins and mineral elements (Wang et al., 2008). Nitrate that accumulates in leafy vegetables originate from residual soil N, as well as from the application of organic and inorganic N fertilizers (Schenk, 1998; Wang et al., 2008).

It was reported in previous studies that under field conditions, soil is aerobic, and ammonium and urea N are rapidly transformed to nitrate-N; therefore, the effect of fertilizer type on nitrate concentration in vegetables usually is not so pronounced (Wang et al., 2008). Phosphorus is an essential macro element necessary for growth and development of plants. Its shortage restricts growth of plants and they remain immature (Hossain, 1990; Sadia et al., 2013). Wang & Li (2004) reported that crop response to P fertilizer depended on soil available P as well as on crop species. In soil that was deficient in available P, crops respond well to P application but in contrast, their results have shown that addition of P fertilizer to a soil containing 109 mg kg^{-1} (Olsen-P) decreased nitrate concentration by 20.1% in green cabbage (Wang & Li, 2004). Increased nitrate concentration (17.3%) was observed in cabbage and on the other hand, phosphorus application had no significant effect on nitrate accumulation in spinach (Wang & Li, 2004). According to Zhou et al. (1989), potassium can accelerate transport of nitrate from roots to aboveground parts. It was reported that compared to control, nitrate concentration in cabbage decreased by 14.6% (from 482 to 412 mg kg^{-1}) with application rate of 75 kg K ha^{-1}.

Effects of K on growth and nitrate accumulation in leafy vegetables vary with crop species, cultivar and environment. Hydroponic experiments showed that the addition of K decreased nitrate concentrations in cabbage by 26.0% compared to the control treatment (Maynard et al., 2003). In contrast, the addition of K increased nitrate concentrations by 8.2% in spinach. Furthermore, application of K to treatments containing high N levels inhibited spinach growth, but had no significant effect on cabbage (Maynard et al., 2003). Scherer (2001) stated that rates of fertilizer should be recommended based on the available element in the soil and the crops' requirement for that element. With an indigenised crop like Mustard spinach (Tshikalange, & Averbeke, 2006; Van Averbeke & Juma, 2006), had previously reported that there is a great deal of variability in the rates at which producers applied nitrogen, phosphorus and potassium.

According to Van Averbeke et al. (2007a), optimum crop growth and yield depend on the adequate availability and accessibility of different plant nutrients. However, application of fertilizers contributes significantly to variables costs of production. It is of utmost of value to consider that those vegetables grown by smallholder farmers are grown with minimum availability of resources. Efficiency in fertilizer application for optimum growth and yield, is always desired if obtained at minimum costs. The objective of this study therefore, was to evaluate the effects of fertilizer application on biomass yield and nutritional quality of Mustard Spinach under field condition. The study would contribute to the production of Mustard Spinach at small-scale farmers' condition because Mustard spinach is one of those leafy vegetables that farmers grow for their home consumption. Integrating Mustard Spinach into mainstream agriculture production has been slow just like any other indigenous and underutilized plant species in South Africa, but the awareness, consumption and demand is currently increasing at rural and urban community. Therefore, there is an acute need for the scaling up and rapid commercialisation of this leafy vegetable crop towards contributing to food and nutritional security at household level in the country.

MATERIALS AND METHODS

A field trial was conducted at the Roodeplaat research farm of the Agricultural Research Council (ARC), South Africa (25°59'S; 28°35'E at an altitude of 1, 200 m.a.s.l.). The experiment was arranged in a randomized complete block design replicated four times. Mustard spinach seed (the cultivar known as 'Florida' broadleaf) were obtained from Starke Ayres seed Pty. Ltd., South Africa) for the experiment. The trial was established on 03 May 2010 and 02 May 2011 in a 200 cavity polystyrene trays filled with a commercial growth medium, Hygromix® (Hygrotech Seed Pty. Ltd., South Africa) and covered with a thin layer of vermiculite after sowing. The soil type at the experimental site is Fernwood as described by Department of Agricultural Development (DAFF) (1991). The seedlings were transplanted on 25 days after sowing; fertilizer was broadcasted by hand in the demarcated experimental plots and being worked into the soil using rakes (Table 1). Limestone Ammonium Nitrate (28% N) was applied as a source of nitrogen, single superphosphate (12.5% P) was applied as a source of phosphorus and potassium was applied in the form of Potassium chloride, KCL (50% K) (Table 1). Nitrogen was split, 60% applied at planting and the remaining 40% was applied as top-dressing after second harvest. A nutrient omission experiments (Table 1) were laid out in a randomized complete block design with five treatments arranged as follows: NPK (full application); N0PK (nitrogen omitted); NP0K (phosphorus omitted); NPK0 (potassium omitted) and N1/2P1/2K1/2 (half of the full

Table 1. Fertilizer treatments (kg ha^{-1}) of Mustard Spinach (*Florida broadleaf*) planted on Fernwood soil form

Fertilizer treatments	Nitrogen (N)	Phosphorus (P)	Potassium (K)
N0P0K0	Control	Control	Control
NPK	100	150	20
N0PK	Omitted	150	20
NP0K	100	Omitted	20
NPK0	100	150	Omitted
N1/2P1/2K1/2	50	75	10

NPK = 100N: 150P: 20K kg ha^{-1} (which was the recommended application rate for N:P:K); N0PK = 0N: 150P:20K kg ha^{-1}; NP0K = 100N:0P:20K kg ha^{-1}; NPK0 = 100N: 150P: 0K kg ha^{-1}).

application) of nitrogen, phosphorus and potassium replicated four times. Drip irrigation system was used and front wetting detectors was installed for monitoring soil water content. Plots were irrigated to field capacity approximately when 30% of soil plant available water is depleted. The treatments denotes: NPK = 100N: 150P: 20K kg ha^{-1}; N0PK = 0N: 150P: 20K kg ha^{-1}; NP0K = 100N:0P:20K kg ha^{-1}; NPK0 = 100N: 150P: 0K kg ha^{-1} and $N_{1/2}P_{1/2}K_{1/2}$ = 50N:75P:10K kg N:P:K ha^{-1}, based on soil analysis including the control = No fertilizer applied(N0P0K0) (Table 1). The total plot size was 4×5 m (20 m^2) and seedlings were transplanted at a row spacing of 0.4 m and in-row spacing of 0.4 m, therefore the total sampled area was 12.8 m^2. Prior to transplanting soil sample was collected to a depth of 0.4 m with 0.2 m intervals. Average monthly rainfall and rainy days (Fig. 1) and minimum and maximum temperatures (Fig. 2) from 2009 to 2012 at the research farm. The climate of the area is semi-arid and approximately 80% of the annual rainfalls in this area occur in the summer months of September to March.

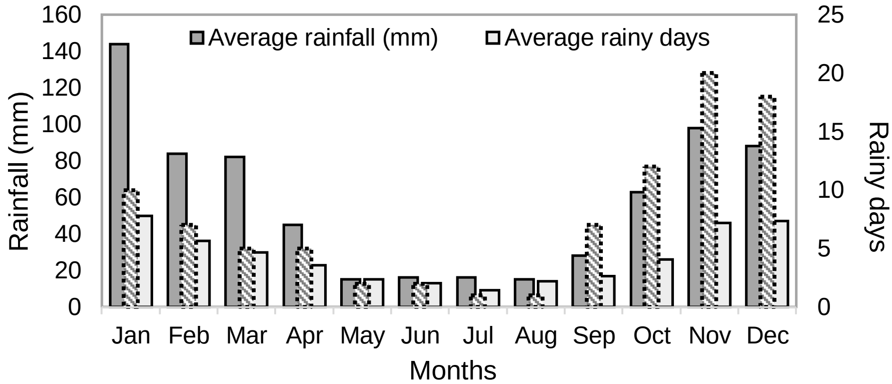


Figure 1. Average monthly rainfall and rainy days during the growth and development of the crop.

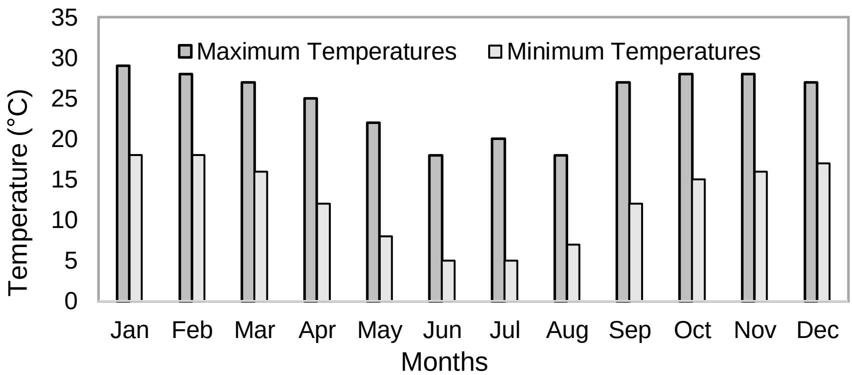


Figure 2. Monthly minimum and maximum temperatures during the growth period of the crop.

Data collection

Soil sampling

Field management practices was carried out as described by Gerrano et al. (2019). The soil samples were collected at a 30 cm depth (Table 2) before planting and collected samples were put in one container and being shaken to mix samples thoroughly. A composite sample was then taken out of the mixed soil sample for laboratory analysis in the analytical laboratory of the ARC (Table 2).

Table 2. Soil chemical content (mg kg^{-1}) and textural analyses (%) at the Roodeplaat experimental site

	Phosphorus		Potassium		Calcium		Magnesium		pH		Texture class	
Year	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011		
Soil depth (cm)											Sand	92
0–20	59.2	61.4	22	32	208	207	78	78	7.74	6.51	Silt	2
21–40	12.4	9.8	19	32	152	193	58	64	6.96	5.8	Clay	6

Sampling for total biomass yield

The first harvest commenced at the eight leaf stage (three weeks after transplanting), when the ninth leaf started to appear. It involved cutting the fifth leaf, which is considered the first marketable leaf, because the first four leaves were too small for marketing purposes (Van Averbeke et al., 2007b). Fresh biomass was determined from harvesting eight plants per plot and put on ADAM® with maximum of 5 kg at d = 0.5 g. Total fresh biomass was determined immediately at harvest. The plants were harvested five times in each growing season when the peduncle elongation starts, which was used as the signal to do the final leaf harvest, because once the plant starts flowering, the leaves become leathery and lose their taste (Tshikalange, 2006). Fresh leaf biomass measurements were done using a portable electronic scale (Scout Pro SPU123®) with a capacity of 5,000 g and an accuracy of 0.001 g, manufactured by Ohaus Corporation.

Sampling of leaves for analysis

Fresh and edible leaves from the same treatment in all replicates were tipped off, combined to constitute a composite sample. Leaves were soaked and thoroughly washed with tap water (four to five times) to remove soil debris and were rinsed with distilled water. Leaves were air-dried on an absorbent paper at room temperature. Leaves were then cut to make a homogenous finer texture using a blender. Samples were transferred to a marked plastic containers, frozen at -20 °C and were sent to the ARC analytical laboratory for ascorbic acid, Dietary fibre, iron and zinc analysis.

Mineral composition analysis

Oven dried (50°C) plant material were analysed for total P, K, Fe and Zn contents according to Campbell & Plank (1991); Kovar (2003) and Wolf et al. (2003) and furthermore, the determination of total N was done according to Gavlak et al. (1996). Plants material were used to determine ascorbic acid content and HPLC-grade acetonitrile (> 99.9%; v/v) from Merck (Darmstadt, Germany) was used. The dietary fiber were analysed by the methods described in AOAC (1990).

Data analysis

Data was subjected to analysis of variance (ANOVA) using the statistical program GenStat® version 11.1 (Payne et al., 2016). Treatment means were separated using Fisher's protected T-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran, 1980). Soil sample analysis (Table 1) for phosphors was carried out using P. Bray 1 method (Bray & Kurtz, 1945; Frank et al., 1998) and for potassium, calcium and magnesium, the Ammonium acetate method was used as described by (Chapman, 1965). Samples collected for mineral composition analysis were combined for each treatment and a composite sample was used per treatment, therefore the results were not subjected to statistical analysis.

RESULTS AND DISCUSSION

Marketable fresh leaves biomass yield

Analysis of variance showed that there was a significant variation among the treatment (Table 3) tested. The highest value obtained in the first harvest was 188 kg ha^{-1}, which was recorded for the first season on full package of NPK (Table 3). The omission of N in this experiment had reduced marketable fresh leaf biomass. The result agrees with what Nemadodzi et al. (2017) reported where accumulation of spinach biomass was significantly reduced when N was not applied. There was a significant difference ($p < 0.05$) between P omitted and K treatments. The increment of marketable fresh leaf biomass yield in the interaction of N and P indicates complementarities of nitrogen in the uptake of phosphorous. The values were 102 kg ha^{-1} when P was omitted and increased to 183 kg ha^{-1} when P was applied in combination with N (Table 3). When K was omitted, there was a significant difference ($p < 0.05$) observed among the treatments (Table 3). Visual differences could be noticed in Fig. 3 indicating that there is variation in growth and development of spinach when compared the control ($N_0P_0K_0$); half ($N_{1/2}$:$P_{1/2}$:$K_{1/2}$); full (N: P: K) and P omitted treatments. In year two of the cropping season, the highest yield was 194 kg ha^{-1} in the first harvest, when NPK was applied in full, there was a significant difference ($p < 0.05$) observed between the treatments (Table 3). Omission of N resulted in yield decrease and was the second after the control (where no fertilizer was applied). The results are in line with those of Soundy et al. (2001b) previous report, who found that omission of N caused reduction in leaf biomass yield. It was further revealed in the findings of Soundy & Cantliffe (2001a) that increasing N concentration in the growth medium resulted in an increased shoot growth of lettuce plantlets. The second harvest also resulted in yield increment for both seasons after the crop has been harvested, the highest value recorded was 382 and 401 kg ha^{-1} respectively, in a full fertilizer application treatment (NPK) with a significance difference ($p < 0.05$) across all treatments. Harvest three had its highest values compared to all other harvests during both cropping seasons indicating that biomass accumulation reached the peak on the sixth week after transplanting. There was a significance difference ($p < 0.05$), when NPK was applied in full, the yield obtained was 935 and 1,686 kg ha^{-1}, respectively. When P (920; 1,400) and K (930; 1,680 kg ha^{-1}) were omitted (Table 3) the values obtained were significantly different ($p < 0.05$) in both seasons. A decreased marketable fresh biomass yield was observed on the fourth and fifth harvest, it decreased from 935 and 1,686 kg ha^{-1} (both cropping season) in the third harvest to 788 and 808 kg ha^{-1} on fourth harvest then 423 and 396 kg ha^{-1} on the fifth harvest respectively. In the case of Brassica rapa subsp. Chinensis, the results of Van Averbeke et al. (2007b) indicated a linear response in fresh and dry biomass of marketable leaves. Increment of fresh and dry biomass reached the peak at 200 kg N ha^{-1} application rate then started to decline. The results of *Brassica rapa subsp. Chinesis* presented in the study of Van Averbeke et al. (2007a) indicated that the availability of P affected mass of leaves below some relatively low critical level and once the critical level of P availability in the soil has been attained, adding more P will have no effect. The application of K had a positive effect on the yield of Mustard spinach. Similarly, *Brassica rapa subsp. Chinesis*, showed fresh biomass increased up to 80 kg K ha^{-1} and declined when this optimum rate was succeeded.

Table 3. Response in marketable fresh leaf biomass yield (kg ha^{-1}) of Mustard Spinach (*Florida broadleaf*) to fertilizer application on a nutrient omitted experiment

Fertilizer treatments	W2		W4		W6		W8		W10	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
$N_0P_0K_0$	52f	48f	126f	142f	110f	114f	106f	102f	82f	83f
NPK	188a	194a	382a	401a	935a	1686a	788a	808a	423a	396a
N_0PK	56e	50d	130e	148e	114e	112e	105e	107e	80e	88e
NP_0K	102d	188b	360c	382c	920c	1400c	664c	640c	368c	241d
NPK_0	183b	186c	364b	392b	930b	1680b	782b	801b	419b	372b
$N_{1/2}P_{1/2}K_{1/2}$	176c	189d	344d	371d	731d	1244d	582d	482d	301d	248c

NPK = 100N: 150P: 20K kg ha^{-1} (which was the recommended application rate for N:P:K); N0PK = 0N: 150P: 20K kg ha^{-1}; NP0K = 100N:0P:20K kg ha; NPK_0 = 100N: 150P: 0K kg ha^{-1} and $N_{1/2}P_{1/2}K_{1/2}$ = 50N:75P:10K kg ha^{-1} N:P:K based on soil analysis including the control = No fertilizer applied($N_0P_0K_0$). $LSD_{(0.05)}$. Means followed by the same lower case letter are not significantly different at 5% level; W2 = week two after transplanting; W4 = week four after transplanting; W6 = week six after transplanting; W8 = week eight after transplanting; W10 = week ten after transplanting.

Figure 3. Field trials showing visual differences between treatments, full and halved NPK application, the omission of P and the control (no nutrient was applied).

Total fresh biomass yield (kg ha^{-1})

Yield accumulation of treatment where nitrogen was applied at 100 kg N ha^{-1} and potassium at 20 kg K ha^{-1} outperformed other treatments significantly ($p < 0.05$) (Table 4).

Values obtained on this treatment were 4,510 and 4,982 kg ha^{-1} total fresh biomass yield in both seasons, respectively (Table 4). However, when comparing treatments omission of P and K treatments had no significant difference ($p < 0.05$) on the first season but on the second season there was a significant difference ($p < 0.05$). Omission of P had 4,510 kg ha^{-1} of total fresh biomass yield while omission of K had 4,506 kg ha^{-1} on the first season. The second season obtained 4,982 kg ha^{-1} of total fresh biomass when P was omitted and 3,769 kg ha-$^{-1}$ total fresh biomass yield when K was omitted.

Table 4. Total fresh biomass yield (kg ha^{-1}) yield of Mustard Spinach for year one (2010) and year two (2011) in a winter field trials with different fertilizer treatments

Fertilizer treatments	Year 1	Year 2
$N_0P_0K_0$	252e	820f
NPK	3,986c	3,784b
N_0PK	244f	832e
NP_0K	4,510a	4,982a
NPK_0	4,506ab	3,769c
$N_{1/2}P_{1/2}K_{1/2}$	3,521d	2,986d

NPK = 100N: 150P: 20K kg ha^{-1} (which was the recommended application rate for N:P:K); N0PK = 0N: 150P: 20K kg ha^{-1}; NP0K = 100N:0P:20K kg ha; NPK0 = 100N: 150P: 0K kg ha^{-1} and $N_{1/2}P_{1/2}K_{1/2}$ = 50N:75P:10K kg ha^{-1} N:P:K based on soil analysis including the control = No fertilizer applied(N0P0K0). $LSD_{(0.05)}$. Means followed by the same lower case letter are not significantly different at 5% level.

Mineral composition of Mustard Spinach

Mineral elements were presented in percentages in the leaves of Mustard Spinach using composite sampling, which was affected by the rate of fertilizer application (Table 5). On the first season, the lowest percentage (2.63%) was obtained when nitrogen fertilizer was omitted. Full NPK application increased the content of N on the leaves of Mustard Spinach up to 3.29%, followed by 3.15% obtained when P was omitted (Table 5). The second season had the highest percentage of 3.11% when full fertilizer was applied and the omission of N decreased N content up to 2.34%, which was the lowest compared to all treatments (Table 5). P content was below one percent across all treatments; however, the highest percentage obtained was 0.69% on the first season in the potassium omission treatment. (Table 5) On the second season both omission of P and K obtained the same percent, 0.65. The lowest value was obtained when nitrogen was omitted in both seasons; it was 0.43% and 0.37%, respectively (Table 5). Concentration of potassium was affected by NPK application as the highest percentage was obtained on this treatment in both seasons, it was 3.59 and 3.55%, respectively (Table 5). The second highest values were obtained on the halved fertilizer application rate, 3.19 and 3.25% were recorded for this treatment (Table 5). The lowest values were obtained in the control and the second lowest values were obtained when K was omitted (Table 5).

Table 5. Mineral composition presented in percentages (%) as affected by N:P:K application

Fertilizer	N		P		K	
treatments	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
$N_0P_0K_0$	2.57	2.41	0.53	0.48	2.03	2.05
NPK	3.29	3.11	0.59	0.56	3.59	3.55
N_0PK	2.63	2.34	0.43	0.37	2.58	2.55
NP_0K	3.15	3.02	0.62	0.65	3.23	3.11
NPK_0	3.06	2.96	0.69	0.65	2.42	2.38
$N_{1/2}P_{1/2}K_{1/2}$	3.07	3.01	0.63	0.59	3.19	3.25

Content of dietary fiber, ascrobic acid and micronutrients

Composite samples were used per treatments for determination of dietary fiber, ascrobic acid and micronutrients content. Dietary fibre was presented in percentage, and on the first and second season the highest percentage was obtained on the K omission (N: P: K_0) treatment followed by halved fertilizer application treatment ($N_{1/2}$ $P_{1/2}$ $K_{1/2}$) (Table 6). The lowest percentage was obtained when N was omitted and the second lowest value was in the omission of P treatment. The values obtained in both seasons were 1.03 and 2.54% when N was omitted in both seasons and when P was omitted, 2.18 and 2.54% (Table 6). In the case of ascrobic acid content, the lowest value obtained was in the control, 126.94 and 117.42 mg 100 g^{-1}, respectively for both seasons. On the first season, the highest value obtained was 175.09 mg 100 g^{-1} and on the second season, 191.32 mg 100 g^{-1} when P was omitted (Table 6). The second highest values were observed on the K omission treatment for both season, they were 166.42 and 175.44 mg 100g^{-1}, respectively (Table 6). Iron was more concentrated on the treatment where K was omitted for both seasons. Values were 544.60 mg kg^{-1} on the first season and 542.8 mg kg^{-1}, followed by halved fertilizer treatment, which obtained 469.2 on the first season and 478.5 mg kg^{-1} iron content. In the first season, zinc content was higher at halved fertilizer application (61.4 mg kg^{-1}) treatment and it was lower in the second season (59.3 mg kg^{-1}) compared to the first season but still the highest when compared to the rest of the treatments (Table 6). N: P: K treatment had the lowest zinc content, which was 26.5 and 23.9 mg kg^{-1}, respectively. The omission of both K and P increased the content of zinc compared to when N was omitted. When fertilizer was applied as recommended, zinc content slightly increased from the lowest values obtained in the control treatment. The values increased from 28.1 to 30.9 mg kg^{-1} on the first season and from 23.9 to 26.7 mg kg^{-1} (Table 6).

Table 6. Dietary fiber, ascorbic acid and micronutrients as affected by fertilizer application

	Year 1				Year 2			
Fertilizer treatments	AC (mg 100g^{-1})	DF (%)	Iron (mg kg^{-1})	Zinc (mg kg^{-1})	AC (mg 100g^{-1})	DF (%)	Iron (mg kg^{-1})	Zinc (mg kg^{-1})
$N_0P_0K_0$	126.94	2.96	266.4	28.1	117.42	2.88	276.8	23.9
NPK	142.59	2.46	378.6	30.7	151.89	3.08	371.9	26.5
N_0PK	130.20	1.03	322.7	27.4	166.57	2.53	328.2	29.6
NP_0K	175.09	2.18	364.8	47.3	191.32	2.54	388.7	49.6
NPK_0	166.42	3.16	544.6	42.0	175.44	4.34	542.8	39.6
$N_{1/2}P_{1/2}K_{1/2}$	142.09	3.01	469.2	61.4	172.92	3.78	478.5	59.3

DF = Dietary fibre; AC = Ascrobic acid, NPK = 100N: 150P: 20K kg ha^{-1} (which was the recommended application rate for N:P:K); N_0PK = 0N: 150P: 20K kg ha^{-1}; NP_0K = 100N:0P:20K kg ha^{-1}; NPK_0 = 100N: 150P: 0K kg ha^{-1} and $N_{1/2}P_{1/2}K_{1/2}$ = 50N:75P:10K kg ha^{-1} N:P:K based on soil analysis including the control = No fertilizer applied($N_0P_0K_0$).

CONCLUSION

In the fertilizer omission experiment the current result indicates that the balance in plant nutrient availability and absorption is paramount. Full application NPK (100N:150P:20K) increased total biomass fresh yield of Mustard Spinach and in this investigation the crop is not a heavy feeder of N, P and K. The synthetic fertilizers are currently expensive due to climate change scenario; such crop may provide a relative economic advantage for smallholder farmers. Application of less amount of inputs on highly nutritious vegetable production will improve the state of food security and nutrition among rural communities and smallholder farmers in South Africa.

ACKNOWLEDGEMENTS. The first author would like to thank to Ms. Pulane Modisane and Ms. Mumsy Mashilane for collecting the first season data. Mr. William Maluleke for technical support in data collection. The Gauteng Department of Agriculture and Rural Development (GDARD) for funding this project and the ARC-VOP staff members for their support in the field to execute the experiment.

REFERENCES

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, Vol. II, 15th edition. Sec.985.29, the Association: Arlington, VA

Bray, R.H. & Kurtz, L.T. 1945. Determination of total organic and available forms of phosphorus in soils. *Soil Science* **59**, 39–43.

Campbell, C.R. & Plank, C.O. 1991. Sample Preparation. In: C.O. Plank (ed.). *Plant Analysis Reference*. Procedures for the Southern Region of the United States. Southern Cooperative Series Bulletin No. **368**, pp. 1–11.

Chapman, H.D. 1965. Cation-Exchange Capacity. In: A.G. Norman (ed.), Methods of Soil Analysis. Part2. Chemical and Microbiological Properties. *Agronomy* **9**, 891–901.

Frank, K., Beegle, D. & Denning, J. 1998. Phosphorus In: JR Brown ed. *Recommended Chemical Soil Test Procedures for the North Central Region*. Research publication No. **221**, pp. 21–30.

Gavlak, R.G., Horneck, D.A. & Miller, R.O. 1996. *Total Nitrogen in Botanical Materials-Automated Combustion Method*. In Western States Laboratory Proficiency Testing ProgrampSoil and Plant Analytical Methods. Version 3.00, pp. 71–72.

Gerrano, A.S., Jansen van Rensburg, W.S., Venter, S.L., Shargie, N.G., Amelework, B.A., Shimelis, H.A. & Labuschagne, M.T. 2019, Selection of cowpea genotypes based on grain mineral and total protein content. Acta Agriculture Scandinavica. Section B. *Soil & Plant Science* **62**, 155–166.

Grubben, G.J.H. & Denton, O.A. (eds). 2004. Plant Resources of Tropical Africa 2: Vegetables, PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands, pp. 123–127.

Hossain, M.Z. 1990. *Effect of nitrogen and sulphur on growth, yield and nutrient content of rice*. An M.Sc. thesis, Department of Soil Science, Bangladesh Agricultural University, Mymensingh, p. 17.

Kovar, J.L. 2003. Method 6.3 *Inductively coupled plasma spectroscopy*. In: J. Peters (ed.) Recommended Methods of Manure Analysis. University of Wiscons in Extension, Madison, WI publication **A3769**, pp. 41–43.

Maboko, M.M. 2013. Effect of Plant Density and Harvesting Frequency on Yield Components of Hydroponically Grown Mustard Spinach (*Brassica juncea*). *Acta Horticulturae* **1007**, 515–522.

Makus, D.J. 1992. Fertility requirements of vegetable amaranth (Amaranthus tricolor L.) in an upland mineral soil of the mid-South. *Acta Horticulture* **318**, 291–297.

Maynard, D.N., Hochmuth, G., Olson, S.M., Vavrina, C.S., Stall, W.M., Kucharek, S.E., Webb, S.E., Taylor, T.G., Smith, S.A.& Simonne, E. 2003. Tomato production in Florida. In: Olson, S.M., Simonne, E. (Eds.), *Vegetable Production Handbook for Florida* 2003–2004. IFAS, Gainesville, pp. 271–283.

Nemadodzi, L.E., Araya, H., Nkomo, M., Ngezimana, W & Mudau, N.F. 2017. Nitrogen, phosphorus, and potassium effects on the physiology and biomass yield of baby spinach (*Spinacia oleracea* L.). *Journal of Plant Nutrition* **40**, 2033–2044.

Payne, RW, Murray, DA, Harding, SA, Baird, D.B. & Soutar, DM. 2016. *GenStat for Windows*. 18th Edition, VSN International, Hemel Hempstead.

Sadia, A.A., Mahasen, M., Shahrin, S., Roni, M.Z.K. & Uddin, A.F.M. 2013. Phosphorus levels on growth and yield of Turnip (*Brassica campestris* var. Rapifera). *Bangladesh Research Publications Journal* **8**, 29–33.

Schenk, M.K. 1998. Nitrogen use in vegetable crops in temperature climates. *Horticulture* **22**, 185–223.

Scherer, H.W .2001. Sulphur in crop production – invited paper. *European Journal of Agronomy* **14**, 81–111.

Smartt, J. & Haq, N. 1997. Domestication, production and utilization of new crops. International Centre for Underutilised Crops, Southampton, UK, 134–146.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical methods. 7th ed., Iowa State, University press. Department of Agricultural Development (DAFF). 1991, Department of Agricultural Development. Soil Classification Working Group. Soil Classification–A taxonomic system for South Africa. *Memoirs on the Agricultural Natural Resources of South Africa* **15**, Pretoria, South Africa.

Soundy, P & Cantlife, D.J. 2001b. Improving lettuce transplant quality in response to nitrogen nutrition in a floatation production system. *Journal of Horticulture Science* **114**, 294–303.

Soundy, P., Cantliffe, D.J., Hochmuth, G.J. & Stoffella, P.J. 2001a. Nutrient requirements for lettuce transplants using a floatation irrigation system. I. Phosphorus. *Horticulture Science* **36**, 1066–1070.

Tshikalange, T.E. 2006. Response of *Brassica rapa L. subsp. chinensis* to nitrogen,phosphorus and potassium in pots. MSc. Pretoria, Tshwane University of Technology 159 pp.

Tshikalange, T.E & Van Averbeke, W. 2006. The cultivation of *Brassica rapa L*. subsp. chinensis in Vhembe, Limpopo Province, South Africa. International Symposium on the Nutritional value and Water Use of Indigenous Crops for Improved Livelihoods. Volume of papers (not edited). 19–20 September. The Centre for Nutrition, University of Pretoria, Pretoria, 355 pp.

Van Averbeke, W. & Juma, K.A. 2006. The cultivation of Solanum retroflexum Dun. InVhembe, Limpopo Province, South Africa. Proc. *International Symposium On the nutritional Value and water use of Indigenous crops for improved livelihoods*. The centre for nutrition, University of Pretoria, Pretoria, South Africa, pp. 19–20.

Van Averbeke, W., Juma, K.A. & Tshikalange, T.E. 2007a. Yield response of African leafy vegetables to nitrogen, phosphorus and potassium: the case of Brassica rapa L. subsp. chinensis and Solanum retroflexum Dun. *Water South Africa* **33**, 355–362.

Van Averbeke, W., Tshikalange, T.E. & Juma, K.A. 2007b. The commodity system *Brassica rapa L. subsp. chinensis* and *Solanum retroflexum* Dun. in Vhembe District of Limpopo Province, South Africa. *Water South Africa* **33**, 349–354.

Van Wyk, B.E. 2005. Food plants of the world, identification, culinary uses and nutritional value. 1st ed. Briza Publication, Pretoria, South Africa, 48 pp.

Wang, Z.-H., Li, S.-X. & Malhi, S. 2008. Effects of fertilization and other agronomic measures on nutritional quality of crops: review. *Journal of the Science of Food and Agriculture* **88**, 7–23.

Wolf, A., Watson, M. & Wolf, N. 2003. Method 5.4 *Nitric and Hydrochloric acid digestion with peroxide*. In: Peters, J. (ed.) Recommended Methods of Manure Analysis. University of Wisconsin-Extension, Madison, WI publication A3769, pp. 35–36.

Zhou, Y., Ren Shunrong, R. & Zhengxiang, W. 1989. *The effect of application of nitrate fertilizer on the accumulation of nitrate in vegetables*. Municipal Academy of Agricultural Sciences (China). Institute of soil and fertilizers, pp. 110–115.

Agronomy Research **18**(1), 267–289, 2020
https://doi.org/10.15159/AR.20.011

Game species fodder conditions in Eastern Siberia and Amur region

A. Senchik[1], Yu. Guretskaya[1,*], M. Bormotov[1], A. Pavlov[1], A. Ryabchenko[1], V. Glushkov[2], Y. Sato[3] and H. Igota[3]

[1]Far-Eastern State Agrarian University, Politehnicheskaja street 86, RU675005 Blagoveshchensk, Amur Oblast, Russia
[2]Russian Scientific Research Institute of Hunting and Fur farming named after Professor V.M. Zhitkov, 79, Engelsa street, RU610000 Kirov, Russia
[3]Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu-shi, JP069-8501 Hokkaido, Japan
[*]Correspondence: gureczkayay@bk.ru

Abstract. Hunting farms can serve as a source of primary data on the state of natural resources. Taking into account the increasing anthropogenic impact on the environment, it is necessary to constantly monitor the situation with food for ungulates and predators in order to be able to take timely measures to improve the quality of habitat for wild fauna. Populations of wild large animals live in the host landscapes of so reduced that animals population become extremely vulnerable and unstable, signs of crisis can be seen in the data on the number of animals. Lack of natural food is a serious deterrent, especially in difficult times of the year, such as winter and spring. An analysis of the situation with food for a particular species will allow hunting farms to properly plan their feeding and other activities throughout the year in order to maintain a population of certain species on their territory and minimize the migration of species outside the protected areas. The aim of our work was to create a baseline for tracking data on the characteristics of wildlife animals. The research results allow hunting farms to use the data of animal feeding preferences during different seasons of the year for better organization of their biotechnical measures and improvement of feeding conditions for such species as red deer (*Cervus elaphus*), elk (*Cervus canadensis*), roe deer (*Capreolus pygargus*) and bear(*Ursus arctos*).

Key words: Amur region, brown bear, Eastern Siberia, fodder base, Siberian roe deer.

INTRODUCTION

The study of wild ungulates is environmental and economic value. Increasing the number of wild ungulates, their preservation, carrying out high-quality biotechnical and protective measures are important tasks of hunting. A detailed study of the food supply of ungulates in different seasons of the year will allow the specialists of the hunting farms to carry out biotechnical measures correctly and in a timely manner, and most importantly, to use the feed needed to maintain the animal in a certain period of the year.

To study feeding situation of wild animals and predators in Eastern Siberia and Amur region in order to develop effective feeding depending on the season. The founders of game management on the territory of the former USSR were Lobachev & Stakhrovsky (1932), Danilov (1960), Danilov & Rusanov (1966). Game management is based on the hunting grounds' quality assessment and calculation of game species appraisal on a five-point scale selected as an approach that is familiar to the local population (i.e. the local secondary schools also use a five-point grading scale). A typology of hunting grounds must be determined first for these computations.

The study and improvement of methods for determining the typology of hunting grounds were carried out by Martynov & Masaytis (2002), Plaksa (2005), and Kozlov (2007).

Various researchers, such as Danilov (1953), Danilov & Rusanov (1966), Dementiev (1973); Kuzyakin (1974), and Kluchev (2003) devoted their work to the calculation of hunting ground appraisal for hunting species. However, there is no consensus regarding the definition of hunting ground quality. According to Danilov & Rusanov (1966) hunting ground quality is determined by the optimal number of animals per area unit; according to Yurgenson (1969) it is an indicator of hunting ground capacity. Kuzyakin (1974) considers ecological standards of species' population to be more correct. For Melnikov & Melnikov (2008) the quality of hunting grounds is the economically feasible density of species population, while Kluchev (2003) and Kozlov (2007) used hunting ground productivity.

We believe that the hunting grounds' appraisal should be calculated using an output expressed by a number of species per one thousand hectares. A natural output should be distinguished from an actual one. The natural output is species population density (number), determined by the hunting grounds' quality without taking biotechnology and poaching into account. It is consonant with the concepts of optimal abundance, hunting capacity and environmental standards of species population.

We investigated the variety and composition of feedstuff during different seasons of the year. We determined the most preferable and less preferable, so called secondary feedstuff, as well as feeds for the most difficult periods of animal life, i.e. 'limiting feeds'. We conducted a comparative analysis of animal populations inhabiting the territories of the Republic of Buryatia and Amur region based on land and forage condition quality in the studied regions of the Russian Federation. The importance of mineral feeding for ungulates has been studied as well.

We believe that any successful conservation program should be based on a clear understanding of the environment in which the species live, i.e., on the systematic collection of qualitative and quantitative data regarding the living conditions of these species. Without this fundamental knowledge needed to conserve any species, it is easy to move away from the main areas of activity necessary for the conservation, preservation and restoration of the animal population. Therefore, our study aimed to outline the current situation, development trends, and characteristics of the given land from the perspective of the carrying capacity of a particular species living in a specific environment. What are the characteristics of the living environment? What are the trends? We are looking for answers to these questions and believe that without them, any conservation program will not be successful.

MATERIAL AND METHODS

Hoofed animals and large predators are valuable objects of the hunting fauna of Eastern Siberia and Amur region. The study of the forage conditions of hunting grounds for these species in different seasons of the year is an important aspect of their ecology. The food base determines the spatial distribution, affects the population indicators of all animals, without exception. An actual output is species population density (number), currently prevailing in the hunting grounds, which depends not only on the quality of the hunting grounds, but also on the anthropogenic impact. We studied scientific works about food supply of ungulates in Yakutia (Antipov, 1976), Krasnoyarsk region (Subbotin, 1980), Irkutsk region (Kozlovskiy, 1997), Amur region (Morozov, 1982; Danilkin, 1999), the Republic of Buryatia (Noskov, 2008; Antropov et al., 2013), the Trans-Baikal region (Sandakov et al., 2015), Khabarovsk (Senchik & Guretskaya, 2017) and Primorsky regions (Smirnov, 2000). Argunov & Stepanov (2011) and Kucherenko (1981) in their studies showed that foodstuff's composition for ungulates includes some other plants that we have not identified. We identified 116 species of plants used by Siberian roe deer for feeding (Antipov, 1976; Korenyuk, 1989).

We studied and analyzed scientific works on brown bear feeding in Yakutia, North-Eastern Siberia, Primorsky, Khabarovsk regions, Kamchatka and Amur region (Chernyavsky & Krechmar, 2001; Seryodkin et al., 2006; Veklich & Darman, 2013; Johansson et al., 2016; Senchik et al., 2017). Special attention was paid to feeds of plant origin, since during bears' active life period their condition is the most stable for this type of species in Amur region.

As a result of the analysis of scientific works, especially of the Far Eastern authors, it has been established that the diet of a brown bear living in adjacent territories also includes some other plant species that also grow in Amur region. However, they were not found by us. We have identified 107 species that can be included in a brown bear diet.

We studied feeding situation in the hunting grounds of Eastern Siberia and Amur region for wild ungulates, such as elk (*Cervus canadensis*), red deer (*Cervus elaphus*), roe deer (*Capreolus pygargus*) as well as large predators – wolf (*Canis lupus*) and brown bear (*Ursus arctos*). Their habitat has been specified, food supplies have been studied, species assessment has been carried out, their population and its density on the given territory have been determined (Forest, 1979; Starchenko, 2008; Treves & Bruskotter, 2014).

Wildlife technicians employed at the hunting farms conducted the field studies within the territory of their employers. They had a walk along the given routes and recorded the traces of animals alongside the coordinates of their detection as well as the landscape features. Using these primary records allowed us to define the status of the environment and its carrying capacity on a larger scale.

We have been investigating food conditions in two regions of Russia for 7 years. Studies were conducted in all seasons of the year. Among other things, we analyzed the data provided by hunting farms, in the appendix to the article one can see examples of the primary working documentation of hunting staff and wildlife officers who made regular rounds of wild lands, recorded and determined the ownership of traces of wild animals and recorded everything in their records. We have laid routes in a large part of Eastern Siberia and Amur region, suitable for ungulates and predators. The routes were covered with the use of motor vehicles and by foot, on skis and on snowmobiles during winter. An average length of hiking routes was 5 to 15 km. More than 20,000 km were covered as a result. More than 6,000 winter record cards, indicating the number of ungulates and wolves met at specific route points, were also analyzed annually (Annex 1, 2, 3, 4, 5, 6, 7). Cards were chosen from each district and from almost all hunting users of Amur region and the Republic of Buryatia. More than 5,000 photos and 1,500 videos were collected and analyzed. These video and photographs provide evidence on the presence of ungulates and predatory species in the area. Animals were captured on camera during feeding, resting, and migration. Some photos and videos were made specifically for this study, some provided by amateur hunters and specialists from hunting farms. The research results allow hunting farms to improve the system of biotechnical and reproductive activities, with the aim of increasing the number of ungulates, as well as improving the quality of hunting territory. A collection of materials on survey data and questionnaires was done. The profile contains data on animal feed options, time when the animal was caught, and stomach content. More than 1,800 hunters were interviewed. For 7 years we annually analyzed 100% of elk, red deer, and roe deer hunting permits, returned by hunters to federal services for protection of wildlife objects of the Republic of Buryatia and Amur region.

To determine the diet of ungulates and predatory animals, the analysis of excrements found in different regions of the Republic of Buryatia and Amur region was carried out. Feeding elements were determined by explicit fragments, as well as by conventional methods. The diet of ungulates was studied according to methods of Novikov (1953), Lebedeva (1965), and Smirnov (1977). The composition of feedstuff was studied using the method of counting animal bites on various trees and shrubs along featured model territories and on cut-tape samples (Novikov, 1953; Lebedeva, 1965; Smirnov, 1977). These studies allowed us to determine the basic feedstuff of elk, red deer, Siberian roe deer, wolf and brown bear, as well as their choice of feeding during different seasons (Vorob'ev et al., 1989; Namzalov et al., 1997; Wäber & Dolman, 2015).

Studies of hunting grounds’ typology have been conducted in Amur region and Eastern Siberia. We have determined hunting grounds division in percentage by quality types and classes as a result. Using Danilov & Rusanov’s (1966) method we determined the weighted average quality assessment of hunting grounds for elk, red deer, roe deer, wolf, bear in two regions. Experts, acknowledged about environmental conditions in the area, classified hunting grounds by the number/size of animals using a common five-point scale, where 1 *is the most valuable for hunting* and 5 *is the least valuable for hunting.*

RESULTS

Flora and climate of the studied region

In this section we give, as far as possible, a general, holistic presentation of the habitat of animals. such a statement may seem redundant, but we wanted to give the picture in its entirety.

Eastern Siberia flora is very rich and diverse. There are all major ecosystems of the northern hemisphere: steppes, forests, wetlands, meadows, alpine tundra and alpine wastelands with unique floristic complexes. The uniqueness of Buryatia biotopes is founded on the paradoxical characteristics of the regional position of the lake Baikal in Eurasian mainland.

Buryatia's flora includes 2,128 species and subspecies belonging to 585 classes and 127 families. This richness can be explained by its natural features - predominance of the mountain relief, diversity of its forms and natural vegetation zones. All that contributed to the preservation of a greater number of relict species (up to 70) and the presence of endemics (about 130).

I. Bald mountain (mountain-tundra) and sub-mountain vegetation types. Sub-mountain soils are represented by the combinations of tundra, dry-peaty, broken stone primitive, as well as a mosaic of mountain-meadow turfy and gley soils of tundra permafrost soils.

II. Baikal-Dzhugdzhursky taiga-mountain type of vegetation is represented by forests, which are mostly distributed on the mountain plateaus and spur slopes, in the large river valleys of Buryatia's main mountain systems in its northern and north-eastern parts, and also in the Eastern Sayan, Zakamensky, Dzhidinsky and Tunkinsky districts. This forest vegetation cover is subdivided according to the landscape.

III. South Siberian types of mountain-taiga vegetation are found in the Central part of the republic in the basins and watersheds of Kurba, Turka, Uda, Selenga rivers, some fragments of these types of vegetation are in the valleys of the northern rivers of Upper Angara, Barguzin.

IV. North Asian steppe plant communities. The vegetation is represented by the west-trans-baikal daurian type of flora, which is found in the valleys and river mouths of Jida and Selenga rivers, in the lower basin of Hilok river in the valleys of Uda, Kurba rivers, some fragments are found in the valleys of Barguzin, Irkut, Dibi, Oka rivers (Shaposhnikov, 1953; Mironova, 2001; The scheme of placement, use and protection of hunting grounds in the territory of the Republic of Buryatia, 2017).

In general, Buryatia's forest soils are relatively barren, which affects forest productivity. Steppe and forest-steppe soil types lack moisture, permafrost soils lack warmth and the soils of light mechanical composition as well as low-productive, stony ones – lack nutrients.

The flora and vegetation of Amur region are characterized by significant wealth and diversity, which is mainly due to the following factors: the main part of the region lies in Amur basin and it is situated on the border of East Asian and Boreal floral territories.

The vegetation is represented by East-Siberian flora (Gmelina larch (*Lárix gmélinii*), Siberian spruce (*Pícea obováta*) and others), Manchurian (Amoor cork tree (*Phellodendron amurense*), Chinese Magnolia vine (*Schizandra chinensis*) and others), Okhotsko-Kamchatky (Ayan spruce (*Picea ajanensis*), Khingam fir (*Abies nephrolepis*) and others), Mongol-Daurian (bush-clover (*Lespedeza cuneata*), feather-grass (*Stipa baicalensis*) and others), Pacific (mountain pine (*Pinus koraiensis*), black-fruited crowberry (*Empetrum nigrum*)).

There are three natural and climatic zones in Amur region:

1. Coniferous forest zone (taiga zone). It covers the territory of Tynda, Zeya, Selemdzhinsky, Skovorodinsky, Magdagachinsky and a part of Shimanovsky municipal districts. This zone is divided into subzones of middle and southern taiga. In the South (below 54 degrees of northern latitude) such broad-leaved representatives as oak, elm, maple, ash-tree are met. In mountainous areas in the lower belt – mountain larch forest, then goes the belt of dwarf Siberian pine; and above 1,300 m - mountain tundra.

2. The zone of mixed coniferous-broadleaved forests. Arkharinsky, Blagoveschensky, Bureysky, Zavitinsky, Mazanovsky, Svobodnensky, Romnensky and part of Schymanovsky municipal districts belong to it. This zone is divided into Amur and Far-eastern provinces. The vegetation composition of Amur province consists of open oak-larch and oak-pine communities. A lot of Daurian (black) birch. Underbrush - heterophyllous hazel and bush-clover. The Far-eastern province is characteristic only for the Eastern part of Bureya and Arkharinsky districts, and is rich floristically: forests of Manchurian type with a mixture of Okhotsk-Kamchatka, East Siberian, Mongolian, Daurian flora. It is characterized by Korean pine, Amur linden, Manchurian ash, Manchurian walnut, Amur cork tree, Mongolian oak, Dahurian larch, Abies Nephrolepis; in the undergrowth – heterophyllous hazel and Manchurian hazel, bush-clover, Eleutherococcus senticosus, spruce and fine-leaved mock oranges. Vines are presented by Chinese Magnolia vine, Amur grape, Actinidia kolomikta.

3. Forest-steppe and forest-meadow zone. Konstantinovsky, Tambovsky, Belogorsky, Ivanovsky, Octyabrsky, Seryshevsky and Mikhailovsky municipal districts are included in this zone. Previously, it was a part of broad-leaved forests, but due to human activity, its forestation decreased and the territory, previously covered with forest, transformed into farmland. Part of it is occupied by floodplain meadows in combination with bushes.

The choice of feeding depends on the physical and geographical distribution of animals, the time of year, the biological condition of an animal and other factors.

Elk

Amur region is a home to Ussuri subspecies of elk (*Alces alces cescameloides*).

Elk is exclusively a forest dweller. Its favorite places are old forest fire-sites, wood-cutting areas, forest river banks, streams, lakes and marshy swamps. Unlike other ungulates, its nutrition is less diverse. In summer elk mainly eats herbaceous near-water plants (*Herbaceum* semi-aquatilium plantis). In autumn mushrooms occupy a certain place in its diet – cepe (*Fungos-albus*), brown cap boletus (Birch), orange-cap boletus (*Aspen*), russule (*Russula*) and even fly agaric (*Fuge agaric*). In winter, the bark and branches of young trees and shrubs (*Cortice et ramis iuvenes arbores et frutices*), mosses (*Muscos*) and lichens (*Lichenas*) serve as fodder for elk. Despite its size, this forest giant feeds very delicately, trying not to touch bushes and trees that were damaged or heavily eaten by other animals.

Elk is distributed throughout Amur region and the Republic of Buryatia, with the exception of some areas. The most preferred habitats during this period are riverine forests in the valleys of Nora river and its tributaries (Meun, Burunda and other rivers). This is due to the fact that their valleys are wide, the floodplain is well expressed, there are islands, canals and bays. All this creates favorable conditions for the development of near – water herbaceous and woody vegetation, and the abundance of water makes it comfortable for the animals. The valleys in the basin of Selemdzha river are narrower, with steep rocky slopes, and their area is small and located mainly along the tributaries (Budaki, Orlovka, Oschmin rivers).

In winter, the area inhabited by elk is reduced as it depends on the snow height. Most animals during this period, as well as in summer, stay in valleys. With a shortage of feedstuff elk begin eating birch shoots, bark, spruce shoots. By the end of winter animals eat mostly tree-bush vegetation. Their diet includes pine, willow, aspen, rowan, bird cherry tree, alder and others.

It should be noted that over the past two years (2016–2017) there were some changes in the species' behavior in winter. According to our observations, animals stayed for a long time on small isolated forest areas, usually in densely grown stalks, or in the thickets of alder on top of small springs with sufficient food base, thus avoiding open swampy complexes, which used to be the main habitats of the animals in the previous years at this time of the year. Animals remained in these places even during severe frosts.

Elk is one of the few deer species that has adapted to the digestion of a significant amount of wood, although it prefers green parts of woody and herbaceous plants, for example: bark, needles, root parts of sedge (Sedge), pond grass (Pondweed), horsetails (Horsetails). The animals eat these feedstuffs during prolonged and volatile months of recent winters.

In the result of our research on classification of hunting grounds and the appraisal of lands of the Republic of Buryatia it was determined that the areas of elk's main habitat belong to the III class of locality quality. Their square size equals 21,305.5 thousand hectors with the exception of Mukhorshibirsky and Tarbagataysky districts where elk does not live (Table 1).

Table 1. Locality quality of hunting grounds for elk in the Republic of Buryatia with limitation factors

No.	Municipal district	Category of land (thousand hectares)			Territories, characteristic for species (thousand ha)	Weighted average indices of land quality	Land/Territory appraisal (average)	Land quality appraisal with limiting factors
		good	satisfactory	bad				
1	Barguzinsky	-	671.8	2.6	674.4	100	III	IV
2	Bauntovsky	-	5,299.1	103.3	5,402.4	98	III	III
3	Bichursky	-	413.1	12.4	425.5	98	III	IV
4	Dzhidinsky	-	463.8	0.8	464.6	100	III	IV
5	Eravninsky	-	2,055.1	4.8	2,059.9	100	III	III
6	Zaigraevsky	-	498	18	516	97	III	V
7	Zakamensky	-	1,354.3	15.9	1,370.2	99	III	IV
8	Ivolginsky	-	190	5.4	195.4	98	III	V
9	Kabansky	-	560.3	3.4	563.7	99	III	III
10	Kizhinginsky	-	590.8	12.5	603.3	98	III	IV
11	Kyahtinsky	-	204.8	3.1	207.9	99	III	V
12	Kurumkansky	-	860.2	6.3	866.5	99	III	IV
13	Mukhorshibirsky	-	-	-	-	-	-	-
14	Muisky	-	1,128.7	149.1	1,277.8	90	III	IV
15	Okinsky	-	1,252	2.3	1,254.3	100	III	IV
16	Pribaikalcky	-	1,215.8	25.3	1,241.1	98	III	III
17	Selenginsky	-	448.9	8.9	457.8	98	III	V
18	Severo-Baikalsky	-	2,593	52.7	2,645.7	98	III	III
19	Tarbagataisky	-	-	-	-	-	-	-
20	Horinsky	-	1,075.7	3.3	1,079	100	III	IV
	Total	-	20,875.4	430.1	21,305.5	98	III	IV

In Amur region, the elk habitat territories mainly belong to the II class of of locality quality. The exceptions are Zeysky, Selemdzhinsky and Tyndinsky districts where hunting performance territories is 1 class lower.

Deer

Deer's modesty in its choice of terrain is also reflected in the choice of food, as it is also not particularly demanding. It eats not only almost everything that grows, but sometimes even the soil and turf. According to most authors, stalks of herbs, branches, needles, bark of trees and bushes, leaves and algae, mosses and lichens are included in the deer's annual diet, where an individual plant's value and their palatability is directly proportional to the types of land, preferred by animals. Still it is necessary to divide all these feeds for the seasons of the year.

With the advent of young green vegetation, red deer completely switches to the spring ration of food, which until the middle of summer consists mainly of herbaceous plants, and also partly of woody fodder and bark. A significant place in the diet is given to sedges and Langsdorf reed grass, which are widely distributed in most areas in combination with all types of birch and mound spaces, forming the basis of the grassy cover of Eastern Siberia and Amur region.

In spring red deer willingly adds bark of young trees to the 'list' of its feedstuff. It is also noted for consuming of young needles of larch, pine, spruce. However, the bark of larch and aspen is also used for food. The fact is that in the spring and autumn, in contrast to the winter, the bark is much softer, and therefore its palatability increases. Although in the literature there are data that in winter the South side of the tree trunk was marked with fresh bites of crust, but, apparently, it is connected in individual cases with the lack of available feed. In Eastern Siberia, deer willingly eats cow parsnip (*Heracleum*), yarrow (*Achillea*), shepherd's bag (*Capsella*), bell (*Campanula*), clover (*Trifolium*) and other plants.

Observations from mid-summer showed that deer switched to leaves and shoots of trees and shrubs, and by the end of August actively eats berries, mushrooms, and in the presence of the acorn harvest in the Amur region gladly goes to it. It seems that such unpretentiousness in the choice of food is caused by the preparation for the rut, before which it is fed up to 30–40 kg of fat and during which it practically eats nothing. But it is known that mushrooms are rich in protein and mineral substances. In the same connection berries are willingly eaten: blueberries, cranberries, strawberries, etc. During the rut bulls eat very little, mainly focusing on the search for females. However, a little 'cooled down' during the rut, red deer switches to tree and shrub forages which are the basis of power the whole autumn-winter period. Thanks to them, the deer in the harsh conditions of winter finds all the necessary organic substances to maintain life. According to our observations, with a high density of the beast, on the willow and aspen fields, the whole young shoots are eaten. It should be mentioned that the animal eats plants at a height of up to 2.5 meters, while the shoots of willow and young aspen deer bites at a height of up to 4 meters, bending branches and making creases.

In the end, in the winter, the importance in choosing the type of food depends on the depth of the snow cover. Herbaceous plants in the form of dried grass and in the autumn-winter remain part of the diet. So, at a small height of snow cover, usually at the beginning of winter, deer in addition to the branch feed, eats a sufficient amount of foot feed, where in addition to dry rags in the ground cover is a large number of plants in green, covered with snow and gladly dug out and eaten by the beast. Such vegetation includes all kinds of sedge, pear and other herbaceous plants. We often found the animal's digging, which he did in the snow to find berries and leaves of blueberries and cranberries. With a significant increase in the height of the snow cover, towards the end of winter, the difficulties in extracting feed resources from under the snow and the almost complete absence of foot vegetation on the surface of the snow cover, make the animal completely switch to the branch feed.

In general, if we give a description of willow-culture, then, in our opinion, and the opinion of many far Eastern researchers, it is out of competition with the rest of the branch feed, being the most favorite, nutritious and affordable type of feed for deer. And undoubtedly refers to the limiting winter feed.

The whole territory of the Republic of Buryatia belongs to the III land quality class for red deer, the area of which is 26,312 thousand hectares (Table 2.) Much of the land in Amur region estimated to have the IV class, which is 14,955.45 thousand hectares of land in two areas of the Amur – Blagoveschensk and Svobodnensky belonging to the third class of land quality, their area equals 48,094 thousand hectares.

Table 2. Land quality of hunting grounds for red deer in the Republic of Buryatia with limitation factors

No.	Municipal district	Category of land (thousand hectares)			Territories, characteristic for species (thousand ha)	Weighted average indices of land quality	Land/Territory appraisal (average)	Land quality appraisal with limiting factors
		good	satisfactory	bad				
1	Municipal district	78	595	174.3	847.3	96	III	IV
2	Barguzinsky	45.8	5,253.3	1,233.4	6,532.5	85	III	IV
3	Bauntovsky	-	413.1	103.2	516.3	83	III	III
4	Bichursky	-	463.8	118.7	582.5	83	III	IV
5	Dzhidinsky	80.7	1,974.4	272.8	2,327.9	95	III	V
6	Eravninsky	-	498	69.7	567.7	90	III	IV
7	Zaigraevsky	-	1,354.3	42	1,396.3	97	III	III
8	Zakamensky	-	190	24.8	214.8	90	III	III
9	Ivolginsky	-	585.6	52.7	638.3	93	III	IV
10	Kabansky	21.1	569.7	96.3	687.1	93	III	IV
11	Kizhinginsky	0	204.8	93.6	298.4	73	III	IV
12	Kyahtinsky	90.2	770	54.3	914.5	110	III	IV
13	Kurumkansky	-	171.7	51.2	222.9	80	III	III
14	Mukhorshibirsky	63.2	1,065.5	671.8	1,800.5	74	III	IV
15	Muisky	-	1,541.9	170	1,711.9	92	III	IV
16	Okinsky	42.8	1,181.7	85.1	1,309.6	99	III	III
17	Pribaikalcky	-	448.9	114.8	563.7	83	III	III
18	Selenginsky	345.5	2,289.4	1,059.3	3,694.2	90	III	V
19	Severo-Baikalsky	-	199.9	71.5	271.4	78	III	V
20	Tarbagataisky	186.3	889.4	138.5	1,214.2	113	III	III
	Total	953.6	20,660.4	4,698	2,631.2	90	III	IV

Other ungulates

The ungulates' feeding is influenced by temperature, anxiety factors, insects. Therefore, ungulates feeding activity is observed in the morning and evening hours.

Siberian roe deer lives almost on the whole territory of the Republic of Buryatia and Amur region and is considered a background species. Due to large areas of unusual land and disturbance factors, the area is not uniform. Siberian roe deer refers to herbivorous animals. Its food base is very diverse. The feed composition of Siberian roe deer includes 599 plant species. In Eastern Siberia, it eats 130–140 species of plants (Table 2). Given the harsh climatic conditions of the Republic, the winter period is very difficult in the animal life. Roe deer is forced to eat scant on the composition of feed. Digging snow with a height of about 30–35 cm front hooves in search of food animals produce leaves, stems, stunted shrubs, fully eating them. More willingly than usual, visits roe deer salted haystack, eating in them plants with developed foliage: clover (*Trifolium*), melilotus (*Melilotus*), alfalfa (*Medicago*), geranium (*Geranium*), vasilistnik (*Thalictrum*), gorichnik (*Peucedanum*), sochevichnik (*Lathyrus vernus*), astragalus (*Astragalus*), alfalfa (*Medicago*) wormwood (*Artemisia*), plantain (*Plantago*), wheatgrass (*Elytrigia*). The diet of roe deer includes tree bark, aspen branches (*Populus tremula*) and birch (*Betula*), pine needles (*Pinus*) and larch (*Larix*). For feeding ungulates species annually harvesting hay:

- in the Republic of Buryatia in 2015 harvested 954 centners, in 2016 – 477 centners, in 2017 – 788 centners;
- in Amur region in 2015, harvested of 67.2 tons, in 2016 – 38.9 tons in 2017 of 70.7 t.

Spring feed of roe deer consists of branches of aspen, birch, larch, willow (*Salix*), Daurian rhododendron (*Rhododendron dauricum*), alder (*Alnus*). With the advent of green vegetation animals eat all green shoots of species such as sedges (*Carex*), veinik (*Calamagrostis*), then go snowdrop (*Galanthus*), dandelion (*Taraxacum*). In late spring, early summer ungulates need quality food, which will depend the development and growth of young. In the summer, the animals move to herbal feed. Due to the fact that in the country the summer is short and the plants wither quickly, the roe deer eats with plants rich in nutrients. In summer the diet roe deer includes and flowers, such as geraniums (*Geranium*), clover (*Trifolium*), yellow lily (*Hemerocallis lilio-asphodelus*), wild lily (*Lilium martagon*), lungwort (*Pulmonaria*), Columbine (*Aquilegia*), Windflower (*Anemone*), vasilistnik, common goutweed (*Aegopodium podagraria*), Hogweed (*Heracleum*), yarrow (*Achillea*), shepherd's purse (*Capsella*), Bellflower (*Campanula*), and others (Sand et al., 2008).

In late summer and early autumn roe deer willingly eat mushrooms and berries. From mushrooms eats brown mushrooms (*Leccinum*), saffron milk caps (*Lactarius*), milk mushrooms (*Lactarius resimus*), Boletus luteus (*Suillus*). However, when opening the stomachs of roe deer we noted that the content of mushrooms did not exceed 20–30% (n = 37 – animals were extracted under permits during the rut, extracted by poachers and delivered to the University by order for forensic biological examinations). In our opinion, this is also due to the severe digestibility of mushrooms. From berries found in the stomachs of cranberries (*Vaccinium vitis-idaea*), blueberries (*Vaccinium myrtillus*), blueberries (*Vaccinium uliginosum)*, strawberries (*Fragaria*). Roe also willing to attend biotechnical fields sown nutritious vegetation . The effectiveness of the attendance of fodder fields depends on their location, season of the year and the abundance of wild vegetation. Typically, the tab field occurs at a distance of 1–2 km from human settlements or forest and field roads, it is connected with the delivery of seed and technology equipment. Sowing of forage fields takes place annually on different areas of land. So, in the Republic of Buryatia in 2015 were sown 109 ha. and in 2016 – more than 31 hectares in 2017 – 62 ha.; in the Amur region in 2015 was planted 1,000 ha., and in 2016 – 655 ha., in 2017 – 517 ha. The hunters and the wildlife sanctuaries of both investigated regions try to diversify the composition of the forage fields and alternate crops. The best forage crops for ungulates are: alfalfa, rapeseed, melon, soybean, rye, vika and other plants that contain large amounts of protein.

Gradually, the animals switch to the autumn diet. The period of rut forces roe deer to go to forest areas with good protective conditions. During this period, we found leaves and stems of shrubs and shrubs: currants (*Ribes*), willow, rose (*Rosa*), Rowan (*Sorbus*), Siberian Apple (*Malus*), Daurian rhododendron, juniper (*Juniperus*) (n = 7 – animals were produced by permission during the rut).

When performing land quality evaluation of the Republic of Buryatia, we concluded that almost all the territory belongs to the III class for Siberian roe deer. The area of such land in the Republic is 24,548.5 ha. When accounting for limiting factors such as forage availability, predation, hunters press changes the class of land quality (Table 3).

Table 3. Hunting land evaluation for Siberian roe deer in the Republic of Buryatia with limitation factors

No.	Municipal district	Category of land (thousand hectares)			Territories, characteristic for species (thousand ha)	Weighted average indices of land quality	Land/Territory appraisal (average)	Land quality appraisal with limiting factors
		good	satisfactory	bad				
1	Municipal district	54.2	656.1	27.4	737.7	108	III	IV
2	Barguzinsky	164.4	5,880.9	103.3	6,148.6	103	III	V
3	Bauntovsky	24.4	388.7	162.6	575.7	82	III	IV
4	Bichursky	25.5	598.5	113.9	737.9	92	III	III
5	Dzhidinsky	-	2,286.8	122.6	2,409.4	96	III	III
6	Eravninsky	23.1	502.5	55.6	581.2	98	III	IV
7	Zaigraevsky	13.6	1,371.8	37.2	1,422.6	99	III	IV
8	Zakamensky	24.5	198.9	19.6	243	108	III	IV
9	Ivolginsky	72.8	532.6	11	616.4	116	III	IV
10	Kabansky	60.8	60	57.7	723.5	106	III	IV
11	Kizhinginsky	51.7	221.7	123.5	396.9	93	III	IV
12	Kyahtinsky	102.2	765.8	70.8	938.8	110	III	IV
13	Kurumkansky	38.6	182.3	111.1	332	89	III	III
14	Mukhorshibirsky	-	1,143	149.1	1,292.1	90	III	V
15	Muisky	-	1,252	2.3	1,254.3	100	III	V
16	Okinsky	17.2	1,198.6	34	1,249.8	100	III	III
17	Pribaikalcky	45.2	494.9	149.9	690	91	III	III
18	Selenginsky	196.5	2,421.7	52.7	2,670.9	109	III	V
19	Severo-Baikalsky	7.8	192.1	94.9	294.8	77	III	IV
20	Tarbagataisky	25.4	1,114.5	93	1,232.9	97	III	IV
	Total	947.9	22,008.4	1,592.2	24,548.5	100	III	IV

Hunting, typical for habitats of Siberian roe in Amur region are divided into 2 classes of class II and III, and their size, respectively, is 10,787.89 and 13,265.02 thousand ha.

The water necessary for the life of ungulates in the regions is located in sufficient quantities. On the territory of the republic of Buryatia there are about 9 thousand rivers and rivers, which belong to the basins of the Yenisei and Lena rivers. In the Amur region more than 29 thousand rivers. We noted that animals prefer running water of rivers, springs, streams.

Every hoofed animal depending on sex and age has a need in minerals associated with the change of feed rations, calving, lactation and rutting, which animals fill up on the salt marshes. In addition, in the literature there are data that the strong alkali substance is used as mechanical stimulus necessary for the normalization of the gastrointestinal tract, especially in the period of transition from rough winter fodder for juicy summer. In most areas of the Amur region and the Republic of Buryatia, there are both artificial and natural saline. The greatest number of them are in the northern regions.

For many decades in the regions under study artificial salt licks have been created and updated by specialists of hunting farms and hunters (Fig. 1; Table 3).

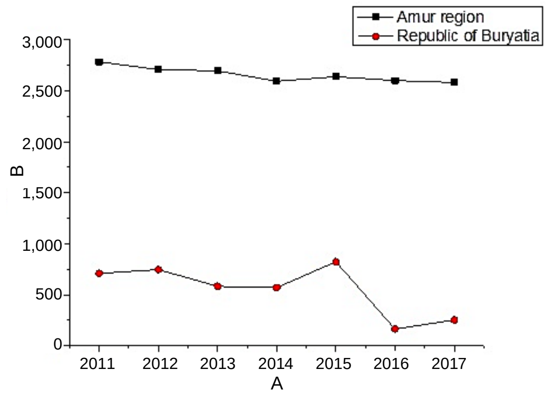


Figure 1. A number of salt licks equipped on the territory of Amur region and the Republic of Buryatia.

Competition among ungulates in Eastern Siberia and Amur region is low and is observed only in snowy winters, when the basis of nutrition is forage. Deer and elk in the winter displaces of the best grassland of the Siberian roe deer. Elk has the least competitive effect on deer because the most serious eating woody food. In addition, in the Republic of Buryatia, its number is small and it does not live throughout the territory. In the southern regions of the Republic there is no competition at all. This is primarily due to the low number of species such as elk and red deer. In Amur region elk on the salt licks compete with deer, displacing them (Figs 2, 3).

It should be noted that the increase in the number of ungulates depends not on the productivity of vegetation, but on the factor of concern and the number of predators. Elk and deer are usually rarely found near settlements. The only exceptions are those villages that are located in a specially protected natural area. Ungulates are more concentrated not in the fields with a good harvest of feed, but in the lands with good protective conditions, where the concern factor is minimal.

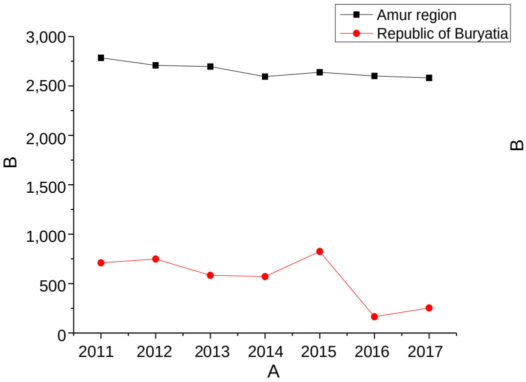


Figure 2. Dynamics of the number of hoofed animals in Amur regioon.

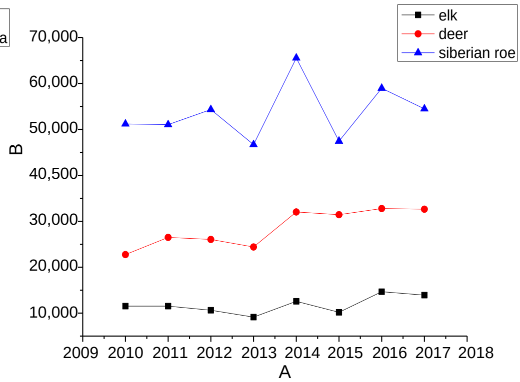


Figure 3. Dynamics of the number of ungulates in the Republic of Buryatia.

Wolf and brown bear

Large predators in Eastern Siberia and Amur region are represented by such hunting species as wolf and brown bear.

Wolf lives throughout both regions. In the Republic, it is most numerous on the Vitimskom plateau, in the Eastern Sayan (Okinskiy and Tunkinsky districts) and in the North of the Republic. The smallest number in the southern and Central regions. The number of wolves is periodically supported by individuals migrating from the territory bordering Mongolia. On the Vitimskom plateau, the high number of predators is explained by the abundance of ungulates, relatively low rainfall in winter. In the Northern regions of the Republic – Severobaikalskom and Muyskom areas, the wolf population is large. Due to the difficult terrain ungulates are concentrated in floodplains, where they become prey.

The basis of the power of the wolf are ungulates: roe deer, red deer, elk, wild boar, reindeer. In ‘hungry years’ predators and domestic animals willingly eat home animals. The damage done by the wolf to hunting and agriculture is great. Every year in the Republic are held events for shooting the wolf.

The food of the wolf is different in different seasons of the year. In the warm season, predators are sedentary and spend less energy. With low temperatures the wolves become more active and move to a nomadic lifestyle.

In spring and summer calving ungulate species in the diet of the wolf includes young roe deer and red deer. Summer prey includes rabbits, mice, badgers, marmots, and masonry upland game. Some sources indicate that the wolf eats insects such as locusts. At the end of the summer, the diet of the predator includes berries.

In the fall, during ungulates’ rutting, when the animals become vulnerable, wolves readily attack adults. With the loss of snow, the choice of feed is significantly reduced. Ungulates become the main food of the predator in winter.

Wolves in Eastern Siberia feed on wild ungulates in the valleys of the river Svetlaya, Barguzin and its tributaries, upstream of Shai, Pravoy Mamy on the rivers Zaza, Kydzhimitu, Conde. Significant damage to wild ungulates wolves inflict on the river valleys of Snezhnaya, Temnik, Dzhida, several other places of ungulates’ concentration.

In Amur region on 30-kilometer routes were found up to 9 roe deer, torn by wolves. In the Far East, two or three predators per day attack up to 12–14 animals, in places of winter concentration, each destroys 20–30 during the snow period, and up to 35–45 individuals per year.

A red deer is an easy prey for a wolf. Wolves hunt for them in large numbers and more often in comparison to elk or roe deer. A large number of individuals die from a predator in snowy winters and during icy period.

Many sources indicate that wolf predation is selective, and depends on the condition, gender and age of the animal. However, during the study period, we recorded cases of ungulates from the wolf of different age groups and regardless of the physical condition of the victim.

The usual habitat of wolf are open areas of steppe and forest-steppe regions. In the forest zone it is characterized by floodplains, wetlands, areas where ungulates concentrate. Our calculations of territory quality for wolves showed that the whole territory of the Republic of Buryatia belongs to the III class. The square area of this territory is 26,881.3 thousand hectares.

Except wolves hoofed animals suffer from bears. Bear is the largest predator. In the Far East this species reaches the largest size. Brown bear refers to omnivorous animals. It eats both large elk and small insects. In the Amur region and Eastern Siberia, brown bear food is both vegetable and animal feed, depending on their availability and the season of the year. The diet of the brown bear includes ungulates such as roe deer, elk, wild reindeer, wild boar, and raisins.

According to survey data received from hunters and our observations there noted some cases of successful hunting of brown bear on wild reindeer in Tyndinskom district, wild boar – in Arkharinskom, Bureyskom, Blagoveshchenskom, Svobodnenskom districts of the Amur region. Cases of attacks on elk and red deer in the Arkharinskom district of the Amur region. Registration of such cases, as a rule, is difficult due to the fact that these species live in remote places, and they can be accurately established only by anatomical study of the extracted individuals.

Insects, mouse-like rodents, fish and other types of feed, which make up a small part in different seasons, are also found in the diet of the bear (Belikov et al., 1993; Chernyavsky & Krechmar, 2001; Brown bear of Kamchatka, 2006; Zimmermann et al., 2014). The fish, which gets into the food of the bear in Siberia and the Amur region, is usually overseas (Veklich & Darman, 2013; State report of the Amur region MPR on environmental protection and ecological situation in the Amur region for 2011, 2012). Facts of expertise to bear on the kind of food we have not yet determined.

It is difficult to name the preferred habitat of the bear, as he leads a wandering life. In winter, the animals go a long distance from the summer area. However, the bear lives where the abundance of berries and nuts.

The bear’s diet depends on the season and harvest. In the spring, after hibernation, the bear eagerly eats asp shoots and dead animals that died during winter. Starts preying on ungulate species. After the snow cover melts, the diet of the bear includes overwintered berries, then growing grass, aspen leaves, small animals and birds.

In summer, eat a variety of berries, pine nuts. In years with a low harvest of these species, the bear goes to feed on oats and maize. Given that the size of the bear is large, it needs a large amount of food. Most of the feed is processed into fat, which is so necessary for the winter of the animal.

The conducted work allowed to establish that in territory of the Amur region main vegetable foods in the diet of the brown bear are parts of plants or their derivatives of the following types: koreyanka toloknyanolistnaya (*Chosenia arbutifolia*), a hidden willow (*Salix abscondita*), goat willow (*Sálix cáprea*), Bebba willow (*Salix bebbiana*), korotonozhkina willow (*Salix brachypoda*), Schwerin willow (*Salix schwerinii*), blueberry willow (*Salix myrtilloides*), willow rosistaya (*Salix rorida*), udskaya willow (*Salix udensis*), trembling poplar (*Pópulus trémula*), Mongolian oak *(Quercus mongolica*), leschina raznolistnaya (*Corylus heterophylla*), edible honeysuckle (*Lonicera edulis*), Manchurian leschina (*Corylus mandshurica*), Amur Linden (*Tilia amurensis*), svida white (*Córnus álba),* strawberry Eastern (*Fragária orientális*), Asian bird cherry (*Padus asiatica*), raspberry (*Rúbus árcticus*), koctyanika schelistnaya (*Rubus humilifolius*), Komarov raspberry (*Rubus komarovii*), Sakhalin raspberry (*Rubus matsumuranus*), krovohlebka lekarstvennaya (*Sanguisórba officinális*), kostyanika ordinary (*Rubus saxatilis*), dudnik Chernyaeva (*Angelica czernaevia*), dudnik Maksimova (*Angelica maximowiczii*), small sedge (*Carex minuta*), dirty sedge (*Carex sordida*), Sedakova sedge (*Carex sedakowii*), sedge gladkachyayschaya (*Carex laevissima*), sedge Schmidt (*Carexsch midtii*), sedge puzyrevataya (*Carex vesicaria*), borschevik rassechenniy (*Heracleum dissectum*), vaccinium topyanoy *(Vaccinum uliginosum*), schisandra chinensis (*Schisándra chinénsis*), Daurian hellebore (*Veratrum dahuricum*), hellebore Maaka (*Veratrum maackii*), cotton grass *(Erióphorum vaginátum*), kedroviy stlannik (*Pinus pumila*), kedr Korean (*Pínus koraiénsis*). We found parts of these plants in the excrement of a brown bear, or observed traces of brown bears eating.

The calculation of the bear habitat appraisal in the Republic of Buryatia in all areas is equal to the III class of bonus. The area of land peculiar to the bear is 23,921.1 ha. The exceptions are Mukhorshibirsky and Tarbagataysky areas where the bear lives. According to our calculations the land of the Amur region for the bear, too, belong to the class III site class, area 1,845,876 ha. The worst hunting grounds for the bear located in Zavetinskom and Oktyabrskom districts of the region, and the territory of the Zeysko-Bureyskoy plain is practically not typical of this type.

DISCUSSION

The highest species composition of the feed suitable for the nutrition of elk, possess the hunting grounds Mazanovskogo, Romnenskogo, Selemdzhinkogo, Tyndinskogo, Skovorodinskogo districts of the Amur region and Severobaikalskogo, Muyskogo and Bauntovskogo regions of Eastern Siberia. This is due to the presence of large areas of wet swampy forest biotopes and secondary forests. These types of land are rich in coniferous young, larch, aspen, with the presence burnt areas and clear cuttings. In addition, there are a sufficient number of rivers and lakes. The choice of animal feed depends on the season. According to our observations, the most preferred elk feed is willow and aspen. Year-round elk eats shoots of deciduous trees. Hay elk eat in exceptional cases. In difficult winter conditions elk goes to shoots and bark of spruce and pine.

Hunting areas with good forage conditions for elk located in Bauntovskom, Zakamenskom, Eravninskom, Muyskom, Pribaikalskom, Okinskom, Severobaikalskom, Horinskom areas of the Republic of Buryatia and in Tyndinskom, Skovorodinskom, Zeyskom, Magdagachinskom, Szymanowskom, Mazanovskomom, Svobodnenskom, Selemdzhinkom, Burejskom, Arkharinskom districts of the Amur region. This is due to the mountain-forest landscape, which has a positive impact on the survival of the animal. Mosaic forest and the presence burnt areas, are also located in sufficient numbers in these areas. In both regions, we carried the stems and leaves belokopitnik, wormwood, sedge, veinik as a favorite food of the red deer. When there is a shortage of feed, the deer pass to tree and shrub plants. The animal eats the hay of any quality.

Hunting grounds of the Republic of Buryatia and Amur region have good feeding conditions for siberian roe deer. The capacity of hunting grounds allows to increase the number at times. However, in winter, the snow cover forces the animals to go to the snow-covered areas. siberian roe deer prefers biotopes with a predominance of pine, larch, birch, as well as burning, cutting, steppe and meadows. Siberian roe deer prefers easily digested feed rich in nutrients. As in Eastern Siberia and in the Amur region, the animals readily eat aspen, willow, birch, fireweed, troelistka, wormwood. With a lack of the favorite forage of deer eats on pine and hay.

The main food of the wolf are ungulates, and the predator chooses habitats with the presence of high density of ungulates. This kind of territory we assign Bauntovskiy, Muyskiy, Severobaikalskiy, Okinskiy, Zakamenskiy regions of Eastern Siberia and Tyndinskom, Skovorodinskom, Magdagachinskom, Mazanovskom, Svobodnenskom, Selemdzhinkom the areas of the Amur region. According to our data, the diet of the wolf from ungulates often comes across Siberian ROE deer. We associate this primarily with the widespread distribution of ROE deer, as well as a relatively high number compared to other ungulates. When the lack of food, the predator eats livestock.

In the Amur region the most wide range of plant feed for the brown bear are presented in Selemdzhinkom, Mazanovskom, Burejskom, Romnenskom, Seryshevskom, Svobodnenskom, Skovorodinskom, Magdagachinskom, Blagoveshchenskom, Belogorskom, Ivanovskom, Zavetinskom, Konstantinovskom, Mikhaylovskom, Tambovskom and Arkharinskom. The best feeding conditions for bears in Eastern Siberia have the Severobaikalskiy, Barguzinskiy, Pribaikalskiy, Kabanskiy, Bauntovskiy, Muyskiy, Okinskiy and Horinskiy areas. The favorite food of the bear is vegetable food. To such feeds we carried nuts, b6. In General, feed conditions in both regions are represented by a wide species composition of vegetation, and for predators there is also a large selection of animal feed. On the territory of the Amur region and the Republic of Buryatia, feed is rich in nutrients and is in sufficient quantity. This is confirmed by the fact that competition among ungulates is minimal and seasonal. The capacity of hunting grounds in Eastern Siberia and the Amur region can significantly increase the number of ungulates. Adverse climatic conditions, namely high snow cover, as well as predation and poaching, do not have a positive impact on the growth of ungulates. However, the strengthening of security measures on the part of hunting farms and state services for the protection of wildlife, conducting high-quality biotechnical measures and activities related to the regulation of the number of predators can lead to a noticeable result of the hunting economy, namely the growth of the population of ungulates.

The present findings may find application within the context of other papers (Miquelle et al., 2010; Lavrillier, 2013; Sonnenburg et al., 2016). This section is aimed to demonstrate how a well-planned research can serve as a framework for the long-term conservation of species. We believe that any successful conservation program should be based on a clear understanding of the environment in which the species live, i.e., on the systematic collection of qualitative and quantitative data concerning their living conditions. Without this fundamental knowledge needed to conserve any species, it is easy to move away from the main areas of activity necessary for the conservation, preservation and restoration of the animal population. This study is an attempt to demonstrate how research and sometimes simple surveys can have important consequences if the information from them is applied for the conservation purposes. However, it is not enough to simply conduct good research and publish the findings. The research should focus on mitigating threats or at least provide information on the relative importance of these threats. Secondly, to make findings contributive, scientists need to come out from the sidelines and make active steps toward the initiation of the conservation efforts. It is the scientific community that understands the significance of their findings to the deepest level and thus should be proactively involved in the enlightening of public and key political figures and in the implementation of environmental measures.

CONCLUSIONS

The results of the research are of great practical use for hunting entities of Eastern Siberia and Amur region, especially for the application of hunting grounds description experience, conducting species hunting appraisal, improving local systems of biotechnical activities aimed at wild ungulates, studying the causes and mechanisms of feeding adaptations of ungulates and predators to the environment and seasons of the year, efficient measures to regulate predator population in Eastern regions of Russia. The research results allow hunting farms to improve the system of biotechnical and reproductive activities, with the aim of increasing the number of ungulates, as well as improving the quality of hunting territory.

The collected data and information can also be used as lecture material in higher and professional educational institutions for the classes in biology, hunting, ecology and zoology.

The obtained data is of particular interest for collective entities and individual hunters who professionally plan their activities in the difficult climatic conditions of Siberia and Far East of Russia, as well as for the non-professional hunters who are interested in biology and ecology of wild ungulates and try to increase their level of knowledge of typology of hunting grounds, technology and production technology, and increase the efficiency of wild ungulates hunting.

Data obtained during this study can be also used when planning and implementing measures to protect the living conditions of small native peoples of Siberia and Amur Region. Native peoples still use natural resources for traditional crafts and thus their lifestyle largely depends on the wildlife situation. Without data present here, the event plans concerned with the native peoples will be considered groundless.

REFERENCES

Antipov, E.I. 1976. Winter feeding of deer and roe deer in the Western Sayan. *Research components of forest biogeocenosis in Siberia*. Krasnoyarsk, Russia, pp. 37–40 (in Russian).

Antropov, V.F., Seredkin, A.D. & Schepin, A.A. 2013. *Forestry of Buryatia*. LLC 'Publishing house 'EKOS', Ulan-Ude, Russia, 184 pp. (in Russian).

Argunov, A.V. & Stepanova, V.V. 2011. Diet structure of the Siberian roe deer in Yakutia. *Ecology* **2**, 144–147. doi:10.1134/S1067413611020032

Belikov, S.E., Chernyavskiy, F.B., Chestin, I.E., Grachev, Y.A., Danilov, P.I., Krechmar, A.V., Kudaktin, A.N., Lavov, M.A., Loskutov, A.V., Mordosov, I.I., Pavlov, M.P., Pazhetnov, V.S., Puchkovskiy, S.V., Revenko, I.A., Rusakov, O.S., Shubin, N.G., Slobodyan, A.A., Sobanskiy, G.G., Tumanov, I.I., Ustiniv, S.K., Vaisfeld, M.A., Yudin, V.G., Zavatzkiy, B.P. & Zhiryakov, V.A. 1993. Bears: brown bear, polar bear, Himalayan bear. In Vaisfeld, M.A. & Chestin, I.E. (eds): *Commercial animals of Russia and adjacent countries and their habitat*. Nauka, Moscow, Russia, 519 pp. (in Russian).

Chernyavsky, F.B. & Krechmar, M.A. 2001. *Brown bear (Ursus arctos L.) in the North-East of Siberia*. IBPS SVNZ DVO RAN, Magadan: Russia, 93 pp. (in Russian).

Danilkin, A.A. 1999. *Deer*. GEOS, Moscow, Russia, 552 pp. (in Russian).

Danilov, D.N. 1953. *The principles of typology and valuation of hunting lands. Trudy VNIIO issue XII. Biology of fur-bearing animals* **12**, 48–90. (in Russian).

Danilov, D.N. 1960. *The hunting grounds of the Soviet Union*. Publishing house, Centrosoyuz, Moscow, Russia, 331 pp. (in Russian)

Danilov, D.N. & Rusanov, Ya.S. 1966. *Basics of game management*. Forest industry, Moscow, Russia, 331 pp. (in Russian).

Dementiev, V.I. 1973. *Evaluation of the hunting lands. Basics of immunology*. Forest industry, Moscow, Russia, pp. 64–67 (in Russian).

Forest, D. 1979. Haltung von Rehen (Capreolus capreolus L/) zu Yersuc hszwecken Ztschr. *Saugeticrk* **3**, 190–199.

Johansson, M., Ferreira, I.A., Støen, O.G., Frank, J. & Flykt, A. 2016. Targeting human fear of large carnivores-many ideas but few known effects. *Biological Conservation* **201**, 261–269. doi:10.1016/j.biocon.2016.07.010

Kluchev, A.G. 2003. *Evaluation of the hunting lands. Hunting*. ISAA, Irkutsk, Russia, pp. 112–130 (in Russian).

Korenyuk, S.N. 1989. The impact of deer on natural regeneration in the Zeya nature reserve. *Ekologiya* **1**, 75–77.

Kozlov, V.M. 2007. Technological evaluation of hunting grounds. *Modern problems of nature management, hunting and animal husbandry: materials of the international scientific practical conference devoted to the 85th anniversary of VNIIOZ*. Kirov, Russia, 195 pp. (in Russian).

Kozlovskiy, I. 1997. Wolves-the eternal problem of Russia. *Hunting and hunting* **12**, 12–14.

Kucherenko, S.P. 1981. *The ungulates of the south of the Far East*. Report from the funds of the Far East Branch VNIIOZ. Khabarovsk, 464 pp. (in Russian).

Kuzyakin, V.A. 1974. *On the evaluation of hunting grounds. Hunting: proceedings of Central research laboratory ìglavohotaî of the RSFSR*. Forest industry, Moscow, Russia, pp. 140–157 (in Russian).

Lavrillier, A. 2013. Climate change among nomadic and settled Tungus of Siberia: continuity and changes in economic and ritual relationships with the natural environment. *Polar record* **49**(3), 260–271. doi:10.1017/S0032247413000284

Lebedeva, L.S. 1965. On the composition of summer forages of wild boar in the Alpine zone of the Caucasian reserve. Scientific notes of MSPI. Potemkin. *Department of zoology* **61**(4–5), 105–271.

Lobachev, S.V. 1932. *In Stahovsky V.G. Upper-Vychegda expedition*. Moscow, Russia, 384 pp. (in Russian)

Martynov, E.N. & Masaitis, V.V. 2002. Typology and appraisal of habitats in the forest hunting animals. *Modern problems of nature, of hunting and fur farming: proceedings of the International scientific-practical conference dedicated to the 80th anniversary of VNIIOZ*. Kirov, Russia, pp. 299–301 (in Russian).

Melnikov, V.K. & Melnikov, V.V. 2008. Optimum number of hunting animals. *Modern problems of the organization of hunting economy of Russia, hunting tourism and the analysis of its legal support in foreign countries and Russia*. Russian Ministry of Agriculture, Moscow, Russia, pp. 61–68 (in Russian).

Miquelle, D.G., Goodrich, J.M., Kerley, L.L., Pikunov, D.G., Dunishenko, Y.M., Aramiliev, V.V., ... & Seryodkin, I.V. 2010. Science-based conservation of Amur tigers in the Russian Far East and Northeast China. In *Tigers of the World*. William Andrew Publishing, pp. 403–423.

Mironova, L. 2001. *Report. State of the environment and environmental activities in the Republic of Buryatia in 2000*. Ulan-Ude, Russia, 205 pp.

Morozov, K.A. 1982. *Experience of mineral feeding of wild ungulates in the Amur region. Increase of productivity of hunting grounds*. Moscow, Russia, pp. 43–49 (in Russian).

Namzalov, B.B., Bogdanov, K.M. & Bykov, I.P. 1997. *Buryatia: the plant world. Issue II*. Publishing house of Buryat State University, Ulan-Ude, Russia, 250 pp. (in Russian).

Noskov, V.T. 2008. *Hunting animals of Buryatia*. Publishing house of the BGSHA, Ulan-Ude, Russia, 224 pp. (in Russian).

Novikov, G.A. 1953. *Field studies of the ecology of terrestrial vertebrates*. Owls. Science, Moscow, Russia, 502 pp. (in Russian).

Plaksa, S.A. 2005. Typology and classification of Dagestan hunting grounds. *Biological resources: state, use, and protection: mater. vseros. science. prod. conferences*. Kirov, Russia, pp. 202–205 (in Russian).

Sand, H., Wabakken, P., Zimmermann, B., Johansson, Ö., Pedersen, H.C. & Liberg, O. 2008. Summer kill rates and predation pattern in a wolf–moose system: can we rely on winter estimates? *Oecologia* **156**(1), 53–64.

Sandakov, S.L., Senchik, A.V. & Bormotov, M. 2015. *Population Dynamics of red deer (Cervus elaphus xanthopygus) and Ussuri moose (Alces alces cameloides) in the Amur region. The Protection and rational use of animal and plant resources*. Irkutsk, Russia, pp. 155–160 (in Russian).

Senchik, A.V. & Guretskaya, Y.S. 2017. *Evaluation of the hunting lands Mukhorshibir district of the Republic of Buryatia in the Siberian ROE deer (Capreolus pygargus Pall). Environmental protection and rational use of small-scale resources*. Proceedings of the IX international forest foorum, pp. 215–219 (in Russian).

Senchik, A.V., Pavlov, A.M. & Sato, Y. 2017. Analysis of brown bear (Ursus arctos) feeding conditions in the Amur region. Theoretical and scientific-practical journal ‘Innovations and food security’. *Publishing center NGAU ‘Golden spike’* **4**(18), 119–126.

Seryodkin, I.V., Paczkowski, J., Shuntov, V.P. & Raygorodetsky, G.R. 2006. *Brown bear of Kamchatka: ecology, protection and rational use.* Dalnauka, Vladivostok, Russia, 148 pp. (in Russian).

Shaposhnikov, F.D. 1953. ‘Solontsovaya’ wild hoofed animals in the mountain taiga of the Altai Republic. *Bull. MOIP. Otd. Biol*. **1**.

Smirnov, M.N. 1977. Morphology and nutrition of Western Transbaikal roe deer. Faunistic and ecological studies in Transbaikalia: Tr. Buryat. *Institute of natural. Sciences (series)* **15**, 171–197.

Smirnov, M.N. 2000. *Roe deer in the upper Yenisei*. Krasnoyarsk State University, Krasnoyarsk, Russia, 154 pp. (in Russian).

Sonnenburg, J., Ryser-Degiorgis, M.P., Kuiken, T., Ferroglio, E., Ulrich, R.G., Conraths, F.J., Gortázar, C. & Staubach, C. 2016. Harmonizing methods for wildlife abundance estimation and pathogen detection in Europe-a questionnaire survey on three selected host-pathogen combinations. *BMC veterinary research* **13**(1), 53. doi:10.1186/s12917-016-0935-x

Starchenko, V.M. 2008. *The flora of the Amur region and problems of its protection: the Far East of Russia*. Nauka, Moscow, Russia, 228 pp. (in Russian).

State report of the Amur region MPR on environmental protection and ecological situation in the Amur region for 2011. 2012. Blagoveshchensk, Russia. (in Russian).

Subbotin, A.M. 1980. Distribution and habitat occupancy of ungulates (Cetacea) and predatory (Carnivora) in the Khamar-Daban (Baikal region). *Fauna and ecology of vertebrates of Siberia*. Science, Novosibirsk, Russia, pp. 115–122 (in Russian).

The scheme of placement, use and protection of hunting grounds in the territory of the Republic of Buryatia. 2017. Ulan-Ude, Russia, (in Russian). https://www.hunting.ru/articles/view/150262/

Treves, A. & Bruskotter, J. 2014. Tolerance for predatory wildlife. *Science* **344**(6183), 476–477. doi:10.1126/science.1252690

Veklich, T.N. & Darman, G.F. 2013. *An illustrated flora of the Zeya nature reserve, Far East Russia*. ‘Studio Art’, Blagoveshchensk, Russia, 378 pp. (in Russian).

Vorob'ev, V.V., Derevianko, A.P. & Schulman, N.K. 1989. *Amur oblast: experience encyclopaedia dictionary*. Khabar. kn. publishing house of the Amur. Edition, Blagoveshchensk, Russia, 415 pp. (in Russian).

Wäber, K. & Dolman, P.M. 2015. Deer abundance estimation at landscape-scales in heterogeneous forests. *Basic and applied ecology* **16**(7), 610–620. doi:10.1016/j.baae.2015.06.005

Yurgenson, P.B. 1969. About feed capacity land. *Hunting and hunting* **6**, 22–23.

Zimmermann, B., Nelson, L., Wabakken, P., Sand, H. & Liberg, O. 2014. Behavioral responses of wolves to roads: scale-dependent ambivalence. *Behavioral Ecology* **25**(6), 1353–1364. doi:10.1093/beheco/aru134

Appendix

Examples of registration cards and statements of calculating the number of animals – the primary materials of hunting farms.

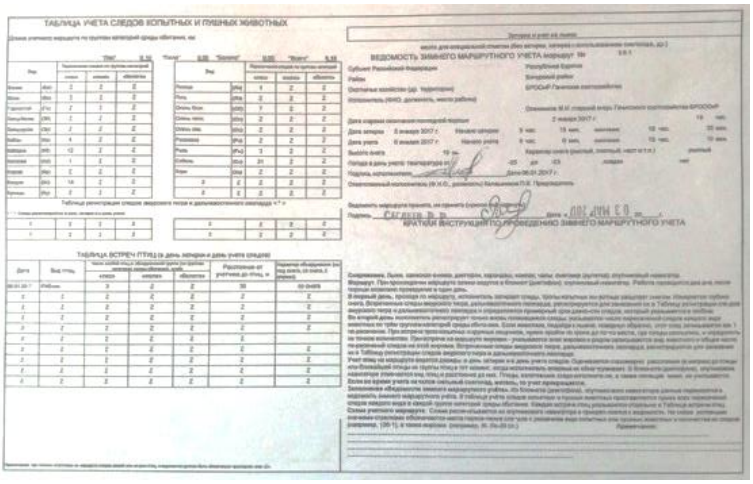


ТАБЛИЦА УЧЕТА СЛЕДОВ КОПЫТНЫХ И ПУШНЫХ ЖИВОТНЫХ

ВЕДОМОСТЬ ЗИМНЕГО МАРШРУТНОГО УЧЕТА

ТАБЛИЦА ВСТРЕЧ ПТИЦ

КРАТКАЯ ИНСТРУКЦИЯ ПО ПРОВЕДЕНИЮ ЗИМНЕГО МАРШРУТНОГО УЧЕТА

Supplementary Fig. 1.
An example of a reporting document. A similar table is filled in by the Wildlife Officer upon return. The table shows the types of animals whose tracks he met on his way, the whole table is divided into several categories, for animals, birds, and traces of the Amur tiger and Far Eastern leopard are placed in a separate category. The number of cases of observation of tracks is entered in the table, the sign 'Z' means the absence of observed tracks. The table indicates the type of animal that left traces, as well as the characteristics of the terrain (swamp, forest, etc.). After fixing the traces in his records, a Wildlife Officer destroys the traces by rubbing them in the snow, thus performing periodic walks; the Wildlife Officer fixes only new cases and thus controls the number and activity of animals.

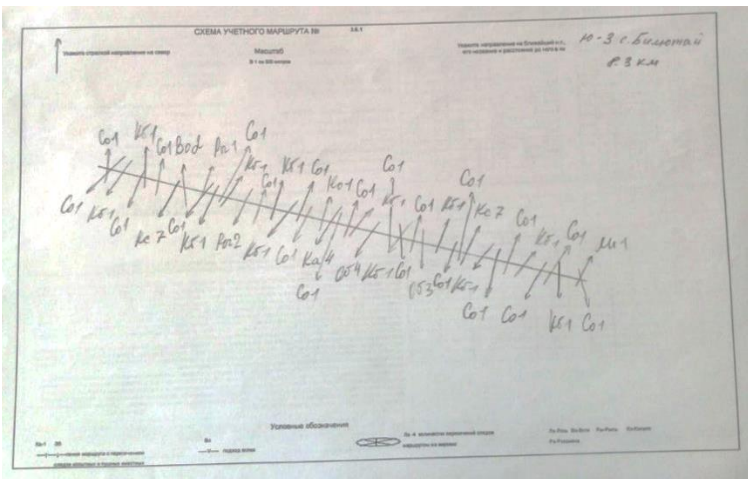
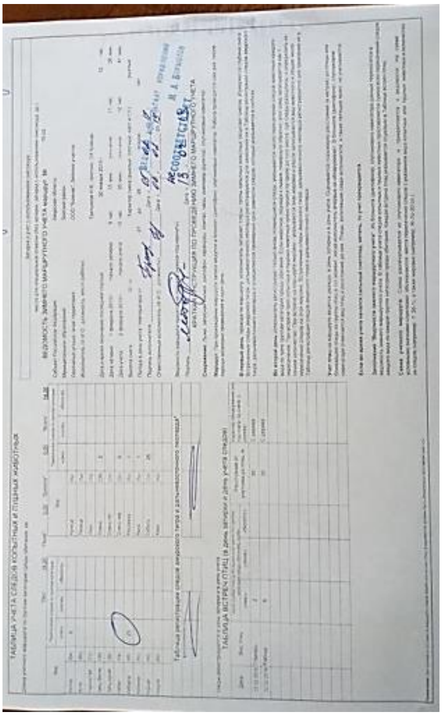


СХЕМА УЧЕТНОГО МАРШРУТА №

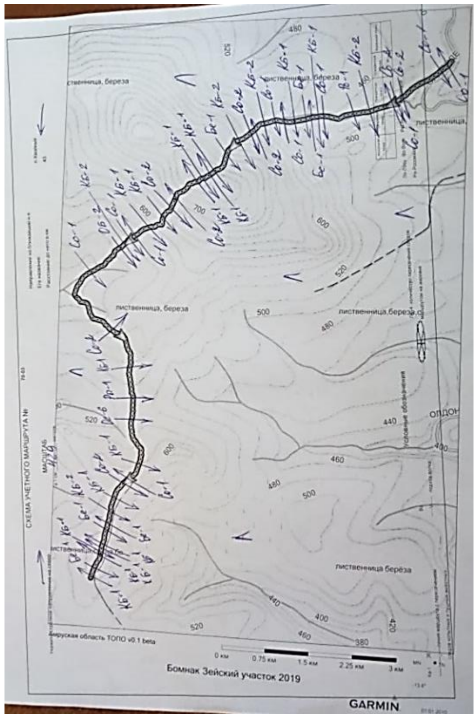
Масштаб

Условные обозначения

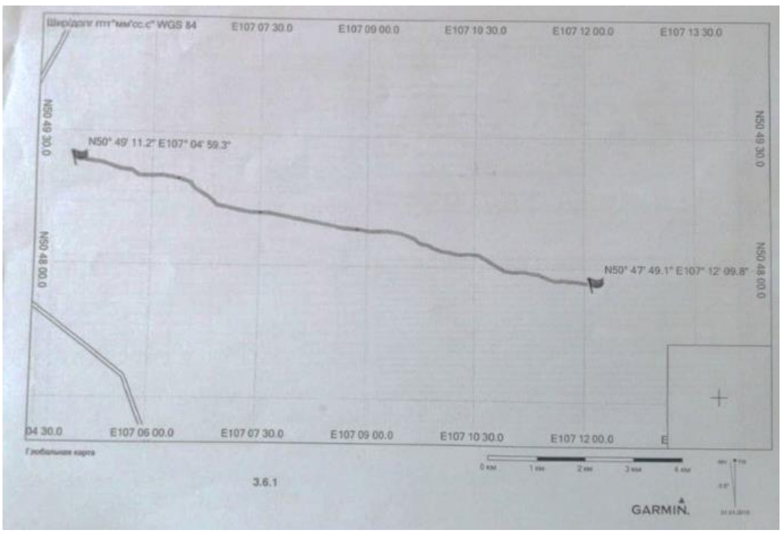
Supplementary Fig. 2.
An example of an officer's travel notes, the direction of the intersection of the path with a chain of traces and the symbol designating a living one that left traces is noted.



Supplementary Fig. 3.
Another example of filling out a table is similar to the first figure in this appendix.

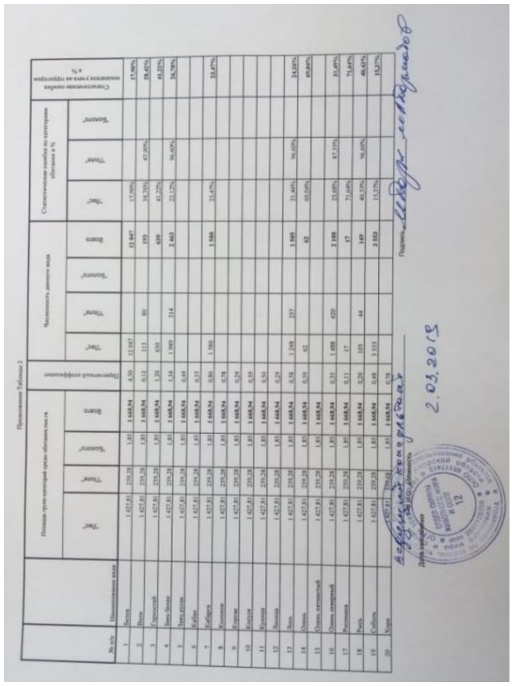


Supplementary Fig. 4.
Another example of more accurate travel notes linked to the area map using GPS.



Supplementary Fig. 5.
Another example of more accurate travel notes linked to the area map using GPS.

Supplementary Fig. 6.
Another example of more accurate travel notes linked to the area map using GPS.



Supplementary Fig. 7.
An example of a statement summarizing all travel notes of all forestry officers on a specific date. This is the data that then will form the basis of statistical generalization.

Supplementary Fig. 8.
The location of the Republic of Buryatia and the Amur Region on a map of the Russian Federation.

Agronomy Research **18**(1), 290–300, 2020
https://doi.org/10.15159/AR.20.026

Production of *Aspergillus oryzae* RCAM 01133 biomass with increased protein and polysaccharides content using by-products of food industry

E. Serba[1], N. Pimenov[2], P. Mochalina[1], M. Overchenko[1], Yu. Borscheva[1], A. Sharikov[1,*] and L. Rimareva[1]

[1]Russian Research Institute of Food Biotechnology – branch of Federal Research Center of Nutrition and Biotechnology, Samokatnaya street 4b, RU111033 Moscow, Russia
[2]Federal State Educational Institution of Higher Professional Education Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Skryabin, 23 Academician Skryabin street, RU109472 Moscow, Russia
[*]Correspondence: anton.sharikov@gmail.com

Abstract. The biomass of filamentous fungi is a promising source of protein and carbohydrate. It can be used as an unconventional substrate in technologies for obtaining food and feed ingredients. The studies show that the fungus *Aspergillus oryzae* synthesizes an insufficient level of protein in the process of solid-state fermentation. The aim of the research was to develop conditions for the production of fungal biomass with a high content of protein and polysaccharides on the basis of solid-state fermentation using by-products of food industry as inexpensive substrate for biomass production. Wheat bran, soybean meal, distilled dry grains with solubles, and brewer's spent grain were used as raw material. Results of fermentation show that the protein content was 73.4% and 82.0%, which is more than 3 times higher than in fungus grown by submerged fermentation. The studies of the biosynthesis properties of the selected strain *A. oryzae* RCAM 01133 confirmed the high biological value of microbial biomass cultivated using food by-products. Fermentation of micromycete on culture media containing soybean meal and DDGS provided an increase of protein content by 1.45 times. Maximum increase of amino acids was observed for isoleucine, leucine, tryptophan, and glutamic acid. The increased content of synthesized polysaccharides related to media containing wheat bran and DDGS. The highest concentrations of polysaccharides were 27.9% and 32.9%, respectively.

Key words: *Aspergillus oryzae,* solid-state fermentation, protein, amino acids, polysaccharides, food by-products.

INTRODUCTION

Active studies of the biochemical and structural-functional properties of fungal biomass are conducted to identify the prospects of its use as a substrate for the production of protein and amino acids additives and functional ingredients (Ward et al., 2006; Feofilova, 2010; Abdel-Gawad et al., 2017).

Filamentous fungi have the ability to synthesize industrially significant biologically active substances. Fungus biomass contains protein, vitamins and valuable polysaccharides, mainly glucan, chitin, mannan (Polizeli et al., 2005; Kumaresapillai et al., 2011; Gow et al., 2017). The composition of mycelial biomass is not constant. The content of metabolized fungi polysaccharides and protein varies considerably. It's related to the genetic affiliation and productivity of the used strains, the conditions of their cultivation and the media composition (Zhong et al., 2018).

The biomass of the fungi can be used for large scale manufacture of bio-based products and active pharmaceutical ingredients (Meyer et al., 2016). The food industry is constantly under the state and social press to reduce wastes damage to environment and become more eco-friendly. Some by-products are included in recipes of food and feed products as inexpensive sources of protein, dietary fibre, phenolic compound and the other biologically active substances (Laufenberg, et al., 2003). In addition to direct use as an ingredients, food by-products and wastes can be used more effectively as substrates for fermentation to produce biomass protein and polysaccharides (Zhang et al., 2008; Jin et al., 2010; Shin et al., 2018).

Solid state fermentation as a process carried out at a low water content has several advantages over submerged fermentation. Among the main advantages, one can note a higher resistance to contamination, lower costs of energy for sterilization and heat treatment, lesser wastewater production, and a higher concentration of target fermentation products. An important aspect is the possibility of using of solid agro-industrial wastes as substrate in their natural form for the production of fungal biomass (Soccol et al., 2017). *Aspergillus oryzae* is one of most used species for utilisation of food industry wastes such as rice bran (Rudravaram et al., 2006; Shin at al., 2019), brewer's spent grains (Bekatorou et al., 2007; Ogunjobi et al., 2011), soybean meal (Hong et al., 2004; Chean et al., 2013), distiller's dried grains with solubles (Lio & Wang, 2012), pea-processing byproduct (Souza Filho et al., 2018), sweet potato beverage residues and peanut shells (Zuo et al., 2018).

Results of investigations of Serba et al. (2016) show that the protein and polysaccharides content in the fungal biomass of *Aspergillus oryzae* RCAM 01133 produced by submerged fermentation is not high enough and corresponds to 18–25% and 25–33%, respectively. The aim of this study is the development of conditions for the production of fungal *Aspergillus oryzae* RCAM 01133 biomass with a high content of protein and polysaccharides as promising feed additives effective for animal production using solid state fermentation (SSF) of food industry by-products and wastes.

MATERIALS AND METHODS

Microbial Culture, Substrates and Fermentation

The object of study was non-pathogenic strain *Aspergillus oryzae* RCAM 01133 from the microorganisms collection of the Russian research institute of food biotechnology. A distinctives features of the strain are its high growth rate with decreases spore formation.

Substrates for solid-state fermentation of the fungus were by-products of food industry such as wheat bran (WB), soybean meal (SBM), distilled dry grains with solubles (DDGS), brewer's spent grain (BSG) and their combinations in various ratios shown in Table 1.

Fermentation was carried out at 30° within 2 and 3 days. Prepared growth media with moisture content of 55–60% was sterilized at 0.1 MPa for 40 minutes. The culture efficiency was estimated by the growth rate of biomass, content of polysaccharides and protein, and hydrolytic enzymes activity.

All treatments and analyses were carried out in two sets.

Table 1. Combination of by-products for fermentation medium

No. of medium	Content of food industry by-products in solid phase of the medium, %			
	SBM	DDGS	BSG	WB
1	100	0	0	0
2	0	100	0	0
3	0	0	100	0
4	0	0		100
5	80	20	0	0
6	20	80	0	0
7	20	0	80	0
8	50	0	0	50
9	80	0	20	0
10	0	80	20	0
11	0	50	50	0
12	0	50	0	50

Chemical Analysis

The content of polysaccharides was determined as total reducing sugars (TRS) after acid hydrolysis measured spectrophotometrically by Nelson-Somogyi method (Nelson, 1944). The total protein content was determined by the Kjeldahl method. Approximately 0.5 g of raw material was hydrolyzed with 15 mL) concentrated sulfuric acid ($H_2SO_4$with catalyst tablet containing 5 g Potassium Sulphate (K_2SO_4) and 0.5 g Copper (II) Sulphate ($Cu_2SO_4 \cdot H_2O$) using Turbotherm digestion unit (Gerhard, Germany) at 420 °C for 2 h. Nitrogen was determined using distillation system Vapodest (Gerhardt, Germany). Protein content was arrived by multipliing amount of total nitrogen in the raw materials by the nitrogen-to-protein conversion factor of 6.25. Amino acids profile was estimared by the liquid chromatographic method using the amino acid analyzer Knauer (Germany) with ultraviolet spectrophotometric detector Knauer Smartline 2500 operated at 570 nm. Before analysis, samples were hydrolyzed with 6 N hydrochloric acid (HCl) in a boiling water bath under reflux for 6 hours followed by subsequent autoclaving for 2 hours at 120 °C. Then hydrolyzates were centrifuged at 6,000×gfor 15 minutes. Supernatant was used for quantitative and qualitative total amino acids analysis. Aminograms were calculated by comparing the areas of the standard and the samples curves.

The concentration of amine nitrogen was determined by the copper method of Pope and Stevens in the absence of ammonium salts (Cowan & Steel, 1993).

Iodine Fuwa method (Fuwa, 1954) was used to measure the α-amylase activity (AA). A 300 µL of enzyme was mixed with 600 µL of 1% (w/v) soluble starch dissolved in 100 mM NaAcetate buffer pH 4,7 at 30 °C. One unit of the α-amylase activity was defined as the amount of enzyme that produced 10% reduction in starch-iodine staining after 10 min of incubation under the experimental conditions. Protease Hemoglobin colorimetric Sigma Aldrich assay was used to determine proteolytic activity (PA). One unit of protease activity will hydrolyze Hemoglobin to produce color equivalent to 1.0 µmole of Tyrosine per minute at pH 4,7 at 30 °C (color by Folin & Ciocalteu's Reagent).

Statistical analysis

Statistical processing of the results was carried out using the one-way ANOVA and the post-hoc Tukey's test for multiple comparison. Differences were considered significant for $\alpha < 0.05$.

RESULTS AND DISCUSSION

The process of biosynthesis the mycelial biomass by solid-state fermentation of fungus *A. oryzae* RCAM 01133 in culture media containing by-products of food industry has been studied. During the growth of the culture on by-products media, an intensive formation of characteristic white mycelium and synthesis of hydrolytic enzymes were occurred. Maximim values of the amylolytic enzymatic activity were noted for WB and SBM media. The difference was not statistically significant for both substrates. The levels of amylolytic and proteolytic enzymatic activity during the cultivation of micromycete on wheat bran media were 105 U g^{-1} and 64 U g^{-1}, respectively. The proteolytic activity decreased to 12.0–29.5 U g^{-1} using SBM, DDGS, BSG as nutrient medium (Table 2).

The enzymatic activity confirms the biosynthetic ability of the micromycete *A. oryzae* RCAM 01133 grown on food by-products. Despite the fact that the target cultivation products were protein and polysaccharides, Shi et al. (2015) noted that synthesis of multiple enzymes can be useful to eliminate the antinutritional components and improve the protein quality using the final product as feed additive.

Table 2. Synthesis of α-amylase and protease by the fungus *Aspergillus oryzae* RCAM 01133 on various media during solid-state cultivation

Nutrient medium	Enzymatic activity, U/g	
	Amylolytic (AA)	Proteolytic (PA)
WB	105.0 ± 5.2[a]	64.0 ± 3.2[a]
SBM	110.3 ± 5.5[a]	29.5 ± 1.5[b]
DDGS	63.1 ± 3.1[b]	12.0 ± 0.6[c]
BSG	43.1 ± 2.2[c]	15.0 ± 0.8[c]

Values of enzymatic activity with the same letters in each column are not significantly different from each other at $\alpha = 0.05$.

The formation of spores was observed during fermentation only on BSG medium. It was noted the formation of yellow-green spores on the 2nd day of the fermentation.

The content of protein and polysaccharides in the surface culture of a fungus grown within 2 days was studied. It has been established that the highest accumulation of protein has been achieved in SBM and DDGS medium (Fig. 1). The level of total protein content in comparison with the initial medium increased in SBM and DDGS media from 56.6% to 82.0% and from 50.7% to 73.4%, respectively. In the study of Hong et al. (2004), fermented soybeans and fermented soybean meals contained 10% more crude protein than initial substrates as a result of 48 hours fermentation with by *Aspergillus oryzae* GB-107. Chen et al. (2013) showed increase of protein content from 50.47 to 58.93% after 36 h of fermentation of soy meal via *A. oryzae* 12892 from American Type Culture Collection. A lower protein gain in these studies could be associated with a shorter period of fermentation.

Lio & Wang (2012) used DDGS as substrates to evaluate the effect of coculturing three different fungi, *Aspergillus oryzae*, *Trichoderma reesei*, and *Phanerochaete chrysosporium*, on enzyme production by SSF. These products also had 3.5–15.1% lower fiber and 1.3–4.2% higher protein contents. Authors suggested a potential feed quality improvement. Since the purpose of their experiment was to study the synthesis of enzymes, it is not entirely correct to compare the increase in protein content with this study. Nevertheless, a general tendency toward an increase in protein content can be noted.

The maximum percentage increase in protein content, which amounted to 115.2%, was noted during fermentation using BSG. The study of Bekatorou et al. (2007) revealed that BSG slurries treated directly using *A. oryzae and A. awamori* at various conditions could increased their protein content by 20–36%. Long-term solid state fermentation of BSG using *Aspergillus oryzae* increased significantly in the percentage protein from 18.22% in the unfermented to 28.33% in the fermented BSG (Ogunjobi et al., 2011).

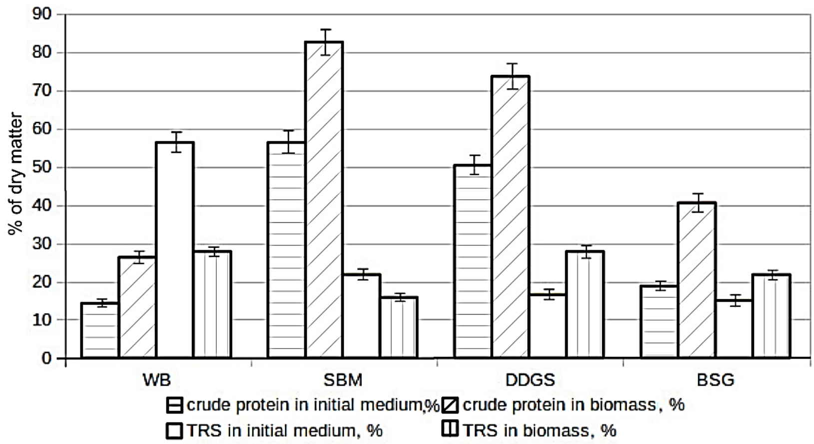


Figure 1. Crude protein and total reducing sugars content in the biomass of fungus *Aspergillus oryzae* RCAM 01133.

Wheat bran as a substrate for cultivation of the micromycete *A. oryzae* RCAM 01133 also showed high protein gain from 14.7% to 26.8%. These results correlated well with the results of Ravinder et al. (2003), who studied the production of single cell proteins from deoiled rice bran by *Aspergillus oryzae* mutants. *A. oryzae* MTCC 1846 and Shan2 mutant increased total nitrogen content from 9.2% to 16.4% and to 28.1% after 3 days fermentation, respectively. Also the protein increase of deoiled rice bran from initial 9.20% to 24.80% was achieved by Rudravaram et al (2006) growing *A. oryzae* MTCC 1846 with optimum growth process parameters such as moisture, pH of the substrate, inoculum size, temperature and nitrogen source. Fermentation of wheat bran with *Candida utilis* and *Rhizopus oligosporus* with optimum growth parameters resulted in a maximum crude protein yield of 41.02% compared with the 4.21% crude protein of the non-fermented wheat bran (Yunus, F.-U et al., 2015).

It should be noted that the protein content in the surface culture of the fungus is more than 3 times higher than in fungus grown by submerged fermentation with *Aspergillus oryzae* RCAM 01133 carried out by Serba et al. (2016).

Analysis of the amino acid composition of the obtained microbial biomass samples with a high protein concentration showed statistically significant increase in the content of all amino acids in comparison with their initial content in medium (Table 3).

Amino acid content in biomass grown on SBM media increased by 1.46 times and amounted to 350.6 mg g^{-1} in comparison with 239.6 mg g^{-1} in initial medium. The maximum increase in mass was observed for isoleucine (from 13.20 to 20.70 mg g^{-1}), leucine (from 21.80 to 36.10 mg g^{-1}), tryptophan (from 3.10 to 18.90 mg g^{-1}), and glutamic acid (from 51.80 to 61.10 mg g^{-1}). In percentage terms, this increase amounted to 56.2%, 65.6%, 509%, and 17.9%, respectively. Histedine showed minimal increase from 7.90 to 10.20 mg g^{-1}. The experimental data correlated with the results of other researchers, although they have a higher degree of increase. Solid-state fermentation of soy meal via *Aspergillus oryzae* by Chen et al. (2013) showed increase of total amino acid content by 13.13%. The contents of methionine, cysteine, threonine, and tryptophan increased by 11.11, 28.57, 18.65, and 6.76%, respectively. Available lysine and valine content increased by 6.21% and 12%.

Table 3. Amino acid composition of initial media and fungal biomass of *Aspergillus oryzae* RCAM 01133 during SSF

Amino acid	Content, mg g^{-1}			
	on SBM		on DDGS	
	in culture medium	in biomass	in culture medium	in biomass
Aspartic acid	31.20 ± 0.96	38.40 ± 1.02	13.60 ± 0.48	20.60 ± 0.41
Serine	0.70 ± 0.03	5.10 ± 0.26	11.40 ± 0.27	15.70 ± 0.29
Threonine	10.30 ± 0.29	14.50 ± 0.52	8.20 ± 0.21	12.10 ± 0.28
Glutamic acid	51.80 ± 1.27	61.10 ± 2.84	34.00 ± 1.03	44.10 ± 1.21
Proline	13.60 ± 0.35	19.80 ± 0.64	27.10 ± 1.16	33.30 ± 1.18
Glycine	11.30 ± 0.31	14.60 ± 0.38	6.10 ± 0.11	9.40 ± 0.17
Alanine	11.80 ± 0.21	18.40 ± 0.74	16.90 ± 0.35	23.50 ± 1.02
Valine	12.70 ± 0.51	16.50 ± 0.53	7.70 ± 0.19	11.50 ± 0.38
Methionine	3.40 ± 0.05	7.90 ± 0.18	2.70 ± 0.06	6.10 ± 0.11
Isoleucine	13.20 ± 0.32	20.70 ± 0.78	6.20 ± 0.17	13.70 ± 0.29
Leucine	21.80 ± 0.75	36.10 ± 1.21	12.40 ± 0.28	26.70 ± 0.57
Tyrosine	10.30 ± 0.26	14.50±0.38	5.60 ± 0.14	9.80 ± 0.19
Phenylalanine	0.80 ± 0.02	7.30 ± 0.27	7.00 ± 0.14	13.30 ± 0.27
Histidine	7.90 ± 0.21	10.20 ± 0.31	4.50 ± 0.13	6.80 ± 0.14
Lysine	15.30 ± 0.32	21.80 ± 0.78	4.40 ± 0.19	9.50 ± 0.28
Tryptophan	3.10 ± 1.24	18.90 ± 0.45	18.30 ± 0.41	38.60 ± 1.33
Arginine	20.40 ± 0.98	24.80 ± 0.74	7.10 ± 0.14	10.80 ± 0.21
Total number	239.60 ± 6.51	350.6 ± 8.53	193.20 ± 4.05	295.50 ± 6.78
Crude protein, % of *absolute dry substance*	56.60 ± 1.24	82.00 ± 2.10	50.70 ± 1.24	73.40 ± 2.67

All changes in amino acid content in culture medium and in biomass are statistically significant for each amino acid and substrate at $\alpha = 0.05$.

Hong et al. (2004) reported increase of glycine, glutamic acid, and aspartic acid content and the lack of significant changes of any essential amino acids content as result of soybean meals fermentation with *A. oryzae* GB-107.

Ravinder et al (2003) showed significant increase of lysine, threonine, cysteine, and tryptophan content for both *A. oryzae* MTCC 1846 and Shan 2 mutant. after fermentation of deoiled rice bran.

Leucine, glutamic acid, and tryptophan were amino acids with maximim increase in content after fermentation of *A. oryzae* RCAM 01133 on DDGS medium. Minimal rise was observed as well as for SBM medium for histidine.

The highest content of polysaccharides was noted during *Aspergillus oryzae* RCAM 01133 fermentation on WB and DDGS medium, it was 27.9% and 28.0%, respectively. These polysaccharides containing a chitin-glucan-mannan complex can be used can be used in technologies of manufacturing bioproducts and additives with predominant content of dietary fiber.

Further, the ability of fungus strain *A. oryzae* RCAM 01133 to synthesize protein and polysaccharides during solid-state fermentation on combined mediums containing mixtures of SBM, WB, DDGS and BSG according to Table 1 was investigated. A comparison of protein and polysaccharide levels in fungus biomass after 3 days of solid state fermentation on combined mediums is presented in Fig. 2.

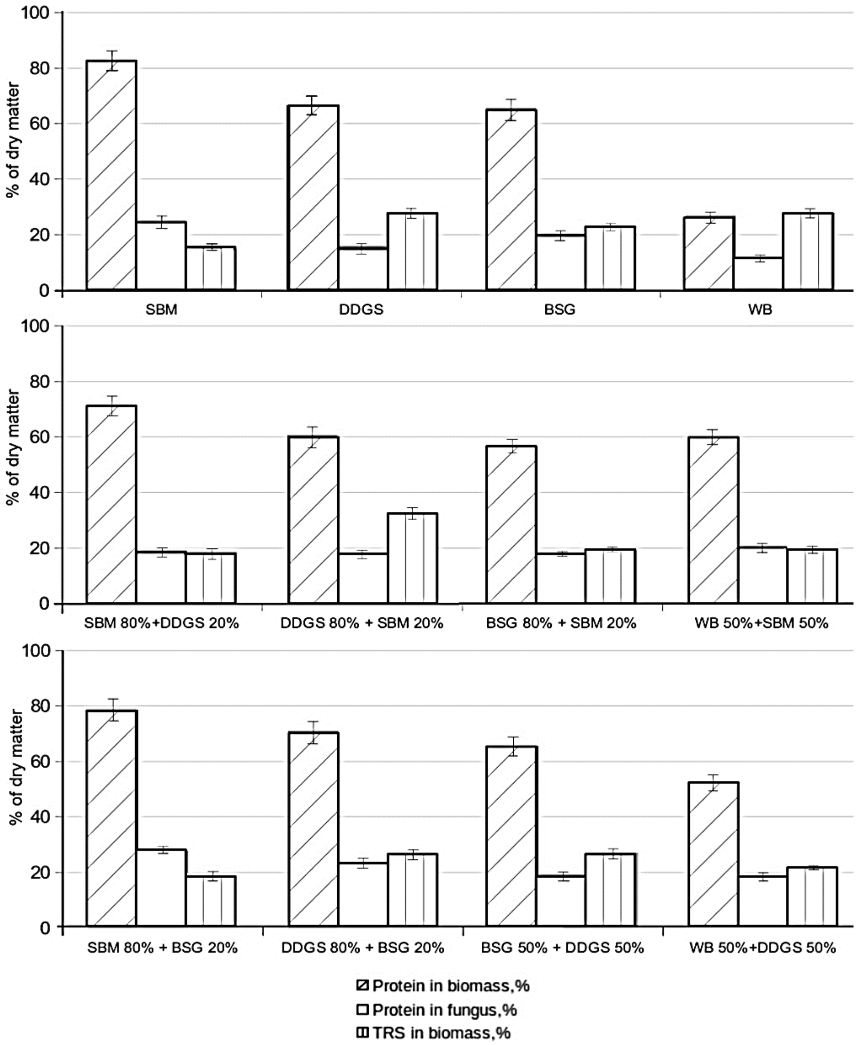


Figure 2. Content of protein and reducing polisaccharides in *A. oryzae* RCAM 01133 fungus biomass after 3 days solid state fermentation.

The results of experimental studies of solid-state fermentation of micromycete made it possible to select culture media that provide the highest level of synthesis of biopolymers in microbial biomass: culture medium No. 4 – 100% wheat bran; culture medium No. 2 – 100% soybean meal; culture medium No. 6 – 20% SBM and 80% DDGS; culture medium No. 5 – 80% SBM and 20% DDGS; culture medium No. 7 – 20% SBM and 80% BSG; culture medium No. 9 – 80% SBM and 20% BSG.

Medium samples No. 4 and No. 6 provided the highest accumulation of polysaccharides (27.9% and 32.9%, respectively) by fungus *A. oryzae* RCAM 01133 (Fig. 3, a) and can be used to produce bio-products with adsorbing properties.

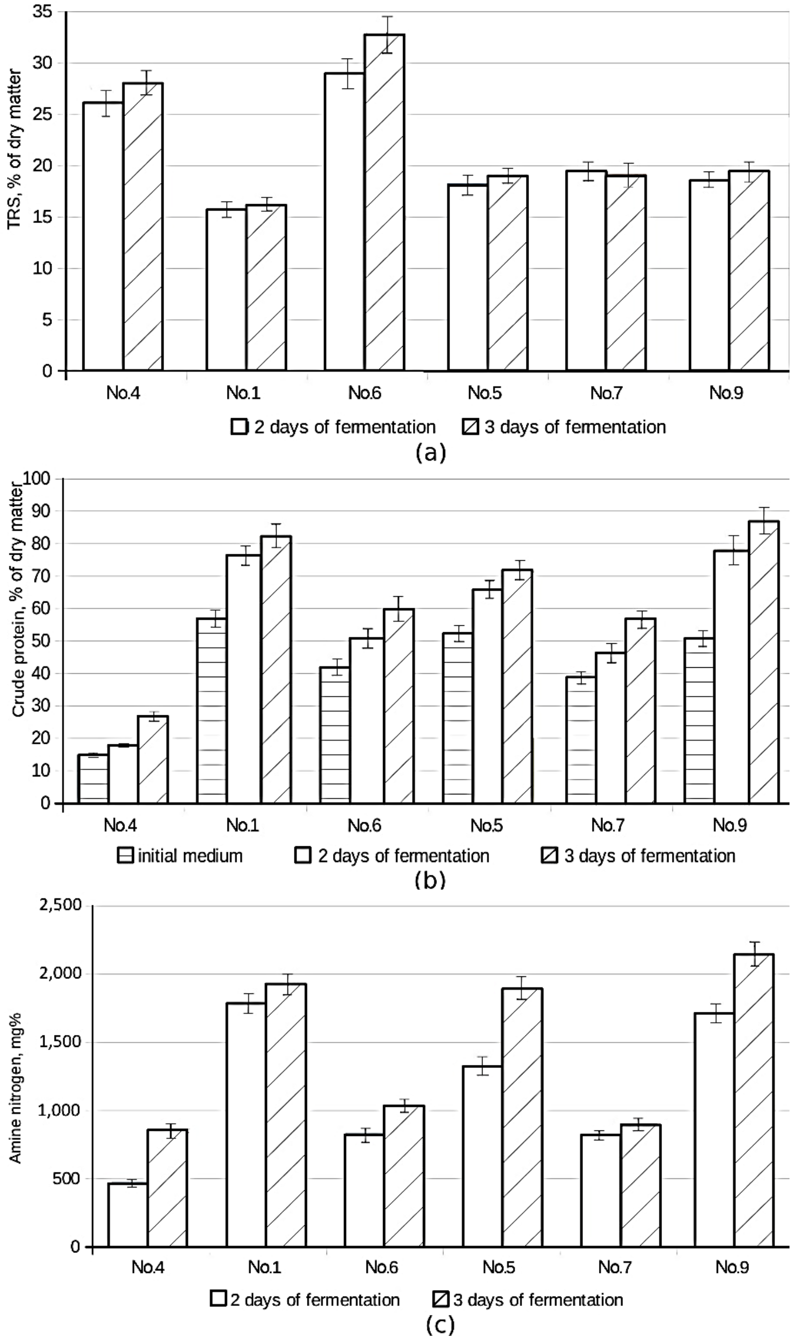


Figure 3. Accumulation of polysaccharides (a), protein (b) and amine nitrogen (c) in the surface culture of the fungus *A. oryzae* RCAM 01133 on selected culture mediums according to Table 1.

Predominant accumulation of protein was observed in culture media No. 1 and No. 9. These medium has a potential to be used in manufacture protein and amino acids food enrichers and feed additives (Figs 3, a and 3, b). A high level of amine nitrogen accumulation indicated an increased synthesis of proteolytic enzymes by fungus *A. oryzae* on these media, especially on the 3rd day of cultivation (Fig. 3, c).

CONCLUSIONS

The obtained experimental data confirmed the possibility of obtaining bio-preparations with the high content of protein substances and polysaccharides based on solid-state fermentation of the fungus *Aspergillus oryzae* on growth medium containing by-products of food industry. Maximum It was found that the protein content in the surface culture of the fungus was 73.4% and 82.0%, which is more than 3 times higher than in fungus grown by submerged fermentation in research of Serba et al. (2016).

The research results of the biosynthetic ability of the *A. oryzae* RCAM 01133 strain confirmed the high biological value of microbial biomass grown on food wastes. Fermentation of micromycete on culture media containing soybean meal and distilled dry grains with solubles provided an increase in the level of protein by 1.45 times. Maximum increase of amino acids for SBM and DDGS media was observed for isoleucine, leucine, tryptophan, and glutamic acid. Higher level of polysaccharides chitin-glucan-mannan complex by *A. oryzae* fungus is achieved on culture media with WB and DDGS (27.9% and 32.9%, respectively), that can be used in technologies for obtaining bio-preparations with predominant content of dietary fiber.

Thus, the results of the study show that fermentation of *Aspergillus oryzae* RCAM 01133 on WB, SBM, DDGS, and BSG culture media increased protein and polisaccharides content. The fungus strain and substrates can be used in technologies for producing protein and amino acid additives and bioproducts as sources of valuable polysaccharides.

ACKNOWLEDGEMENTS. The research was carried out at the expense of the grant for the fulfillment of the state task within the framework of the Program of Fundamental Scientific Research of the State Academies of Sciences (topic No. 0529-2019-0066).

REFERENCES

Abdel-Gawad, K.M., Hifney, A.F., Fawzy, M.A. & Gomaa, M. 2017. Technology optimization of chitosan production from Aspergillus niger biomass and its functional activities. *Food Hydrocolloids* **63**, 593–601.

Bekatorou, A., Bountas, Y., Banat, I.M. & Kanellaki, M. 2007. Upgrading brewer's spent grains by treatment with Aspergillus species. *Chemical Industry and Chemical Engineering Quarterly* **13**(2), 72–78.

Cowan, S.T. & Steel, L.J. 1993. Manual for the identification of medical bacteria, 3rd edition. Edited and revised by G.I. Barrow and R.K.A. Feltham. Cambridge University Press. 211.

Chen, L., Madl, R.L. & Vadlani, P.V. 2013. Nutritional enhancement of soy meal via Aspergillus oryzae solid-state fermentation. *Cereal Chemistry* **90**, 529–534.

Feofilova, E.P. 2010. The cell wall of fungi: modern views on the structure and biological function. *Mikrobiologiya* **79**, 723–733 (in Russian).

Fuwa, H. 1954. A new method for microdetermination of amylase activity by the use of amylose as the substrate. *The Journal of Biochemistry* **41** (5), 583–603.

Gow, N.A.R., Latge, J.-P. & Munro, C.A. 2017. The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiol. Spectrum* **5**, 1–25.

Hong, K.J., Lee, C.H. & Kim, S.W. 2004. Aspergillus oryzae GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *Journal of Medicinal Food* **7**(4). 430–435.

Jin, B., Yu, Q., van Leeuwen, J.H. & Hung, Y.T. 2010 An integrated biotechnological process for fungal biomass protein production and wastewater reclamation. In: *Wang L., Tay, J.H., Tay, S., Hung YT. Environmental Bioengineering. Handbook of Environmental Engineering, vol 11. Humana Press, Totowa, NJ.*

Kumaresapillai, N., Basha, R.A. & Sathish, R. 2011. Production and evaluation of chitosan from Aspergillus niger MTCC strains. *Iranian journal of Pharmaceutical Research* **10(**3), 553–557.

Laufenberg, G., Kunz, B. & Nystroem, M. 2003. Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresource Technology* **87**, 167–198.

Lio, J. & Wang, T. 2012. Solid-state fermentation of soybean and corn processing coproducts for potential feed improvement. *Journal of Agricultural and Food Chemistry* **60**(31), 7702–7709.

Meyer, V., Andersen, M.R., Brakhage, A.A., Braus, G.H., Caddick, M.X., Cairns, T.C., De Vries, R.P., Haarmann, T., Hansen, K., Hertz-Fowler, C., Krappmann, S., Mortensen, U.H., Peñalva, M.A., Ram, A.F.J. & Head, R.M. 2016. Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. *Fungal Biology and Biotechnology* **3**, 6.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *The Journal of Biological Chemistry* **153**, 375–380.

Ogunjobi, A.A., Mejeha, O.K. & Fagade, O.E. 2011. Protein enrichment of brewery spent grains using Aspergillus oryzae. *AU Journal of Technology* **15** (1), 53–56

Polizeli, M.L., Rizzatti, A.C.S., Monti, R., Terenz, H.F., Jorge, J.A. & Amorim, D.S. 2005. Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology* **67**, 577–591.

Ravinder, R, Venkateshar, R.L., Ravinder, P. 2003. Studies on Aspergillus oryzae mutants for the production of single cell proteins from deoiled rice bran. *Food Technology and Biotechnology* **41**(3), 243–246.

Rudravaram, R., Chandel, A., Linga, V., Ravindra, P. 2006. Optimization of protein enrichment of deoiled rice bran by solid state fermentation using Aspergillus oryzae MTCC 1846. *International Journal of Food Engineering* **2**(4).

Serba, Ye.M., Rimareva, L.V., Overchenko, M.B., Sokolova, Ye., Pogorzhelskaya, N.S., Ignatova, N.I. & Borshcheva, Yu.A.. 2016. Filamentous fungi - promising source of hydrolasas and valuable polymers. *Vestnik of Russian agricultural science* **4**, 41–43 (in Russian).

Shi, C., He, J., Yu, J., Yu, B., Huang, Z., Mao, X., Zheng, P. & Chen, D. 2015. Solid state fermentation of rapeseed cake with Aspergillus niger for degrading glucosinolates and upgrading nutritional value. *Journal of Animal Science and Biotechnology* **6**, 13.

Soccol, C.R., Scopel, E., Alberto, L., Letti, J., Karp, S.G. & Woiciechowski, A.L. 2017. Recent developments and innovations in solid state fermentation. *Biotechnology Research and Innovation* **1**(1), 52–71.

Souza Filho, P.F., Nair, R.B., Andersson, D., Lennartsson, P.R. & Taherzadeh, M.J. 2018. Vegan-mycoprotein concentrate from pea-processing industry byproduct using edible filamentous fungi. *Fungal Biology and Biotechnology* **5**, 5.

Shin, H. Y., Kim, S. M., Lee, J.H. & Lim, S.T. 2018. Solid-state fermentation of black rice bran with Aspergillus awamori and Aspergillus oryzae: Effects on phenolic acid composition and antioxidant activity of bran extracts. *Food Chemistry* **272**, 235–241.

Ward, O.P., Qin, W.M., Dhanjoon, N.J., Ye, J. & Singh, A. 2006. Physiology and biotechnology of Aspergillus. *Advances in Applied Microbiology* **58**, 1–75.

Yunus, F.-U., Nadeem, M. & Rashid, F. 2015. Single-cell protein production through microbial conversion of lignocellulosic residue (wheat bran) for animal feed. *Journal of the Institute of Brewing* **121**, 553–557.

Zhang, Z.Y., Jin, B, Bai, Z.H. & Wang, X.Y. 2008. Production of fungal biomass protein using microfungi from winery wastewater treatment. *Bioresource Technology* **99**(9), 3871–3876.

Zhong, Y., Lu, X., Xing, L., Ho, S. & Kwan, H.S. 2018. Genomic and transcriptomic comparison of Aspergillus oryzae strains: a case study in soy sauce koji fermentation. *Journal of industrial microbiology & biotechnology* **45**(9), 839–853.

Zuo, S., Niu, D., Ning, T., Zheng, M., Jiang, D. & Xu, C. 2018. Protein enrichment of sweet potato beverage residues mixed with peanut shells by Aspergillus oryzae and Bacillus subtilis using central composite design. *Waste and Biomass Valorization* **9**, 835–844.

Agronomy Research **18**(1), 301–306, 2020
https://doi.org/10.15159/AR.20.019

Prebiotic properties of licorice root extracts

L. Tsirulnichenko[*] and Yu. Kretova

South Ural State University, Higher School of Medicine and Biology, Department of Food and Biotechology, 85 Lenina Avenue, RU454080 Chelyabinsk, Russia
[*]Correspondence: tcirulnichenkola@susu.ru

Abstract. The study objective is to investigate effect of licorice root extract on growth of probiotic microorganisms. As fructan percentage in licorice roots is 27.8–28.8%, this value is sufficient to enable the raw material suitability as a prebiotic ingredient. The licorice root extract (0.1%, 1% and 10% of medium volume) was added to media. The same media without added extract were considered as controls. Effect of the licorice root extract on growth of probiotic microorganisms was studied in 2 commercial probiotic drug Bifiform (Denmark) and Bifidobacterin forte (Russia). Licorice root extract provides probiotic bacteria an opportunity to tolerate acidity/alkalinity gradient of model media well and to decrease their count slower. In vitro studies showed, what 1% extract provides more favorable conditions for microorganisms that 10% one.

Key words: licorice root, fructans, functional food products, diabetes, intestinal microflora.

INTRODUCTION

It is well known that intestinal microflora indicates functional microorganism status. This microflora produce great number of enzymes (e.g., proteases, lipases, amylases, cellulases, etc.) involved in protein, lipid, carbohydrate, bile acid and cholesterol metabolism, as well as water-electrolyte metabolism. Also, it provides absorption of calcium, iron and vitamin D. So, it plays a key role in consumed food-based power production (Bâckhed et al., 2005).

It should be noted that human intestinal microflora is very unstable system liable to variations. It depends on number of factors such as endogenous (neurological status, food, body temperature, etc.) and exogenous (season, atmospheric oscillations, etc.) ones.

Gastrointestinal diseases have been an increasing global health problem in recent years (Faghfoori et al., 2015). These diseases act directly on the destruction or decline of beneficial gut microbiota as a consequence of physiological and metabolic disturbances induced by a stressful lifestyle, diet modifications, antibiotic consumption and age-related events, i.e., reduction of functionality of the immune system (Biagi et al., 2012). Diets based on prebiotics have been increasingly accepted for improving intestinal health (Gionchetti et al., 2005; Boirivant & Strober, 2007; Hatakka & Saxelin, 2008; Lutgendorf et al., 2008).

Fructans represents a category of natural prebiotic compounds that includes fructose polymers synthesized from sucrose and fructose molecules (Banguela & Hernández, 2006). linked by fructose-fructose glycosidic β-(2 → 1) and β- (2 → 6) bonds and having one terminal glucose unit. Due to the structure and type of linkage-β, fructans are not metabolized by host enzymes in the upper gastrointestinal tract, reaching the lower tract where they become available for the resident microbiota to use as substrates. Therefore, fructans stimulate the proliferation of beneficial bacteria, mainly *Lactobacillus* and *Bifidobacterium*, associated with health-promoting effects and the production of short-chain fatty acids (SCFAs), mainly acetic, propionic, and butyric acids, whose increase antagonizes the growth of some pathogenic bacterial strains and favors mucin production in the colon (Andrade et al., 2019). Licorice root can be one of advantageous sources of probiotic substances, including fructans (Banguela & Hernández, 2006).

Three polysaccharides (i.e., glycyrrhizans UA, UB and UC) were extracted from *Glycyrrhizae uralensis* root, and several polysaccharides (glycyrrhizans GU and GA) were derived from a stolon (i.e., an elongated side shoot) of *Glycyrrhizae glabra var glandulifera*. Structure of glycyrrhizan GA is based on β-1,3-bound galactose residues. α-arabino-β-3,6-galactan (L-arabinose, D-galactose, L-rhamnose, D-galacturonic and D-glucuronic acids – 22:10:1:2:1) is a main structural unit of the glycyrrhizan. Glycyrrhizan UA consists of L-arabinose, D-galacturonic acid, D-galactose and L-rhamnose (molar ratio – 20:3:1:14). β-1,3-bound galactose whose residues contain α-1,5-bound L-arabinose (position 6) in a side chain is a structural unit of glycyrrhizan UA. The glycyrrhizan consists of L-arabinose, L-rhamnose, D-galactose and D-glucose (molar ratio – 12:20:1:10:10). Also, it contains few O-acetyl groups and approximately 10% and 35% of glycyrrhizan UA and UB residues, respectively, peptide residues and D-galacturonic acid as methyl ethers. Glycyrrhizan UC (i.e., a neutral polysaccharide) consists of L-arabinose, D-galactose, L-rhamnose and D-glucose (molar ratio – 10:30:1:27). It may be determined as arabino-3,6-galacto-glucan. Galactose, xylose, arabinose, glucose and mannose were found in polysaccharide hydrolisates. According to several studies presented herein, about 15% of mono- and oligosaccharides were extracted from licorice roots with 82% ethanol. These compounds contained glucose, galactose, mannose, sucrose and glucofructan (Denisova, 2000).

Shen et al. (2015) derived and described a water-soluble polysaccharide GIP1 from *Inflata* licorice roots. Mocanu et al. (2009) obtained a novel probiotic product named ROSALACT®, prepared from pasteurized milk with rosehip and licorice extractusing a mixed culture of probiotic bacteria. *L. plantarum*, was found to be capable of rapidly utilizing liquorice root extract for probiotic cell cultivation. From the results of this study, it can be concluded that the liquorice root extract could be used as a non-dairy raw material for probiotic lactic acid bacteria (Mousavi, Z.E. & Mousavi, M., 2019). In terms of abovementioned data, we can consider the plant as a potential source of substances stimulating growth of probiotic microorganisms.

The study objective is to investigate effect of licorice root extract on growth of probiotic microorganisms.

MATERIALS AND METHODS

Licorice root extraction

At the first stage, extracts were obtained from four samples of licorice root produced by various pharmaceutical companies in Russia (Sample 1–4).

To remove fructose, sucrose and other oligosaccharides in the first stage, extraction was carried out with a 95% solution of ethyl alcohol. Then, water extraction was repeated under the same conditions (80 °C, 150 minutes).

The presence of fructosans in solution was determined using acid hydrolysis by spectrophotometric method. In an acidic environment, these compounds are capable of forming the product of ¾ 5-hydroxymethylfurfural in the wavelength range from 280 to 380 nm. In literary sources, the greatest formation of this compound is noted at wavelengths of 283 and 285 nm (Ananyina, 2008).

The quantitative determination of the content of fructans (X) in terms of fructose in% was calculated by the formula 1.

$$X = \frac{D \cdot 100 \cdot 25}{298 \cdot m \cdot 1} \quad (1)$$

where X – the content of fructosans, %; D – the optical density of the test solution; 298 – specific absorption rate of the fructose transformation product after acid hydrolysis; m – the mass of the plant sample in the studied extract.

The licorice root extract (0.1%, 1% and 10% of medium volume) was added to model media. The same media without added extract were considered as controls.

Microorganism growth study

Effect of the licorice root extract on growth of probiotic microorganisms was studied in 2 commercial probiotic drugs described in the Table 1.

Table 1. Description of probiotic drugs

S/N	Drug name, country of origin	Microorganism species composition	Bacterial count
1	Bifiform, Denmark	*B. longum*, *E. faecium*	$1 \cdot 10^7$ bacteria/dose
2	Bifidobacterin forte, Russia	*B. bifidum*	$5 \cdot 10^7$ bacteria/dose

Probiotic microorganisms were cultivated in solid media with recommended composition (Likhacheva et al., 1992; Darmov et al., 2011). During the microaerophilic cultivation we applied Anaerobic system Mark III – LE003 (Hi Media Laboratories Pvt. Ltd, Mumbai, India) and Hi Anaero Gas Pacet gas-producing bags. Probiotic microorganism survival rate was tested *in vitro* simulating human digestive conditions.

Model media were prepared on basis of citrate phosphate buffer and enzyme drugs (Acidin-pepsin, Panzinorm forte 20000). After interaction with enzyme drugs along with incubation of model media and bacterial suspensions living microorganism count in probiotic drugs and suspensions was evaluated by inoculation of appropriate 10-fold serial dilutions of test drugs and suspensions in solid media in Petri dishes and colony counting after incubation.

The main point of these tests was to incubate microorganisms of investigated probiotic drugs and licorice root extract in acidic model medium with Acidin-pepsin (pH = 2.3) and alkaline model medium with Panzinorm forte 20000 (pH = 7.2) successively during mean time of mixed meal presence in stomach and intestine, respectively. Then, we counted survived microorganisms as compared with their initial number. Viable probiotic microorganism count per a drug dose (CFU mL^{-1}) was evaluated initially (0 h), in 4 hours after incubation in acidic model medium with Acidin-pepsin (0.5 mg mL^{-1}) and in 12 hours after incubation in alkaline model medium with Panzinorm forte 20000 (2.5 mg mL^{-1}) (Darmov et al., 2011).

Statistical analysis

All analyses were carried out in triplicate. The results were presented as means ± standard deviation of three replicates of independent experiments. Statistical analysis was performed using the SPSS 17.0, a value of $p < 0.05$ was considered statistically significant.

RESULTS

Oligosaccharides (i.e., fructans and raffinose) are the most important water-soluble prebiotic carbohydrates. See fructans level in licorice root extracts produced by various manufacturers in the Fig. 1.

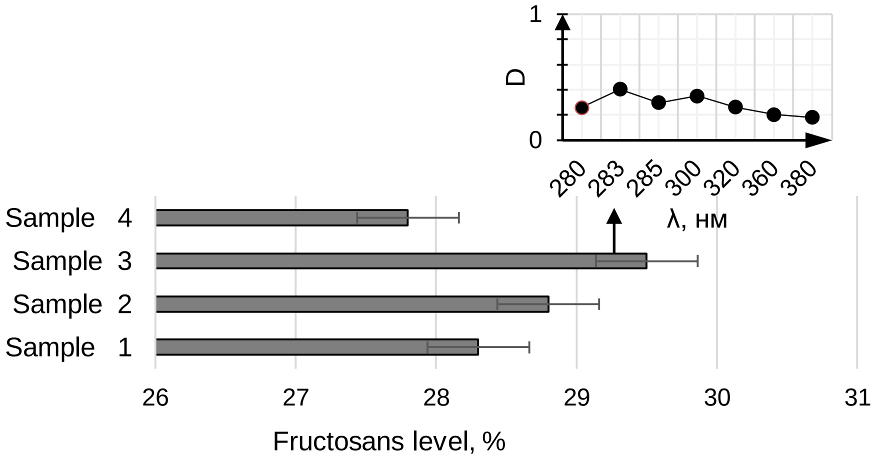


Figure 1. Fructans level in licorice root extracts (equivalent to fructose. Statistically significant differences ($p < 0.05$) between test and reference samples by the Kruskal-Wallis criteria.

As fructan percentage in licorice roots is 27.8–28.8%, this value is sufficient to enable the raw material suitability as a prebiotic ingredient (Isbrucker & Burdock, 2006). On average, the level of fructans in different plants varies from 5 to 30% (Judprasong et al., 2011).

To assess licorice root extract effect on growth of probiotic microorganisms, we simulated human gastrointestinal medium at the next stage of the study.

See results related to the study of licorice root extract effect on probiotic microorganism growth in the Table 2.

The Table 2 demonstrates that all the probiotic microorganisms are exposed to negative effect of factors related to model media simulating human gastrointestinal factors. Bacterial count decreases both in acidic model medium with Acidin-pepsin (4 h) and in alkaline model medium with Panzinorm forte 20000 (i.e., an enzyme drug).

Table 2. Effect of licorice root extract on probiotic microorganism growth

Licorice extract level in medium, %	Bacterial count in a sample during the testin ... hours, CFU mL^{-1} ($X \pm I_{95}$)				
	Bifiform			Bifidobacterin	
	0	4	12	4	12
0.1	$(1.0 \pm 0.1)\cdot 10^7$	$(3.0 \pm 0.2)\cdot 10^4$	$(1.5 \pm 0.2)\cdot 10^3$	$(9.0 \pm 0.5)\cdot 10^5$	$(1.5 \pm 0.2)\cdot 10^3$
1.0	$(1.0 \pm 0.1)\cdot 10^7$	$(1.5 \pm 0.3)\cdot 10^5$	$(5.0 \pm 0.4)\cdot 10^4$	$(6.0 \pm 0.5)\cdot 10^5$	$(2.0 \pm 0.4)\cdot 10^4$
10	$(1.0 \pm 0.1)\cdot 10^7$	$(1.0 \pm 0.4)\cdot 10^5$	$(7.0 \pm 0.2)\cdot 10^3$	$(2.2 \pm 0.4)\cdot 10^5$	$(3.9 \pm 0.4)\cdot 10^3$
control	$(1.0 \pm 0.1)\cdot 10^7$	$(3.0 \pm 0.2)\cdot 10^4$	$(1.5 \pm 0.2)\cdot 10^3$	$(9.0 \pm 0.5)\cdot 10^4$	$(1.5 \pm 0.2)\cdot 10^3$

Licorice root extract provides probiotic bacteria an opportunity to tolerate acidity/alkalinity gradient of model media well and to decrease their count slower. B. Bifidum cells were more sensitive to licorice extracts than B. Longum and E. Faecium cells. This may be due to the differences in growth conditions and adaptation of the strain to the new environment (Mousavi & Mousavi, 2019). The viability of B. Bifidum cells after 4 hours (pH-2,3) is an order of magnitude higher compared to the control sample, even with a minimum concentration of the added extract (0.1%). According to the literature, the optimum condition for growth of Bifidobacterium is at pH 6.5–7.0 (Cronin et al., 2011). This tendency to maintain high viability for bifidobacteria cells is preserved in an alkaline medium after 12 hours.

Along with this, 1% extract provides more favorable conditions for microorganisms that 10% one. The effect can be attributed to glycerritic acid (i.e., a licorice root ingredient) that can inhibit growth of both gram-positive and gram-negative microorganisms (Astafieva, 2013).

CONCLUSION

Nowadays pre- and probiotics are common tools to manage gastrointestinal diseases, immune disorders and allergic diseases.

Licorice root can be one of advantageous sources of probiotic substances, including fructans. Licorice root extract has a sufficient fructan level to provide good resistance of probiotic bacteria to acidity/alkalinity gradient.

In general, 1% licorice root extract can be considered the minimum effective dose in which the higher growth of probiotic microorganism is stimulated. In addition, our results corroborate the prebiotic concept that suggests that fructolygosaccharides stimulate the growth of one or a limited number of bacteria in the colon, improving the health of the host.

ACKNOWLEDGEMENTS. This article was created on a grant for financial support of the basis part of the government task under the contract №19.8259.2017/BCh.

REFERENCES

Andrade, A.I.C., Bautista, C.R., Cabrera, M.A.R., Guerra, S.R.E., Chávez, G.E., Ahumada, F.C. & Lagunes, G.A. 2019. Agave salmiana fructans as gut health promoters: Prebiotic activity andinflammatory response in Wistar healthy rats. *International Journal of Biological Macromolecules* **136**, 785–795.

Ananyina, N.A. 2008. Polysaccharides tubers dahlias simple. *Khimiya rastitel'nogo syr'ya* **2**, 135–136 (in Russian).
Astafieva, O.V. 2013. *Use of Glycyrrhiza glabra L., Achillea micrantha willd. and Helichrysum arenarium L. for the development of biologics with antibacterial properties*. Dissertation, Stavropol Research Anti-Plague Institute, Astrakhan, Russia, 193 pp.
Báckhed, F., Ley, R.E. & Sonnenburg, J.L. 2005. Host-bacterial mutualism in the human intestine. *Science* **307**, 1915–1920.
Banguela, A. & Hernández L. 2006. Fructans: from natural sources to transgenic plants. *Biotecnol. Apli.* **23**, 202–210.
Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi, C. & Brigid, P. 2012. Ageing of the human metaorganism: the microbial counterpart, *Age* **34**, 247–267.
Boirivant, M. & Strober W. 2007. The mechanism of action of probiotics. *Curr Opin Gastroentero* **23**, 679–692.
Cronin, M., Ventura, M., Fitzgerald, G.F. & van Sinderen, D.2011. Progress in Genomics. *Metabolism and Biotechnology of Bifidobacteria* **149**, 4–18.
Darmov, I.V., Chicherin I.Yu., Erdyakova A.S., Pogorelskii I.P. & Lundovskih I.A. 2011. Comparative assessment of the survival of probiotic microorganisms in commercial preparations in vitro. *Ekspertiza i klinicheskaya gastroenterologiya* **9**, 96–101 (in Russian).
Denisova, S.B. 2000. *Liquid-solid phase extraction of the main classes of biologically active substances of licorice root*. PhD. Institute of Organic Chemistry, Ufa Federal Research Center, Russian Academy of Sciences, Ufa, Russia, 166 pp.
Faghfoori, Z,. Bahram Pourghassem Gargari, B.P. & Gharamaleki, A.S. 2015. Cellular and molecular mechanisms of probiotics effects on colorectal cancer. J. Funct. Food 18, 463–472.
Gionchetti, P, Lammers K.M., Rizzello F. & Campieri, M. 2005. Probiotics and barrier function in colitis. *Gut* **54**, 898–900.
Hatakka, K. & Saxelin, M. 2008. Probiotics in intestinal and non-intestinal infectious diseases-clinical evidence. *Curr. Pharm. Des.* **14**, 1351–1367.
Isbrucker, R. & Burdock, G. 2006. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regul. Toxicol. Pharmacol*. **46**, 167–192.
Judprasong, K., Tanjor, S. & Puwastien, P. 2011. Investigation of Thai plants for potential sources of inulin-type fructans. Journal of Food Composition and Analysis 24(4–5), 642–649.
Likhacheva, A.Yu., Bondarenko, V.M. & Sokolova, K.Ya. 1992. Current status of the taxonomy of bacteria of the genus Lactobacillus. *Zhurnal mikrobiologia* **9**(10), 74–78 (in Russian).
Lutgendorff, F, Akkermans, L.M. & Soderholm, J.D. 2008. The role of rnicrobiota and probiotics in stress-induced gastroinstetinal dam-age. *Curr. Mol. Med.* **8**, 282–298.
Mocanu, G.D., Rotaru, G. & Botez, E. 2009. Sensory evaluation and rheological behavior of probiotic dairy products with Rosa canina L. and Glycyrriza glabra L. extracts. *Innov. Rom. Food Biotechnol*. **4**, 32–39.
Mousavi, Z.E. & Mousavi, M. 2019. The effect of fermentation by Lactobacillus plantarum on the physicochemical and functional properties of liquorice root extract.*LWT - Food Science and Technology* **105**, 164–168.
Shen, H., Zeng G., Sun, B. & Cai, X. 2015. A polysaccharide from Glycyrhiza inflate Licorice inhibits proliferation of human oral cancer cells by inducing apoptosis via mitochondrial pathway. *Tumor. Biol*. **36**, 4825–4831.

INSTRUCTIONS TO AUTHORS

Papers must be in English (British spelling). English will be revised by a proofreader, but authors are strongly urged to have their manuscripts reviewed linguistically prior to submission. Contributions should be sent electronically. Papers are considered by referees before acceptance. The manuscript should follow the instructions below.

Structure: Title, Authors (initials & surname; an asterisk indicates the corresponding author), Authors' affiliation with postal address (each on a separate line) and e-mail of the corresponding author, Abstract (up to 250 words), Key words (not repeating words in the title), Introduction, Materials and methods, Results and discussion, Conclusions, Acknowledgements (optional), References.

Layout, page size and font

- Use preferably the latest version of **Microsoft Word**, doc., docx. format.
- Set page size to **ISO B5 (17.6 × 25 cm)**, all **margins at 2 cm**. All text, tables, and figures must fit within the text margins.
- Use single line spacing and **justify the text**. Do not use page numbering. Use **indent 0.8 cm** (do not use tab or spaces instead).
- Use font Times New Roman, point size for the title of article **14 (Bold)**, author's names 12, core text 11; Abstract, Key words, Acknowledgements, References, tables, and figure captions 10.
- Use *italics* for Latin biological names, mathematical variables and statistical terms.
- Use single ('…') instead of double quotation marks ("…").

Tables

- All tables must be referred to in the text (Table 1; Tables 1, 3; Tables 2–3).
- Use font Times New Roman, regular, 10 pt. Insert tables by Word's 'Insert' menu.
- Do not use vertical lines as dividers; only horizontal lines (1/2 pt) are allowed. Primary column and row headings should start with an initial capital.

Figures

- All figures must be referred to in the text (Fig. 1; Fig. 1 A; Figs 1, 3; Figs 1–3). Use only black and white or greyscale for figures. Avoid 3D charts, background shading, gridlines and excessive symbols. Use font **Arial, 10 pt** within the figures. Make sure that thickness of the lines is greater than 0.3 pt.
- Do not put caption in the frame of the figure.
- The preferred graphic format is Excel object; for diagrams and charts EPS; for half-tones please use TIFF. MS Office files are also acceptable. Please include these files in your submission.
- Check and double-check spelling in figures and graphs. Proof-readers may not be able to change mistakes in a different program.

References

- **Within the text**

In case of two authors, use '&', if more than two authors, provide first author 'et al.':

Smith & Jones (1996); (Smith & Jones, 1996);

Brown et al. (1997); (Brown et al., 1997)

When referring to more than one publication, arrange them by following keys: 1. year of publication (ascending), 2. alphabetical order for the same year of publication:

(Smith & Jones, 1996; Brown et al., 1997; Adams, 1998; Smith, 1998)

- **For whole books**

Name(s) and initials of the author(s). Year of publication. *Title of the book* (*in italics*). Publisher, place of publication, number of pages.

Shiyatov, S.G. 1986. *Dendrochronology of the upper timberline in the Urals*. Nauka, Moscow, 350 pp. (in Russian).

- **For articles in a journal**

Name(s) and initials of the author(s). Year of publication. Title of the article. *Abbreviated journal title* (*in italic*) volume (in bold), page numbers.

Titles of papers published in languages other than English, should be replaced by an English translation, with an explanatory note at the end, e.g., (in Russian, English abstr.).

Karube, I. & Tamiyra, M.Y. 1987. Biosensors for environmental control. *Pure Appl. Chem.* **59**, 545–554.

Frey, R. 1958. Zur Kenntnis der Diptera brachycera p.p. der Kapverdischen Inseln. *Commentat.Biol.* **18**(4), 1–61.

Danielyan, S.G. & Nabaldiyan, K.M. 1971. The causal agents of meloids in bees. *Veterinariya* **8**, 64–65 (in Russian).

- **For articles in collections:**

Name(s) and initials of the author(s). Year of publication. Title of the article. Name(s) and initials of the editor(s) (preceded by In:) *Title of the collection (in italics)*, publisher, place of publication, page numbers.

Yurtsev, B.A., Tolmachev, A.I. & Rebristaya, O.V. 1978. The floristic delimitation and subdivisions of the Arctic. In: Yurtsev, B. A. (ed.) *The Arctic Floristic Region*. Nauka, Leningrad, pp. 9–104 (in Russian).

- **For conference proceedings:**

Name(s) and initials of the author(s). Year of publication. Name(s) and initials of the editor(s) (preceded by In:) *Proceedings name (in italics)*, publisher, place of publishing, page numbers.

Ritchie, M.E. & Olff, H. 1999. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olff, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38th Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

Please note

- Use ‘.’ (not ‘,’) for decimal point: 0.6 ± 0.2; Use ‘,’ for thousands – 1,230.4;
- Use ‘–’ (not ‘**-**’) and without space: pp. 27–36, 1998–2000, 4–6 min, 3–5 kg
- With spaces: 5 h, 5 kg, 5 m, 5 °C, C : D = 0.6 ± 0.2; $p < 0.001$
- Without space: 55°, 5% (not 55 °, 5 %)
- Use ‘kg ha^{-1}’ (not ‘kg/ha’);
- Use degree sign ‘ ° ’ : 5 °C (not 5 ^{O}C).