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Investigating *Calotropis Procera* natural dye extracts and PEDOT:PSS hole transport material for dye-sensitized solar cells

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Abstract. In this work, natural dye extracts from *Calotropis Procera* are used as the main dye-sensitizer in solar cells. The *Calotropis* plant is a non-food item capable of surviving the harsh climate of the United Arab Emirates. Its incorporation into dye-sensitized solar cells is tested by constructing various cells, whose performance was also compared to that of more common chlorophyll-based dye extracts (i.e. spinach) as well as compared against a baseline cell sensitized with a synthetic ruthenium dye. The performance of the *Calotropis*-based cells was in general better than those with other natural sensitizers, but of course scored lower efficiency results when compared to cells built with synthetic dyes (0.075% compared to 5.11%). The advantage in using a natural sensitizer include facile extraction and preparation, low cost and abundance, since the *Calotropis* source has no competing applications in terms of food, livestock feed, etc. The figure-of-merit of cell output vs. cost for such cells makes them a good contender for further research and development effort to overcome the obvious drawbacks of stability and service longevity. Adding a hole-transport material to the cells in the form of PEDOT:PSS was also attempted to assess the enhancement it could provide to the cells. This did not yield the desired results and more experiments have to be done to better understand the interaction of each added layer to the original cell design.

Key words: *Calotropis Procera*, dye-sensitized solar cells, natural sensitizer, third generation photovoltaics.

INTRODUCTION

Solar energy, the energy emitted from the sun, is a promising renewable energy resource that is widely available, easily accessible, and non-exhaustible, in addition to that it is utilized in various applications (Kibria et al., 2014; Ushasree & Bora, 2019). Different materials and natural resources can be readily adapted for various eneryg applications, such in solar collectors (Palabinskis et al., 2008), electromagnetic shielding (Mironovs et al., 2014) or for anticorrosion coatings (Dastpak et al., 2020). Given these innumerable advantages, researchers are racing towards enhancing the efficiencies and economies of collecting and storing solar energy, specifically using solar cells. The creation of solar cells underwent several stages. Each stage, known as a generation, was developed to enhance the development process of solar cells (Alami et al., 2019). First generation solar cells, known as the conventional silicon-based solar cells, are the most widely used solar cells and account for almost 80% of all the developed solar cells in the world. Silicon-based solar cells have high efficiency and are durable; however, lately it surfaced that the market share of silicon-based solar cells is declining (Aghaei, 2012). This is due to the amount of energy required to develop solar cells which in return requires huge investments. Later on, second generation solar cells were developed utilizing thin film semiconductors, which led to a huge reduction in the required investments but also reduced the overall solar cell efficiency (Alami et al., 2019). To utilize the full potential of solar cells it is essential to find an optimal combination of the associated costs of solar cells and its relative efficiencies, this indicates that either a solution must be provided for better utilizing the silicon material or a replacement for the silicon material that optimizes the solar cells' efficiencies and significantly lowers its costs must be found. Consequently, third-generation solar cells were developed to acquire the silicon-based solar cells' high efficiencies and the costs of the thin film solar cells (Bharam & Day, 2012). This combination will allow for an efficient and cost-effective production of solar cells that will facilitate an easier process of fabricating the solar cells (Kibria et al., 2014). The current mainstream third-generation solar cell types are: organic/polymer based solar cells, perovskite based solar cells, and dye sensitized solar cells (DSSC) (Sharma et al., 2015). Fabricating organic based solar cells requires low amounts of energy in contrast to inorganic solar cells (Yuan et al., 2011). Organic based solar cells are mechanically flexible, lightweight (Ganesh et al., 2013), and have low processing and materials costs. However, the resulting efficiencies of the organic based solar cells have been found to be relatively low (Yuan et al., 2011). The advantages offered of developing organic based solar cells make them attractive for portable applications and other domestic usages (Ganesh et al., 2013). Another type of third-generation solar cells, perovskites based solar cells, have gained significant attention because of its relatively low costs (Zhou et al., 2018) and high efficiencies that can reach up to 25.5% (NREL, Best Research-Cell Efficiencies, (2021). https://www.nrel.gov), but it struggles to maintain these efficiencies as perovskites based solar cells tend to have issues in their stability and durability. A recent investigation have revealed that perovskites based solar cells will have an essential role in future electric automobiles batteries (Sharma et al., 2015). Dye Sensitized Solar Cells (DSSC), another type of third-generation solar cells, have reached efficiencies of about 13% and have had extensive attention from researchers and significant developments because of their relatively low costs, easy process, and high efficiencies (Zhou et al., 2018). Moreover, their fabrication process can use several low cost organic materials and eliminates the incorporation of silicon within the process (Alami et al., 2019). A dye sensitizer, semiconductor electrode (p-type NiO, and n-type TiO_2), counter electrode, and the mesoporous media are the most essential parts of a DSSC. DSSCs utilize dye molecules in-between electrodes (Sharma et al., 2015). The most well-known type of DSSC is the n-type titanium dioxide (TiO_2) DSSC, where sunlight is absorbed by the surrounding molecular dye of the TiO_2. Once the sunlight penetrates the electrode passing through the molecular dye the electrons get excited and pass to the conduction band of the TiO_2. The electrode will collect the excited electrons as they pass through to power a load, afterwards the electrons re-enter the DSSC electrolyte on a counter electrode. The DSSC electrolyte brings the electrons to the dye molecules again (Tian et al., 2019). The photosynthesis process of the TiO_2 and the transparent dyes enhances the DSSC efficiency to higher than 10% (Sharma et al., 2015).

In this work we report on various designs of DSSC with a natural dye sensitizer extracted from Calotropis procera. The incorporation of the natural Calotropis in DSSCs is very attractive, since Calotropis is abundant in the Emirate of Sharjah and is known to be a non-food plant that grows wildly during all seasons. Baseline cells with ruthenium-based dyes and another chlorophyll-based dye (spinach) were also built to compare the efficiencies and power output with those having the natural dye. Besides the standard DSSC structure, we also investigated the effects of conductive polymer poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) on both types of cells. The hole-transport material, PEDOT:PSS, was deposited on the counter electrode to examine if it would increase cell performance, with the possibility of replacing the conventional electrolyte setup. This would be an important advantage done since forgoing the electrolyte can enhance the service time of the cells and reduce its sensitivity to adverse operating conditions of heat and humidity that negatively affects electrolyte and cause evaporation and leakage. Our studies on the Calotropis natural sensitizer and DSSC designs with PEDOT:PSS polymer can provide insights into developing green, low cost and more stable solar cells.

MATERIALS AND METHODS

Experimental Setup

Preparation and deposition of TiO_2 on photoelectrode

The photoanode was bought from Techinstro, which consists of the fluorine-doped tin oxide (FTO) with dimensions of 25 mm × 25 mm × 2.2 mm and sheet resistivity ≤ 7 ohms/sq. The cleaning process was done in two phases and started with cleaning the glass sheets with ethanol and placing it in ultrasonic bath for 15 minutes, and with DI water for another 15 minutes, and then by rinsing it with DI water and ethanol, before which the glass sheets were dried. The sheets were kept for UV-ozone treatment for 15 minutes. After measuring the conductive side using a multimeter, the TiO_2 active side was restricted with an area of 0.25 cm^2 using a plastic tape on three sides of the glass sheet. 50 mg of TiO_2 with 10–25 nm nanoparticles size was grinded with 50 mg of polyethylene glycol using a mortar and pestle for 30 minutes to obtain a homogenous titanium oxide paste. The paste was then spread on a FTO glass sample using doctor blading method. Lastly, a heating process was done to solidify the paste in a furnace by varying the temperature, starting at 325 °C, 375 °C, 450 °C, and finally to 500 °C (each temperature step took 20 minutes). The glass sheets were then allowed to cool to 60 °C, before being immersed in an Ru dye sensitizer and in natural dye solutions extracted from *Calotropis Procera* or spinach, storing it under dark for 24 hours for the dye adsorption. The cell structure and *Calotropis Procera* plant are shown in Fig. 1, a, b.

a) b)

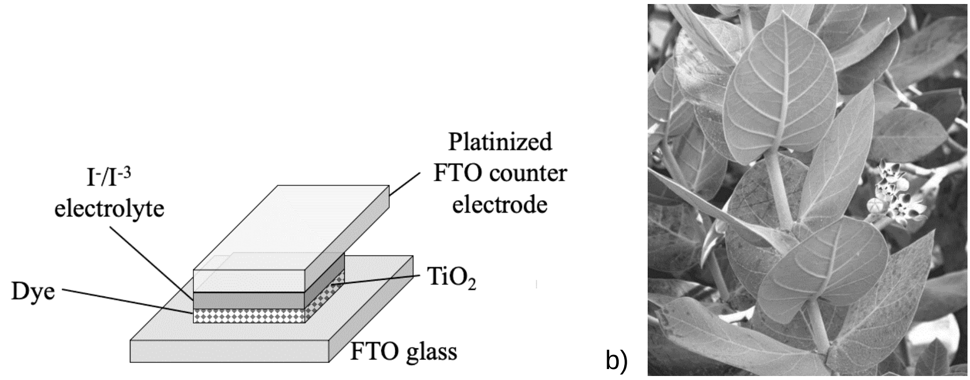


Figure 1. (a) Cell structure and (b) *Calotropis Procera* plant. Note that in (a) the PEDOT:PSS would be placed between the platinized electrode and the dye.

Dye extraction and FTIR analysis

For extracting and preparing natural dyes, leaves from *Calotropis Procera* or spinach were first washed with tap water and then rinsed with deionized water. For *Calotropis* dye extraction, the leaves were then cut into small pieces and kept to dry, the total mass is 36.3041 g which was immersed into a beaker before which the ethanol was added with a mass ratio of 3:1 of ethanol to leaves in balance, which resulted in 108.9123 g of ethanol. The beaker was then kept in an ultrasonic bath for one hour after covering it with parafilm. The spinach dye extraction for comparison with another natural sensitizer followed identical route. Lastly, a PTEF syringe with a porosity of 0.45 μm was used to filter the solution from any solid contaminants and the beaker was covered in a dark, cool environment to prevent dye oxidation.

Optical characterization

After putting the dye in cuvettes of transparent material (Ocean Optics Ultra Micro Cell with a range of 220–900 nm) and then on a cuvette holder, the optical absorbance of the solution was measured by a Maya 2,000 - Pro high-resolution spectrometers (Ocean Optics) with aHL - 2,000 tungsten halogen light source. Three Maya 2,000 spectrometers were used with software signal for measuring the UV-to-Infrared spectrum range (wavelength range from 200 nm to 1,100 nm @ 0.2 nm resolution). The same procedure was then replicated for spinach leaves to compare the dye pigments absorptivity.

As for dyes on TiO2, the optical assessments were also conducted using the same spectrometer. An ISP-REF integrating sphere with a WS-1 reflectance standard for calibration obtained from OceanOptics was used for the measurement of reflectance. The dyed titania electrodes transmissivity was estimated with an HL-2,000 tungsten halogen light source before it was converted into absorbance using the built-in software.

An integrating sphere (also from OceanOptics ISP-REF with an aperture of 0.4 inch, with a built-in tungsten halogen light source (Ocean Optics LS-1-LL) is used to measure specular and scattered reflectance. The baseline absorptance (0%) spectra were stored using the reflectance standard to assist comparison between the different compositions. To find the optical bandgap for each electrode, Tauc formula was then used.

A PerkinElmer spectrophotometer, USA in pressed-disc method using KBr pellets was used to record the Fourier Transform Infrared (FTIR) spectra. The FTIR range of operation was adjusted from 4,000 to 500 cm^{-1}, with 10 scans being signal-averaged having a resolution of 1.0 cm^{-1}.

Solar Cell manufacture

The solar cell was assembled by first joining the TiO_2 deposited electrode with platinum counter electrode (Solaronix.com) using binder clips. Injecting the drilled counter electrode with Iodolyte AN 50 electrolyte obtained from Solaronix (Solaronix.com) using a syringe. Next, for using the conductive PEDOT solution from Sigma Aldrich (sigmaaldrich.com) with or instead of the Iodolyte, the electrode was deposited with the PEDOT solution using a pipette and spin coating for 1 minute at 600 RPM. The electrode is then placed on a hotplate at 100 °C for 30 minutes and then allowed to cool down.

Cell characterization and impedance properties

Under an ABET SunLite solar simulator with AM1.5G and 100 mW cm^{-2} irradiance at a room temperature of 25 °C, the DSSCs current density-voltage (*J*-*V*) measurements were done using a Keithley 2400 SourceMeter.

Impedance analyzer potentiostat (VSP-300) under the sun simulator irradiation was used to obtain impedance spectra. 14.2 mV AC signal was supplied to the cell under 1-Sun conditions. The frequency range was changed between 100 kHz and 0.1 Hz with the bias voltage of the open circuit potential of the DSSC kept unchanged with a potential resolution of 20 μV. The Z-Fit analysis software from Bio-logic was used to fit the spectra. The tests are repeated three times for each cell and the average curve is reported and discussed.

RESULTS AND DISCUSSION

Dye FTIR analysis

For information on functional groups existent in the dye extract, FTIR is the appropriate tool. These peaks are often evidence for the existence of certain bonds and are shown in Fig. 2. All FTIR peaks present have been reported elsewhere (Ahliha et al., 2017). In the wave number range of 3,600–3,200 cm^{-1}, the O-H bond (polar group) manifests creating both intramolecular and intermolecular hydrogen bonds. Weak bonds at wave numbers 1,420–1,330 cm^{-1} were also observed due to the weakening of the in-plane O-H bending. Finally, infrared absorption shows at wave number 1,725–1,705 cm^{-1} causing the C=O stretching vibration. In general, the FTIR readings are all compatible with a green natural sensitizer with chlorophyl as a main pigment.

Dye optical properties

The Absorption tests of the dye solutions are shown in Fig. 3. It is noted that the *Calotropis* dye extract compares well with spinach, which is another dye based on the chlorophyll pigment. It is noted, however that the absorptivity of the Calotropis dye is better in the visible range (~ 400–700 nm). This is expected result in better performance of the assembled DSSC as more photons are likely to enhance the photogeneration within the cell.

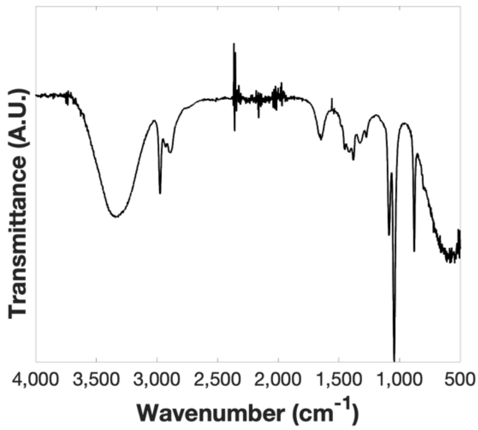


Figure 2. FTIR spectra of extracted *Calotropis Procera* dye.

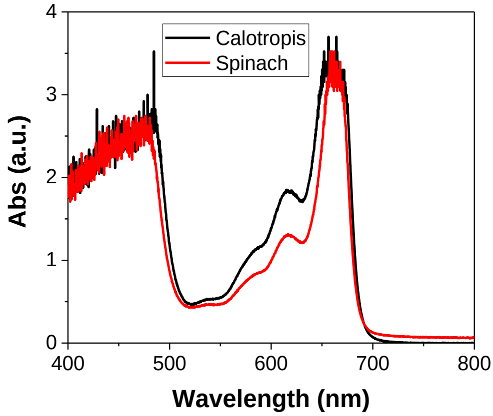


Figure 3. Absorption tests of *Calotropis* vs. spinach dye extracts

Absorptivity on TiO_2

Another optical assessment was done after extracting the dye from *Calotropis* leaves by adding the dye on a photoelectrode which consists of FTO with the deposited TiO_2 prior to cell assembly and testing. As shown in Fig. 4, the bandgap results are shown for one uncovered TiO_2 reference, an immersed electrode with a chlorophyll II pigment source (spinach) and also for the *Calotropis* dye. These results compare well with known natural sensitizer that is used in literature (Ahliha et al., 2017). The absorbance results are also shown inset.

For both spinach and *Calotropis* dyes at the violet-blue zone, the absorptivity graphs shown in Fig. 4 show a higher spectral absorption, with the former plateauing at the onset of the visible regime while the latter falls off towards the infrared. Considering that in Fig. 3 where *Calotropis* dye

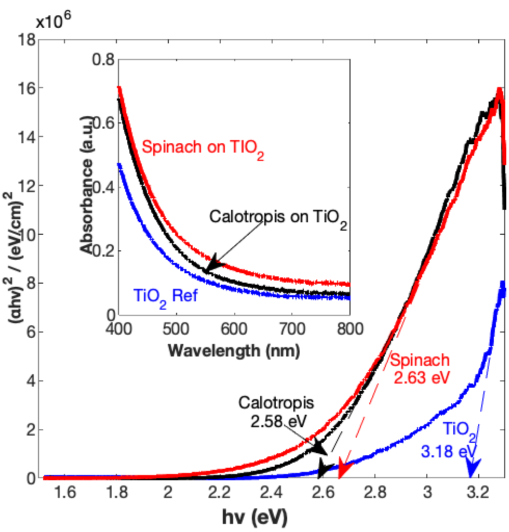


Figure 4. Bandgaps for prepared dye solutions loaded on TiO_2 material, calculated using Tauc formula for the *Calotropis Procera* dye, bare titania reference and another immersed in spinach. These values are calculated from absorbance values (inset).

solution shows higher absorptivity, the lower optical absorbance of *Calotropis* dye on photoelectrode compared to spinach is indicative of its insufficient adsorption onto the TiO_2 photoelectrode, which requires further improvement in the extraction method.

Cell testing results

Impedance testing

The impedance and frequency response results for the tested DSSC with *Calotropis* dye are shown in Fig. 5. The general shape of the obtained spectrum is the one normally obtained for DSSC at open circuit potential (Alami et al., 2019). A peak is found at the high frequency range, shown as an inset of Fig. 5, while a broad semicircle at the intermediate frequency range and lastly a tail at low frequencies. The behavior at high frequencies (inset) depicts the interface of the counter electrode and electrolyte. The charge transfer process occurs causing the shown perturbation in signal. Its small magnitude is an indication of the continued activity of the electrode and the potential of its enhancement for better performance of the cell. For intermediary frequencies, the peak is wide and is known to be due to electron diffusion in TiO_2 as well as the transfer of electron at the interface between TiO_2 and electrolyte. The electrolyte will degrade under illumination, causing its viscosity to change and hence hinder ion transport. This would be evident by the disappearance of this peak. Finally, a small tail appears at low frequencies which is known to correspond to the slow diffusion process of I_3^- in the electrolyte. For the present tests, the tri-iodide species concentration is low when compared to iodide ones which is advantageous in reducing losses due to recombination.

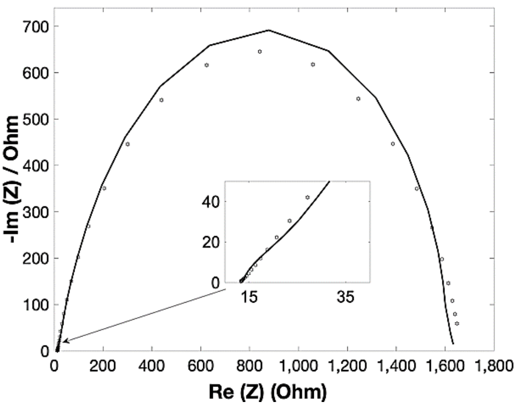


Figure 5. Impedance (Nyquist) test for the cell sensitized with *Calotropis* with the high frequency range results shown inset.

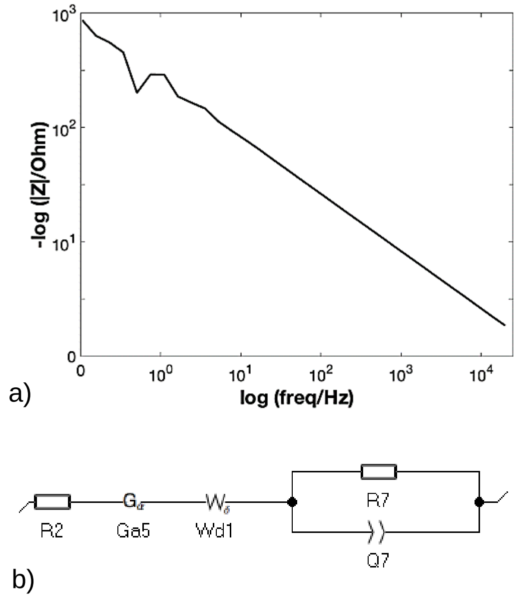
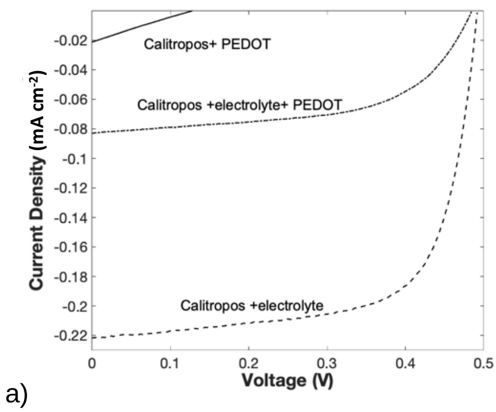


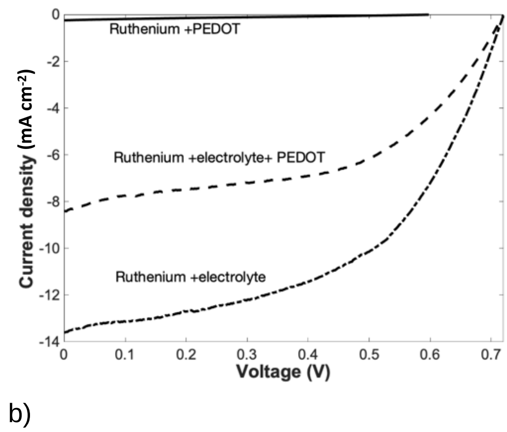
Figure 6. (a) The frequency response and (b) the proposed equivalent circuit for the cell sensitized with *Calotropis.*

The Bode plot for the naturally sensitized cells is shown in Fig. 6, a. There are no wide scale peaks as a general linear trend is observed at low frequencies. This indicates poor operational conditions of low rate of charge transfer at TiO_2/dye/electrolyte interface. This gives hope that better results can be obtained from the cell by applying better conditions of illumination, temperature or concentration. It is noted that the EIS data was fitted into the circuit model shown in Fig. 6, b. The components are as follows: R2 corresponding to the ohmic sheet resistance of the electrolyte, Ga5 resembling an electrochemical system with reflecting boundary where the distributed capacitance is replaced by distributed constant phase element (CPE) to account for non-ideality in the diffusion-recombination processes. The finite-length Warburg element (Wd1) was attributed to the transmission line model with short-. circuit terminus. Also, R7, Q7 are present at the counter electrode/electrolyte interface. It is important for a high functioning cell that the electron transport or diffusion resistance (R_d) be as small as possible and the electron recombination resistance be as large as possible and with a high diffusion coefficient, D

DSSC J-V characterizations

The standard structure DSSC (FTO / TiO_2:dye / I^-/I_3^- electrolyte / Pt / FTO) were assembled following the procedures detailed in the experimental section. The current density - voltage (J-V) characteristics of the various DSSCs are presented in Fig. 7 (Table 1 shows the summarized photovoltaic parameters).





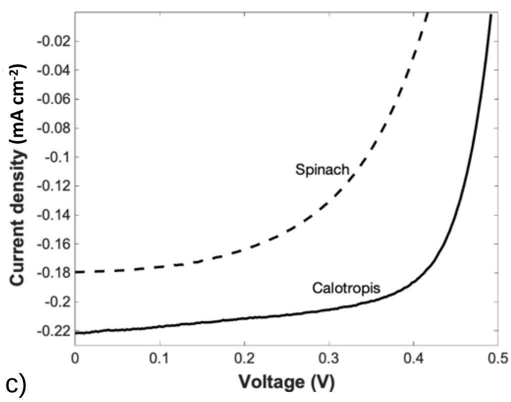


Figure 7. Characteristic curves of tested cells with different dyes and the hole-transport material (PEDOT:PSS) (a) *Calotropis* dye (b) Ruthenium dye and (c) natural sensitizers: spinach and *Calotropis*.

Comparing the standard cell structures in Fig. 7, a, the synthetic Ru-based sensitizer N719 showed good device performance in baseline DSSC, with a power conversion efficiency (PCE) of 5.1%. Differently, in the same device structure, the natural dyes extracted from the leaves of *Calotropis* and spinach both exhibited much lower cell performance (0.075% PCE). While the *Calotropis* cells demonstrated a slightly higher fill factor (FF) (~ 68% vs. ~ 52% for Ru-based cells), they are mainly limited by the small photocurrent density (0.22 mA cm^{-2}), which is more than one order less than the N719 dye. As such, the priority in future optimization for *Calotropis* DSSCs is increasing the dye loading ratio on TiO_2 photoelectrode, while adopting more efficient dye extraction methods. The smaller open-circuit voltage of the *Calotropis* cells is indicative of a smaller bandgap of the sensitizer.

Table 1. Summary of cell performance sorted by dye

Cell	Dye	Electrolyte	FF	PCE (%)	J_{sc} $(\frac{\text{mA}}{\text{cm}^2})$	V_{oc} (V)
1	Ru	Iodolyte	0.52	5.11	13.6	0.72
2	Calotropis	Iodolyte	0.68	0.075	0.22	0.49
3	Ru	PEDOT	0.23	0.033	0.24	0.60
4	Calotropis	PEDOT	0.24	0.0006	0.022	0.13
5	Ru	PEDOT+Idolyte	0.51	3.083	8.41	0.72
6	Calotropis	PEDOT+Idolyte	0.57	0.023	0.083	0.486
7	Spinach	Iodolyte	0.52	0.04	0.18	0.41

As a further improvement to cell design and functionalities, the p-type conductive polymer PEDOT:PSS has been proposed to be an alternative for the Pt catalyst counter electrode (Lee et al., 2012), Hong et al. (2008), or replacing the liquid electrolyte for a quasi-solid-state cell design (Biancardo et al., 2007). Such incorporation can benefit DSSCs by reducing production cost, and/or mitigating issues from using liquid electrolyte (such as evaporation and leakage) and extending the cell lifetime. As a test trial, we further investigate the effects of PEDOT:PSS on the natural dye cells, with and without the presence of I^-/I_3^- electrolyte. The results are shown in Fig. 7, b, c. It can be seen for both *Calotropis* and Ru-based cells that the addition of PEDOT:PSS polymer did not produce a direct benefit for the performance, as the J-V curves for both cells are considerably reduced in short-circuit current density (J_{SC}) when using both I^-/I_3^- electrolyte and PEDOT:PSS. Furthermore, attempts at using PEDOT:PSS to replace the I^-/I_3^- electrolyte entirely contributed to the further decline of device performance, as shown by the black curves in Fig. 7, b, c. The observation of the preliminary studies here suggests the need for future work on modifying the PEDOT:PSS material and investigating the interplay between PEDOT:PSS and the DSSC structure. On the other hand, the main advantage of using natural sensitizer would be the figure-of-merit that compares the efficiency of the cell with its potential cost. The ruthenium-based dye, bought from solaronix.com and 1 g of the material cost CHF 137 ($150). The natural dye that is extracted from a non-food plant that is abundant even in the harsh summers of the Gulf region, with costs of less than ~$1.5 per gram to extract the dye (cut the leaves, use ethanol at 1 gram of dye to 3 liter of ethanol). With these numbers, the figure of merit for a naturally sensitized solar cell would be around (0.075/1.5 = 0.05) compared to that of a Ru-sensitizer: (5.11/150 = 0.032). These numbers are the same order of magnitude but with a clear advantage towards the naturally sensitized ones.

CONCLUSIONS

This work presented the construction of a dye-sensitized solar cell with the use of a natural sensitizer from a local non-food plant. The *Calotropis Procera* is a plant abundant in the United Arab Emirates, and its adaptation to the harsh weather and strong solar radiation makes it worthy of investigation for solar cell development. The dye extracted from the plant mainly contains chlorophyll pigments, but when compared to another natural sensitizer (spinach) it scored around 46% enhancement in efficiency. The *Calotropis* plant cannot be consumed by livestock or humans due to its skin irritation qualities, and hence it has this advantage as well over green plants such as spinach. A baseline cell was also constructed using a ruthenium-based sensitizer (N719) to ensure that the DSSC built would score acceptable results and is constructed well. At 5.11% efficiency, the cells did perform as hoped. Another track was also followed by adding PEDOT:PSS, which is a conductive polymer in place and in conjunction with the liquid electrolyte for a potential quasi-solid DSSC. This development, however, did not yield acceptable results and in fact was detrimental to the current extracted from the cells as well as their efficiency. More work will have to be done to overcome the potential limitations of such cells, such as the compatibility of the cells structures and other conductive materials at the counter electrode. Better sealing options can also be devised to limit electrolyte leakage and evaporation and keep dye material from degrading in the process.

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Evolution of production and forage quality in sown meadows of a mountain area inside Parmesan cheese consortium

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Abstract. Sown meadows, encompassing alfalfa and grass-legume mixtures, are the forage crops on which is based Parmesan cheese production system in the mountain area of the Apennines (central Italy). These grassland types experience, during their development, deep changes in terms of production potentiality, botanical composition and forage quality, thus these meadows have to be periodically renewed to guarantee adequate productive and qualitative performances. To have an accurate assessment of this evolution along time, a survey was performed in different mountain farms inside the Parmesan cheese consortium, analysing alfalfa meadows and grass-legumes mixtures of different ages. Grasslands were monitored during 2019, performing three samplings during growing season. Aboveground biomass production, botanical composition and crude protein content were collected during the survey. Results permitted to evaluate the level of production decrease along years, the evolution of analysed parameters among cutting dates and the dependence of productive and qualitative features on botanical composition and presence of sown species in the swards. Results were useful to hypothesize the composition of future mixtures, to improve management issues and to delineate the possible duration of sown meadows for the area with respect to different purposes in terms of desired productive or qualitative objectives.

Key words: alfalfa, botanical composition, forage, grass-legume mixtures, mowing.

INTRODUCTION

Parmesan cheese is one of the food excellences of Italy and is exported all over the world (Lovarelli et al., 2019). Its production district is limited to a very restricted area almost completely occurring in Emilia-Romagna region (central Italy) that encompasses farms rearing mainly dairy cattle of Holstein Friesian breed. Most of these dairy farms are located in the plane, but about 100 of them are in mountain and less favoured areas (Mancini et al., 2019). In this territory, main forage resources employed are artificial crops cut for hay production of high quality (Tabacco et al., 2020). Occurrence of wide surfaces devoted to grasslands inside a specific territory allows also the provision of specific ecosystem services that go beyond the primary productive function, such as soil protection, preservation of landscape heterogeneity, C-footprint reduction, biodiversity conservation and reduced surplus of nutrients (Giustini et al., 2007; Argenti et al., 2011;

Hao et al., 2017; Pulina et al., 2017; Bengtsson et al., 2019; Viira et al., 2020). Furthermore, the quality of hay and the maximization of their inclusion in the animal diet represent the basis of dairy cow nutrition, for the following milk and dairy products quality but they also play a role in the improving of animal health and welfare (Mordenti et al., 2017).

Among sown meadows inside the studied area, alfalfa (*Medicago sativa*) is one of the most spread mainly for its adaptation to local environmental condition, the high nutritive value and the remarkable potential productivity (Pacchioli & Fattori, 2014). Employment of this species in artificial meadows permits to provide forages with high concentrations of protein, reducing external inputs, economic costs and environmental impacts (Tabacco et al., 2018; Kic, 2019). Other forage resources occurring in the area are represented by mixtures based on grasses and legumes. Species belonging to these two botanical families, chosen according to peculiar pedo-climatic conditions (Movedi et al., 2019), are combined in many different ways, from very simple, based only on two species, a grass and a legume, such as *L. perenne* mixed with *Medicago sativa*, *Trifolium repens* or *Lotus corniculatus*, as reported by Høgh-Jensen et al. (2006), to very complex, encompassing many different species with the aim to establish a forage resource able to became more resilient to adverse weather conditions, to yield at higher rates with respect to monocultures and to provide a more balanced forage quality (Lüscher et al., 2014). Species used for these complex mixtures are represented by productive grasses (such as *Lolium perenne*, *Dactylis glomerata*, *Festuca arundinacea*, *Phleum pratense*) and by already cited legumes, and in some cases also by species belonging to other botanical families, such as *Cichorium intybus* (Skinner, 2008; Sanderson, 2010; Roca-Fernández et al., 2016). Advantages from mixtures in comparison to monocultures are attributed to the presence of species with a very high different behaviour that in turn will result in optimized resource utilization through niche complementarity (Lüscher et al., 2014). In both above mentioned cases, a careful utilization is essential for a proper management of grasslands and to maintain them in an efficient way (Argenti et al., 2020).

Assessment of botanical composition of sown grasslands and its evolution is of extreme importance as it is closely related to yield and forage quality (Chataigner et al., 2010; Targetti et al., 2018). Floristic composition can change remarkably in terms of ground cover of sown species and presence of native species (Sanderson et al., 2005; Ponzetta et al., 2010). Assessment of this development can determine the more appropriate moment for reseeding (Mocanu et al., 2017), when productive and qualitative features fall under specific thresholds. The main aims of this study are: i) to assess the development of botanical composition in sown grasslands along time; ii) to evaluate evolution of main productive characteristics in meadows and how they are affected by botanical composition; iii) to provide technical recommendations for a proper management of artificial forage crops for the studied territory.

MATERIALS AND METHODS

The investigated area is inside the Consortium 'Terre di Montagna', that gathers about 100 farms producing Parmesan cheese in Apennines mountain areas, at an altitude over 600 m a.s.l. and comprising some municipalities inside the provinces of Bologna and Modena (Emilia-Romagna Region, central Italy). Soil types mainly developed on sandstone, limestone, and marl, with a very different range of pH (from acidic to calcareous). Climate of meteorological station of Montese, roughly in the centre of the studied area (44,268844N, 10,942369E), is characterized by a mean annual temperature of 10.1 °C and by an average precipitation of 930 mm, generally without drought during summer (Regione Emilia-Romagna, 2020).

Inside the territory belonging to the Consortium, 6 sites of artificial meadows devoted to hay production were chosen. Sites were selected to be representative of the main forage resources occurring in the area. They differed for environmental location, sown crop (4 monocultures of *Medicago sativa* and 2 grasses-legumes mixtures) and age of meadows, ranging from 2 to 12 years since sowing (Table 1).

Table 1. Main characteristics of investigated sites

Site	Altitude (m a.s.l.)	Average slope (%)	Forage crop	Years since sowing
1A	740	15	Alfalfa	2
2A	870	12	Alfalfa	4
3A	750	8	Alfalfa	8
4A	690	4	Alfalfa	12
5M	780	5	Mixture	2
6M	740	14	Mixture	4

Inside each site, a survey was conducted during 2019, with 3 sampling dates along growing season: in spring, summer and late summer, namely 30−31 May, 15−16 July, and 28−29 August. During each sampling date, three replications inside each experimental area were randomly chosen. Plots were represented by a square of 0.5×0.5 m, according to Mikhailova et al. (2000), and in each plot above ground biomass was harvested using battery-operated grass clippers, stored in sealable bags and then took back to laboratory and oven-dried at 80 °C for 48 hours to constant weight, in order to obtain dry matter production (Wang et al., 2019). On the same sample, chemical analyses were conducted to obtain percentage of crude protein (CP) content (AOAC, 2012) as a main drivers of forage quality (Berauer et al., 2020).

Botanical composition was assessed inside the same sample plots for each cutting. Proportion of each occurring species was estimated visually as percentage contribution to the herbage mass according to Boob et al. (2019). In following elaboration, herbaceous species were grouped in grasses, legumes and other forbs as usually performed in forage research (Wilson et al., 2020). Moreover, knowledge of introduced species, *i.e.* alfalfa or those present in original mixtures, permitted the discrimination of sown species from weeds deriving from natural recolonization in following elaboration.

Results were utilized to compare areas seeded by different crops by means of ANOVA, eventual means separation was performed adopting Tukey test. Moreover, obtained results were used to find out possible relationships among investigated variables and to evaluate evolution of different forage characteristics along time. All analyses were performed using statistical software SPSS (release 26, IBM, 2019).

RESULTS AND DISCUSSION

Productive trends in studied meadows

The trend in production capacity along the season is the same for all investigated sites as the first cut is always the most productive in comparison to the others, with a range from just over 2 tons per hectare of dry matter (oldest sites 3A and 4A) to about 5, in the case of the two-year alfalfa of site 1A (Fig. 1). The decrease in production between the first and second cutting is considerable, but it is much less in the cases of the most recent meadows, regardless of whether they are alfalfa or mixtures (sites 5M and 1A). Occurrence of sown species, more productive, stable, and more represented in the first years since sowing, produced a sort of stabilization effect on the loss of productivity as the growing season progresses. The last cut is the least productive (in some cases around a ton per hectare) and this condition characterizes the oldest alfalfa meadows, referable to sites 3A and 4A of the trial.

Results give evidence of the importance of management in meadows and its impact on their evolution, especially if located at reduced altitudes (such as in our study) as in higher altitude forage resources characteristics are more forced by environmental factors (Pierik et al., 2017). Findings are consistent with those reported in the research of D'Ottavio & Ziliotto (2003), in which first cut represented almost the 50% of total annual dry matter production. Similar results are reported by Borreani et al. (2005) for grasslands in North Italy at altitude similar to ours, in which the first cut represents a percentage of 40−60% of the total annual harvested biomass. Decline of production along years depicted in our research is coherent with results of Bélanger et al. (2017) that provided evidence of a stable production for at least the first 5 years since seeding.

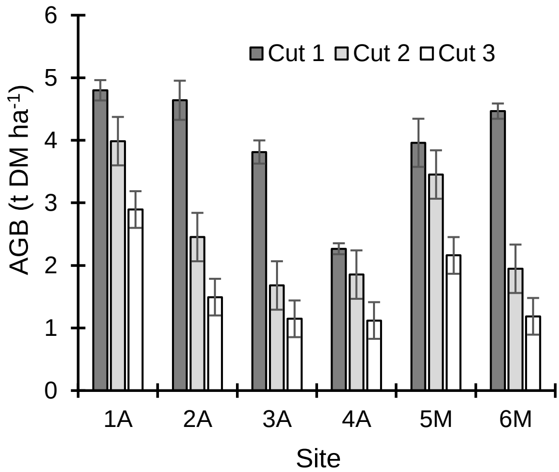

Figure 1. Above ground dry matter biomass (ABG, t ha^{-1}) for each cut in the investigated sites. Bars on each column represent standard errors.

Botanical evolution in studied meadows

Proportion of legumes is constantly increasing in all sites along the growing season, accounting a remarkable presence also in mixtures (site 5M and 6M), in many cases not significantly different from what observed in pure stands of alfalfa, and roughly ranging between 75−90% in second and third cut (Table 2). The complete list of species observed along all botanical samples is reported in Table S1 (Supplementary materials). Main legumes occurring in the samples are *Medicago sativa* (largely the predominant legume) followed by *Trifolium repens* and *Trifolium pratense*. Presence of forbs (mainly represented by *Taraxacum officinale*, *Geranium dissectum*, *Plantago lanceolata*, *Ranunculus arvensis* and *Rumex obtusifolius*) is very limited with exception of oldest sites, and this is especially true for the 12-years old meadow (4A), with a maximum value of 32.3 at second cut. Higher proportion of grasses (such as *Dactylis glomerata*, *Arrhenatherum elatius* and *Lolium* sp.) in the swards at the first cut is due to their general fast growth after winter at reduced temperatures, roughly 4−6 °C as reported by Brum et al. (2009), and this is true also for alfalfa stands, in which ground cover of grasses is higher than legumes at first cut, except for the most recent meadow in site 1A. To confirm this, Pacchioli & Ligabue (2013) consider the first cut of alfalfa a sort of forage mixture due to high presence of grasses. Infestation of alfalfa is a generalized issue, and our data confirms previous research. Mseddi et al. (2017) found a constant increase of spontaneous species in alfalfa pure stands and in fourth year since sowing the density of weeds was higher than the sown species, with a corresponding decrease in productivity and forage quality. Also, environmental conditions play a crucial role in determining potential productivity of alfalfa crops, as on poor soil crop decline begins after 3−4 years (Lazarev & Starodubtseva, 2015).

Relationships among productive and qualitative characteristics and botanical composition and age of meadows

Evolution of crude protein content along different cuts is clearly linked to production and botanical composition already analysed (Fig. 2). In almost all investigated meadows there is a remarkable effect of sampling dates on this parameter, with a very reduced value for old alfalfa meadows and for forage mixtures at first sampling date. Second and third cut presented a remarkable increase in protein content, with average values always over 20%. In sites 1A and 2A, represented by recently sown pure stand of *Medicago sativa*, CP content is more constant among cuts, especially in the youngest one.

The narrow relationship between presence of legumes and forage quality in studied meadows is testified by the relationship among occurrence of legumes in the swards

Table 2. Percentage presence grouped for grasses, legumes, and forbs in different sites for each cutting period. Values are shown as average ± standard error. Values, within the same column followed by the same letters are not significantly different at $P < 0.05$ according to Tukey test

Site	Grasses	Legumes	Forbs
Cut 1			
1A	16.3 ± 0.3^{c}	78.4 ± 4.4^{a}	5.3 ± 0.7^{b}
2A	51.3 ± 5.0^{b}	35.7 ± 2.9^{b}	13.0 ± 2.6^{ab}
3A	77.3 ± 5.5^{a}	10.7 ± 3.5^{c}	12.0 ± 2.1^{ab}
4A	66.3 ± 9.2^{ab}	15.0 ± 6.1^{c}	18.7 ± 4.3^{a}
5M	45.3 ± 0.7^{b}	45.0 ± 1.7^{b}	9.7 ± 1.2^{ab}
6M	80.3 ± 1.4^{a}	11.7 ± 1.6^{c}	8.0 ± 2.0^{ab}
Cut 2			
Site	Grasses	Legumes	Forbs
1A	11.0 ± 4.0^{c}	79.0 ± 3.7^{a}	10.0 ± 2.5^{bc}
2A	4.7 ± 0.8^{c}	90.7 ± 2.9^{a}	4.7 ± 2.0^{c}
3A	36.7 ± 4.9^{ab}	48.7 ± 3.6^{b}	14.7 ± 2.4^{b}
4A	42.0 ± 8.9^{a}	25.7 ± 8.4^{b}	32.3 ± 1.9^{a}
5M	14.7 ± 5.5^{bc}	81.7 ± 6.3^{a}	3.7 ± 1.4^{c}
6M	15.7 ± 2.9^{bc}	79.3 ± 3.2^{a}	5.0 ± 0.6^{c}
Cut 3			
Site	Grasses	Legumes	Forbs
1A	1.3 ± 0.9^{d}	90.0 ± 1.7^{a}	8.7 ± 1.7^{c}
2A	0.7 ± 0.3^{d}	93.7 ± 2.0^{a}	5.7 ± 1.7^{c}
3A	40.7 ± 3.5^{a}	42.0 ± 3.7^{c}	17.3 ± 0.3^{b}
4A	38.3 ± 6.0^{a}	33.3 ± 4.4^{c}	28.3 ± 3.3^{a}
5M	7.3 ± 1.3^{c}	84.0 ± 2.5^{a}	8.7 ± 1.2^{c}
6M	18.7 ± 5.7^{b}	74.7 ± 3.0^{b}	6.7 ± 2.8^{c}

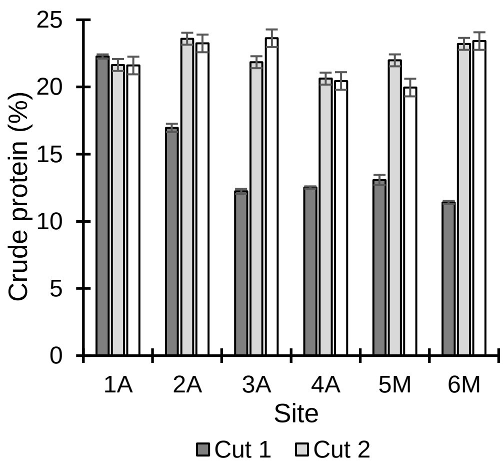

Figure 2. Crude protein content in above ground biomass (% on dry matter) for each cut in the investigated sites. Bars on each column represent standard errors.

(mainly alfalfa) and crude protein content in each cut (Fig. 3). At increasing level of this botanical family in the sward there is a corresponding increase of percentage of protein in forage biomass, even if no significant increase of protein content is observed at high percentage presence of legumes due to the logarithmic form of the regression line.

Evolution of forage quality along season observed in the study is in relation to climatic conditions that strongly affect grassland botanical composition in mountain areas (Dibari et al., 2015), with a higher presence of legumes in summer and, in turn, a higher forage value in this period (Cervasio et al., 2016). This evolution toward a richer presence of legumes is of extremely importance in mountain areas as pointed out by D'Ottavio & Ziliotto (2003), whose results are similar to ours, with a remarkable increase of crude protein of forage biomass along growing season. Results are consistent also with data of Borreani et al. (2005) that observed an increase of protein content from 4% to 8% from first to third cut along the vegetative season in mountain grasslands. Presence of legumes in semi-natural or sown grasslands can be considered an elementary driver of quality and a key factor in the diet of animals in order to improve their performances (Lüscher et al., 2014); this is especially true for dairy cows considering both the increase of individual production and the protein quote of milk composition that occurred in the last years (Mordenti et al., 2017). In a mixture, percentage of legumes in the sward ranging between 40−60% can be considered optimal to achieve effective benefits in terms of forage quality, higher productivity and reduced cost of feed (Phelan et al., 2015). Importance of botanical composition of meadows for crude protein content has already pointed out in many works. For instance, Reiné et al. (2020) found a narrow relationship between percentage of legumes in natural meadows in Spain and crude protein content in forage biomass. Moreover, Deak et al (2007) reported that legume proportion in some grasslands is able to explain a high amount of variability of crude protein in forage samples (r^2 = 0.85). Similar results were found by Wróbel & Zielewicz (2018) for Polish artificial meadows in which crude protein content of hay increased with the proportion of sown legumes occurring in the sward (represented in these cases by *Lotus corniculatus* and *Trifolium pratense*) in a very highly significantly way.

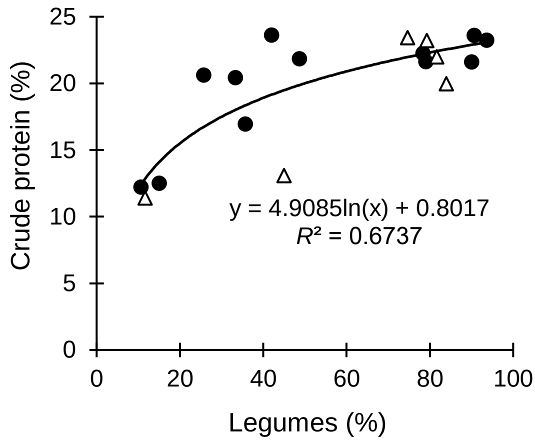

Figure 3. Regression between presence of legumes in the sward (%) and crude protein content in above ground biomass (%). Alfalfa meadows: solid circles; mixtures: triangles.

Aboveground biomass collected yearly was in relation with both years since sowing (Fig. 4) and occurrence of sown species collected during samplings (Fig. 5).

Reduction in productive potentiality is well correlated with age of meadows, and after some years since sowing (in both cases of monocultures and mixtures) annual dry matter production is sensibly reduced. In our case, acceptable annual productions (for instance, setting a threshold of 8 t ha^{-1}) are provided by meadows with at maximum 5 or 6 years since sowing (Fig. 4). This evolution towards grasslands characterized by reduced productivity should be attributed to development in the canopy of native species, that are characterized by reduced productivity and low forage quality. In fact, in our case, less productive meadows are those with a very low proportion of sown species (roughly 20−30%) represented by old grasslands (Fig. 5). On the other hand, recent meadows with a very high occurrence of sown species (at least 70−75%) have remarkable yield. The importance of presence of sown species on productive parameters in artificial forage resources has been already highlighted previously (e.g. Cavallero & Talamucci, 2002). Even if the persistency of seeded plants can reach notable duration in restored meadows (for instance 20 years, Švamberková et al., 2019), the efficient productive role can deplete in few years in sown resources that should be adequately renewed periodically with appropriate species in oder order to reduce loss of quality and potential yield and, in turn, of animal performances (Eriksen et al., 2014; Jing et al., 2017). Penetration of native species in sown grasslands is a natural process that affects all types of forage resources, monocultures and mixtures, even if the former is considered generally more sensible to weed invasion (Helgadóttir et al., 2018). Our data is consistent with previous research. For instance, Sturludóttir et al. (2013) report a constant decrease of productivity along years in an analysis conducted on artificial temperate grasslands. Persistency of sown species, with a higher presence in youngest meadows, had a stronger influence on productive performances, as reported by Adamovics et al. (2017) for different Latvian sown grasslands. Also Bélanger et al. (2017), analysing different binary mixtures in Canada for some years after sowing, highlighted the importance of sown species on forage features, and especially how

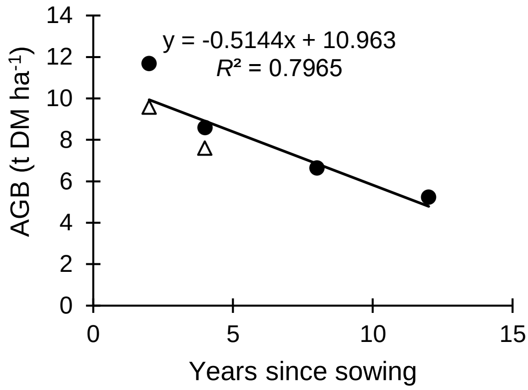

Figure 4. Regression between number of years since sowing and annual above ground biomass (t DM ha^{-1}). Alfalfa meadows: solid circles; mixtures: triangles.

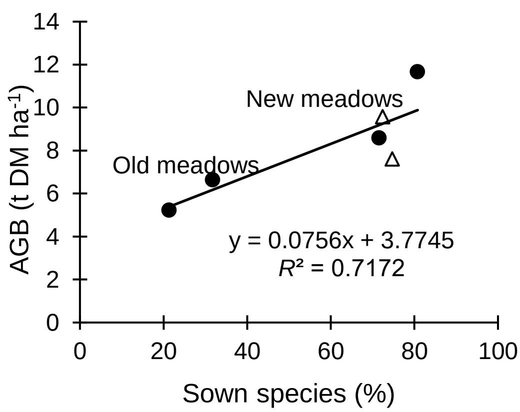

Figure 5. Regression between occurrence of sown species in the sward (%) and annual above ground biomass (t DM ha^{-1}). Alfalfa meadows: solid circles; mixtures: triangles.

legumes proportion, generally decreased along time, affects qualitative characteristics of harvested biomass.

CONCLUSIONS

The research permitted a proper and accurate characterization of forage resources employed in the investigated area, in terms of evolution along years and during the growing season. Even if they are based on different types (monocultures and mixtures) it was possible to depict the general development of main forage features, such as productivity and quality and how these characteristics are closely related to botanical composition.

The results allowed not only to describe possible development along years but, from a technical point of view, they were useful to foresee hypothetical management of meadows for the area in relation to specific objective. For instance, the productive decreasing resulted highly correlated to age of meadows, and to achieve an average dry matter yield of at least 8 t ha^{-1} per year, the appropriate duration can be scheduled to be about 5−6 years. The quality of forage is closely linked to a specific component of botanical composition (presence of legumes) and is conceivable that quality reduction is faster than loss of productivity, as legumes are generally characterized by reduced persistency compared to grasses. For these reasons an appropriate duration, having as key guideline forage quality, can be foreseen in 3−4 years since sowing, in order to obtain at least 20% of crude protein content as yearly average. Concerning mixtures, data collected permitted also to assess potential composition of meadows. Among legumes, *Medicago sativa* confirmed its high adaptability to the area, even if also some clovers could be useful employed to enhance legumes presence in the long term, mainly *Trifolium repens*. Among grasses, *Dactylis glomerata* was the species more suitable to be utilized, followed by *Arrhenaterum elatius*, *Festuca arundinacea* and *Lolium* sp.

As resulted by previous literature analysis, the appropriate duration of a sown meadow and its composition along time are the most important issues in artificial forage crops and they have to be adequately considered to achieve high performances, under a quantitative and qualitative point of view, for Parmesan cheese production.

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Table S1 (Supplementary material). List of species recorded along all the botanical samples grouped for grasses (G), legumes (L) and forbs (F) and their average percentage presence as classes (up to 1%, 1−5%, more than 5%) in alfalfa meadows or mixtures

Species	Botanical family	Alfalfa meadows	Mixtures
Agropyron repens	G	1−5%	< 1%
Agrostis tenuis	G	< 1%	-
Arrhenatherum elatius	G	1−5%	< 1%
Bromus hordeaceus	G	< 1%	< 1%
Bromus sterilis	G	< 1%	-
Cynosurus cristatus	G	< 1%	-
Dactylis glomerata	G	1−5%	>5%
Festuca arundinacea	G	< 1%	1−5%
Hordeum murinum	G	< 1%	-
Hordeum vulgare	G	-	< 1%
Lolium multiflorum	G	1−5%	1−5%
Lolium perenne	G	< 1%	< 1%
Phleum pratense	G	< 1%	-
Poa pratensis	G	< 1%	< 1%
Poa trivialis	G	< 1%	1−5%
Medicago sativa	L	> 5%	> 5%
Trifolium pratense	L	1−5%	1−5%
Trifolium repens	L	< 1%	1−5%
Achillea millefolium	F	< 1%	-
Amaranthus retroflexus	F	< 1%	-
Angelica archangelica	F	< 1%	-
Artemisia vulgaris	F	< 1%	-
Bellis perennis	F	< 1%	< 1%
Brassica napus	F	< 1%	-
Capsella bursa-pastoris	F	< 1%	< 1%
Cerastium arvense	F	< 1%	-
Cichorium intybus	F	< 1%	< 1%
Convolvulus arvensis	F	< 1%	-
Dianthus carthusianorum	F	< 1%	-
Erigeron annuus	F	< 1%	-
Foeniculum vulgare	F	< 1%	-
Geranium dissectum	F	< 1%	< 1%
Geum urbanum	F	< 1%	-
Hypericum perforatum	F	< 1%	-
Lamium purpureum	F	< 1%	< 1%
Leucanthemum vulgare	F	< 1%	-
Malva sylvestris	F	< 1%	-
Mentha arvensis	F	< 1%	-
Myosostis arvensis	F	< 1%	< 1%
Ornithogalum umbellatum	F	-	< 1%
Papaver rhoeas	F	< 1%	-
Picris hieracioides	F	< 1%	< 1%
Plantago lanceolata	F	< 1%	< 1%
Potentilla erecta	F	< 1%	-
Ranunculus arvensis	F	< 1%	< 1%
Rumex obtusifolius	F	1−5%	1−5%
Senecio vulgaris	F	< 1%	< 1%
Silene vulgaris	F	< 1%	-
Taraxacum officinale	F	1−5%	1−5%
Verbena officinalis	F	< 1%	-
Veronica persica	F	< 1%	-

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Relationship between somatic cell count in goat milk and mature Kashkaval cheese parameters

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Abstract. It is challenging to ensure Kashkaval cheese consistent quality during the production process which is directly correlated to the somatic cell count (SCC) and bacterial presence. This is one of the most popular and widely discussed topic areas in the dairy industry. SCC is used to limit the inflammatory process and to predict the health status of the animal's mammary glands. The objective of this study was to evaluate the quality characteristics of mature Kashkaval cheese was produced from goat milk with different SCC (below 1,200 thous cells mL^{-1} - group I (low), above 1,750 thous cells mL^{-1} - group II (high) and up to 1,600 thous cells mL^{-1} - group III (medium)) and samples were evaluated on the 1st and 60th day of ripening by chemical, microbiological and sensory profile. The results showed a significant relation ($P < 0.05$) between the levels of SCC and Kashkaval cheese water content during ripening. For all analysed samples, the total lactic acid bacterial count was the highest between the 15th and 45th day of ripening and reached values up to 6.0 log cfu g^{-1}. Pathogenic microorganisms (*Listeria monocytogenes*, *Coagulase-positive staphylococci*) and coliforms were not detected. The highest number of psychrotrophic microorganisms was observed in Kashkaval samples with high SCC. The sensory evaluation revealed a higher score for cheese samples with low and medium SCC in comparison to the cheese sample with a high SCC.

Key words: cheese, chemical profile, microbiological profile, quality, ripening, sensory profile.

INTRODUCTION

In recent years, there is a steady increase in the development of dairy goat farms in Bulgaria (Stankov, 2020). Goat milk provides many specific benefits (Zhou et al., 2016; Metodieva et al., 2018; Sousa et al., 2019). It is rich in proteins with high biological quality, as well as carbohydrates, vitamins, minerals and milk fat with specific composition of medium–chain fatty acids and bioactive compounds which determines its nutritional and functional properties (Zenebe et al., 2014; Bergilos-Meca et al., 2015; Clark & García, 2017; Verruck et al., 2019).

The intensive dairy goat breeding and the increased animal productivity are associated with an increase in inflammatory processes of the mammary gland (Gouveia et al., 2015; Tančin et al., 2017). Mastitis is one of the most common diseases in lactating animals which main indicator of appearance is the elevated somatic cell count (SCC) in milk (Abebe et al., 2016; Franceschi et al., 2020). The SCC is accepted as a criterion for udder health (Stocco et al., 2019a, Pisanu et al., 2020) and is used worldwide to describe the hygiene control implemented in milk production (Hanuš et al., 2018). According to the European legislation (Regulation 853/2004), the total number of microorganisms in milk from other species than cow's milk is up to 1,500 thous cfu mL^{-1}, but the total number of somatic cells is not mentioned.

Goat milk can be used successfully for production of different kinds of dairy products such as – set or stirred yoghurt, cheese, beverages, cheeses etc. (Aryana & Olson, 2017; Pal et al., 2017; Fazilah et al., 2018; Miller & Lu, 2019; Sepe & Argüello, 2019). Marcinkoniene & Ciprovica (2020) show a clear relation between the charge of SCC in goat milk and its suitability for cheese production. Kashkaval cheese is produced primarily in Bulgaria, but the same type of cheeses, called pasta–filata cheeses include a wide range of cheese variants which are also produced in Central and Southeast Europe (Medved'ová et al., 2020). The specific characteristics of Kashkaval cheese are those shared by all hard cheeses: low cooking temperature, cheddarization, and stretching of the cheese curd in hot brine. Factors related to the quality of goat milk, the technological steps in the cheese–making process, and the ripening period are of essential importance in the formation of flavour, aroma compounds and texture of Kashkaval cheese. There is still not enough information about the microbiological quality of goat milk, and its relation on cheese quality.

The aim of this study was to evaluate the changes of Kashkaval characteristics, produced from goat milk with different levels of SCC, during ripening.

MATERIALS AND METHODS

Milk collection: Each sample of bulk milk (from 19 goats) was taken at the morning milking once a month, each month, throughout the whole-lactation period from 'Plovdiv 1' - farm, located in the village of Orizare, municipality of Plovdiv, Bulgaria. The bulk milk samples were collected at early (March - May), middle (June - August) and late (September - November) lactation, from the breed Bulgarian White Dairy Goat. According to the values of SCC, milk samples were categorized into three groups, as follows: group I goat milk collected in May with below 1,200 thous cells mL^{-1} SCC (low); group II goat milk collected in August with above 1,750 thous cells mL^{-1} SCC (high); group III goat milk collected in November with up to 1,600 thous cells mL^{-1} SCC (medium).

Cheese preparation: Goat milk samples, from the three groups, were processed into Kashkaval cheese according to a traditional cheese-making technology (Kozhev & Kozhev, 2009) as follows: KL - Kashkaval cheese produced from group I goat milk; KM - Kashkaval cheese produced from group III goat milk; KH - Kashkaval cheese produced from group II goat milk. The milk was further clarified (at 35–45 °C), thermized (at 63 ± 1 °C for 15–20 s), cooled (at 33 ± 1 °C) and a starter culture in amount of 0.35% as well as calcium dichloride solution (50%) in amount of 30 mL per 100 L of milk (previously diluted in water in 1:10 ratio) and rennet (previously diluted in water in 1:10 ratio) in such amount that the coagulation started 10 ± 2 min after enzyme addition were added. A set coagulum was formed after 45 min. The obtained coagulum was cut at two stages - into 5–6 cm grains and after 5–6 min into 6–7 mm grains. The curd was stirred and the grains were stabilized for 5–6 min. They were further heated (at 41–42 °C for

50 ± 5 min). They were drained (separated whey titratable acidity increased by 3–4 °Th). The curd was collected for pressing (5–6 min at pressure 6 atm, 2 kg weight for 1 kg curd) and cut in 50–60 cm parallelepiped slices. The cheddaring process took place (40–60 min, until pH of the curd reached 5.20–5.25) and the cheddared curd was milled (slices with width of 2–3 mm) and salted in a hot water solution (at 70 °C and 14% salt content). The curd was formed in 0.5 kg mould and stabilized the forms by 4–5 turning. The stabilized fresh cheese (at 8–10 °C for 12–16 h) was unmould and further dried (at 6–8 °C for 2–3 days), packed and ripened (at 6–7 °C and relative humidity 70–75% for 60 days).

The chemical composition of the milk samples (dry matter, solid non-fat, milk fat, proteins, lactose, and minerals) as well as density and freezing point were determined by Lactoscan SFP Options Milk Analyzer (Milkotronic Ltd., Bulgaria). Titratable acidity was determined according to Thorner's method (BS 1111:1980) and pH was measured by a pH meter (model MS 2000, Mycrosist, Plovdiv, Bulgaria). Screening for residual antibiotics in milk was performed with BetaStar® S Combo rapid tests (Chr. Hansen, Denmark). The microbiological analysis of raw goat`s milk for SCC was determined by Ekoscope–FPS1 (Bultech 2000 Ltd, Bulgaria) and the total bacterial count (TBC) was determined by (ISO 4833-1:2013).

Cheese samples were analysed on the 1^{st} and 60^{th} day of ripening for the following chemical parameters: water content and dry matter (BS 1109:1989); fat content (ISO 3433:2008); total nitrogen by the Kjeldahl method (BS EN ISO 8968-1:2001); sodium chloride content (BS 8274:1982); titratable acidity (BS 1111:1980) and potentiometric pH measurement. Cheese samples were evaluated for the following microbiological indices: total lactic acid bacteria count (IDF Standard 117B:1997) and verified by (IDF Standard 122C:1996); psychrotrophic microorganisms (ISO 17410:2019); yeasts and moulds (BS EN ISO 6611:2006); coliforms (ISO 4831:2006); *L. monocytogenes* (BS EN ISO 11290-1:2017); *coagulase-positive Staphylococci* (BS EN ISO 6888-1:2005). The sensory evaluation of the cheese samples was carried out by (BS 15612:1983) according to the following parameters - taste and aroma, appearance, texture, cut surface and color.

Statistical analysis was done by computer processing of the results was performed using Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by LSD method. The results are presented as mean values ± SD (n = 12, per every 3-month analysed period which refers to 3 milk samples of each 2 cheese samples were produced of each 2 analysis were made) and were considered as statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The influence of seasonal variations on the chemical and microbiological composition of raw goat milk for the production of Kashkaval cheese is presented in Table 1.

During the study period, the indicators of dry matter, solid non-fat, proteins and minerals remained almost unchanged, and a similar tendency was observed for the indicator of density. In the summer months of the year, milk fat content reached its lowest value. These results were consistent with the results of Pamukova et al. (2020) for the period May - September 2017, where the percentage of fat in the milk from local goats averaged 3.5%, solids non-fat were about 8.2%, total protein - 3.1%, and the dry matter - was 11.8%. Comparable results were reported by Paskas et al. (2020) where the fat content of milk reached the lowest values during the summer period, followed by an increase, especially toward the end of lactation. A similar tendency was observed for the concentrations of dry matter and solid non-fat which was in agreement with the data described by Marcinkoniene & Ciprovica (2019). The lowest carbohydrate content recorded in goat milk in August was directly related to the higher somatic cell count in the milk. Kalevska & Kocoski (2013) explained this fact with the proliferation of somatic cells in milk. May be the reason why SCC were elevated (pathological or metabolic process) was a reason for that result. It can be suggested that the seasonal variations on milk is more accented than SCC. The freezing point is related to other properties of the milk e.g. dry matter. This indicator was relatively higher during the transitional periods of lactation, which was due to the higher milk fat content. The values of titratable acidity and active acidity remained within the normal limits during the studied period. The results for the variations in the chemical composition of raw goat milk during lactation were in agreement with the results reported by Sulejmani & Hayaloglu (2017). The rapid test did not detect any inhibitors in the milk, which was an important fact for the production of Kashkaval cheese.

Table 1. Chemical and microbiological properties of raw goat milk

Properties	May	August	November
SCC (thous cells mL^{-1})*	1,200 ± 1,440^{a}	1,750 ± 1,750^{b}	1,600 ± 1,600^{c}
Dry matter (%)	12.75 ± 0.34	12.00 ± 0.25^{a}	12.63 ± 0.30
Solid non-fat (%)	8.80 ± 0.10	8.50 ± 0.04^{a}	8.60 ± 0.05
Milk fat (%)	3.95 ± 0.05	3.50 ± 0.06^{a}	3.90 ± 0.05
Proteins (%)	3.40 ± 0.10	3.20 ± 0.11	3.30 ± 0.10
Lactose (%)	4.60 ± 0.10	4.45 ± 0.04^{a}	4.60 ± 0.08
Minerals (%)	0.80 ± 0.01	0.80 ± 0.02	0.80 ± 0.01
Density (g mL^{-1})	1.029 ± 0.002	1.029 ± 0.001	1.028 ± 0.001
Freezing point (°C)	-0.570 ± -0.001	-0.567 ± -0.001^{a}	-0.569 ± -0.001
Titratable acidity (°Th)	18 ± 1	17 ± 2	17 ± 1
pH	6.78 ± 0.03	6.72 ± 0.03	6.76 ± 0.01
Antibiotics and inhibitors	Negative	Negative	Negative
TBC (thous cfu mL^{-1})*	680 ± 250^{a}	910 ± 320^{b}	740 ± 300^{c}

$^{a-c}$ Means with different letters within a row are significantly different ($P < 0.05$); * The term thousand refers only for the first value.

The use of raw goat milk with known microbiological indices such as total bacterial count (TBC) and somatic cell count (SCC) ensured a high-quality cheese. The results indicated that the TBC in the milk samples varied in the range from 680 to 910 thous cfu mL^{-1} (Table 1). From the onset to the middle of the lactation period, this indicator tended to increase, then decreased again and reached a value of 740 thous cfu mL^{-1} at the end of the study period. The total number of somatic cells in the studied milk was found to increase with progressing lactation. The SCC at the beginning of lactation was relatively low - 1,200 thous cells mL^{-1}, and reached its highest value during the summer months of the year - 1,750 thous cells mL^{-1}, while at the end of the period it began to decline again, reaching 1,600 thous cells mL^{-1} (Table 1). The obtained data were in agreement with those reported by Paskas et al. (2020) who stated that SCC and TBC were the most variable indices during lactation and by Marcinkoniene & Ciprovica

(2020) who established that the SCC increased in the second lactation. According to Skeie (2014) the frequency of intramammary infection was due to different factors, such as – lactation phase, environmental condition, month of milking, parity of lactation, etc. In contrast, Sulejmani & Hayaloglu (2017) observed that the value of SCC was lower in summer goat milk. However, Puggioni et al. (2020) concluded that mastitis should be preferably monitored by testing goat milk at the peak of lactation.

The chemical composition of Kashkaval cheese samples during the ripening period is given in Table 2.

Table 2. Chemical composition of Kashkaval cheese during ripening

Properties	KL		KH		KM	
	1st day	60th day	1st day	60th day	1st day	60th day
Water content (%)	44.50 ± 0.24^{aA}	43.60 ± 0.23^{bA}	46.8 ± 0.22^{aB}	45.00 ± 0.23^{bB}	44.90 ± 0.23^{aA}	43.90 ± 0.25^{bA}
Dry matter (%)	55.50 ± 0.33^{aA}	56.40 ± 0.24^{bA}	53.90 ± 0.20^{aB}	54.80 ± 0.24^{bB}	55.10 ± 0.22^{aA}	56.10 ± 0.20^{bA}
NaCl (%)	2.20 ± 0.05^{aA}	2.30 ± 0.04^{bA}	2.20 ± 0.05^{aA}	2.50 ± 0.06^{bB}	2.10 ± 0.04^{aC}	2.20 ± 0.05^{bC}
Fat content (%)	26.00 ± 0.10^{aA}	26.50 ± 0.10^{bA}	25.00 ± 0.10^{aB}	25.50 ± 0.10^{bB}	27.00 ± 0.10^{aC}	27.50 ± 0.10^{bC}
Total protein (%)	27.50 ± 0.05^{aA}	26.50 ± 0.04^{bA}	25.00 ± 0.05^{aB}	24.00 ± 0.04^{bB}	26.00 ± 0.04^{aC}	25.00 ± 0.05^{bC}
Titratable acidity (°Th)	160 ± 2^{aA}	169 ± 2^{bA}	152 ± 2^{aB}	178 ± 1^{bB}	155 ± 2^{aC}	162 ± 1^{bC}
pH	5.10 ± 0.02^{aA}	5.02 ± 0.02^{bA}	5.20 ± 0.03^{aB}	4.95 ± 0.05^{bB}	5.20 ± 0.04^{aC}	5.03 ± 0.03^{bA}

$^{a\text{-}c}$ Means with different letters within columns, for the same sample but for a different ripening period, are significantly different ($P < 0.05$); $^{A\text{-}C}$ Means with different letters within columns, for the same ripening period, are significantly different ($P < 0.05$).

The quality characteristics of Kashkaval cheese was largely determined by the water content, as it affected by the metabolic activity of lactic acid bacteria from the starter culture during ripening. The number of somatic cells reflected on some chemical parameters of the final product. In the KH samples, a more intensive process of acid formation was observed due to the higher percentage of water content in the end of the ripening period, also connected with the accelerated physicochemical and biochemical processes. The dynamics in the development of acidity predetermined the increased hydrophilic properties of the milk protein, as a result of which the KH sample had a higher water content compared to the KL and KM samples. The results for the dynamic of water content during cheese ripening were comparable to the conclusions of Ivanov et al. (2018) and Mateva et al. (2020). A similar tendency was established by Niro et al. (2014) who found that for a period of 60th days of ripening, the values of the water content of Caciocavallo cheese decreased by 5.7%. During ripening, the salt content in all samples slightly increased. The intensity of the salting process was closely related to the water content of the samples and the acid formation. The salt content increased by 0.1% for samples KL and KM, and by 0.3% for KH. Similar results about NaCl content in Kashar cheese were also observed by Kavak (2020). According to Marinova et al. (2016) the physicochemical parameters of Kashkaval cheese from cow milk did not change significantly during the process of ripening. Almost similar pH values and salt content to the present study, but lower dry matter was reported for Kashar cheese ripened up to 60 days (Temizkan et al., 2014). At the same time, closer fat content but a higher protein value was found in Caciocavallo cheese (Perna et al., 2015). Cheeses obtained from milk with high SCC were with increased recovery of lactose in the curd and water retention (Stocco et al., 2019b). Our study revealed that the pH decreased during ripening in contrast to the results reported by Darnay et al. (2019) where the pH values increased slightly and remained higher than pH 5.0 during ripening. The fat content in cheese samples remained practically almost constant throughout ripening, without any significant differences being observed ($P > 0.05$). The chemical composition, microbial properties and types of milk, cheese–making steps and ripening conditions may well explain the above differences. Maybe not SCC directly influenced cheese parameters but milk composition changes, and SCC was just related indicator to this different outcome.

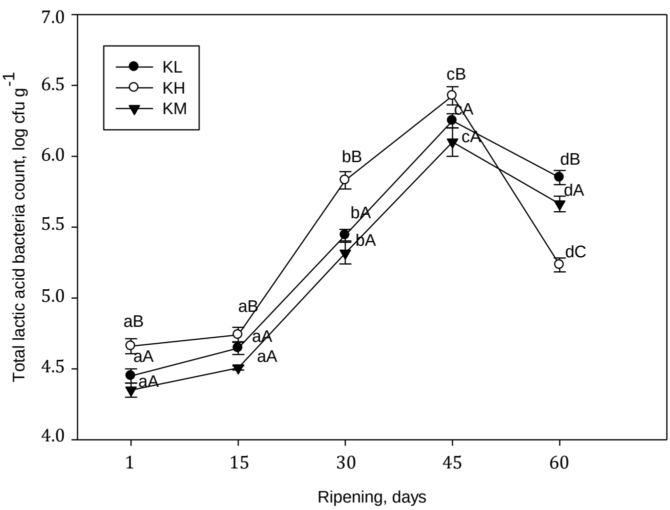

Figure 1. Change of total lactic acid bacteria count in Kashkaval cheese from goat milk during ripening.

$^{a-d}$ Means with different letters within a range are significantly different ($P < 0.05$); $^{A-C}$ Means with different letters within different series are significantly different ($P < 0.05$).

Fig. 1 illustrates the dynamics in the development of lactic acid microflora during ripening of the studied samples of Kashkaval cheese from goat milk during ripening. The results showed that at the beginning of the ripening period the total number of lactic acid bacteria in samples KL and KM was approximately 4.4 log cfu g^{-1}, and in KH it was 4.6 log cfu g^{-1}. These lower values of the total number of lactic acid bacteria in the three Kashkaval cheese samples were due to the hot salting technological process, leading to a significant reduction of the population of these microorganisms. In all samples, an increase in the amount of lactic acid microflora was recorded after 15 days of ripening. This was a result of the longer time required for the starter culture adaptation to the ripening temperature. In samples KL and KM, a gradual increase in the bacterial population was observed between the 15th and 45th days, but at the end of the studied period its levels decreased and reached a value of approximately 5.6 log cfu g^{-1}. A more intensive process of acid formation and a higher initial number of lactic acid bacteria were found in the KH samples. Thus, the reported higher values for water content in the KH samples (Table 1) correlated with the increase in the hydrophilicity of milk protein, leading to accelerated ripening process and faster accumulation of bacterial biomass between the 15th and 45th days of ripening. These results were similar to the data obtained by Sulejmani & Hayaloglu (2016) who studied the same influence on Turkish Kashkaval-type cheese Kashar. Our results were in agreement with Talevski et al. (2017) who concluded that a slight decrease in the active acidity after the 30th day could be noticed due to the presence of residual lactose fermented by active lactic acid bacteria at the ripening temperature from 10–12 °C. The count of lactic acid bacteria decreased after 45 days of maturation probably because of the depleted amount of lactose and the low pH.

The results about the change in nonstarter microflora in samples of Kashkaval cheese from goat milk during ripening are given in Table 3.

Table 3. Survival of nonsarter microflora in Kashkaval from goat milk during ripening

Nonstarter microflora	KL		KH		KM	
(log cfu g^{-1})	1st day	60th day	1st day	60th day	1st day	60th day
Psychrotrophic microorganisms	2.04 ± 0.30^{aA}	3.36 ± 1.00^{bA}	3.32 ± 1.18^{aB}	4.53 ± 1.65^{bB}	2.11 ± 0.70^{aC}	3.32 ± 1.11^{bC}
Yeasts and moulds	< 1	< 1	< 1	< 1	< 1	< 1
Coliforms	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	ND	ND	ND
Coagulase-positive staphylococci	ND	ND	ND	ND	ND	ND

$^{a-b}$ Means with different letters within columns, for the same sample but for a different ripening period, are significantly different ($P < 0.05$); $^{A-C}$ Means with different letters within columns, for the same ripening period, are significantly different ($P < 0.05$).

It was found that during ripening the number of psychrotrophic microorganisms increased significantly ($P < 0.05$) in the three samples of Kashkaval cheese. The data in Table 2 showed that the higher levels of titratable acidity and the lower pH values favoured the development of psychrotrophic microorganisms. According to Faccia et al. (2013) *Pseudomonas* spp. counts were similar to those commonly found in bovine mozzarella cheese with high moisture content. The presence of moulds and yeasts in the tested cheese samples was significantly low ($P > 0.05$), which suggested that their growth was inhibited during the ripening period. Our findings coincided with the results described by Pappa et al. (2020) who found low levels of moulds and yeast in samples of Kashkaval cheese produced in the mountainous region of Pindus, Greece. No coliform bacteria, coagulase-positive staphylococci or listeria monocytogenes were detected in this study. Mastromatteo et al. (2014) concluded that the right control of the growth and activity of these microorganisms during cheese ripening were essentially important because they caused the quality alteration of cheese. This was in accordance with the hygienic quality of Fior di latte cheese from goat milk stated by Faccia et al. (2015). These findings may be awarded to the good microbiological quality of the raw goat milk and the thermal effect of the stretching process, which had a preservative effect on Kashkaval cheese (Pappa et al., 2019; Samelis et al., 2019). According to the results obtained in this study, it can be concluded that the combination of good quality goat milk (samples KL and KM), which was subjected to thermisation and subsequent heat treatment of the curd during the stretching process at 72 °C possibly had a positive preservative effect on Kashkaval cheese.

The sensory profiles of Kashkaval samples from goat milk were evaluated at the end of the ripening process and are presented in Fig. 2.

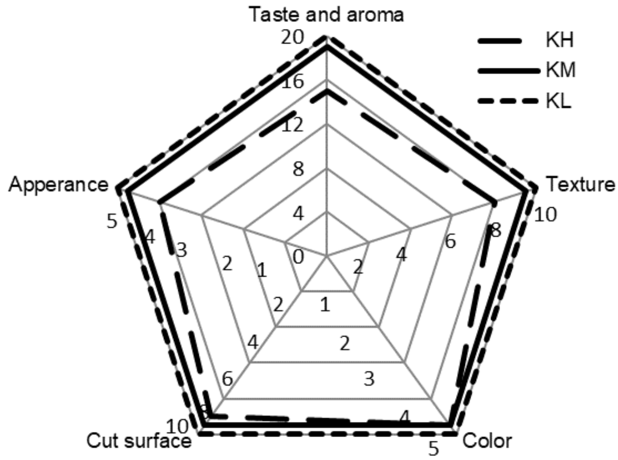

Figure 2. Sensory evaluation of Kashkaval cheese from goat milk after ripening.

Samples KL and KM were characterized by better flavour and aroma, specific for the respective type of cheese, while the KH samples were given lower sensory scores. According to Chen et al. (2010) high SCC (1,000 thous cells mL^{-1} < SCC < 1,500 thous cells mL^{-1}) in goat milk seemed to affect the sensory characteristics and quality of matured cheeses. As a result, KL and KM had a smooth, homogeneous structure, uniform creamy-yellow colour, dense and elastic consistency, distinct aroma of mature Kashkaval cheese, specific for the type of milk from which it was produced. The lower scores awarded to the indicators flavour and aroma, cut surface and consistency were a result of the intensity with which the biochemical reactions occurred during the ripening period in the KH samples. At the end of the study period, the KH samples had a very strong specific aroma, slightly pungent taste, soft texture and rich amber colour. These data were in agreement with the findings of Semjon et al. (2019), who associated the modification of the elasticity of cheese due to a more pronounced lactic acid bacteria activity and resulting proteolysis. Bezerra et al. (2020) also reported that a high SCC in raw milk had a significant effect on the quality of pasteurized milk and Coalho cheese as it presented lower sensory acceptance.

CONCLUSIONS

The results showed that the high number of somatic cells was related to some chemical parameters such as fat and lactose content, as well as the freezing point. The high number of somatic cells, in combination with the increased total number of microorganisms, led to increased water content in the samples of Kashkaval produced from the correspondent milk which made it worse compared to the other variants. Reduced active acidity favoured the development of a great number of psychrotrophic microorganisms in the sample with high number somatic cells. Significant differences in the sensory profile of the samples containing high levels of somatic cell count were observed. Its appearance, texture, taste and aroma were deteriorated. However, further work is required to evaluate the relation between SCC and the level of proteolysis and lipolysis during the ripening and storage periods.

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Research into geometric parameters of digging shares used for lifting sugar beet roots from soil with assistance of vibration

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Abstract. One of the important conditions in securing the high quality, when performing the work process of vibrational root lifting, is to avoid damaging the roots. It is obvious that the greatest probability of damaging and even breaking the lifted root arises, when the tool interacts with the root body during their first contact and in the time of the root passing in the throat between the operating shares. The aim of the study is to substantiate the rational design length for the working throat of the vibrational root lifter in its interaction with the sugar beet root while lifting the latter from the soil. As a result of the completed research, the minimum permissible tool oscillation frequencies have been determined for the specific values of the lifter's translational velocity and the working throat rear part length, at which the event of the vibrational lifting tool gripping the root will occur at least one time. For example, when the length of the lifter's working throat rear part is equal to 0.1 m and the oscillation frequency is equal to $\nu = 20.3$ Hz, the satisfactory quality of the vibrational root lifting process is ensured, when the velocity of the translational motion performed by the vibrational lifter stays within the range of 1.3–2.55 m s^{-1}. In order to ensure the good quality of the vibrational root lifting process at the lifter's translational velocity equal to $V = 2.0$ m s^{-1} and the frequency of its tool's oscillations equal to $\nu = 10$ Hz, it is necessary that the length of the lifter's working throat rear part is equal to 0.2 m, at a tool oscillation frequency of 6.7 Hz - 0.3 m. As a result of the completed numerical calculations, the permissible values have been determined for the tool oscillation frequency, which can be recommended for the translational velocities within the range of 1.3–2.2 m s^{-1}, taking into account the limitation set for the tool oscillation frequency by the pre-condition of the guaranteed gripping of each root by the digging shares.

Key words: amplitude, frequency, oscillation, sugar beet root, vibrational lifting tool.

INTRODUCTION

Not damaging sugar beet roots and preventing their loss are important conditions in securing the high quality, when performing the work process of vibrational sugar beet root lifting from the soil (Dobrovsky, 1968; Vasilenko et al., 1970; Sarec et al., 2009; Schulze Lammers, 2011). The greatest probability of damaging the roots and even breaking them arises during the interaction between the digging tool and the root body in the time of their vibrational contact and when the root moves in the throat between the faces of the vibrational lifter. In view of the fact that the above-mentioned interaction takes place during the first contact between the vibrational lifting tool and the root body, when the latter is still strongly bonded with the surrounding soil and a side face of the lifter positively acts on it (one or both faces), damage to the roots appears to be even inevitable because of the lifter's translational motion velocity and the velocity of its oscillatory motion, especially in case the roots are lifted from the dry and firm soil (Bulgakov et al., 2014; 2015a, 2015b). During such a contact within a rather short time interval, the conditions arise that can result in the complete break-off and loss of the root's tail part.

An important issue in the theoretical investigation of the work process under consideration is to determine the length of the vibrational lifter's working throat rear part that begins at the point of its first contact with the beet root and ends at the end of the working throat (where the final lifting takes place), that is, the length of the lifter's working faces, within which their continuous contact with the root and the latter's direct lifting from the soil occur. The said length can vary (taking into account the difference in the design solutions that take into consideration the irregular sizes of the roots, their depth of sitting in the soil etc.), however, it has to have some mean value l, which could in the future be used in the designing of such vibrational tools for sugar beet root lifting that are appropriate for the successful use in state-of-the-art root harvesters.

Hence, a topical problem arises of researching into the above-mentioned interaction between the sugar beet root body sitting in the soil and the digging shares of the vibrational lifting tool, which would enable determining the rational design and kinematic parameters of the tool, under the pre-condition of not damaging (no break-off and no loss of) the roots during the said interaction that takes place in the time of their vibrational lifting.

Sufficiently thorough studies on the process of lifting sugar beet roots from the soil with the use of vibration are presented in the papers (Vasilenko et al., 1970; Vermeulen & Koolen 2002; Bulgakov, 2005; Bulgakov et al., 2005; Gruber, 2005, 2007; Bulgakov & Ivanovs, 2010; Gu et al., 2014). Among other issues, these studies examine in detail the interaction between the vibrational lifting tool and the sugar beet root body, when the latter is imparted a vibrational action with the parameters that ensure its lifting from the soil. In many vibrational lifting tool designs, the construction features stipulate that the direct lifting of the root from the soil takes place in its interaction with different parts of the lifter, in particular, in the time of the root being gripped and during its further translation along the tapered working throat. The vibrational lifting tools installed in many root harvesters feature lifting faces (digging shares) not only of different sizes, but also with working edges (blades) of different shapes and having different positions, inclinations in space etc. (Schulze Lammers & Schmittmann, 2013; Boson et al., 2019).

In the above-noted articles, the interaction between the sugar beet root body and the shares of the vibrational lifting tool is discussed for only one or two points of contact, while in reality the sugar beet root body in the process of its lifting from the soil makes in a successive order contact with different points of the digging shares during the translational motion of the vibrational lifting tool. The points of contact change their positions along both the longitudinal and the vertical axes of the shares gripping the root body on its both sides. Hence, the progression of the sugar beet root body along the tapered throat of the vibrational lifting tool, first of all, in terms of the basis for determining the length of the working throat in the vibrational lifting tool is not discussed in the papers.

Only the studies (Pogorely et al., 1983; Pogorely & Tatyanko, 2004) provide some results of the experimental investigations on the frequency of the interaction between the root body and the lifting tool, which depends not only on the translational velocity of the lifter, but on the length of the working throat in the vibrational lifting tool as well.

All the described circumstances make it necessary to carry out the theoretical research that would enable establishing the geometrical and kinematic parameters of the digging shares, which form a vibrational lifting tool for sugar beet roots.

The aim of the research is to justify the rational geometric parameters of the new design of digging shares for the vibrational lifting of sugar beet roots from the soil in relation to the frequency and amplitude of their oscillation and the velocity of their translational motion.

MATERIALS AND METHODS

The authors have developed a design of the vibrational lifting tool for sugar beet roots (Bulgakov et al., 2020). The 3D model and the schematic process model of the design are presented in Fig. 1. This lifting tool has been subjected to comprehensive production testing, which has proved the tool's high efficiency in lifting sugar beet roots from the soil. In order to validate the design and kinematic parameters of the discussed vibrational lifting tool that would ensure minimising the damage to and loss of sugar beet roots, the authors have carried out theoretical and experimental investigations of the tool.

In the new design, the digging shares 1 of the vibrational tool under consideration are linked with the use of the kinematic tie link 3 with the vibration generation device (not shown in Fig. 1) that imparts to the digging shares 1 oscillatory motion in the longitudinal and vertical plane with pre-set amplitudes and frequencies. The vibration generation device provides for setting different amplitudes and frequencies of the oscillations performed by the digging shares 1 in the above-mentioned plane, depending on the condition of the soil at the time of lifting sugar beet roots. In order to prevent the fallout and loss of sugar beet roots lifted from the soil at the final stage of their lifting from the soil, on the back sides of the posts 3, immediately above the rear parts of the digging shares 1, the cylinder-shaped extension pins 4 are installed with appropriate spacing. The sets of extension pins 4 placed on each of the posts 2, i.e. on both sides of the rear part of the tapered throat, effectively make a grill-shaped extension of the throat, which travels during the lifter's operation above the soil surface and at the same time performs oscillatory motion in the longitudinal and vertical plane. The kinematic tie link 3 has such a design that not only it is possible to impart to the posts 2 and the digging shares 1 the parameters of the vibration process, but also the shares 1 are capable of self-aligning (within relatively narrow limits) with respect to the row of planted sugar beet roots.

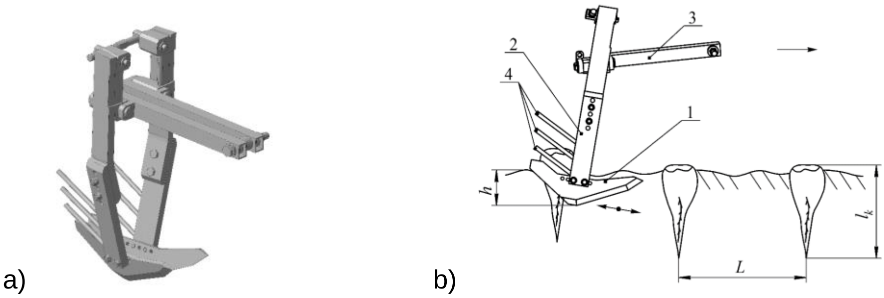

Figure 1. 3D model (a) and design and process schematic model (b) of vibrational lifting tool for sugar beet roots: 1 – digging shares; 2 – posts; 3 – kinematic tie link between posts and vibration generation device; 4 – extension pins.

The digging shares of the vibrational lifting tool under consideration run at the pre-set depth h in the soil, the value of which is within the range of 0.06–0.1 m. The depth, through which the sugar beet roots sit in the soil, i.e. the actual length of the roots, is designated h_k and is equal to 0.23–0.30 m for the majority of sugar beet varieties. In fact, the said root length represents the sugar-bearing mass content in the root body, therefore, any loss of or damage to the sugar beet roots in the process of their vibrational lifting are undesirable. The spacing between any two adjacent beet root bodies in the sowed row designated L is a random value. In order to provide for the optimum yield, it has to stay within the range of L = 0.16–0.23 m. In that case, one running metre of planted sugar beets contains 4–6 pcs of sugar beet roots.

For the purpose of theoretically substantiating the design parameters of the vibrational lifting tool, the principal dimensions of one of its digging shares and of the working throat in the lifting tool as a whole are shown in Fig. 2.

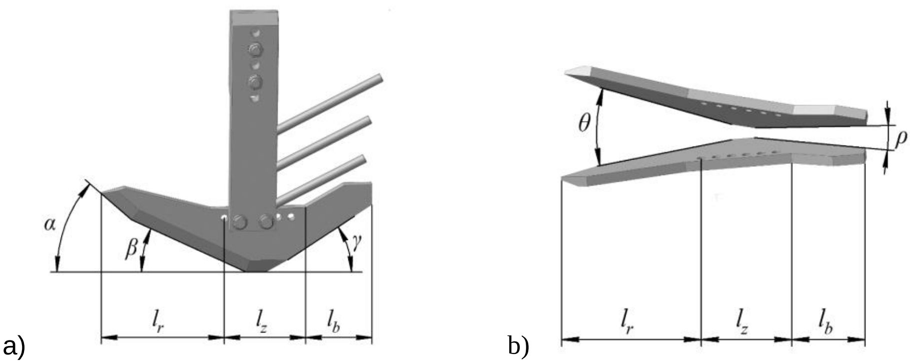

Figure 2. Structural dimensions of digging share (a) and working throat (b) of vibrational lifting tool.

As can be seen in the schematic model in Fig. 2, the most forward part of the digging share defined by the length l_r and featuring a complex shape in the longitudinal and vertical plane has a tapered cutting edge of the blade defined by the following two angles: α – angle of inclination to the horizon of the blade's pointed end section, β – angle of inclination to the horizon of the front blade's lower edge. At the same time, the angle of inclination to the horizon α of the blade's pointed end edge is of greater importance, than the angle of inclination to the horizon β of the blade's lower edge. It is exactly in the forward section of the vibrational lifting tool, where the distance between the fore ends of the shares in the horizontal plane is the greatest, and, correspondingly, which is the part that forms the angle θ of the fore part (mouth) in the working throat of the vibrational lifting tool, and this angular part ensures catching all the roots positioned in the row with some deviations from its centreline. The middle section of the share defined by the length l_z is, effectively, the area of the first contact between the share and the sugar beet root body, which by this moment is still fixed in the soil. Within this length not only the first contact with the root body is made, but also the root becomes firmly gripped in the working throat that gradually gets narrower and entrained in the movement and moves further along the whole narrowed throat of the vibrational lifter. It is exactly that part of the share, where the throat is at its narrowest, and which has a tilt with respect to the longitudinal and vertical plane. These features of the part make it possible to pull the root body out from the soil within a short time interval due to the upward motion of the shares' working faces in their vibrational oscillations. Finally, the rear parts of the digging shares are defined by the length l_b, the shortest of the three lengths, and are inclined upward at an angle of γ to the horizon, while the working throat here features some divergence (at an angle of ρ), because exactly at this part of the vibrational lifting tool the sugar beet root body must be released from the vibrational lifter for its prompt feeding onto the cleaning tool.

In order to substantiate the design parameters of the vibrational lifting tool, first of all, of its working shares, it is necessary to analyse the above-mentioned contact between the root body and the tool and to find the analytical relation between the number of oscillations performed by the vibrational lifting tool in its interaction with the root within the time, when the latter resides in the area of the working throat, on the one hand, and the length of the working throat's rear part, the oscillation frequency and the translational velocity of the lifter, on the other hand (Babakov, 1968; Schmitz & Smith, 2012). If l – distance from the point of the first contact with the root to the end of the lifter's working throat and V – velocity of the translational motion performed by the lifter, the duration of the period, when the root resides in the said area of the working throat, is equal to:

$$t_p = \frac{l}{V} \cdot \tag{1}$$

Within this time, the tool performs the following number of oscillations:

$$k = \nu \frac{l}{V}, \tag{2}$$

where ν – vibrational tool oscillation frequency (Hz).

For example, assuming in accordance with Pogorely & Tatyanko (2004), that $V = 2$ m s^{-1}, $\nu = 20$ Hz, $l = 0.1$ m (the minimum possible value of the length), the vibrational lifting tool will perform one oscillation ($k = 1$) within the time that the root is found in the rear part area of the lifter's working throat.

The next task is to determine the number of times that the vibrational lifting tool grips the root within the time that the latter resides in the rear part area of the lifter's working throat, if $k = 1$, that is, in case the tool completes one full oscillation within the above-mentioned time.

The process under consideration can follow two possible scenarios.

The first scenario. The first direct contact between the tool and the root occurs at the moment, when the tool is in its upward movement from its lowest position to the highest one. The period of the tool's oscillation is designated τ. As, in this scenario, the perturbing force is vectored upward, the above-mentioned first contact between the tool and the root is also the first time, when the tool grips the root, which initiates the process of breaking up the bonds between the root and the soil. The gripped condition continues up to the moment, when the tool reaches its highest position. The duration of this condition is designated t_1. It is obvious that:

$$t_1 = s_1 \tau \, , \tag{3}$$

where $0 \le s_1 \le 0.5$ – factor that indicates, during what part of the oscillation period the first gripping of the root by the tool takes place.

For example, if $s_1 = 0.5$, it means that the first gripping of the root starts in the lowest position, therefore, $t_1 = 0.5\tau$. If $s_1 = 0$, it implies that the first contact starts in the highest position, consequently, $t_1 = 0$. All the other values of s_1 that comply with the above in equation correspond to the start of the gripping at any instant of time during the movement of the tool from its lowest position to the highest one.

Having reached the highest position, the tool starts moving downwards. In view of the conical shape of the root, the perturbing force stops acting on the root, hence, the gripping of the root is absent. However, the loss of contact between the root and the tool is rather unlikely in view of the translational motion of the lifter and the tapered shape of its working throat. As the perturbing force no longer acts on the root, the latter will attempt returning into the vertical position due to the elastic stiffness of the soil and its own elastic stiffness. In this case, the root will possibly have a small forward inclination as a result of the lifter's translational motion. The described situation will be observed within the period of time $t_2 = 0.5\tau$, during which the tool moves from its highest position to the lowest one. After that, the tool again switches to moving upwards from its lowest position to the highest one.

Hence, within the period of time $t_3 = \tau - (t_1 + t_2)$, the second instant of the root being gripped by the tool will take place, which will initiate the further process of disrupting the bonds between the root and the soil right up to the direct lifting of the root. In case the time of the root residing in the rear part of the working throat (after the first contact) does not exceed τ, the root must without fail be completely lifted from the soil within the time of the second gripping. Otherwise, the root will be left in the soil, i.e. either it will be sheared off by the shares or it will obstruct the lifter's working throat.

But if the root is fixed in the soil not that strongly, it is not improbable that its lifting from the soil can take place immediately during its first gripping by the vibrational lifting tool.

The second scenario. The first direct contact between the tool and the root occurs at the moment, when the tool is in its downward movement from its highest position to the lowest one. This will take place during the period of time $t_1 = s_1\tau$, where $0 \le s_1 \le 0.5$.

In this case, no perturbing force generated by the vibrational tool acts on the root. After the tool reaches its lowest position, it starts moving in the opposite direction, i.e. upwards from its lowest position to the highest one. At this instant of time, the root is gripped by the tool for the first time and this gripping lasts for a time of $t_2 = 0.5\tau$, until the tool reaches its highest position. After that, the tool starts its downward movement and, within the time period of $t_3 = \tau - (t_1 + t_2)$, again no perturbing force acts on the root, i.e. there is no gripping of the root within this time interval.

In the second scenario, within the time period of $t_1 + t_2 + t_3 = \tau$, the root is gripped by the tool only once. If in this scenario the time of the root residing in the rear part of the working throat (after the first contact) does not exceed τ, the complete lifting of the root from the soil must take place during this single gripping. Otherwise, the root will be left in the soil not lifted.

Solely the act of gripping can be insufficient for digging out the root that has strong bonds with the soil. In such a case, it is necessary to increase significantly the gripping force. However, that can result in the disintegration and rupture of the root body itself. It is quite obvious that a single gripping of the root at a certain depth, which promotes its separation from the surrounding and holding it soil, and the further movement of the root in the narrowed throat of the lifting tool on the inclined faces of the shares are sufficient for the complete lifting of the root.

In case of $k < 1$ (vibrational lifting tool has no time to complete one full oscillation within the time of the root residing in the rear part of the working throat), under the first scenario only one instant of the root being gripped by the tool can occur, under the second scenario - none. Hence, the root has to be lifted from the soil either within one act of gripping by the tool or, in the last resort, during its movement in the narrowed working throat of the lifter due to the latter's translational motion (as happens in the standard share-type lifting tool). However, an attempt to lift a root firmly fixed in the soil through its movement in the narrowed throat due to the lifter's translational motion can result in the sudden tilting of the root in the direction of the lifter's motion and its breaking off. Also, at $k < 1$ a root firmly fixed in the soil can be not lifted within one act of gripping by the vibrational lifting tool.

The relation between the vibrational lifting tool's oscillation frequency, its translation velocity and the length of the working throat must be such that the tool is capable of performing more than one full oscillation within the time of the root residing in the rear part of the working throat, that is, the condition $k > 1$ must be satisfied.

For example, if $k = 2$ (when the length l of the rear part of the lifter's working throat is equal to 0.2 m) and the first scenario takes place (the first contact between the tool and the root occurs during the tool's upward movement), the tool will grip the root twice within the period of the first oscillation and once - within the period of the second oscillation. In case the second scenario takes place (the first contact between the tool and the root occurs during the downward movement of the tool), the tool will grip the root once within the periods of both the first and the second oscillations. That is, at $k = 2$, the digging shares of the vibrational tool will grip the root either three times or, at worst, two times.

In the general case, at $k = n$, where n – some natural number, it follows from the above considerations that the tool can perform $n + 1$ or n acts of gripping the root.

The greater k is, the smoother and higher-quality the process of lifting the root from the soil by the vibrational lifting tool will be, because in case of greater numbers of oscillations per root it is possible to apply smaller perturbing forces for lifting the roots and, accordingly, to reduce the probability of rupturing the root bodies. Moreover, the greater k is, the greater number of oscillations the root performs together with the tool and, consequently, the better it is cleaned from the soil stuck to it.

An increase in the value of k can be achieved either by increasing the tool's oscillation frequency and the length of its working throat or by reducing the velocity of the lifter's translational motion. Still, even in case of $k = 1$, where the tool effects only two acts of gripping the root within the time that the root resides in the rear part of the lifter's working throat, the oscillatory process takes place and the root is displaced by the perturbing force, then returns to the original position under the action of the restoring forces (elastic force of the soil and elastic force of the root itself). At $k > 1$, the oscillatory process that promotes the guaranteed lifting of the root from the soil takes place.

After specifying the number k ($k \geq 1$) of the oscillations performed by the vibrational lifting tool during the time, in which one root passes the lifter's working throat, it is always possible to find the relation between the parameters ν, l and V in accordance with the expression (2). For specific values of l and V, the following can be derived from the expression (2):

$$\nu = \frac{kV}{l} \cdot \qquad (4)$$

In this way, the minimum vibrational lifting tool oscillation frequency that ensures the rational performance of the vibrational root lifting process is determined. If $k = 1$ (one oscillation of the tool per root), the following is derived from the expression (4):

$$\nu = \frac{V}{l}. \qquad (5)$$

When the frequency of the oscillations performed by the vibrational lifting tool is set below the value obtained in the expression (4), the conditions required for the vibrational lifting of roots are not provided. That means that some roots will not be gripped by the tool in the vibrational process and, therefore, will remain not lifted or will be broken in their tail parts, which will be left in the soil. All that will result in the undesirable loss of the roots in the process of their lifting.

RESULTS AND DISCUSSION

The unjustified relation between the parameters k, ν, l and V is one of the principal reasons for the loss of part of the roots during their vibrational lifting with the existing beet harvesters.

As may be inferred from the graphs (Fig. 3) generated in accordance with the expression (4), the growth of the lifter's translational velocity results in the increase of the minimum permissible tool oscillation frequency that ensures the single gripping of the root by the tool.

In order to analyse in more detail the relation between the minimum permissible frequency ν of the oscillations performed by the vibrational lifting tool and the length l of its working throat rear part, the diagram of the relation has been generated for the specific values of the velocity V of the translational motion performed by the lifting tool (Fig. 4).

As is proved by the graphs (Fig. 4), at a pre-set velocity *V* of the translational motion performed by the vibrational lifting tool, an increase in the length *l* of its working throat rear part results in the decrease of the value of the minimum permissible frequency *ν* in accordance with the hyperbolic law.

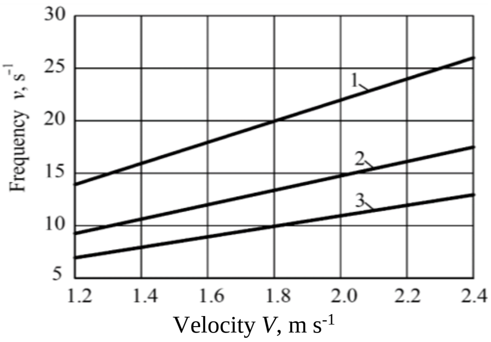

Figure 3. Relation between minimum permissible frequency *ν* of oscillations performed by vibrational lifting tool and velocity *V* of its translational motion at following values of length of working throat rear part: 1) $l = 0.10$ m; 2) $l = 0.15$ m; 3) $l = 0.20$ m.

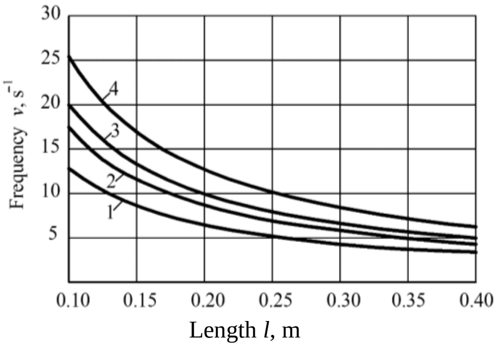

Figure 4. Relation between minimum permissible frequency *ν* of oscillations performed by vibrational lifting tool and length *l* of its working throat rear part at following values of tool's translational motion velocity: 1) $V = 1.30$ m s^{-1}; 2) $V = 1.75$ m s^{-1}; 3) $V = 2.00$ m s^{-1}; 4) $V = 2.55$ m s^{-1}.

The use of the presented graphic relations provides a possibility to determine the minimum permissible frequencies *ν* for various values of the length *l* at the pre-set velocity of translational motion $V = 2.0$ m s^{-1}, which corresponds to the operational rate of travel of the state-of-the-art root harvesters. The following is obtained:

at $l = 0.1$ m: $\nu = 20$ Hz;
at $l = 0.2$ m: $\nu = 10$ Hz;
at $l = 0.3$ m: $\nu = 6.7$ Hz;
at $l = 0.4$ m: $\nu = 5.0$ Hz.

At a translational velocity of $V = 2.0$ m s^{-1}, the values obtained for the minimum frequency *ν* of the oscillations performed by the vibrational lifting tool ensure that the digging shares perform at least one act of gripping and impart to the root body the upward vibrational motion, which guarantees the proper performance quality of the vibrational lifting of the roots from the soil.

For each specific value of the velocity *V* of the lifter's translational motion and the length *l* of its working throat rear part, the specific value of the minimum frequency exists, below which the vibrational root lifting process is impaired, that is, some roots are not lifted by the vibrational lifting tool. As is seen in the graph (Fig. 3), at $l = 0.1$ m a frequency of $\nu = 20$ Hz ensures the satisfactory vibratory process of lifting roots from the soil for all values of the velocity *V* of the lifter's translational motion below 2.0 m s^{-1}, while at $l = 0.15$ m a frequency of $\nu = 20$ Hz ensures the satisfactory vibratory root lifting process for all values of the velocity *V* below 3.0 m s^{-1}.

At $l = 0.1$ m, if it is necessary to ensure the operation at the lifter's translational velocity equal to $V = 2.0$ m s^{-1}, all the permissible frequency value ranges obtained under the pre-condition of not damaging the roots during their force interaction with the tool must be limited from below by the frequency value $\nu = 20$ Hz.

On the other hand, in case the frequency values permissible for impact interaction obtained for some kinematic conditions are below $\nu = 20$ Hz, they automatically do not meet the requirements to the satisfactory performance of the vibratory root lifting process at the length of the working throat rear part $l = 0.1$ m and the lifter's translation velocity $V = 2.0$ m s^{-1}.

The results obtained in the theoretical investigations were verified by conducting experimental investigations under field conditions. The experimental investigations were carried out in a plot with sugar beet roots planted in the soil, the physical and mechanical properties of which had already been analysed by the authors. The primary properties of the soil, in which the sugar beet roots were sitting, determined by the authors were its moisture content and hardness. The soil parameters had been measured in multiple replications as in the inter-row spacing so in the rows.

During the investigations, the soil moisture content W was equal to 18%, its value was determined with the use of the weight method based on the dehumidification of the soil sample and its further weighing. According to the said method, the soil moisture content W (%) was found with the use of the following formula:

$$W = \frac{m_1 - m_2}{m_2 - m} \cdot 100, \qquad (6)$$

where m_1 – mass of humid soil complete with container and lid (g); m_2 – mass of dry soil complete with container and lid (g); m – mass of empty container (g).

The hardness of the soil was determined with the use of the Revyakin hardness tester and the standard method (Medvedev, 2009) that stipulates measuring the hardness as the magnitude of the force needed for the indenter with known parameters to penetrate into the soil. Hence, the soil hardness T (Pa) was obtained with the use of the following expression:

$$T = \frac{P}{S}, \qquad (7)$$

where P – mean force of the soil's resistance to the penetration of the indenter into it (N); S – area of indenter (m^2).

In accordance with the scale proposed by Medvedev (2009), the hardness value of 1.8 MPa obtained by the authors for the soil in the analysed beet root field plot corresponded to a firm soil, which is typical for heavy loam soils in the autumn season, that is, the period of sugar beet harvesting.

The data of the theoretical conclusions have been rather accurately proved by the experimental investigations carried out by the authors for determining the mass of the lost sugar beet roots. It has been established that at the velocity of the translational motion performed by the vibrational lifting tool $V = 2.1$ m s^{-1} and the frequency of the oscillations performed by its digging shares $\nu = 20.3$ Hz the mass of the lost sugar beet roots amounts to 0.64%, at the frequency $\nu = 15.7$ Hz – 2.2%, at the frequency 8.5 Hz – 3.48% (Table 1).

At the velocity of translational motion equal to $V = 2.1$ m s^{-1}, the oscillation frequency $v = 20.3$ Hz ensures the satisfactory performance of the vibrational sugar beet root lifting from the soil, while the frequencies $\nu = 15.7$ Hz and $v = 8.5$ Hz deliver the insufficient quality of the beet root lifting from the soil, i.e. some roots are not lifted by the tool or get broken off in their tail parts. That is supported by the calculations carried out in accordance with the expression (4).

The same patterns are observed also at the lifter's translational motion velocities $V = 1.3$; 1.75; 2.55 m s^{-1} (Table 1).

Table 1. Mass of lost sugar beet roots (%)

Translational velocity (m s^{-1})	Oscillation frequency (Hz) (X_1)								
	8.5			15.7			20.3		
	Running depth in soil (m) (X_2)								
	0.06	0.09	0.12	0.06	0.09	0.12	0.06	0.09	0.12
1.3	2.7	2.7	1.9	1.7	0.5	0.4	0.4	0.1	0.2
	2.9	2.7	1.8	1.9	0.5	0.5	0.4	0.3	0.4
	2.8	2.8	1.9	1.7	0.4	0.5	0.3	0.4	0.4
	2.6	2.7	1.8	1.8	0.5	0.3	0.4	0.4	0.3
	2.7	2.6	1.8	1.8	0.6	0.4	0.4	0.5	0.4
1.75	2.9	1.8	2.0	1.9	0.4	0.6	0.3	0.4	0.5
	2.9	2.0	2.1	2.0	0.5	0.6	0.6	0.4	0.5
	3.0	1.9	2.6	1.9	0.5	0.7	0.5	0.5	0.6
	3.2	2.0	2.0	1.8	0.4	0.8	0.4	0.3	0.4
	2.8	1.9	2.4	1.9	0.5	0.7	0.3	0.4	0.5
2.1	3.5	0.6	2.7	2.3	0.2	0.9	0.7	0.5	0.6
	3.5	0.4	2.4	2.2	0.4	0.8	0.6	0.6	0.6
	3.2	0.6	2.7	2.1	0.2	0.9	0.6	0.4	0.4
	3.6	0.5	2.6	2.2	0.3	1.0	0.6	0.5	0.5
	3.6	0.6	2.8	2.2	0.2	0.9	0.7	0.5	0.3
2.55	4.2	0.9	3.1	2.6	0.6	1.2	0.9	0.8	0.3
	4.3	0.9	3.2	2.1	0.8	1.1	1.1	0.5	0.6
	4.2	0.8	2.9	2.4	1.2	1.2	1.4	0.6	0.4
	4.5	0.9	2.8	2.5	0.7	1.3	1.2	0.7	0.6
	4.3	0.9	2.9	2.6	0.8	1.2	1.1	0.7	0.6
(X_3)	(Y_1)								

When the translational velocity is equal to $V = 1.3$ m s^{-1}, at the oscillation frequency $v = 20.3$ Hz the mass of the lost beet roots amounts to 0.38%, at the frequency $v = 15.7$ Hz – 1.78%, at the frequency $v = 8.5$ Hz – 2.74%. The frequencies $v = 20.3$ Hz and $v = 15.7$ Hz provide for the satisfactory performance of the vibrational root lifting, the frequency $v = 8.5$ Hz does not.

When the translational velocity is equal to $V = 1.75$ m s^{-1} and the running depth in the soil is equal to 0.06 m, at the oscillation frequency $\nu = 20.3$ Hz the mass of the lost roots amounts to 0.42%, at the frequency $v = 15.7$ Hz – 1.9%, at the frequency $v = 8.5$ Hz – 2.96%.

If the velocity of translational motion is equal to $V = 2.55$ m s^{-1} and the lifter's running depth is the same, at the oscillation frequency $\nu = 20.3$ Hz the mass of the lost roots will amount to 1.14%, at the frequency $\nu = 15.7$ Hz – 2.44%, at the frequency $\nu = 8.5$ Hz – 4.3%.

This pattern can be seen rather clearly in the results of the experimental investigations carried out by the authors, as presented in Table 2, where the mass of the lost roots (%) is determined for the tool oscillation frequency $\nu = 8.5$ Hz.

The obtained research results provide grounds for stating that, when the running depth in the soil of the vibrational lifting tool is equal to 0.06 m, the mass of the lost roots has the following (mean) values:

at $V = 1.4$ m s^{-1} – 1.2%;
at $V = 1.65$ m s^{-1} – 4.9%;
at $V = 2.1$ m s^{-1} – 6.2%.

When the running depth in the soil of the lifting tool is equal to 0.08 m, the following results have been obtained with regard to the root loss rate:

at $V = 1.4$ m s^{-1} – 1.2%;
at $V = 1.65$ m s^{-1} – 2.1%;
at $V = 2.1$ m s^{-1} – 4.2%.

The above losses arise from breaking off the tail parts of sugar beet roots and outright not lifting some part of them due to the fact that the tool oscillation frequency $\nu = 8.5$ Hz does not ensure the proper gripping of each root by the digging shares.

It is obvious that the root loss rate at the running depth of the vibrational lifting tool's digging shares in the soil equal to 0.06 m is greater than at the running depth in the soil equal to 0.08 m. This is due to the increase in the number of the roots not lifted from the soil.

Table 2. Mass of lost sugar beet roots (%) at oscillation frequency equal to 8.5 Hz (when soil penetration resistance is equal to 1.8 MPa (at the maximum digging share running depth in the soil equal to 0.12 m), soil moisture content is equal to 18.0% (mean value at the digging share running depth in the soil))

Translational velocity (m s^{-1})	Running depth in soil (X_2) (m)			
	0.06	0.08	0.10	0.12
1.4	3.0	1.0	0.0	0.0
	3.0	1.5	0.0	0.5
	2.9	1.0	1.0	1.0
	3.1	1.2	0.0	0.0
	3.0	1.3	1.0	1.0
1.65	5.0	2.0	0.0	0.0
	4.9	2.5	1.0	0.0
	4.8	2.0	1.0	1.0
	4.9	2.0	0.0	0.0
	5.0	2.0	1.0	0.5
2.1	6.0	4.0	2.0	0.0
	6.5	4.5	2.5	1.0
	6.0	4.0	2.0	0.0
	6.2	4.5	2.0	1.0
	6.1	4.2	1.8	1.0
(X_1)	(Y_4)			

According to Pogorely & Tatyanko (2004), in the course of the rapid development of vibrational lifting tools for the beet harvesters produced by the leading manufacturers, the frequency of the oscillations performed by the vibrational lifting tools has grown from 3.3–6.0 Hz to 10 Hz, that is, under production conditions, oscillation frequencies above 10 Hz are still not achievable in view of the insufficient reliability of the tool oscillation generating devices. The above calculations imply that the satisfactory conditions for performing the process of the vibratory sugar beet root lifting from the soil at the lifter's translational velocity equal to $V = 2.0$ m s^{-1} and the tool oscillation frequency $\nu = 10$ Hz will be ensured, if the relations between the geometrical parameters of the tool provide for the length of the lifter's working throat meeting the condition $l \geq 0.2$ m. Otherwise, at the velocity of translational motion equal to $V = 2.0$ m s^{-1}, the vibrational lifting process will be impaired.

The next step is to select from the results obtained by the completed numerical calculations the permissible vibrational lifting tool oscillation frequency values that could be recommended for use within the translational motion velocity range of 1.3–2.2 m s^{-1}, taking into account the limits set for the tool oscillation frequency by the requirement of each root being gripped without fail by the digging shares. This can be done, and for that purpose the reduced mass of the vibrational lifting tool must also be taken into account:

– for the reduced mass of the tool equal to m = 0.8 kg: at a running depth in the soil of 0.08 m and an oscillation amplitude of 0.008–0.024 m, the permissible oscillation frequency is equal to 21.2 Hz; at a running depth of 0.10 m - 10.0 Hz; at a running depth of 0.12 m - 9.0 Hz;

– for the reduced mass of the tool equal to m = 1.0 kg: at a running depth in the soil of 0.08 m and an oscillation amplitude of 0.008–0.024 m, the permissible oscillation frequency is equal to 16.4 Hz; at a running depth of 0.10 m and an oscillation amplitude of 0.008–0.018 m, the permissible oscillation frequency is equal to 10.0 Hz, at an amplitude of 0.020–0.024 m - 8.3 Hz;

– for the reduced mass of the tool equal to m = 1.5 kg: at a running depth in the soil of 0.08 m and an oscillation amplitude of 0.008–0.024 m, the permissible oscillation frequency is equal to 10.0 Hz; at a running depth of 0.10 m and an oscillation amplitude of 0.008–0.010 m, the permissible oscillation frequency is equal to 10.0 Hz, at an amplitude of 0.012 m - 8.0 Hz;

– for the reduced mass of the tool equal to m = 2.0 kg: at a running depth in the soil of 0.08 m and an oscillation amplitude of 0.008–0.016 m, the permissible oscillation frequency is equal to 10.0 Hz, at an oscillation amplitude of 0.018–0.020 m - 8.1 Hz.

In view of the requirement to minimise the damage to and loss of sugar beet root bodies in the process of their vibrational lifting from the soil, it is necessary to take into account and comply with the conditions presented in Table 3 in the process of designing advanced lifting tools for state-of-the-art root harvesters.

Table 3. Recommended oscillation frequencies v (Hz) for vibrational lifting tools

Reduced mass of tool m (kg)	Tool running depth in soil (m)								
	0.08			0.10					0.12
	Oscillation amplitude (m)								
	0.008–0.016	0.018–0.020	0.008–0.024	0.008–0.024	0.008–0.018	0.020–0.024	0.008–0.010	0.012	0.008–0.024
0.8	–	–	21.2	10.0	–	–	–	–	9.0
1.0	–	–	16.4	–	10.0	8.3	–	–	–
1.5	–	–	10.0	–	–	–	10.0	8.0	–
2.0	10.0	8.1	–	–	–	–	–	–	–

Accordingly, if the results obtained by the authors in their experimental investigations aimed at determining the rate of sugar beet root loss in vibrational lifting are compared to the results of other investigations and tests, it becomes obvious that, at the recommended length of the rear narrowed part of the working throat l_z and l_b equal to 0.1 m and the lifter's translational motion velocity equal to 2.0 m s^{-1}, the root loss rate decreases to 1.0%, which is 2–3 times lower than the similar indices obtained for the known vibrational lifters (Zuyev, 1970; 1980; 1989; Pogorely et al., 1980; 1983;

Bulgakov, 2005). Taking into account the fact that the research accomplished by the authors and the relations established for the geometric parameters of the digging shares in the vibrational lifting tool for harvesting sugar beet roots are tied up with the characteristics of the vibrational action applied to the roots, the obtained results open up opportunities for designing also other different tools, which will deliver virtually zero root loss and damage rates. That said, the above-mentioned parameters can be easily changed (before the start of the harvesting operations) in accordance with the properties of the soil, in which the sugar beet roots (or other root crops) are cultivated, the dimensions and shape of the roots, the travel rate that the multiple-row root harvester can maintain, the engine power rating etc.

The literature currently available in the world does not contain any theoretical studies on the relation between the sugar beet root harvesting quality and the length of the working throat between the digging shares, their oscillation frequency and amplitude as well as their translation velocity.

Also, it should be noted that, in view of the earlier mentioned fact that sugar beet roots are often spaced within the row at different distances (L – some average distance between the roots shown in Fig. 1), it can be expected in some cases that the following root is situated quite close to the root already being lifted at the moment. As a result of that, it can happen so that at the instant of time, when the lifted root is still in the area of the lifter's working throat rear part, the digging shares will already grip the next root. Before the next root reaches the rear part of the working throat, the lifted root will already depart from the lifter's working throat, moving back and upwards, and move to the area of the cleaning tools, freeing the space in the working throat rear part for the movement there of the next root. The lengths l_z and l_b of the rear narrowed part of the working throat are just what provides for such consecutive lifting of beet roots from the soil despite their positions in the row close to each other. In the worst case, if the previous root remains not extracted from the soil, all the same, it will depart from the working throat due to the lifter's translational motion - the tool will crush it pressing it down. In such a case, the root will become lost, but the working throat will least likely be obstructed by not lifted roots. However, even in this case the loss of the roots in view of their close spacing in the row will be minimal, because the angles of inclination in space of the working throat in the vibrational lifting tool under consideration, specifically in its rear narrowed part defined by the lengths l_z and l_b, are such that they promote the advancement of the root bodies backward and upward, i.e. operate on the principle of the conventional share lifter.

CONCLUSIONS

1. The relation between the number of the oscillations completed by the vibrational lifting tool within the time of the sugar beet root residing in the lifter's working throat, on the one hand, and the tool oscillation frequency, the length of the working throat rear part and the lifter's translational velocity, on the other hand, under the pre-condition of ensuring the good performance quality of the vibrational sugar beet root lifting process, has been obtained.

2. The minimum permissible tool oscillation frequencies, which ensure at least one instant of the root being gripped by the vibrational lifting tool, have been determined for several specific values of the lifter's translational motion velocity and the length of the working throat rear part.

3. When the length of the lifter's working throat rear part is equal to 0.1 m, the oscillation frequency $\nu = 20.3$ Hz ensures the satisfactory performance of the vibratory root lifting process for the lifter's translational motion velocity within the range of 1.3–2.55 m s^{-1}, the oscillation frequency $\nu = 15.7$ Hz - for the lifter's translational motion velocity within the range of 1.3–1.75 m s^{-1}, while the frequency $\nu = 8.5$ Hz does not ensure the satisfactory performance of the vibratory root lifting process for the above-mentioned ranges of translational velocity, that is, some part of the sugar beet roots will not be lifted by the tool or they will be broken off in their tail parts.

4. In order to ensure the good quality of the vibrational sugar beet root lifting from the soil at the lifter's translational motion velocity equal to $V = 2.0$ m s^{-1} and the tool oscillation frequency equal to $\nu = 10$ Hz, it is necessary for the length of the lifter's working throat rear part to be equal to 0.2 m, at a tool oscillation frequency of 6.7 Hz - 0.3 m.

5. As a result of the PC-assisted numerical calculations, the values of the permissible tool oscillation frequencies, which can be recommended for the translational velocity range of 1.3–2.2 m s^{-1}, taking into account the limitation of the tool oscillation frequency under the pre-condition of each root being gripped without fail by the digging shares, have been determined.

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Enzymatic activity of podzolized chernozem contaminated by pollutants during its detoxification

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Abstract. The soil is an indicator of the general technogenic situation. In terms of the scale of pollution, as well as the impact on biological objects, heavy metals occupy a special place among pollutants. One of the priority pollutants are Pb, Cd, Zn, Cu. In assessing the ecological state of the environment, the study of the soil cover plays an important role. The most informative integral characteristics of the biological activity of the soil is the activity of soil enzymes. In a lysimetric experiment with podzolized chernozem, we studied the change in the biological activity of soil in terms of dehydrogenase, catalase, urease, invertase and phosphatase enzymatic activity under the complex influence of heavy metals under conditions of the use of detoxification agents. The soil at the experimental site had the following characteristics: pH_{KCl} 6.2; humus content - 3.2%, P_2O_5-229 mg kg^{-1}, K_2O-250 mg kg^{-1} of soil. Organic and mineral fertilizers in various combinations were used as detoxifiers. According to the obtained data, the redox enzyme - dehydrogenase and hydrolytic enzymes urease and phosphatase are the most sensitive to soil pollution. The best decontamination effect is obtained when using a system of organo-mineral fertilizers, what contributes to an increase in the activity of soil urease by 3.38 times, invertase - by 2.47 times, phosphatase - by 1.48 times, dehydrogenase - by 1.46 times, catalase - by 1.60 times. Changes in the activity of these enzymes can be used to diagnose the effectiveness of the use of various fertilizer systems on soil contaminated by heavy metals.

Key words: podzolized chernozem, detoxification, heavy metals, dehydrogenase, catalase, urease, invertase, phosphatase.

INTRODUCTION

Heavy metals that enter the environment as a result of human production activities (industry, transport, etc.) are one of the most dangerous pollutants. These metals tend to be fixed in individual links of the biological cycle, accumulate in the biomass of microorganisms and plants and enter the body of animals and humans through trophic chains, negatively affecting their vital activity (Gromakova et al., 2017; Bansal, 2018).

Heavy metals are among the most dangerous toxicants for human health. They are classified as thiol poisons that block sulfhydryl groups of proteins and disrupt metabolic processes in the body (at low doses), in large doses they can act as blockers and other functionally active groups of proteins - amine, carboxylic, etc (Godwill et al., 2019).

Soil is a unique natural formation in which the interaction of living and non-living matter is most intense, where biological and biogeochemical cycles of substances are formed.

Of the many indicators of the biological activity of the soil, soil enzymes are of great importance. Their diversity and richness make it possible to carry out successive biochemical transformations of organic residues entering the soil.

In terms of enzymatic diversity and enzymatic pool, the soil is the most diverse system. The transformation of animal and plant residues into humic substances is a complex biochemical process that takes place with the participation of extracellular enzymes immobilized by the soil, as well as various groups of microorganisms. A direct relation between enzymatic activity and the intensity of humification is shown (Hagmann et al., 2015; Kouchou et al., 2017).

The enzymatic activity is influenced by a number of natural factors - the chemical and physical composition of the soil, moisture, acidity (pH), temperature, etc. However, in recent years, due to the growth of anthropogenic load on soil, anthropogenic factors have an increasingly intense effect on enzymatic activity (Aiju et al., 2013, Nwaogu et al., 2014; Naimi, 2018).

The research of monitoring and diagnostics of soil cover by biochemical methods, in particular, indicators of the enzymatic activity of soil, show maximum efficiency. This diagnostic indicator provides high sensitivity, ease of determination and, low experimental error (Hagmann et al., 2015.)

Hydrolases are widespread in soil and play an important role in enriching them with nutrients that are mobile and sufficient for plants and microorganisms, destroying high molecular weight organic compounds. This class includes the following enzymes: urease, invertase, phosphatase, etc., whose activity is an important indicator of the biological activity of soil and is widely used to assess anthropogenic impact (Sharma et al., 2019).

Urease is an enzyme involved in the regulation of nitrogen metabolism in the soil, catalyzing the hydrolysis of urea to ammonia and carbon dioxide, causing the hydrolytic cleavage of the bond between nitrogen and carbon in the molecules of organic substances. Urease is found in all soils, and its activity correlates with the activity of all the main enzymes of nitrogen metabolism. The rate of hydrolysis of urea in the soil is influenced by temperature and soil acidity. Heavy metal salts, as well as aliphatic amines, dehydrophenols, and quinones, significantly inhibit urease.

In soil in the form of organic compounds, there is a large amount of phosphorus, coming with dying remains of plants, animals and microorganisms. The release of phosphoric acid from these compounds is carried out by a relatively narrow group of microorganisms with specific phosphatase enzymes. Phosphatase activity of the soil is determined by its genetic characteristics, physicochemical properties and the level of farming culture (Micuti et al., 2017).

Invertase catalyzes the hydrolytic cleavage of sucrose into equimolecular amounts of glucose and fructose, also affects other carbohydrates to form fructose molecules - an energy product for the life of microorganisms and catalyzes fructose transferase reactions. The research by many authors has shown that the invertase activity reflects well the level of fertility and biological activity of soil (Maddela et al., 2017).

Enzymes belonging to the class of oxidoreductases catalyzes redox reactions that play a leading role in biochemical processes in the cells of living organisms, as well as in the soil. The most common oxidoreductases in soil are catalase and dehydrogenases, the activity of which is an important indicator of the genesis of soil.

The research by various authors (Mikanová et al., 2001; Makádi et al., 2019) has established that the activity of soil enzymes can serve as an additional diagnostic indicator of soil fertility and its changes as a result of anthropogenic impact. The use of enzymatic activity as a diagnostic indicator is facilitated by a low experimental error and a high stability of enzymes during storage of samples.

Thus, in the conditions of technogenic soil pollution, the search for measures to reduce the negative impact on the biosphere. One of the effective methods of reducing the negative consequences of soil contamination with heavy metals is the development of optimal systems for the use of fertilizers.

The purpose of this research was to study the effect of complex pollution by heavy metals on the enzymatic activity of podzolized chernozem under conditions of its sanitation.

MATERIALS AND METHODS

Developing agrochemical methods for decomposition of contaminated soil, in a stationary lysimetric experiment, a preliminary stage was carried out, in which the total content of Zn, Cu, Pb, Cd in podzolized chernozem, its hydrolytic acidity (pHhyd) in each lysimeter was studied and acidity was neutralized.

The soil at the experimental site has the following characteristics: pH_{KCl} 6.2; humus content - 3.2%, P_2O_5 - 229 mg kg^{-1}, K_2O - 250 mg kg^{-1} of soil. The studies were carried out on the Meshchera ecopolygon (Polkovo, Ryazan district), in four-fold repetition.

According to the regional gradation of soil pollution levels, compiled on the basis of the geochemical background, the content of Cu of 90 mg kg^{-1}, Zn - 110 mg kg^{-1}, Pb - 40 mg kg^{-1}, Cd - 0.6 mg kg^{-1} in the soil represent increased pollution.

Modeling of the increased complex level of soil contamination was carried out by adding to the soil. In this case, chemically pure salts were used: $Zn(CH_3COO)_2{\cdot}2H_2O$; $CuSO_4{\cdot}5H_2O$; $Pb(CH_3COO)_2$; $CdSO_4$.

For this, a soil layer of 20 cm depth was selected from the lysimeter. The calculated dose of salts of Cu, Zn, Pb, and Cd was thoroughly mixed with this soil and placed in the same lysimeter.

The effect of the following fertilization systems was studied: organic (cattle manure), organo-mineral and mineral, where double superphosphate was used periodically and annually in increased doses. For podzolized heavy loamy chernozem, the manure rate is 100 t ha^{-1} (Table 1). The norms of mineral fertilizers, depending on the crop, are adopted according to the recommendations for our zone (Ivanov & Derzhavin, 2008).

The calculated fertilizer rates were evenly and manually distributed over the surface of the lysimeter, and then the soil was dug to a depth of 12–15 cm. The soil surface was levelled. A mixture of herbs (fescue, timothy, clover) was sown as an equalizing crop.

As mineral fertilizers were used the urea (N - 46%), double superphosphate (P_2O_5 - 44%), potassium sulphate (K_2O - 48%).

Table 1. The scheme of carrying out the field lysimetric experiment

No of variants	Variants	Abbreviations in variant name tables
1	Control (without heavy metals and detoxification agents)	Control
2	Soil contaminated by a complex of heavy metals	Pollutants
3	Periodic application Cattle manure 100 t ha^{-1}	C100
4	Periodic application Cattle manure 100 t ha^{-1} + N60(N90)P60K60(K120) – annually depending on the crop (kg ha^{-1})	C100N1P1K1
5	P2 - periodic application of phosphorus, once every 2 years at a dose of 120 kg ha^{-1}, annual use N60(N90)K60(K120)	P2N1K1
6	P4 - periodic application of phosphorus, once every 4 years at a dose of 240 kg ha^{-1}, annual use N60(N90)K60(K120)	P4N1K1
7	P2 (e) - annual application of an increased dose of phosphorus (120 kg ha^{-1}) and optimal doses N60(N90)K60(K120)	P2(e)N1K1

The collection and analysis of soil samples for enzyme activity was carried out by methods generally accepted in enzymology and soil science. For comparative characteristics of different types of soils in terms of enzymatic activity, samples were taken at the end of the growing season (in september), in order to exclude the influence on the enzymatic activity of plants. An average soil sample from the top layer with a thickness of 0–5 cm was taken from each site. Uncontaminated soil without the use of fertilizers, was served as a control. The activity of catalase was determined by the gasometric method by the volume of released oxygen, based on measuring the rate of decomposition of hydrogen peroxide during its interaction with soil (Devyatova & Kramareva, 2008.)

To determine the dehydrogenase activity, colorless tetrazolium salts (2,3,5-triphenyltetrazolium chloride (TTC), which are reduced to red formazan compounds (triphenylformazan (TPF)), were used as a hydrogen acceptor. Determination of invertase activity was carried out by photocolorimetric method, which is based on taking into account the reducing sugars formed during the breakdown of sucrose (Khaziev, 2005). Phosphatase and urease activity was assessed using the Gaponyuk’s and Malakhov’s scale (Zvyagintsev, 1978) in mg P_20_5 10 g 1 h^{-1} and in mg $NH_4{}^+$ 10 g 24 h^{-1}, respectively.

The statistical processing of the results obtained was carried out by conventional methods (assessment of significance according to Fisher's and Student's criteria) using the Microsoft Excel software package.

RESULTS AND DISCUSSION

Effect of detoxification on hydrolytic enzymes of podzolized chernozem

Urease is one of the enzymes for the transformation of nitrogen compounds in the medium (Bansal, 2018). We noted a significant change in the urease activity of the soil against the background of its contamination by heavy metals and the use of detoxification (Table 2). In the control variant, the urease activity was 7.6 μg NH_4 10 g^{-1}

soil. In a soil sample with complex pollution, a sharp decrease in the activity of urease was observed - to 1.5 μg NH_4^+ g^{-1} of soil, that is less than the control by 80.26% (Table 3). This is due to the strong inhibitory effect of pollutants on urease. A decrease in urease activity to a minimum level indicates a weakening of the biochemical processes of the exchange of nitrogen-containing compounds, which may be the result of inhibition of the vital processes of microorganisms that synthesize urease.

According to the scale for assessing the degree of soil enrichment with enzymes (Table 4), this variant is characterized as very poor.

The application of various fertilizers contributed to the restoration of urease activity. However, it should be noted that the most effective method, according to our research, is an organo-mineral complex of fertilizers, the introduction of which made it possible not only to stop the negative effects of heavy metals, but also to increase the urease activity of the soil by 3.38 times (Table 2), i.e. it was 25.7 μg NH_4 10 g^{-1} of soil - medium enriched with this enzyme.

Table 2. Hydrolytic enzymes of podzolized chernozem contaminated by heavy metals using sanitation methods

Variants	Urease, mg NH_4 per 10 g for 24 h	Invertase, mg of glucose per 11 g for 24 h	Phosphatase mg P_2O_5 per 10 g for 1 h
Control	7.6	19.1	3.3
Pollutants	1.5	9.3	0.6
C100	18.1	47.1	4.1
C100N1P1K1	**25.7**	**51.2**	**4.9**
P2N1K1	8.7	26.3	3.2
P4N1K1	9.2	28.0	3.1
P2(e)N1K1	8.9	25.2	3.0
$LSD_{0.95}$	0.04	0.04	0.03

Table 3. Changes in enzyme activity in podzolized chernozem (in % compared to control)

Variants	Urease	Invertase	Phosphatase	Catalase	Dehydrogenase
Pollutants	-80.26	-53.03	-81.81	-50.00	-85.71
C100	+144.73	+36.87	+24.24	+19.44	+20.00
C100N1P1K1	+238.16	+158.59	+48.48	+55.56	+45.71
P2N1K1	+14.47	+32.83	-03.03	-8.33	-11.73
P4N1K1	+21.05	+41.41	-06.06	-11.11	-8.57
P2(e)N1K1	+17.1	+27.27	+27.27	-11.11	-11.43

The invertase activity of the control variant of the experiment is characterized as average - 19.1 mg of glucose per 1 g for 24 h. Especially strong depression of invertase is observed in the variant without the introduction of sanitation means, where its decrease in comparison with the control variant was by 53.03%. The use of fertilizers has led to a decrease in the negative impact on the soil of complex pollution by heavy metals. The best effect was obtained when using C100N1P1K1, the invertase activity here was 47.1 mg of glucose per 1 g for 24 h, which is higher by 158.59% in comparison with the control variant of the experiment. Probably, the enzymes show a higher resistance due to the organomineral complex, which stabilizes them in the soil environment.

The total phosphatase activity of the soil depends on the content of humus and organic phosphorus, which is a substrate for the enzyme. Soil contamination led to a decrease in phosphatase activity in comparison with the control variant by 81.81% and amounted to 0.6 mg P_2O_5 per 10 g per 1 hour, which characterizes the chernozem as very poor (Table 3) in terms of the degree of enrichment with this enzyme. When using detoxification, an increase in phosphatase activity occurred from 3.0 (variant P2 (e) N1K1) to 4.9 mg P_2O_5 (variant D100N1P1K1) per 10 g per 1 h.

Table 4. Scale for assessing the degree of soil enrichment with enzymes according to D.G. Zvyagintsev (Zvyagintsev, 1978)

Soil enrichment rate	Catalase, O_2 cm^3 g^{-1} for 1 min	Dehydrogenase, mg TPF per 10 g for 24 hours	Invertase, mg of glucose per 1 g for 24 h	Urease, mg NH_4, per 10 g for 24 h	Phosphatase, mg P_2O_5 per 10 g per 1 h
Very poor	< 1	< 1	< 5	< 3	< 0.5
Poor	1–3	1–3	5–15	3–10	0.5–1.5
Average	3–10	3–10	15–50	10–30	1.5–5.0
Rich	10–30	10–30	50–150	30–100	5–15
Very rich	> 30	> 30	> 150	> 100	> 15

The influence of chemical compounds in the composition of fertilizers on the enzymatic activity can be either direct (inhibitors or activators of the action of enzymes), or indirect (influence on the growth and development of soil organisms and plants that produce enzymes). A number of authors (Khaziev, 1982; Gianfreda et al., 2005) say that, in general, fertilizers have a powerful effect on the enzymatic activity of soils. On all soils, complex mineral fertilizer (NPK) was more effective, especially when combined with organic fertilizers, as opposed to individual types. At the same time, the effect of mineral fertilizers on various enzymes is not the same (Burns et al., 2013).

High doses of phosphorous fertilizers reduced the activity of phosphohydrolytic enzymes, and at low doses their activity increased. For nitrogen fertilizers, the activity of all enzymes, especially phosphatase and invertase, increases (Khaziev, 2018). Stable high enzymatic activity is achieved by systematic application of fertilizers to the soil, especially organic fertilizers together with mineral fertilizers, which corresponds to our research.

The effect of detoxification on oxidoreductases in podzolized chernozem

Catalase plays an important role in the neutralization of hydrogen peroxide, toxic to soil living organisms, which enters the soil as a result of their high physiological activity during unfavorable living conditions. Researchers have noted the sensitivity of this enzyme to the content of heavy metals (Murali & Patel, 2017; Jaworska & Lemanowicz, 2019; Aponte et al., 2020).

Soil contamination by a complex of heavy metals led to a 2-fold decrease in catalase activity compared with the control variant of the experiment and amounted to 1.8, O_2 cm^3 g^{-1} per 1 min, which characterizes it as poor in the degree of enrichment with this enzyme (Table 5).

Table 5. Oxidoreductases of podzolized chernozem contaminated by heavy metals using sanitation methods

Variants	Catalase, O_2 cm^3 g^{-1} per 1 min	Dehydrogenase, mg TPF per 10 g for 24 h
Control	3.6	3.5
Pollutants	1.8	0.5
C100	4.3	4.2
C100N1P1K1	5.6	5.1
P2N1K1	3.3	3.1
P4N1K1	3.2	3.2
P2(e)N1K1	3.2	3.1
$LSD_{0.95}$	0.04	0.03

The use of mineral fertilizers promoted the activation of catalase, however, it did not fully compensate for their negative effect and was 8.33–11.11% lower than in the control variant. The use of C100N1P1K1 led to an increase in catalase activity by 55.56% and amounted to 5.6 O_2 cm^3 g^{-1} per 1 min, which classifies the soil as a medium supplied with this enzyme (Table 4).

Dehydrogenases are enzymes that are involved in the respiration process, splitting off hydrogen from oxidized substrates. The activity of dehydrogenases is an informative indicator, since it depends on the intensity of the processes of nitrification, nitrogen fixation, respiration, and oxygen absorption by the soil. Therefore, even with a low level of technogenic load on the soil, its dehydrogenase activity decreases. This makes it possible to use indicators of the activity of these enzymes in the diagnosis of soil pollution by heavy metals (Wiatrowska et al., 2015, Łukowski & Dec, 2018)

Soil microorganisms have different resistance to heavy metals and are in constant interaction with each other and the soil. Therefore, the reaction of the microbiocenosis to heavy metals is determined by their interaction with the soil, their effect on microorganisms and on the competitive relationships of microorganisms (Micuti et al., 2017).

In general, these processes, according to Wolińska & Stepniewska (2012), are reflected in the change in the level of dehydrogenase activity, that is, in its inhibition, depending on the specific ecotoxicological situation. Explaining the reasons for the decrease in the enzymatic activity of the soil (Burns et al., 2013, Khaziev, 2018) under the influence of heavy metals, they are attributed to blocking the respiration chains and delaying the synthesis of enzymes of microorganisms while suppressing their growth.

According to the obtained data, presented in the Table 5, the activity of dehydrogenases in the variant 'Pollutant' decreased by 7 times compared to the control and amounted to 0.5 mg TPF per 10 g per 24 hours, which characterizes chernozem as very poor in the degree of enrichment with this enzyme. The use of mineral fertilizers made it possible to reduce the toxic effect of heavy metals, however, these sanitation measures did not allow to return to the indicators of clean, uncontaminated soil. The organo-mineral complex of fertilizers had the best effect. In this variant, there was an increase in dehydrogenase activity by 45.71% in comparison with the control variant.

Adding fertilizers to the soil not only improves the nutrition of plants, but also changes the conditions for the existence of soil microorganisms, which also need mineral elements. Under favorable climatic conditions, the number of microorganisms and their activity after fertilizing the soil increases significantly (Godwill et al., 2019).

CONCLUSIONS

In conditions of soil contamination with heavy metals, it is important to find ways to overcome their negative impact on agrocenoses. One of the methods can be the means of agrochemistry-organic and mineral fertilizers. The use of detoxicants helps to reduce the negative effect of toxicants on the studied indicators of the enzymatic activity of the soil. In the conducted studies, it was revealed that the organomineral system of fertilizers had the greatest protective effect on the enzymatic activity, which allowed to increase the activity of soil urease by 3.38 times, invertase - by 2.47 times, phosphatase - by 1.48 times, dehydrogenase - by 1.46 times, catalase - by 1.60 times.

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Effect of superabsorbent on soil moisture, productivity and some physiological and biochemical characteristics of basil

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Abstract. The study was carried out in 2019–2020, in the conditions of the Right-Bank Forest-Steppe of Ukraine. The results on the influence of absorbents in gel and powder forms on the productivity of basil plants (*Ocimum basilicum* L.) are presented. For research were used field, laboratory, statistical and calculation-analytical methods. Absorbent in the form of a gel was used while transplanting: dip the roots of the plant in the solution and then transplant in the field. Absorbent in the form of a powder - 5 kg ha^{-1}, application of the absorbent into the soil layer 20–25 cm^{-1}. Absorbents contributed to a slight decrease of sugar content (-0.86–2.68% in the cultivar of Badioryi, -1.48–2.35% in the cultivar of Rutan), significantly decrease ascorbic acid (-8.6–20.1%) and content of the essential oil (8.0–19.4%) and indirectly increased essential oil yield by increasing fresh weight yield in both varieties. The activity of APX, CAT, SOD, tended to decrease in all variants of the experiment, regardless of the form of the absorbent. APX (-12.8–35.1%), CAT (-10.9–22.0%), SOD (-11.9–17.0%). Higher yields were observed in the version with the introduction of the absorbent in the form of a gel. Thus, the yield of the cultivars of Badioryi and Rutan exceeded the control by 52.67 and 50.05%, in accordance. The productivity of basil is increased with the use of superabsorbent polymers. This practice can be recommended to agricultural producers who grow vegetables, in particular, basil in areas of unstable or insufficient moisture.

Key words: antioxidant activity, *Ocimum basilicum*, chlorophyll, yield.

INTRODUCTION

The stability of the production of vegetable greens in the forest-steppe of Ukraine depends mainly on weather conditions. The main factors limiting the productivity of vegetables are climate aridity, which has increased in recent years.

Optimization of the water regime of soils, preservation of moisture reserves in it, leveling of moisture deficit are urgent problems of agrocenoses. Ensuring optimal soil moisture is one of the main conditions for increasing fertility, their sanitary protection functions.

Application of hydrogels consists in rough powders of polyacrylamide or potassium polyacrylatematrix sold with a huge range of names (Plant-Gel, Super Crystals, Water-Gel Crystals) and used as long term reservoir of water for plant growth in gardening, and industrial horticulture (Chalker-Scott, 2015)

Superabsorbent polymers are hydrophilic polymer networks that have the potential to absorb and hold a large amount of water and aqueous solution sintheirn et work Zohuriaan-Mehr &Kabiri (2008), Milani et al. (2017), Das et al. (2020). ‘As a class of material, hydrogels are unique, they consist of a self-supporting, water-swollen three-dimensional (3D) viscoelastic network which permits the diffusion and attachment of molecules and cells’ Chirani et al. (2016).

The hydrogels play an important role in agriculture Dehkordi (2017). Over the last decade, hydrogels have been widely used to improve water penetration for plants, by increasing water retention properties, the use of hydrogel polymers can be an effective means of improving water and fertilizer efficiency Dehkordi et al. (2013), Skrzypczak et al. (2020) it can absorb and store water many times more from its own volume and perform the function of reservoirs with water and increase the efficiency of irrigation, as well as superabsorbent polymers improve some physical properties of the soil.

The hydrogel polymer was developed as a retarder to reduce groundwater losses and increase crop yields Hüttermann et al. (1999); Bahram et al. (2016), Ovalesha et al. (2017).

The hydrogel products can be classified as: Based on source (Zhao et al., 2013; Sandhya et al., 2021):

Natural hydrogels;

Synthetic hydrogels;

Semi-synthetic hydrogels.

Synthetic hydrogels based on acrylates and acrylamides show high mechanical strength and the potential to absorb significant quantities of water. Due to the problem of their biodegradability, they are being attempted to replace with biopolymers such as alginate, agar, cellulose, chitosan, and starch (Skrzypczak et al., 2020)

Hydrogels may be obtained from natural sources or they can be prepared synthetically. Alginate, Xanthan, Dextran, Pullulan, Hyaluronic acid, Guar gum, Okra gum, and Locust gum, Gellan, Xyloglucan, Pectin and Scleroglucan are some of the examples of natural polymers capable of forming hydrogels. Some of the examples of synthetic polymeric gelators are Poly (vinylalcohol), Polymethacrylic acid (PMAA), Polyacrylic Acid (PAA) Poly N-vinylpyrrolidone) PVP), polyethyleneglycol diacryl ate/dimethacrylate PEGDA/PEGDMA), Polyethyleneglycol acrylate/methacrylate (PEGA/PEGMA), and Poly (styrene) (PS) (Saini, 2016).

A productive life of hydrogels of 5 years that is, the effect of the absorbent after application to the soil is observed for 5 years. The recommended doses vary from 5 to 25 kg ha-1, depending on the type of soil, crop and climate, according to the manufacturer (SNF Inc., 2011; Pande, 2017). In addition to the effect of water retention in the soil, these products improve aeration and maintain temperatures that promote better plant development, with the consequent effect on yield, as has been shown experimentally in crops such as Glycine max (Galeş et al., 2012), *Apium graveolens* (Kosterna et al., 2012), potato (Faried et al., 2014; Starovoitova et al., 2020), *Vigna Unguiculata* L. (Lopes et al., 2017), *Pisum sativum* (Norodinvand et al., 2019), wheat (Shaikh et al., 2020). Söylemez et al. (2020) it has been determined that total yield, total fruit number, average fruit weight, root, shoot and leaf dry weights, plant height, stem diameter and chlorophyll content generally increased with use of waterpad, but these increases were statistically insignificant.

The hydrogel polymers are used as a water-retaining material in arid, semi-arid or in regions with unstable rainfall Montesano et al. (2015). Moreover, previous studies indicate a good ability of the hydrogel polymer to increase water retention, which helps reduce water stress in plants and ensure high plant productivity, which leads to increased growth and yield Belen-Hinojosa et al. (2004). The research should focus to reduce utilization of water in agricultural sector as it uses a large amount of water. This problem can be minimized by the use of hydrogels (Shubhadarshi & Kukreja, 2020).

Therefore, the main purpose of this study was to evaluate the effect and effectiveness of various forms of superabsorbents on plant growth, chlorophyll content, activity of antioxidant enzymes and basil productivity.

MATERIALS AND METHODS

The research of the influence of different forms of superabsorbent was carried out in 2019–2020 on the experimental field of the Department of Vegetable Growing of Uman National University of Horticulture (Right-Bank Forest Steppe of Ukraine) in accordance with national methods Bondarenko & Yakovenko (2001), Ulianych et al. (2019, 2020).

The soil was black, puddle, heavy loam with a well developed humus horizon (about 2.9% of humus) in the deep of 40–45 cm^{-1}. Soil pH was determined in water (soil water ratio 1:1). Electrical conductivity (ECe) of the soil suspension was measured using the conductivity meter. The P, K were determined by AB-DTPA method Ryan et al. (2001). Chemical properties of soil: organic carbon - 2.2%, pH 6.0 -6.2, P - 102 mg kg^{-1}, K - 123 mg kg^{-1}, NO_3-N - 64 mg kg^{-1}.

Planting was carried out by the scheme of 50×30 cm. The total area for the experiment was 400 m^2, for the plot it was 100 m^2, and for the sampling - 10 m^2. The experiment was performed as a Factorial Randomized Block Design with four replications.

The two-factor experiment consisted of the use of superabsorbent of the company ‘Maxi Marin’ in the form of gel (anionic polyacrylamide copolymer) and powder with granulometry < 0.5 mm (cationic polyacrylamide copolymer).

Application methods. Superabsorbents were applied before planting seedlings to a depth of 15 cm. Absorbent in the form of a gel was used while transplanting: Thoroughly mix 2 g (or according to recommended rate) of hydrogel per litre of water to prepare a free-flowing solution; allow it to settle for half an hour. Dip the roots of the plant in the solution and then transplant in the field. Absorbent in the form of a powder - 5 kg ha^{-1} before planting seedlings, locally in the furrows (according to the manufacturer's recommendations), application of the absorbent into the soil layer 20–25 cm^{-1}.

Biometric research. The leaf length and width, leaf blade area and total leaf area per plant on the 60th day after planting (DAP) (BBCH 55) were determined; the plant height and the number of leaves per plant were calculated, and the leaf blade area was determined by a calculated (linear) method, using the parameters of length and width of the leaf by the formula:

$$Sn = 0.74 \times ab \quad (1)$$

where Sn – single leaf area, cm²; a – the largest leaf width, cm; b – leaf length, cm; 0.74 is the leaf configuration coefficient.

We studied the effect of various forms of superabsorbent on the basil cultivars (enzymes activity, productivity of plants, pigments contents in leaf, vitamin C). All analyzes were performed in three replicates.

Plant material. Basil (*Ocimum basilicum* L.) cv. Badioryi and Rutan.

Determination of chlorophylls. The plant material was washed with distilled water and air-dried in a shady, well-ventilated room at the temperature of 22–25 °C. Then, 0.10–0.05 g of the sample was weighed and triturated with 5 mL of the solvent (ethanol) for 15 min. The obtained suspension was filtered into a volumetric flask (capacity of 10 mL) and supplemented with ethanol. Concentrations ($\mu g\ mL^{-1}$) of chlorophylls a and b, were determined using equations published by and Lichtenthaler & Buschmann (2001):

$$\text{Chlorophyll a} = 13.36A_{664.1} - 5.19A_{648.6} \quad (2)$$

$$\text{Chlorophyll b} = 27.43A_{648.6} - 8.12A_{664.1} \quad (3)$$

Activity measurements of antioxidant enzymes

A sample of 0.5 g^{-1} of fresh sweet basil seedling leaves was macerated in liquid nitrogen to obtain the extract for measurements, as proposed by Waterhouse (2002). The method used to measure antioxidant activity was based on the scavenging activity of the 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH, 60 μM), as proposed by Rufino et al. (2009). The absorbance was measured at 515 nm^{-1} using a spectrophotometer (UV-VIS Beckman 640 B). The readings were monitored every 30 min to a total of four readings, while the absorbance reduction was observed until stabilization. All measurements were performed in triplicate. The results were expressed as the percentage of free radical scavenging activity (% FRS), according to the equation:

$$aa = [(A_{control} - A_{test})/A_{control}] \times 100, \quad (4)$$

where aa is the antioxidant activity (%); $A_{control}$ is the absorbance of the control solution, and A_{test} is the absorbance of the extract samples.

The enzymatic extract was obtained by maceration of 200 mg of fresh leaf tissue in liquid nitrogen, with the addition of 1.5 mL of 400 mm potassium phosphate extraction buffer (pH 7.8), 1.0 mm EDTA, and 200 mm^{-1} ascorbic acid. The suspension was centrifuged at 12,000 rpm for 15 min^{-1} at 40 °C and the supernatant collected Bonacina et al. (2017).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by its ability to inhibit the photochemical reduction of nitroblue tetrazolium, as described by Giannopolitis & Ries (1977). The absorbance of the reaction mixture was read at 560 nm^{-1}, and one unit of SOD activity (UA) was defined as the amount of enzyme required to cause 50% inhibition of the nitroblue tetrazolium photoreduction rate. The results are expressed as UA mg^{-1} fresh weight (FW) min^{-1}.

Catalase (CAT, EC 1.11.1.6) activity was determined, as described by Havir & Mchale (1987), by the H_2O_2 consumption, which was monitored by reading the absorbance at 240 nm at the moment of H_2O_2 addition and 1 min^{-1} later. The difference in absorbance was divided by the H_2O_2 molar extinction coefficient (36 M^{-1} cm^{-1}) Anderson et al. (1995). CAT activity was expressed in mmol H_2O_2 g^{-1} FW min^{-1}.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was detected according to the method described previously by Nakano & Asada (1981). The absorbance was read at 290 nm 1 min^{-1} after H_2O_2 was added to the reaction solution. The APX activity was quantified using a molar extinction coefficient of 2.8 mm^{-1} cm^{-1}. The results were expressed in mmol ascorbate g^{-1} FW min^{-1}. All enzymes were evaluated using 96-well flat-bottomed ELISA plates. In all assays, three technical replicates and three biological replicates were used. The absorbance was read using a spectrophotometer (UV-VIS Spectra Max Plus®) with the Soft Max Pro program, version 6.5.1.

Dry matter (%). The average dry matter weight (g^{-1}) of leafs after curing were measured by drying 10 randomly sampled leafs in an oven with a forced hot air circulation at 70 °C until a constant weight was obtained. The percentage of leafs dry matter was calculated by taking the ratio of the dry weight to the fresh weight of the sampled leafs and multiplying it by 100.

Extraction of basil essential oils. A sample (10 g) of air dried basil leaf were used for essential oil distillation. Basil oil was extracted by hydrodistillation using an Electrothermal type (UK) apparatus. The duration of this procedure was 2 hours. The yield (v/w) of the obtained essential oil was expressed as a percentage of absolute dry weight (Grytsaenko et al., 2003).

Analysis of vitamin C. Lyophilized samples (each 0.2 g^{-1}) were ground and added to 30 mL^{-1} of 3% metaphosphoric acid solution and homogenized at 11,000 rpm for 2 min^{-1} using a T25 basic ULTRA–TURRAX homogenizer (IKAWerke GmbH & Co. KG, Staufen, Germany). The volume was made up to 50 mL with 3% metaphosphoric acid solution. The extract (2 mL^{-1}) was centrifuged at 12,000 rpm for 3 min^{-1}, and the supernatant filtered through a 0.45 lm polyvinylidene difluoride (PVDF) membrane filter (Whatman I nternational Ltd., Maidstone, UK). All samples were immediately analyzed using an HPLC system, equipped with a PU (2089 pump), an AS (2057 auto injector), and a MD (2010 UV) vis variable wavelength detector (JASCO Corp., Tokyo, Japan). Separation was carried out in a Crest Pak C18S column (15,094.6 mm, i. d., 5 lm, JASCO Corp.), and the isocratic elution was carried out with 0.1% trifluoroacetic acid in distilled water as a mobile phase for 15 min^{-1} (flow rate 0.8 mL^{-1} min^{-1}). The peak was read at 254 nm using an UV detector and quantification was determined via external calibration against ascorbic acid.

Soil moisture. The research was conducted on the chernozem of clay granulometric composition. The study of the productive moisture reserves in was conducted on six sample plots in three replicates, laid on the levelled area of arable land. The moisture content was determined by the thermogravimetric method, the density of the composition by Kachynski method:

$$W_{spm} = W_t - W_{pm} \tag{5}$$

where W_{spm} – stock of productive moisture (mm); W_t – total water supply in the soil at the time of sampling, mm; W_{pm} – water supply in the soil, which accords to the wilting point, mm.

Total water supply in the soil at the time of sampling. was determined by thermostatic weight method according to the formula:

$$W_t, \% = \frac{A - B}{B - C} \times 100 \qquad (6)$$

where A – the mass of the box with raw soil, g; A – weight of the box with dry soil, g; C – the mass of the empty box, g.

The weather conditions in the years of research. According to the Uman meteorological station, hydrometeorological conditions in 2019 were characterized by slightly less precipitation compared to long-term averages. The amount of precipitation for this period in 2020 was much higher than in 2019. Most of them fell in June, which allowed the plants to form better leaf mass (Fig. 1).

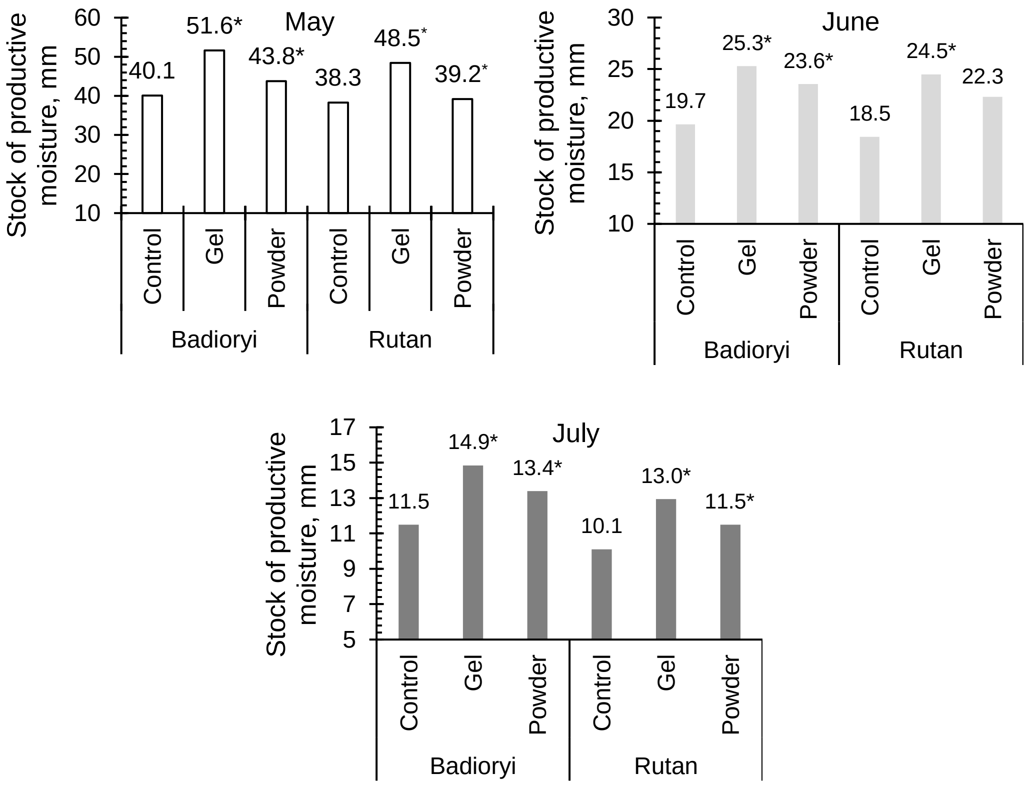

Figure 1. Dynamics of stock of productive moisture (mm) in the root zone (in the soil layer 0–30 cm) of basil cultivars vs. various form of superabsorbent.

Results of statistical processing	May	June	Jule
$LSD_{0.05}$ A	1.51	0.57	0.29
B	1.85	0.70	0.35
A×B	2.62	0.99	0.50

* show significance at the $P \leq 0.05$ probability levels.

The air temperature in 2019–2020 at the time of planting was slightly higher than the perennial one, which had a positive effect on the development of true basil plants (Table 1).

Table 1. The weather conditions during the growing season of basil (2019–2020)

Month	Precipitation, mm			Temperature °C		
	2019	2020	Average for many years	2019	2020	Average for many years
May	35.6	101.0	55.0	17.0	12.5	14.6
July	69.8	70.4	87.0	23.4	20.9	17.6
June	33.8	21.4	87.0	20.0	21.6	19.0
August	19.2	17.1	59.0	20.7	21.2	18.2
September	30.6	27.4	43.0	15.6	17.8	13.6

Statistical analysis. For the food and chemical composition, three samples were analyzes were performed in three replicates. The results were expressed as averages. The antioxidant activity, and chemical composition were analyzed using analysis of variance, with $P \leq 0.01$, for yield, weight of plant and stock of productive moisture $P \leq 0.05$ using statistical analysis program (SAS) v. 9.1.3.

RESULTS AND DISCUSSION

Since the main root mass of basil plants is located in the arable soil layer (0–30 cm), the productivity of this crop largely depends on its moisture content. In 2020, the reserves of productive moisture accumulated during the winter-spring period were higher than in 2019 due to heavy rainfall in May. The superabsorbent in the form of a gel helped to increase the reserves of productive moisture. On average over two years, the application of the gel increased this indicator relative to control by 27–29% in May; 25–37% in June; 28–29% in July. The application of the powder was much lower. The inter-varietal difference in moisture reserves was also revealed. The soil in the variants with the Badioryi cultivar was characterized by more reserves of moisture, which indicates its lower need for water and, accordingly, higher drought resistance. (Fig. 1).

Similar results are reported by Godunova et al. (2014) in studies with winter wheat and different hydrogel norms and Revenko & Agafonov (2018) in studies with soybeans and winter wheat, where productive moisture reserves increased significantly compared to controls.

The plant growth and the leaf area formation (Table 2). The absorbent in the form of a gel contributed to a significant increase in the height of plants of the cultivars of Badioryi and Rutan (+ 10.71 and 8.45% to control). The use of the absorbent in the form of a gel had a more positive effect on the increase of the diameter of the basil plant bush of both studied varieties (+ 16.14 and 14.10% to control). The absorbent in the form of a gel contributed to a significant increase in the number of leaves on plants of the cultivars of Badioryi and Rutan (+ 8.76 and 5.96% to control).

The use of the absorbent in the form of a gel contributed to a significant increase in the number of leaves on the plant (+ 8.76% in the cultivar of Badioryi and 5.96% in the cultivar of Rutan), the absorbent in the form of powder was less effective and caused a slight increase (+ 3.59 and 1.58% in accordance) of this indicator in both varieties. Plant height and number of leaves was increased due to high retention of moisture in soil and nutrients availability in the root zone of the crop, where it might have helped to enhance the activity of cell, causing increment in plant height and number of leaves per plant. The result is in conformity with the result of Al-Harbi et al. (1999) in cucumber.

Determination of the basil leaf area showed that it significantly increases with the use of the absorbent in the form of a gel (+ 7.26% in the cultivar of Badioryi, + 8.20% in the cultivar of Rutan).

Table 2. Plant growth and leaf area of the basil cultivars vs. various forms of superabsorbent (2019–2020), (BBCH 55)

Cultivar (factor A)	Super-absorbent (factor B)	Plant height, cm	Plant diameter, cm	Number of branches per plants, pcs.	Number of leaves, pcs.	Leaf area, cm²
Badioryi	Control	40.68	37.08	10.01	185.06	25.24
	Gel	45.04 ns	43.06*	10.53*	201.27 ns	31.00*
	Powder	42.43 ns	40.51 ns	10.41 ns	192.28 ns	27.40*
Rutan	Control	40.90	37.48	6.73	186.04	17.37
	Gel	44.35*	42.76*	7.73*	197.13 ns	22.34*
	Powder	42.09 ns	40.35 ns	7.64*	188.98 ns	19.54*
	$LSD_{0.05}$ A	1.12	1.40	0.24	5.54	0.52
	B	1.38	1.71	0.30	6.78	0.64
	A×B	1.95	2.43	0.42	9.59	0.90
	CV%	4.2	6.3	18.8	3.3	21.3

* show significance at the $P \leq 0.05$ probability levels.

Biochemical parameters (Table 3). In the control variants, the dry matter content in the in the cultivars of Badioryi and Rutan was 8.88 and 9.23%, in accordance, that is the use of absorbents in the cultivation technology causes an increase of yield, but the dry matter content is decreased.

Table 3. Biochemical parameters of the basil cultivars vs. various forms of superabsorbent (2019–2020), (BBCH 55)

Cultivar (factor A)	Super-absorbent (factor B)	Total sugar, mg 100 g^{-1} FW	Dry matter, %	L-ascorbic acid, mg 100 g^{-1} FW	Essential oil, %	Essential oil yield, kg ha^{-1}
Badioryi	Control	9.10	8.88	13.17	1.09	14,25
	Gel	9.02 ns	8.71 ns	10.51 ns	0.99*	17,02*
	Powder	8.86 ns	8.76 ns	12.26*	1.01*	15,39*
Rutan	Control	18.66	9.23	15.76	1.51	13,94
	Gel	18.38*	8.98 ns	12.65*	1.31*	16,52*
	Powder	18.22*	9.07 ns	14.41*	1.35*	15,24*
	$LSD_{0.01}$ A	0.51	0.36	0.60	0.05	0.65
	B	0.63	0.44	0.74	0.06	0.80
	A×B	0.89	0.62	1.03	0.09	1.13
	CV%	37.7	2.2	13.8	17.4	7.9

* show significance at the $P \leq 0.01$ probability levels.

The use of absorbents caused a slight decrease in sugar content, but the difference between the varieties was very significant: the cultivar of Badioryi - 8.86–9.10 mg 100 g^{-1} FW, the cultivar of Rutan - 18.22–18.66 mg 100 g^{-1} FW. The content of ascorbic acid, regardless of the form of the absorbent decreased significantly (6.9–20.1% in the cultivar of Badioryi and 8.6–19.7% in the cultivar of Rutan) in all variants of the experiment, but the use of the absorbent in powder form contributed to its largest and less decreased significantly content.

It has been suggested that under stress a higher density of oil glands due to the reduction in leaf area results in an elevated amount of oil accumulation. Studies on the effects of irrigation, which indicate a decrease in the content of essential oil in other crops with optimal and excessive moisture: Oregano (Virga et al., 2020), *Ocimum spp.* (Khalid, 2006), *Salvia officinalis* (Bettaieb et al., 2009).

In our study, growth parameters of basil improved after the use of water preservative compounds in soil and indirectly increased essential oil yield by increasing fresh weight yield.

The absorbent in the form of a gel contributed to a significant increase in the yield of the essential oil in both varieties. The use of the absorbent in powder form caused a less significantly increase of yield (8.00–9.33%) and absorbent in the form of a gel caused a significantly increase of yield (18.51–19.44%) of cultivar Badioryi and Rutan in the yield of the essential oil per unit of the area. The results of our research are similar to those obtained Shi et al. (2010), Kalamartzis et al. (2020), Beigi et al. (2020) reported about increased essential oil yield by increasing water availability in soil.

The activity of antioxidant enzymes and the pigment complex of leaves (Table 4). The activity of APX, CAT, SOD, tended to decrease in all variants of the experiment, regardless of the form of the absorbent.

The studied cultivars had significantly lower activity of APX and SOD (-12.84–24.13% APX and 11.85–11.97% SOD - in the cultivar of Badioryi - 21.15–35.12% APX and 16.01–16.99% SOD - in the cultivar of Rutan), but the activity of CAT had insignificant reduction of this indicator for both cultivars in all variants of the experiment (-10.89–18.43% - in the cultivar of Badioryi and 13.11–21.95% in the cultivar of Rutan).

Table 4. Antioxidant enzyme activity in leaves and leaf's total chlorophyll (*a* + *b*) content of the basil cultivars vs. various form of superabsorbent (2019–2020), (BBCH 55)

Cultivar (factor A)	Super-absorbent (factor B)	APX (mM ascorbic acid g^{-1} FW min^{-1})	CAT (mM H_2O_2 g^{-1} FW min^{-1})	SOD (U SOD mg^{-1} FW min^{-1})	Chlorophyll content, mg g^{-1} FW		
					a	*b*	∑ of chlorophyll
Badioryi	Control	0.23	0.40	74.75	1.05	0.38	1.43
	Gel	0.17*	0.32*	65.90*	1.29*	0.45*	1.74*
	Powder	0.20*	0.35*	65.80*	1.23*	0.38ns	1.61*
Rutan	Control	0.15	0.33	67.75	0.99	0.43	1.43
	Gel	0.10ns	0.26*	56.23ns	1.23*	0.52*	1.75*
	Powder	0.12*	0.29*	56.90ns	1.09*	0.48*	1.57*
	$LSD_{0.01}$ A	0.007	0.013	2.506	0.05	0.046	0.053
	B	0.010	0.016	3.069	0.06	0.056	0.070
	A×B	0.013	0.022	4.341	0.09	0.079	0.093
	CV%	30.1	15.1	10.9	10.3	12.3	8.9

* show significance at the $P \leq 0.01$ probability levels.

The obtained results indicate that the highest physiological activity is shown by SOD, where the activity of the antioxidant complex is significantly higher both against control and against other experimental options. The results of this study showed that increasing the activity of antioxidant enzymes in control variants of basil cultivars indicates their resistance to the drought.

The use of absorbents helped to increase the content of chlorophyll a + b, but with the use of the absorbent in the form of a gel, the increase was most significant to control (+ 21.31% in the cultivar of Badioryi, 22.51% in the cultivar of Rutan). The absorbent in the form of powder also contributed to a significant increase in the concentration of chlorophyll a + b of an average by 12.45% in the cultivar of Badioryi and by 10.29% in the variety Rutan. The inter-varietal difference according to this indicator was insignificant: the cultivar of Badioryi accumulated 1.43–1.74%, the cultivar of Rutan - 1.43–1.75% of dry matter.

Mass and yield of plants (Fig. 2). The results of the study indicated a significant effect of absorbents on the weight change of basil plants, regardless of the form of the absorbent in all variants of the experiment. The application of the absorbent in the form of a gel was more effective (+ 23.73 g^{-1} in the cultivar of Badioryi and 19.24 g^{-1} in the cultivar of Rutan). The absorbent in a powder form also caused a significant increase (+ 12.99 and 11.75 g^{-1}, respectively). of this indicator in both cultivars.

The yield, is the most important indicator of the effectiveness of cultivation technology. The use of absorbent of Maxi Marin in the form of granules increased the yield of basil of the cultivar of Badioryi by 2.89 and the cultivar of Rutan by 2.4 t ha^{-1} relatively to the control.

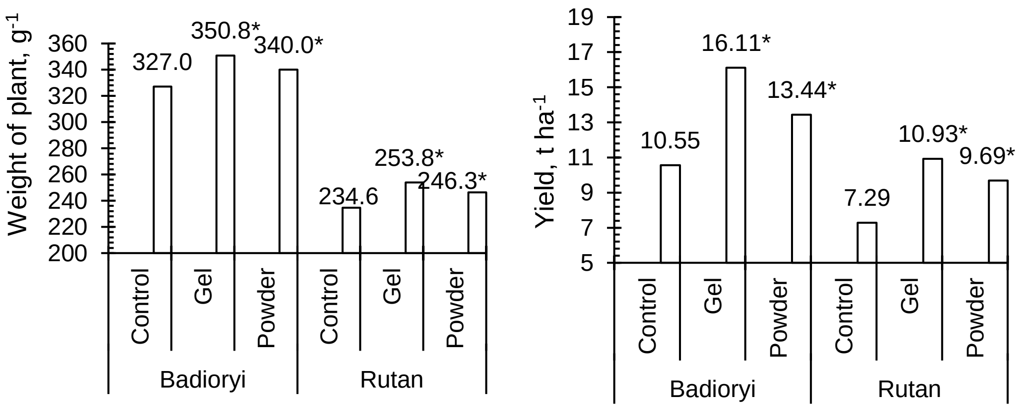

Figure 2. Weight of the plant (g^{-1}) and yield (t ha^{-1}) of the basil cultivars vs. various form of superabsorbent, (BBCH 55).

Results of statistical processing	Weight of the plant (g^{-1})			Yield (t ha^{-1})		
$LSD_{0.05}$ A	6.55	4.62	8.46	0.480	0.701	0.166
B	8.02	5.67	10.36	0.588	0.344	0.096
A×B	11.34	8.01	14.65	0.832	1.214	0.118

* show significance at the $P \leq 0.05$ probability levels.

The application of the absorbent in the form of a gel was more effective. Higher yields were observed by applying the absorbent in the form of a gel. Thus, the yield of the cultivars of Badioryi and Rutan was at the level of 16.11 and 10.93 t ha^{-1}, which exceeded the control by 5.56 and 3.64 t ha^{-1}, in accordance.

The use of superabsorbent polymer improves plant growth. For example, the total amount of raw cucumber biomass (*Cucumis sativus L.*) and fruit biomass increased by 840 and 494 g^{-1} per plant, in accordance Montesano et al. (2015). Another study with different varieties of potatoes (*Solanum tuberosum L.*) found an increase in tuber yield using superabsorbents locally in the furrows to a depth of 25 cm^{-1} Salavati et al. (2018). A similar result was found with the application of 60 and 90 kg ha, which increased the yield of potatoes by 38.2 and 50.5% relatively to the control when applying superabsorbents to a depth of 20 cm^{-1} Hou et al. (2017). Although the use of superabsorbents can improve plant growth, the depth of its use can significantly affect its effectiveness.

In the conditions of insufficient moisture, superabsorbents have a greater impact on plant productivity Fazeli Rostampour et al. (2013), Egrinya Eneji et al. (2013). The dry matter of sorghum (*Sorghum bicolor L.* Moench) increased only when there was a shortage of the water in the sandy loamy soil Fazeli Rostampour et al. (2013). In a 3-year study, the use of superabsorbents increased wheat yield relatively to the water-deficient years Grabinski et al. (2019). Similar results were obtained with beans (*Phaseolus vulgaris L.*) grown with superabsorbents Satriani et al. (2018).

CONCLUSION

The results of this study showed that increasing of the activity of antioxidant enzymes (catalase, superoxide dismutase and ascorbate peroxidase) in basil varieties indicates their resistance to the drought, and this is accompanied by an increase in chlorophyll content. Thus, the use of superabsorbent, which has the ability to absorb significant amounts of the water, improves growth processes and physiological responses of plants and can help plants in conditions of the water shortage.

That is, with the improvement of the water regime, the concentration of sugar, ascorbic acid, essential oil decreases.

The productivity of basil is increased with the use of superabsorbent polymers. Higher growth, yield, were observed with the introduction of the absorbent in the form of a gel compared to the control. This practice can be recommended to agricultural producers who deal with vegetables, in particular, basil in the areas of unstable or insufficient moisture.

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Evaluation phenology, yield and quality of maize genotypes in drought stress and non-stress environments

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Abstract. The aim of the study is to examine the effect of agrometeorological indices (growing degree days, GDD; heliothermal unit, HTU; photothermal unit, PTU; hydrothermal unit, HYTU) on the phenology and yield (GY) of the Sushi (FAO 340) and Fornad (FAO 420) maize hybrids. Furthermore, it was also analysed how the amount of nitrogen and its application time affected the productivity and protein content (GP) of maize under drought stress (DS) and non-stress (NS) conditions. There were seven fertilizer treatments in the scope of the field experiment. Non-fertilized treatment (A_0) spring basic treatment with 60 and 120 N ha^{-1} (A_{60}, A_{120}), and following the basic treatments, 30 kg N ha^{-1} top-dressing was applied in the V6 ($V6_{90}$, $V6_{150}$) phenophase and then another 30 kg N ha^{-1} in the V12 ($V12_{120}$, $V12_{180}$) phenophase. Based on the GDD and PTU, length of the vegetation period of maize hybrids can be predicted. Under DS, the largest GY and GP was recorded in the same treatment for Sushi ($V6_{150}$ kg N ha^{-1}), and at different nutrient levels under NS: GY (A_{120}) and GP ($V6_{150}$). The highest GY of Fornad hybrid under DS was achieved with the A_{120} treatment while the highest GP with the $V6_{150}$; in the case of NS $V6_{150}$ kg N ha^{-1} was the most effective for both GY and GP. The + 30 kg ha^{-1} N fertilizer applied in the V12 phenophase did not improve GY and GP in either hybrid during the two growing seasons. The findings provide useful help for farmers to prepare for future environmental changes and to operate successfully.

Key words: agrometeorological indices, phenology, nitrogen fertilization, grain yield, grain protein content.

INTRODUCTION

In addition to wheat and rice, maize is the crop produced in the highest volume on global scale (Serna-Saldivar, 2019; Santpoort, 2020). It is also a key component of animal feed (Shiferaw et al., 2011; Malaviarachchi et al., 2014) and its role in human nutrition is also extremely important. Of the 300,000 edible plant species, 200 are consumed, of which maize, wheat and rice make up 60% of the diet. In countries struggling with hunger, 80–90% of maize is used for human consumption. Therefore, increasing the average yield, yield stability and quality of maize is of utmost importance.

There is still potential for the global increase of maize production, for which maize yields need to improve by at least 18% by 2030 to meet the growing demand for food for a growing population and a changing diet (FAO, 2017; Listman & Ordóñez, 2019) despite increasingly extreme environmental conditions due to climate change.

Currently, adverse weather extremes caused by climate change, drought and water scarcity are critical barriers to plant growth and development, yields and quality worldwide (Xu et al., 2008; Iversen & Norby, 2014; Lobell et al., 2014; Avramova et al., 2015; Song et al., 2018).

Of the three important climatic parameters, temperature, precipitation, and light, temperature is the primary factor influencing plant development (Marton et al., 2005; Girijesh et al., 2011; Hatfield & Prueger, 2015), which is responsible for potential productivity for yield and quality (Nagy, 2008; Hawkins & Sutton, 2011). Achieving a certain heat unit accumulation is required to reach each phenological stage of maize hybrids (Nandini & Sridhara, 2019; Ahmed & Saikia, 2020). The duration of phenophases determines the rate and distribution of dry matter accumulation in different parts of the plant (Hao et al., 2016; Mirosavljević et al., 2018; Shrestha et al., 2018) Temperature-based agrometeorological indices of (GDD, HTU, PTU, and HYTU) are widely used for plant growth, phenological development, and harvest time estimation (Rajput et al., 1987; Wurr et al., 2002; Roy et al., 2005). These indices are based on the idea that the rate of phenological development is linearly related to temperature in the range between base temperature and optimal temperature (Monteith, 1981). Quantifying heat use efficiency (HUE) is useful for assessing the yield potential of a plant in different environments (Singh et al., 2018). Even under the most favourable agro-climatic conditions, the total heat and radiation energy available to the plant is not completely converted to dry matter. HUE depends on sowing time, genetic factors, and the applied agro-technology (Rao et al., 1999; Rani et al., 2012).

Fertilizer use plays a central role in increasing maize yield (Nagy, 2008; Lucas et al., 2019). Nitrogen is the primary limiting factor for maize plant growth and yield (Berzsenyi 2009; Liu et al., 2013; Thomsen et al., 2014; Du et al., 2020). Nitrogen is an essential building block of plant proteins and as such is one of the most important influencers of quality parameters in addition to quantity (Mamatha et al., 2017; Litke et al., 2019; Széles et al., 2019b).

Nitrogen uptake is lowest during maize emergence and then intensifies as of the 6–7 leaf stage, after stem elongation and is the highest during silking (Ciampitti & Vyn, 2013). Nitrogen uptake and incorporation are also significant during grain filling (Blackmer & Schepers, 1996; Ciampitti & Vyn, 2013). Applying the right amount of spring N basic and top-dressing fertilizer in the appropriate time reduces nitrogen loss, increases the efficiency of nitrogen supply, and improves economical nutrient supply, yield amount, and production efficiency (Muthukumar et al., 2007; Sitthaphanit et al. 2010; Ványiné & Nagy, 2012; Széles et al., 2019a).

The quality of maize grains is determined by climatic factors (Izsáki, 2007; Hegyi et al., 2008; Széles et al., 2018; Butts-Wilmsmeyer et al., 2019). Temperatures above 35 °C negatively affect protein production and alter the chemical structure of proteins (Monjardino et al., 2005; Rahman, 2005; Ristic et al., 2009).

The aim of the present study was to determine the heat demand and heat utilization efficiency of two different FAO number maize hybrids by means of various agrometeorological indices (GDD, PTU, HTU, HYTU). Furthermore, the intention was to explore the correlation between weather factors and nitrogen basic and top dressing, as well as the yield and quality of maize in the given crop production environment.

MATERIAL AND METHODS

Site description

The experiments were performed in the eastern part of Hungary, at the Experimental Site of the University of Debrecen (47° 33 'N, 21° 26' E, altitude 111 m), in moderately warm, dry growing area, on calcareous chernozem soil (Mollisol-Calciustoll or Vermustoll, clay loam; USDA) in a multivariate, four-replicate, stripped small-plot field trial in 2018 and 2019 with natural precipitation, involving hybrids with different genetic composition and different FAO numbers (Fornad, FAO 420 and Sushi, FAO 340).

Soil data

The average pHKCl of the soil is 6.6 (weakly acidic). In the upper (20 cm) layer of the soil Arany's plasticity index is 39, carbonated lime content in the upper 80 cm of the soil is around 0% (lime deficient) but from 100 cm it is 12% (moderately calcareous). The organic matter content in the upper 20 cm layer of the soil is 2.3% and at a depth of 120 cm it does not exceed 1.0%. The soil has a good potassium supply and a medium P supply. The soil has a favourable water absorption and significant water retention capacity. In the soil profile (0–2 m), which is decisive for the water supply of the plants grown in the long-term experiment, the soil is able to retain about 600–700 mm of water, of which approximately 65% is the amount of water available. The average depth of groundwater in the experimental area is 3–5 m (Pepó & Csajbók, 2014).

Weather data

To evaluate the weather conditions of the maize production experiments, the daily data of an automatic meteorological station operated by the Agrometeorological and Agroecological Monitoring Centre of the University of Debrecen near the experimental plots (500 m distance) was used. The climate data of the Debrecen Airport Station of the National Meteorological Service for the period of 1981–2010 (OMSZ, 2020) served as a reference for the examination of the deviations from the multi-year average.

The research included the examination of the cumulative values of different agrometeorological indices, growing degree days (GDD), photothermal units (PTU), heliothermal units (HTU) and hydrothermal units (HYTU) for each phenological stage of the experimental maize stocks.

In addition to the important emergence-tasseling (VE-VT) and emergence-physiological maturation (VE-R6) phases, emergence-6 leaf (VE-V6), emergence-12 leaf (VE-V12), emergence-silking (VE-R1), emergence–dough (VE-R4), tasselling-physiological maturity (VT-R6), silking-physiological maturity (R1-R6) are also evaluated.

Growing degree days (GDD)

$$\mathrm{GDD} = \sum \left(\frac{(\mathrm{T_{max}} + \mathrm{T_{min}})}{2} - \mathrm{T_b} \right) \quad (1)$$

where, T_{max} (°C) is the daily maximum temperature, T_{min} (°C) is the daily minimum temperature, T_b (°C) is the base temperature. If the daily average temperature is lower than the base temperature, i.e if $\frac{(T_{max}+T_{min})}{2} < T_b$ then $\frac{(T_{max}+T_{min})}{2} = T_b$ was used for the calculation, thus the given daily value of thermal heat unit is 0 (McMaster & Wilhelm, 1997). T_b is the temperature below which the rate of development is considered 0. The heat sum was calculated with T_b = 10 ºC in accordance with the scientific literature data (Davidson & Campbell, 1983; Gallagher, 1979).

Photothermal units (PTU)

$$PTU = \sum DL \cdot \left(\frac{(T_{max}+T_{min})}{2} - T_b\right), \quad (2)$$

where, DL (hours) is the length of day. The daily value of PTU is the product of the daily heat unit and the length of the given day (McMaster & Smika, 1988).

Heliothermal units (HTU)

$$HTU = \sum SH \cdot \left(\frac{(T_{max}+T_{min})}{2} - T_b\right), \quad (3)$$

where, SH (hour) is the daily duration of sunlight, the value of HTU is the product of the daily heat unit and the sunny hours of the given day. Data from direct sunlight measurements (with a Campbell-Stokes measuring device) were not available, thus sunlight duration data calculated from the global radiation by the Debrecen station of the National Meteorological Service were used.

Hydrothermal units (HYTU)

$$HYTU = \sum RH \cdot \left(\frac{(T_{max}+T_{min})}{2} - T_b\right), \quad (4)$$

where, RH (%) is the daily mean value of relative humidity, the daily value of HYTU is the product of the daily heat unit and the average relative humidity of the given day. The daily mean value of relative humidity was calculated from hourly data.

Energy use efficiency: Knowing the average yield of each treatment, the value of heat unit efficiency indices were calculated from the value of the previously presented agrometeorological indices summarized for the growing season (VE-R6) as follows:

$$\text{Heat Use Efficiency (HUE, kg ha}^{-1}\ °\text{C}^{-1}\ \text{day}^{-1}) = \text{Yield/GDD} \quad (5)$$

where, Σ GDD = Cumulative growing degree days

$$\text{Photothermal Use Efficiency (PTUE, kg ha}^{-1}\ °\text{C}^{-1}\ \text{day}^{-1}\ \text{hr}^{-1}), = \text{Yield/PTU} \quad (6)$$

where, Σ PTU = Photothermal units

$$\text{Heliothermal Use Efficiency (HTUE, kg ha}^{-1}\ °\text{C}^{-1}\ \text{day}^{-1}\ \text{hr}^{-1}), = \text{Yield/HTU} \quad (7)$$

where, Σ HTU = Heliothermal units

$$\text{Hydrothermal Use Efficiency (HYTUE, kg ha}^{-1}\ °\text{C}^{-1}\ \text{day}^{-1}\ \%^{-1}) = \text{Yield/HYTU} \quad (8)$$

where, Σ HYTU = Hydrothermal units.

Experimental details

In the field experiment, in addition to the treatment without fertilization (control), the fertilizer doses were applied as basic and top fertilizer divided as follows:

– Base fertilization: $A_{(0)}$ = non-fertilized control; A_{60} = 60 kg N ha^{-1}; A_{120} = 120 kg N ha^{-1},

– Top-dressing in the V6 phenophase: $V6_{90} = A_{60} + 30$ kg N ha^{-1}; $V6_{150} = A_{120} + 30$ kg N ha^{-1},

– Top-dressing in the V12 phenophase: $V12_{120} = V6_{90} + 30$ kg N ha^{-1}; $V12_{180} = V6_{150} + 30$ kg N ha^{-1}

The number of plants was 73 thousand plants ha^{-1}, the green crop was maize. The maize was sown on 23/04/2018 and 10/04/2019. The harvest was took place on 27/09/2018 and 09/10/2019. Harvested grain yield was corrected for 14% moisture content.

From the yield of maize hybrids in both years, samples were collected from each treatment and the protein content of the grains was determined with a Foss Infratec™ 1241 device based on the near-infrared-transmittance (NIT) measurement method.

Statistical analysis

The effect of treatments on yield was examined using a general linear model (GLM) (Huzsvai & Vincze, 2013). Within GLM, evaluation was performed based on the Repeated Measurement Model. Mean values of the treatments were compared by means of the Duncan's test to avoid accumulation of Type I error. Within the homogeneous group, the yields did not differ with a significance level of 5%. The evaluation was performed with the statistical software package SPSS for Windows 21.0.

RESULTS AND DISCUSSION

Development of agrometeorological indexes

The 2018 growing season of maize was characterized by high mean temperature and low amount of precipitation, while drought stress (DS) was developed. It was 1.9 °C warmer than the 30-year average (17.5 °C) and had a precipitation deficit of 30 mm compared to the average (346 mm). The weather in 2019 was non-stress (NS), its average temperature (17.8 °C) was almost the same as the temperature characteristics of the years of the region, its precipitation supply was above average (+ 43 mm).

GDD, PTU, HTU, and HYTU developed differently for each hybrid under DS and NS conditions (Table 1).

The combined effect of cultivation conditions and hybrids on heat unit (GDD) was significant in all phenological phases, similar to the results of Malo & Ghosh (2018). Under DS conditions, the longer maturity Fornad maize hybrid required a higher amount of heat (GDD) than the shorter maturity Sushi maize hybrid during the entire growing season to reach each developmental stage. The largest difference was in the vegetative (VE-V12) phase, the accumulated GDD value difference being 84 day °C, coupled with only a 7 mm precipitation excess (Fig. 1). Silking is related to the average air temperature and acts as an important factor on flower formation (Iannucci et al., 2008). The Sushi hybrid reached the R1 phenophase with a lower GDD (594 day °C) than the Fornad hybrid (621 day °C). The difference in GDD between the two hybrids was also significant in the reproductive phase. The difference between the GDD value was 54 day °C in the VT-R6 phase and 46 day °C in the R1-R6 phase. There was a significant difference in precipitation during these phenophases. The Fornad hybrid had a 62 mm precipitation surplus. From emergence to physiological maturity (VE-R6), the Fornad hybrid required 1,446 day °C, while the Sushi hybrid required 1,373 day °C.

Table 1. Development of agrometeorological indices in the phenological stages of maize hybrids of different genotypes under drought stress (DS) and non-stress (NS) conditions

Phenological phases	2018 (DS)				2019 (NS)			
	Accumulated							
	GDD (day °C)	PTU (day °C hour)	HTU (day °C hour)	HYTU (day °C %)	GDD (day °C)	PTU (day °C hour)	HTU (day °C hour)	HYTU (day °C %)
	Sushi hybrid							
VE-V6	186	2,754	2,109	9,967	193	2,916	1,574	14,191
VE-V12	466	7,155	4,966	28,400	463	7,216	4,831	32,935
VE-VT	580	8,970	5,892	35,827	644	10,100	7,268	44,157
VE-R1	594	9,198	5,944	36,812	682	10,693	7,692	46,162
VT-R6	793	11,967	8,071	47,727	780	11,374	8,247	49,225
R1-R6	779	11,740	8,019	46,742	742	10,781	7,823	47,221
VE-R4	832	12,954	8,357	51,231	961	14,980	10,597	65,053
VE-R6	1,373	20,937	13,964	83,554	1424	21,474	15,515	93,383
	Fornad hybrid							
VE-V6	202	3,005	2,288	11,019	214	3,251	1,797	15,680
VE-V12	550	8,493	5,549	34,075	488	7,621	5,129	34,846
VE-VT	598	9,272	6,000	37,104	667	10,456	7,582	45,273
VE-R1	621	9,626	6,179	38,552	700	10,990	7,890	47,056
VT-R6	847	12,668	8,632	50,989	777	11,279	8,082	49,201
R1-R6	825	12,314	8,453	49,541	743	10,746	7,774	47,418
VE-R4	882	13,735	8,702	54,849	1,007	15,670	11,029	68,389
VE-R6	1,446	21,940	14,633	88,093	1,444	21,735	15,665	94,474

Under NS conditions, the cumulative GDD of the Fornad hybrid was higher until the end of the vegetative developmental stage than that of the Sushi hybrid. The difference ranged from 21 day °C to 25 day °C. In the reproductive stage, the difference became balanced. For the entire growing season, the Fornad hybrid accumulated a higher GDD value (1,444 day °C) than the Sushi hybrid (1,424 day °C). There was no difference in the amount of precipitation among the developmental stages, except for the VE-V12 stage, however that it was not significant (6 mm).

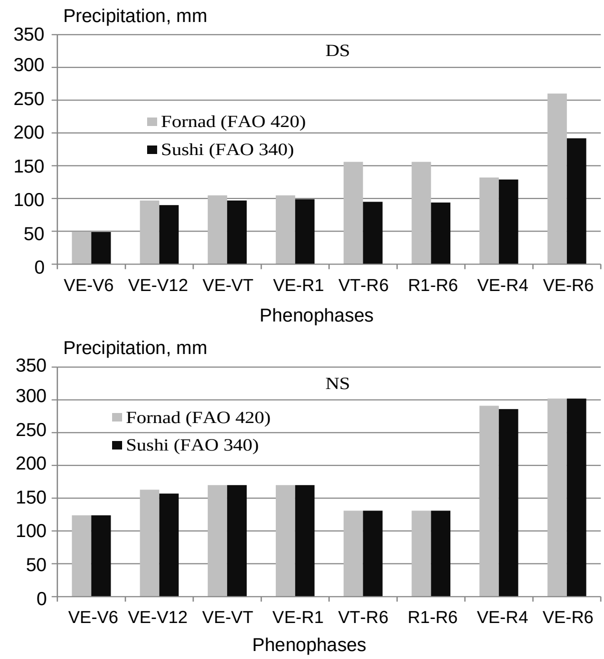

Figure 1. Amount of precipitation in the phenological phases of maize hybrids of different genotypes under drought stress (DS) and non-stress (NS) conditions.

PTU, HTU, and HYTU were higher in the case of the Fornad hybrid than the Sushi hybrid during the growing season (VE-R6) under both DS and NS conditions. Between the vegetative (VE-VT) and reproductive (R1-R6) stages, more PTU, HTU, and HYTU accumulated under DS conditions for the Fornad hybrid, while under NS conditions there was more accumulation for the Sushi hybrid.

Abiotic stress tolerance and the effect of basic fertilization and top-dressing on maize yield

Under DS, yield of the Fornad maize hybrid without fertilization was 7,114 t ha^{-1}, the 60 kg N ha^{-1} applied as a base treatment increased the yield by 40.9% ($P < 0.05$), while the 120 kg ha^{-1} by 78.5% ($P < 0.05$) (Table 2). The 26.7% difference between A_{60} and A_{120} treatments was significant ($P < 0.05$). In the V6 and V12 phenophases, additional fertilizer application did not result in a significant yield increase. The maximum yield was ensured by the $V12_{120}$ treatment (13.614 t ha^{-1}), but based on the Duncan's test, the 12.695 t ha^{-1} result of the A_{120} treatment proved to be the best. The Sushi maize hybrid responded very strongly to the A_{60} treatment, with a yield increase of 71.2% ($P < 0.05$). Increasing the 60 kg N ha^{-1} applied as a base treatment in the V6 and V12 phenophases by an additional 30 + 30 kg N ha^{-1} did not result in a significant yield increase. The highest yield and the statistically confirmed maximum yield coincided in the case of the Sushi hybrid, which was achieved as a result of the $V6_{150}$ treatment (13.167 t ha^{-1}; $P < 0.05$).

Based on the *t-test*, a significant difference between the two hybrids was observed as a result of the A_{120} and $V12_{120}$ treatments. The longer maturity Fornad hybrid outperformed the shorter maturity Sushi hybrid by 1.393 t ha^{-1} ($P < 0.01$) in the A_{120} treatment and by 2.201 t ha^{-1} ($P < 0.001$) in the $V12_{120}$ treatment.

Table 2. Effect of base fertilization and top-dressing on grain yield of maize hybrids under DS and NS conditions

Hybrids	Year	Treatments						
		A_0	A_{60}	A_{120}	$V6_{90}$	$V6_{150}$	$V12_{120}$	$V12_{180}$
Sushi	DS	6.039a	10.341bc	11.303c	11.707bc	13.167d	11.414	12.945bc
	NS	8.910a	11.157c	13.527e	11.328c	12.062d	10.565bc	10.109b
		***	ns	***	ns	ns	***	***
Fornad	DS	7.114a	10.023b	12.695c	9.767b	13.105c	13.614c	12.939c
	NS	9.161a	12.124bc	12.711c	11.808bc	14.023d	11.033b	12.227bc
		***	**	ns	ns	**	***	ns

Note: Within each line, different lowercase letters indicate the difference between fertilizer treatments under DS and NS conditions based on *Duncan's test* ($P < 0.05$). Within the columns, based on the *t-test*, *** $P = 0.001\%$, ** $P = 0.01\%$, ns = non-significant notations indicate the difference between DS and NS.

Heat use efficiency was influenced by different weather conditions and nutrient levels as confirmed by the findings of Rao et al. (1999) and Malo & Ghosh (2018). For the Fornad hybrid, it ranged from 4.92 kg ha^{-1} $°C^{-1}$ day^{-1} (non-fertilized) to 9.42 kg ha^{-1} $°C^{-1}$ day^{-1} ($V12_{120}$). For the Sushi hybrid, the values are between 4.40–9.59 kg ha^{-1} $°C^{-1}$ day^{-1} (non-fertilized and $V6_{150}$). Due to the shorter maturity and the lower heat demand, it was possible that despite the lower average yield, the maximum value of heat use was higher than in the case of the Fornad hybrid. The lowest PTUE, HTUE, and HYTUE values for both hybrids were recorded in the non-fertilized (A_0) treatment, while the highest PTU, HTUE, and HYTUE values were recorded in the

$V12_{120}$ for the Fornad hybrid and in the $V6_{150}$ treatment the Sushi hybrid. For the Fornad and Sushi hybrids, PTUE varied between 0.32–0.62 to 0.29–0.63 kg ha^{-1} °C^{-1} day^{-1} hr^{-1}, HTUE was 0.49–0.93 to 0.43–0.94 kg ha^{-1} °C^{-1} day^{-1} hr^{-1}, and HYTUE ranged from 0.081–0.155 to 0.072–0.158 kg ha^{-1} °C^{-1} day^{-1}%$^{-1}$ for each fertilization treatment (Table 3).

Table 3. Thermal use efficiencies of maize hybrids in terms of grain yield, under DS and NS conditions

	2018 (DS)				2019 (NS)			
	Accumulated							
Treatments	HUE (kg ha^{-1} °C^{-1} day^{-1})	PTUE (kg ha^{-1} °C^{-1} day^{-1} hr^{-1})	HTUE (kg ha^{-1} °C^{-1} day^{-1} hr^{-1})	HYTUE (kg ha^{-1} °C^{-1} day^{-1} %$^{-1}$)	HUE (kg ha^{-1} °C^{-1} day^{-1})	PTUE (kg ha^{-1} °C^{-1} day^{-1} hr^{-1})	HTUE (kg ha^{-1} °C^{-1} day^{-1} hr^{-1})	HYTUE (kg ha^{-1} °C^{-1} day^{-1} %$^{-1}$)
	Sushi hybrid							
A_0	4.398	0.288	0.432	0.072	6.259	0.415	0.574	0.095
A_{90}	7.532	0.494	0.741	0.124	7.837	0.520	0.719	0.119
A_{120}	8.232	0.540	0.809	0.135	9.502	0.630	0.872	0.145
$V6_{120}$	8.527	0.559	0.838	0.140	7.957	0.528	0.730	0.121
$V6_{150}$	9.590	0.629	0.943	0.158	8.473	0.562	0.777	0.129
$V12_{150}$	8.313	0.545	0.817	0.137	7.421	0.492	0.681	0.113
$V12_{180}$	9.428	0.618	0.927	0.155	7.101	0.471	0.652	0.108
	Fornad hybrid							
A_0	4.920	0.324	0.486	0.081	6.346	0.421	0.585	0.097
A_{90}	6.932	0.457	0.685	0.114	8.398	0.558	0.774	0.128
A_{120}	8.781	0.579	0.868	0.144	8.805	0.585	0.811	0.135
$V6_{120}$	6.755	0.445	0.667	0.111	8.180	0.543	0.754	0.125
$V6_{150}$	9.064	0.597	0.896	0.149	9.714	0.645	0.895	0.148
$V12_{150}$	9.416	0.621	0.930	0.155	7.636	0.507	0.704	0.117
$V12_{180}$	8.949	0.590	0.884	0.147	8.470	0.563	0.781	0.129

Under NS conditions, the Fornad hybrid responded to the A_{60} base treatment with a 32.2% yield increase. The yield difference resulting from the two base treatments is not significant. An additional 30 kg of N ha^{-1} ($V6_{150}$) in the V6 phenophase proved to be effective after the A_{120} treatment ($P < 0.05$). For the Sushi hybrid, both base treatments significantly increased yield compared to the control treatment, however, the application of additional N was not effective. The A_{120} treatment (13.527 t ha^{-1}) is considered justified (Table 2).

The Fornad hybrid was more effective than the Sushi hybrid in the $V6_{150}$ treatment with 1.961 t ha^{-1} ($P < 0.001$) and in the $V12_{180}$ treatment ($P < 0.01$). The *t-test* for the other treatments showed no significant difference between the hybrids.

Under NS conditions, there was little difference in the length of the cultivation period of the two hybrids, thus the differences in the individual accumulated heat unit values are also negligible. HUE values differed less between treatments, ranging from 6.35 to 9.71 for the Fornad hybrid and 6.26–9.50 kg ha^{-1} °C^{-1} day^{-1} for the shorter maturity genotype. The lowest PTUE, HTUE, and HYTUE values for both hybrids were recorded for the non-fertilized (A_0) treatment, while the highest PTU, HTUE, and HYTUE values for Fornad hybrid were achieved in the $V6_{150}$, while the Sushi hybrid reached it in the A_{120} treatment. For Fornad and Sushi hybrids, PTUE was 0.42–0.64 and 0.42–0.63 kg ha^{-1} °C^{-1} day^{-1} hr^{-1}, HTUE was 0.59–0.90 and 0.57–0.87 kg ha^{-1} °C^{-1} day^{-1} hr^{-1}, and HYTUE was 0.097–0.148 and 0.095–0.145 kg ha^{-1} °C^{-1} day^{-1}% $^{-1}$ for each fertilization treatment (Table 3).

The effect of weather factor was the most significant in the case of both hybrids in the control treatment. Under the influence of NS, the natural nutrient utilization capacity of the Sushi hybrid was 47.5% higher ($P < 0.001$), while that of the Fornad hybrid was 28.8% ($P < 0.001$) higher than under DS conditions (Table 2). In the case of the Fornad hybrid, the lowest applied base treatment of 60 kg N ha^{-1} (A_{60}) ($P < 0.01$) and the $V6_{90}$ N ha^{-1} ($P < 0.001$) treatment resulted in a yield increase of 21–21% under NS conditions. In the case of the Sushi hybrid, the effect of NS was significant in the A_{120} treatment (19.7%, ($P < 0.001$)). Utilization of the fertilizers applied in the V6 and V12 phenological phases was not significantly helped by NS.

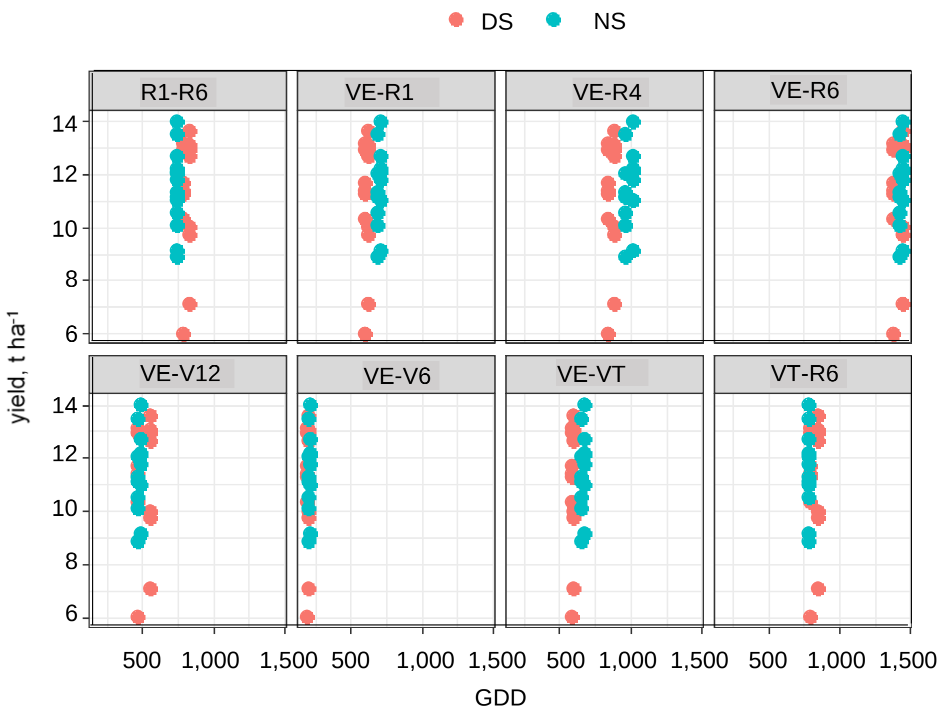

Figure 2. Development of GDD and yield in the average of maize hybrids under drought stress (DS) and non-stress (NS) conditions.

In the control treatment, the heat utilization efficiency of the Sushi hybrid was 42% higher under NS conditions than in the drought stress (DS) crop year. Due to the higher heat sum in the NS crop year, this increase is below the increase of natural nutrient utilization capacity. In the case of the Fornad hybrid, due to the nearly identical heat sum of the two crop years, the 29% difference in HUE is essentially the same as the difference in yield averages. Mainly due to the more favourable water supply, HYTU was 11–12% higher in the NS growing season than in the DS growing season. Consequently, HYTUE differs by 32% in the two crop years in the non-fertilized treatments of Sushi and by 20% in the treatments of the Fornad hybrid, which is significantly below the differences in the average yield. Under more favourable conditions, heat efficiency is higher, but not as much as the increase in natural nutrient utilization.

Overall, the study confirmed the results of Nandini & Sridhara (2019) and Ahmed & Saikia (2020), namely that nearly the same GDD is required for each phenological phase every year (Fig. 2).

Abiotic stress tolerance and the effect of basic fertilization and top-dressing on the protein content of maize grains

Under DS conditions, GP ranged from 6.9 to 9.6 g (100 g dm.)$^{-1}$ (Table 4). As a result of N fertilizer applied at different times and doses GP increased compared to control treatment. The rate of growth varied among the applied hybrids. The hybrid GP of Fornad responded to the A_{120} fertilizer dose with a reliable difference (11.4%, $P < 0.05$). An additional 30 kg N ha^{-1} ($V6_{90}$) applied after 60 kg N ha^{-1} A_{60} base treatment in the V6 phenophase resulted in a 26.0% ($P < 0.05$) increase in GP. The A_{120} treatment which was the most effective in terms of GP, was 18.0% lower than the $V6_{90}$ treatment which provided the highest GP. For Sushi, both base treatments reliably increased GP compared to the control treatment. The 120 kg N ha^{-1} (A_{120}) was more effective, the growth rate was 23.2% ($P < 0.05$). The application of + 30 kg N ha^{-1} after the 120 kg base treatment in V6 phenophase resulted in an additional 11.8% ($P < 0.05$) increase. This treatment provided the highest GP statistically.

Table 4. Effect of basic fertilization and top-dressing on the grain protein content of maize hybrids under DS and NS conditions

Hybrids	Year	Treatments						
		A_0	A_{60}	A_{120}	$V6_{90}$	$V6_{150}$	$V12_{120}$	$V12_{180}$
Sushi	DS	7.0a	7.3a	7.8b	9.2d	8.6c	8.7c	8.5c
	NS	6.9a ns	7.4b ns	8.5c ***	8.5c *	9.5e ***	9.1d *	9.6e ***
Fornad	DS	7.9a	9.4bc	8.8b	9.3bc	9.9c	9.9c	10.0c
	NS	8.1a ns	8.7b ns	9.3c ***	9.9d *	10.2e ns	10.2e **	10.0de ns

Note: Within each line, different lowercase letters indicate the difference between fertilizer treatments under DS and NS conditions based on *Duncan's test* ($P < 0.05$). Within the columns, based on the *t-test*, *** $P = 0.001\%$, ** $P = 0.01\%$, *$P = 0.05\%$, ns = non-significant notations indicate the difference between DS and NS.

There was no significant difference between the two hybrids in the control and A_{60} treatments based on the *t-test*. The GP value of the Sushi hybrid was reliably higher than that of the Fornad hybrid, except for the $V6_{90}$ treatment. The largest difference was in the $V6_{150}$ treatment (10.5%, $P < 0.001$).

As a result of NS, there was an increase in GP, ranging from 7.9 to 10.2 g (100 g dm.)$^{-1}$ (Table 4). For the Fornad hybrid, the A_{60} treatment was more effective than the control (19%, $P < 0.05$) of the basic treatments. There was no significant difference between the two basic treatments, however, increasing the 120 kg N ha^{-1} dose in the V6 phenophase by 30 kg N ha^{-1} significantly improved GP (+12.5%, $P < 0.05$). The amount of N applied in the V12 phenological phase no longer had a GP-increasing effect. In the case of the Sushi hybrid, both basic treatments reliably increased GP compared to control, the A_{120} (14.8% $P < 0.05$) treatment increased it by a higher degree. The GP value was significantly affected by the + 30 kg N ha^{-1} applied after the basic 120 kg N ha^{-1} (A_{120}) treatment in the V6 stage; the growth rate was 9.7% ($P < 0.05$). The amount of N fertilizer applied in the V12 phenophase did not further increase the GP value.

The *t-test* showed significant difference between the hybrids in the A_{120}, $V6_{90}$ and $V12_{120}$ treatments. In all cases, the GP value of the Sushi hybrid was higher. The largest difference was recorded in the $V6_{90}$ treatment (6.5%, $P < 0.05$)

The *weather factor* greatly affected both hybrid GPs. In the non-fertilized (control) treatment, the GP value of the Fornad hybrid under NS conditions was 12.9% ($P < 0.001$) higher than under DS. In the case of the Sushi hybrid, the effect of NS was more significant (17.4%, $P < 0.001$). For the Fornad hybrid, the largest GP modifying effect of NS was recorded in the A_{60} (28.8%, $P < 0.01$) treatment. There was no significant difference between DS and NS in the $V6_{90}$ treatment, in other treatments the effect of NS was reliably higher ($P < 0.001$) in all cases (Table 4).

The GP value of the Sushi hybrid significantly increased in both the basic and top-dressing treatments under the influence of NS. The rate of increase was significant at 0.1% level, with the exception of the $V12_{180}$ treatment. The largest difference, similar to the Fornad hybrid, was recorded in the A_{60} (17.6%) treatment.

CONCLUSIONS

Examining the heat sum values for the whole growing season, it can be stated that there was no significant difference between the years (Sushi 3.7%), and in the case of the Fornad hybrid (0.1%) there was essentially no difference. These findings are in line with the essence of the heat sum concept, i.e. the occurrence of a phenological phase is expected when the value of the heat sum reaches the heat demand of the given plant (species, variety, hybrid) required for the given phenological phase (Rao et al. 1999; Malo & Ghosh, 2018; Bonhomme, 2000).

The difference in growing time between hybrids (FAO 420-FAO 340) was shown by the heat sum, although under NS conditions it was lower than under DS conditions. Although the hybrid with a higher FAO number required a higher amount of heat to achieve silking than the hybrid with a lower FAO number, there was no difference in the amount of heat required in the reproductive phase. Under DS conditions, the need for a larger amount of heat in the case of the Fornad hybrid was demonstrated in both subphases.

Photothermal units can be used to estimate phenophase length for some plants with less error than GDD (McMaster & Smika, 1988; Bouzo & Favaro, 2014). In the two years of the experiment, the values of PTU for the growing season were also almost the same in the two years, the differences (Sushi hybrid 2.6%, Fornad hybrid 0.9%) were significantly lower than the relative differences in the number of growing days. Consistent with the conclusion of Malo & Ghosh (2018), the findings suggest that this agrometeorological index may also be suitable for estimating the length of the phenological phase in the case of maize. However, the limited validity and applicability of PTU remains, as it does not take into account the change in photoperiodic sensitivity over time, the critical length of day, or even significant differences in sensitivity between genotypes. More complex, well-parameterizable formulas than PTU can be used to describe the combined effect of photoperiod and temperature (Birch et al., 1998; Yan et al., 1998; Bonhomme, 2000). Large differences in HTU and HYTU units (7–12%) between the two growing seasons suggest that they are not suitable for estimating the length of phenological phases.

Heat use efficiency (HUE) for grain yield was similar to that described by Rao et al. (1999) and Malo & Ghosh (2018), it showed a difference between the hybrids. The minimum HUE for both hybrids was shown by the unfertilized treatment regardless of weather factors. The maximum HUE was developed for different fertilizer treatments in the case of the two hybrids hybrid.

The suggested amount of N-fertilizer and time of application to achieve the highest yield developed differently for the two hybrids, which was also influenced by environmental factors. In the case of the Fornad hybrid, the A_{120} treatment is recommended under DS and the $V6_{150}$ treatment under NS conditions. However, for the earlier maturity Sushi hybrid, the $V6_{150}$ treatment is suggested under Ds and the A_{120} treatment under NS conditions. DS caused the highest yield loss in the case of the later maturity Fornad hybrid.

The studied maize hybrids adapted to DS conditions by reaching physiological maturity in a shorter time, thereby minimizing the effect of DS.

N top-dressing promoted GP growth of maize grains. Under drought stress (DS), the Fornad hybrid provided the highest GP up to 90 kg N ha^{-1} ($V6_{90}$) and the Sushi hybrid up to 150 kg N ha^{-1} ($V6_{150}$). Under non-stress (NS) conditions, increasing the base dose of 120 kg N ha^{-1} for both hybrids in theV6 phenological phase by an additional + 30 kg N ha^{-1} proved to be an appropriate treatment ($V6_{150}$) to achieve high GP.

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Soil Phosphorus management based on changes in Olsen P and P budget under Long-term fertilization experiment in fluvo-aquic soil

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Abstract. Excessive input of phosphorus (P) in agricultural production and its finite resources is becoming a global concern for sustainable P management. In this study, the annual P input and output were calculated in 27 Fluvo-aquic soil sites of wheat and maize agriculture cropping system in Henan province central-east of China during the period from 1998 to 2016, to quantify soil Olsen P (OP) levels and P budget at the experimental sites and calculate the optimum P fertilizer application. The maize and wheat (Triticum aestivum) recorded 14.2 and 13.6 mg kg^{-1} respectively. The change in soil OP was positively linearly correlated with the P budget ($P < 0.01$), and an increase of 2.8 mg kg^{-}1 in soil Olsen P for each 100 kg ha^{-1} of P budget in the 0–20 cm soil layer. Based on ACV of soil OP with P budget and the critical level of soil OP to ACV in the study area for the next five years, the recommended rate model of soil OP for maize and wheat in the study area was determined. The application amount of P fertilizer ought to be in the range of 64–85 kg P ha^{-1}. This information can help to optimize crop yield, reduce the accumulation of P in soil, and reduce the potential risk of water pollution. More research is needed about the main factors influence on P available (OP).

Key words: Olsen P, agronomic critical value, P budget, P fertilizer, maize, wheat.

INTRODUCTION

Phosphorus (P) is a very important nutrient required for optimum crop production. However, when the P fertilizer application is more than plant uptake, the residual P is accumulated in the soil (Grant et al., 2005; Rubæk et al., 2013), this may lead to environmental problems. For example, P can become soluble in water and mobile, as it enters surface water and causes algae and other unwanted plants to grow(Anju et al., 2010). Herbicides are used for weed control (Zhang, 2003). This reduces the quality of water, fish, and desirable aquatic plants (Li et al., 2010; Jarvie et al., 2013). Global P fertilizer consumption increased from approximately 4.6 to 17.5 Tg P yr^{-1} from 1961 to 2013 (Bai et al., 2013; Lu 2000). In China, P fertilizer application consumption has increased from 1.8 to 8.9 Tg P yr^{-1} from 2000 to 2013 (Yuan et al., 2017). The mean Olsen P (OP) has risen from 8–21 mg kg^{-1} during the period 1980–2011 (Wang et al., 2016). The OP ranges between 20 and 40 mg kg^{-1} in the 90% dominant Chinese arable land, whereas 9% of the agriculture soil have soil OP more than 40 mg kg^{-1}, which poses a threat to the environment (Zhong et al., 2004; Li et al., 2010).

It's useful to keep the optimal yield and properly manage the reasonable OP-value for reducing the risk of environmental pollution (Karamesouti & Gasparatos 2017). Scientists are exerting many efforts to achieve optimal OP values, thus achieving the highest productivity while maintaining the environment (Yan et al., 2013; Karamesouti & Gasparatos 2017). The definition of ACV is the level of OP after which the crop's response to P fertilization is poor or virtually nonexistent (Tang et al., 2009; Bai et al., 2013). Agronomic critical value varies with crops and sites due to the different crop P requirements and the soil properties (Colomb et al., 2007). Li et al. (2011) revealed that the critical value of soil OP extends between 4 to 15.5 mg kg^{-1} for maize and 5 to 20 mg kg^{-1} for wheat, and this coincides with the findings by (Wu et al., 2018) found that discovered the average ACV of OP for maize and wheat were 14.2 mg kg^{-1} and 14.4 mg kg^{-1}, respectively, utilizing both Mitscherlich and Linear-plateau models in Shandong province central of China. The soil OP level is generally affected by P budget; many studies found the soil OP and P budget gave significant positive linear correlation (Messiga et al., 2010; Cao et al., 2012).

Yang et al., 2015 found that in a Fluvo-aquic soil in Tianjin, China, under different treatments of P application rate, the soil OP was increased in the ranges between 1.2 and 3.6 mg kg^{-1} for every 100 kg ha^{-1} of P surplus. Also, Yang et al. (2015) found that the soil OP in the Fluvo-aquic soil at Shanxi province in China was increased by a range of 4.2–17 mg kg^{-1} for every 100 kg ha^{-1} P surplus. In Henan province, the soil OP increased by 1.2 mg kg^{-1} for every 100 kg ha^{-1} P surplus (Yuan et al., 2017). Numerous studies have shown a significant linear correlation between changes in soil OP and P budget (Cao et al., 2012; Zhan et al., 2015; Blake et al., 2000). However, they tried to determine the ideal P application rate depending on the reaction of OP in Fluvo-aquic soil to changes in P budget, considering both the ACV and environmental risks (Cao et al., 2012; Zhang et al., 2019). The aims of this research were (i) to estimate the annually P input-output during the period of the experiment (1998–2016), (ii) to calculate the optimum P fertilizer rate through the correlation between soil OP and P budget and (iii) the wheat and maize response to P fertilizer application, which will be helpful for the sustainable management of P fertilizer application in the research area.

MATERIALS AND METHODS

Experimental location and soil properties

A long-term experiment was conducted in Henan Province, Central-east of China to study the yield response of the wheat and maize system to P fertilizer application. The experiments were established in 1998 on Fluvo-aquic soil, located at Luoyang, Sanmenxia, Anyang, Nanyang, Zhoukou, Xinxiang, Kaifeng, Shangqiu and Puyang, with latitude (N) and longitude (E) range of 33°48'–34°40' N and 111°20–112°44′ E, respectively (Fig. 1). The climatic condition is classed as semi-tropical with the mean annual temperature 14 °C, and the annual precipitation ranges from 600–1,200 mm during the experimental period 1998–2016 (Table 1).

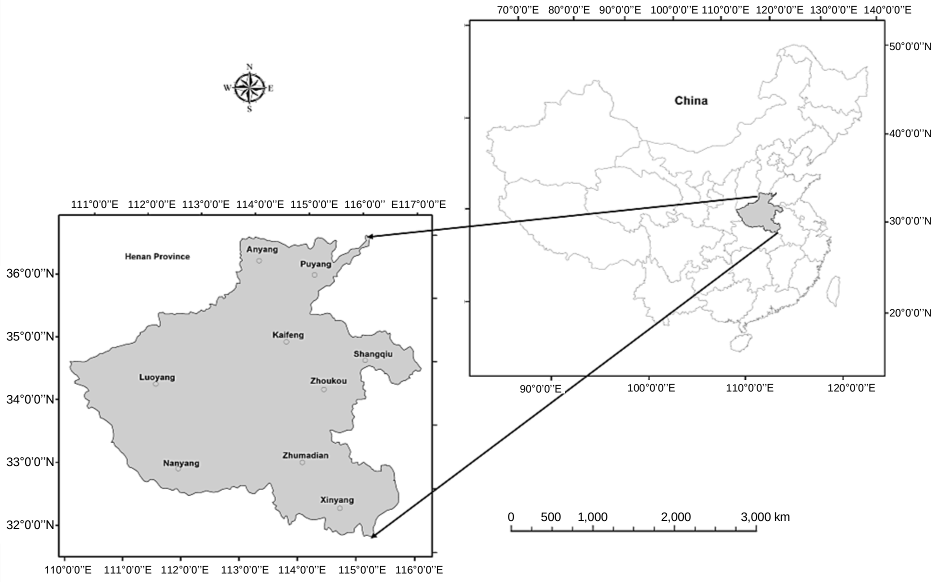

Figure 1. Sampling locations in nine cities in Henan province, central east China in the period between 1998–2016.

Soil and Plant analysis

Table 1. The initial soil properties (average value) in the study area in Henan province, central east China

Item	Unit	The average value
Organic C	g kg^{-1}	14.8
Total N	g kg^{-1}	0.96
Available N	mg kg^{-1}	79.0
Olsen P	mg kg^{-1}	11.6
Available K	mg kg^{-1}	118.0
Soil pH	-	7.8

Soil samples were gathered from depth 0 to 20 cm in the observing seasons after wheat harvest in autumn. Five soil samples were collected from each plot and thoroughly mixed to form a composite sample, air-dried at room temperature (20–25 °C) and sieved through a 2.0 mm sieve. Shake a 2.5 g soil sample with 50 ml 0.5 mol L^{-1} $NaHCO_3$ at 25 °C for 30 minutes, filter the suspension, and use the molybdate ascorbic acid method to determine the P concentration in the filtrate (Murphy & Riley 1962). Soil samples were analyzed for soil organic C (Walkley & Black, 1934), available P (Olsen, 1954), total P (Lu, 2000) and pH in water (1:2.5 soil to water ratio (Thomas, 1996). Total nitrogen was determined using micro-Kjeldahl digestion. Crop samples (grains and straw) were taken at harvest (maturity stage) plants were collected from the center strip of every plot. After drying, the crop samples were ground to pass 0.15 mm sieve to use for chemical analyses. To determine the total P content, plant powder samples were digested with H_2SO_4 H_2O_2, and the concentration in the digestion solution was determined by molybdenum blue colorimetric method (Scudder et al., 1996).

Data processing and calculation

P budget and Olsen P levels

The P budget in this research is related to the direct input and output of P. The processes for estimating P budget and OP level changes were similar at all locations. At the location level, the P budget was calculated by subtracting P output from P input (Messiga et al., 2010). The P uptake is determined according to P concentration in aboveground biomass (straw and grain). The seasonally P budget for every study area was calculated. The P loss caused by runoff and erosion is negligible. The change of OP was obtained by linear regression with the times. A long-term experiment was conducted to establish a prediction model of soil OP accumulation in wheat and maize cropping system, by using the amount of phosphate fertilizer, crop yield and cultivation time. The change of OP in the soil can be described by the following equation:

$$OP_f = OP_i + D \cdot (P_m - C_m Y_m) \cdot t \quad (1)$$

where OP_f – the final OP level; OP_i – the initial OP level; t – the time of cultivation; P – the P fertilizer addition rate; C – the concentration of P in crop grain. Y – the total crop yield per year. The $_m$ – indicates the mean of P, C, and Y during the agriculture period. D – the change in OP (C_{OP}) resulting from a one-unit change in the soil P budget (PB), which is estimated as follows:

$$C_{OP} = f + D \cdot P_B \quad (2)$$

where C_{OP} – the change in OP (mg kg^{-1}); P_B – P budget (P input – P output) kg ha^{-1} and f – the intercept parameter. The mean P fertilizer requirement was calculated by the following equation:

$$P_r = \frac{OP_f - OP_i}{D_t + P_h} \quad (3)$$

where Pr – the mean P fertilizer requirements; D is the slope of the line from the correlation between change in OP and P budget. The (Table 2) below shows the application rate of NPK in all experimental sites during the period of study.

Table 2. The application rate of the annual average Nitrogen (N), Phosphorus (P) and Potassium (K) NPK fertilizers (kg ha^{-1}) in the study area from 1998 to 2016 in Henan province, central east China

Site	Year	N	P	K
	(kg ha^{-1} yr^{-1})			
Luoyang (n = 3)	2004–2016	355 ± 23	83 ± 9	116 ± 16
Zhumadian(n = 3)	2003–2016	408 ± 30	78 ± 9	133 ± 24
Anyang (n = 2)	2000–2016	405 ± 22	81 ± 6	137 ± 9
Nanyang (n = 3)	1998–2016	335 ± 17	88 ± 13	120 ± 17
Zhoukou (n = 2)	2005–2016	418 ± 20	77 ± 9	121± 20
Xinxiang (n = 1)	1998–2016	458 ± 23	74 ± 2	126 ± 4
Kaifeng (n = 5)	1999–2016	273 ± 23	61 ± 7	86 ± 7
Shangqiu (n = 6)	1988–2016	386 ± 18	96 ± 8	144 ± 13
Puyang (n = 2)	1998–2016	414 ± 19	84 ± 9	101 ± 10

n is the number of sites observation.

Model description

To eliminate differences between years and locations, the relative yield is calculated as follows:

$$Y_r = Y_i \div Y_m \cdot 100 \quad (4)$$

where Y – the relative yield; Y_i – the annual crop yield of every treatment (kg ha^{-1}) and Y_m – the high annual crop yield (kg ha^{-1}).

Crop Agronomic critical value (ACV) can be simulated by Mitscherlich and linear-linear fitting.

The ACV of crops can be simulated by the Mitscherlich model (Johnston et al., 2013) as follows:

$$Y = \alpha \cdot [1 - exp(-bx)] \quad (5)$$

where Y – foreseen relative yield; a – high achievable yield; when x – unrestricted; b – the response factor and x is the OP concentration in soil (mg kg^{-1}).

The linear-linear model is defined by Eqs (6) and (7)

$$Y = A_1 + B_{1x}, \quad if\ x < C \quad (6)$$

$$Y = A_2 + B_{2x}, \quad if\ x \geq C \quad (7)$$

where A_1 (or A_2) – the intercept parameter; B_1 (or B_2) is the slope parameter; x – the soil OP concentration (mg kg^{-1}) and C – the critical value of soil OP.

Statistical analysis

Data processing

Mitscherlich and Linear- linear models for calculating crop ACV were simulated with sigma plot 12.5 (SYSTAT Software, San Jose, California, USA). Data were analyzed using one-way ANOVA to check its significance and averages were separated according to Duncan test at $P \leq 0.05$. SPSS 20.0 was used to normalize the experimental data to ensure the reasonable distribution of data.

RESULTS

The grain yield and experimental year

Fig. 2 shows a grain yield of maize and wheat for 19 years under long-term fertilization experiment in the study area. Only the significant differences in the grain yield of maize and wheat during 2014–2016 were observed compared with other periods.

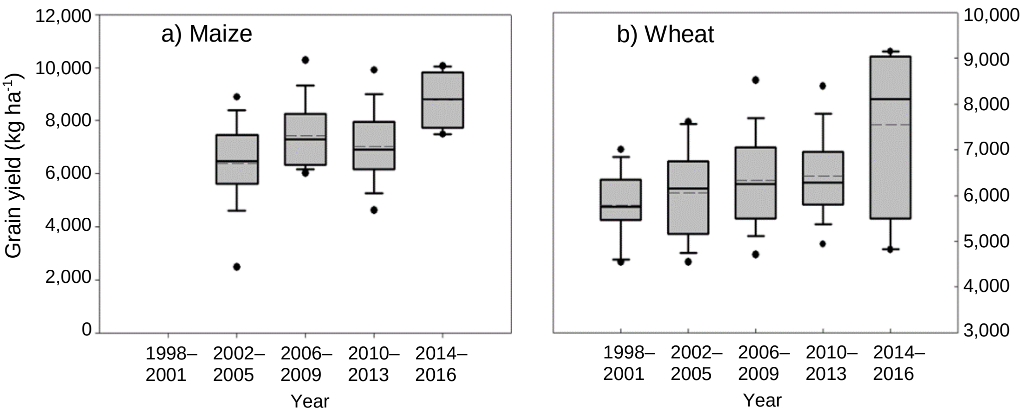

Figure 2. Simulated mean and median grain yield of maize (a) and wheat (b) over 19 years of observation in the study area under long-term fertilization experiment. black solid and The gray dash lines, lower and upper edges, and bars in or outside the boxes represent median and mean values, 25th and 75th, 5th and 95th, and, < 10th and > 90th percentiles of all data, respectively. Different lower-case letters denote significant differences in the grain yield (Duncan's multiple range tests; $p < 0.05$).

The maximum and minimum grain yield of maize was 11,550 and 2,122.5 kg ha^{-1} in 2014–2016 and 2010–2013 respectively, meanwhile the maximum and minimum grain yield of wheat were 9,150 and 4,125 kg ha^{-1} in 2014–2016 and 2006–2009 respectively. The variation in the yield due to the change in the ACV from low to optimum by applied fertilizer. The mean grain yield for wheat and maize significantly increased with time and more NPK fertilizer application as shown in (Tables 3 and 4).

Table 3. The grain yield of wheat during the study period 1998–2016 with NPK fertilizer application

Period	Means grain yield (kg ha^{-1})
1998–2001	5,736
2002–2005	5,944
2006–2009	6,166
2010–2013	6,360
2014–2016	7,060

Table 4. The grain yield of maize during the study period 1998–2016 with NPK fertilizer application

Period	Means grain yield (kg ha^{-1})
1998–2001	6,440
2002–2005	6,570
2006–2009	7,310
2010–2013	7,005
2014–2016	8,894

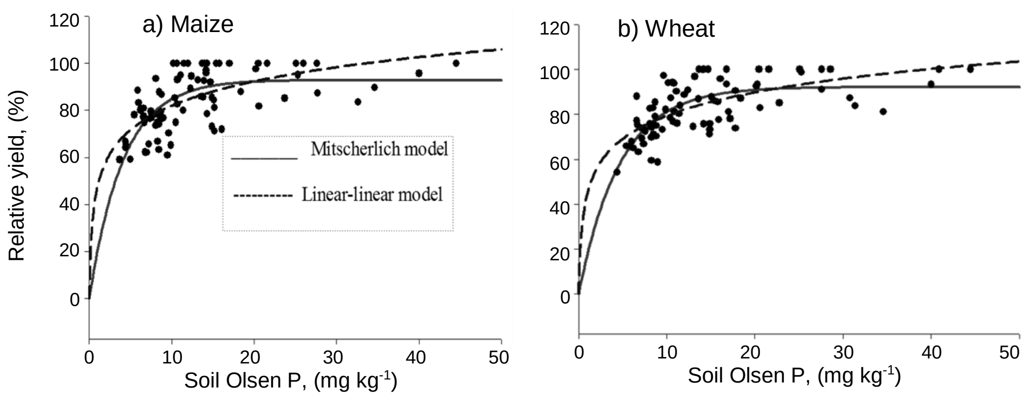

Figure 3. The grain yield of maize (a) and wheat (b) response to soil Olsen P content based on the Mitscherlich and Linear-linear model for all of the experimental sites.

To Determination ACV of OP, the crop yield was affected to soil OP level as appeared in Fig. 3, and the data of all test locations (27 locations) are consistent with Mitscherlich and linear-linear models as shown in (Table 5). The ACV value of OP for wheat and maize was 15.2 and 14.7 mg kg^{-1}, respectively, for the Mitscherlich model. The ACV amounts of OP for maize and wheat were 13.6 and 11.9 mg kg^{-1} respectively, for Linear-linear model. We observed the ACV of OP by the Mitscherlich model was higher than that value by Linear- Linear model. The average ACV of OP for maize and wheat were 14.2 and 13.6 mg kg^{-1} respectively, for both models.

Table 5. The agronomic critical value of Olsen P (mg P kg^{-1}) for all experimental sites during the period (1998–2016) in Henan province, central east China

	Mitscherlich		Linear-Linear			
Crop	Value	R^2	Value	R^2	Mean value	*n*
Maize	14.7	0.43**	13.6	0.39**	14.2	84
Wheat	15.2	0.47**	11.9	0.42**	13.6	84

R^2 is the coefficient of determination for Mitscherlich and Linear- Linear models (** represents $P < 0.01$); *n* is the number of observations used to fit the equation.

The Olsen P level and P budget

Changes in OP level and P budget in 27 experimental locations appeared in (Table 6). The mean annual P input of 101.2 kg P ha^{-1} was recorded as highest in Luoyang site, whereas the lowest average annual P input of 60.8 kg P $ha^{-1}$was recorded on Kaifeng. It was observed that Zhumadian recorded the highest average annual P output (76.3 kg P ha^{-1}), while the least mean annual P output (46.2 kg P ha^{-1}) was on Kaifeng. The average annual P budget ranged between 3.8 and 38 kg P ha^{-1} in Xinxiang and Shangqiu respectively. The initial OP level ranged between 10.4 and 20.4 mg kg^{-1}, and the final OP level ranged between 14.6 and 28.8 mg kg^{-1} as shown in (Table 6).

Table 6. Annual average P input-output, P budget and change in Olsen P in wheat-maize systems different sites in Henan province during (1998 to 2016)

Site	P input	P output	P budget	Initial OP	Final OP	Change OP
	(kg P $ha^{-1}yr^{-1}$)[a]			mg kg^{-1}	mg kg^{-1}	(mg $kg^{-1}yr^{-1}$)[b]
Luoyang (*n* = 3)	101.2	64	37.2	10.4	17.6	0.28
Zhumadian (*n* = 3)	88.7	76.3	12.4	12.8	15.5	0.21
Anyang (*n* = 2)	84.6	74.7	9.9	12.2	28.8	0.14
Nanyang (*n* = 3)	92.1	65.4	26.7	10.7	24.9	0.46
Zhoukou (*n* = 2)	61.9	47.8	14.1	12.5	20.4	0.18
Xinxiang (*n* = 1)	74.5	70.7	3.8	20.4	27.3	0.19
Kaifeng (*n* = 5)	60.8	46.2	14.6	18.7	27	0.31
Shangqiu (*n* = 6)	95.9	57.9	38	19.2	20	0.34
Puyang (*n* = 2)	84	72.8	11.2	10.4	14.6	0.1

n is the number of sites observation; [a] The annual average P input, P output and P budget (kg P ha^{-1} $year^{-1}$); [b] Change in Olsen P(OP) was the slope values getting from the linear regressing of dynamic of Olsen P with years (mg $kg^{-1}year^{-1}$).

Fertilizer P Recommended based on Olsen P and P budget

Table 7. Annual average P fertilizer application rate Recommended for wheat and maize cropping systems at different sites in the study area during (1998 to 2016)

Site	Duration years	P input	P output	OP_m	OP_p	Range in OP	Recommended P in next 5 years
		(kg P $ha^{-1}Yr^{-1}$)[a]		mg kg^{-1}			(kg P $ha^{-1}Yr^{-1}$)[a]
Luoyang (*n* = 3)	12	101.2	64	17.6	27.7	15.240	64.0
Zhumadian (*n* = 3)	13	88.7	76.3	15.7	22.9	15.240	76.3
Anyang (*n* = 2)	14	84.6	74.7	28.8	32.1	15.2 40	74.7
Nanyang (*n* = 3)	12	92.1	65.4	24.9	25.7	15.2 40	65.4
Zhoukou (*n* = 2)	12	61.9	47.8	20.4	15.8	15.2 40	47.8
Xinxiang (*n* = 1)	17	74.5	70.7	27.3	23.2	15.2 40	70.7
Kaifeng (*n* = 5)	13	60.8	46.2	27	19.7	15.2 40	46.2
Shangqiu (*n* = 6)	15	95.9	57.9	20	15.6	15.2 40	57.9
Puyang (*n* = 2)	14	84	72.8	14.6	8.6	< 15.2	85.5

[a] The annual average P input, P output budget and Recommended Fertilizer P rate in next 5 years (kg P ha^{-1} $year^{-1}$) Olsen P_p was the predicted Olsen P and Olsen P_m was the measured Olsen P in 2016 (mg kg^{-1}).

The model was tested using existing data sets collected from 27 locations. Until 2016, only one location in Puyang City had current OP levels below than the ACV 15.2 mg kg^{-1} as shown in (Table 7). To raise the low OP level to the ACV in the next five years from 2016, the P fertilizer addition amount was estimated based on Eq. (3). For other locations, the OP level was more than 15.2 mg kg^{-1} (ACV) but less than the environmental critical level of 40 mg kg^{-1} (Li et al., 2011). The level of soil OP should be maintained by calculating the annual P addition rate. Basing on the absorption of P by crops, the utilization efficiency of P should be improved to obtain the best crop production.

A model of P fertilizer recommendation rate that integrated values of the change in soil OP in response to P budget and the ACV of OP was used, to adjust current levels of soil OP to the ACV at the experimental sites over the next five years, P fertilizer application rate should be in the range of 64–85 kg P ha^{-1}.

DISCUSSION

Determination agronomic critical value of Olsen P

The critical level of OP can be definited as 'soil P status, beyond which crop yield is not affected by P fertilizer application' (Tang et al., 2009; Bai et al., 2013; Sucunza et al., 2018). In our study, the mean ACV of OP for maize crop was 14.2 mg kg^{-1}, inside the range from 7–18 mg kg^{-1} as defined by others (Bollons & Barraclough 1999; Colomb et al., 2007; Bai et al., 2013; Johnston et al., 2013; Poulton et al., 2013). The average of ACV of OP for wheat was13.6 mg kg^{-1} as shown in (Table 5) is similar to the higher limit of 7–11 mg kg^{-1} as reported in the previous studies, by Mitscherlich and Liner-Liner models for both crops (Colomb et al., 2007; Bai et al., 2013). The change of the critical value of the same crop in different studies was caused by the difference of test type (data type), soil properties (pH value, clay content, soil structure, organic carbon, etc.) and climatic factors (Tang et al., 2009; Johnston et al., 2013). These factors will eventually affect P recommended to farmers. In this study, the ACV of OP from the Linear-linear model is lower than that of the Mitscherlich model, which is consistent with former researches. (Mallarino & Schepers 2005; Tang et al., 2009). The ACV of soil OP obtained by using the Mitscherlich model for maize 14.7 mg kg^{-1} and wheat 15.2 mg kg^{-1} were closer to the average critical levels determined by the three models noted by (Tang et al., 2009), with an average OP level for maize and wheat was 15.2 and 16.2 mg kg^{-1}, indicating that the Mitscherlich model can be used to estimate ACV of Fluvo-aquic soil.

The Olsen P level influencing by P budget

Many researchers have determined the correlation between change in soil OP and P budget for different soil types and climate. Cao and others (2012) reported that the OP level increased from 1.5 to 5.8 mg kg^{-1} per 100 ha^{-1} P surplus at different locations. The change of soil OP in different locations was varied according to the P budget. This difference is caused by different planting systems and environmental factors (Cao et al., 2012), and soil properties (soil pH, temperature, clay content and organic matter) all affect the adsorption and desorption of P (Zhang et al., 2009). Also, the soil P adsorption capacity in warm regions is stronger than that in temperate zones (Malhi et al., 2009). For example, in warm climate regions, OP increased by 2 mg kg^{-1} for each 100 kg ha^{-1} P surplus in the soil (Aulakh et al., 2007), In temperate soils, OP increased by 6 mg kg^{-1} for every 100 kg ha^{-1}. the experimental location had a warm temperate soil and it's affected by the air temperature in the study area (mean annual temperature 14 °C), in our experiment, the soil OP increased by 2.8 mg kg^{-1} for every 100 kg ha^{-1} P surplus, as mentioned above, it is in the range of 2 to 6 mg kg^{-1}. Yang et al. (2015) noted in a long-term experiment, the soil OP increased by 2.4 mg kg^{-1} for each 100 kg ha^{-1} P surplus for Fluvo-aquic soil in Shaanxi Province in China (geographically close to our study area), which agree with our results.

Fertilizer Phosphorus Recommended based on Olsen P critical value

The best management of P fertilizers is very important for high crop yield and environmental protection. Many studies focused on estimating rational P fertilizer application rates in China: appropriate application rate of phosphorus fertilizer was calculated based on high yield and high P use efficiency of soybean in Heilongjiang province (Li et al., 2010). Ma et al. (2011) determined the appropriate amount of P depended on the effect of P fertilizer on rice yield increasing. There were also studies estimating P fertilizer application rates based on changes in soil Olsen P. Li et al. (2012) utilized a model to calculate P fertilizer required improving the OP level to 40 mg kg^{-1}, but the ACV of OP was not considered. In this study, according to ACV and environmental critical value of soil OP, and using the calculated values of soil P budget, we put forward suggestions on the addition of P fertilizer in Fluvo-aquic soil.

Li et al. (2011) adopted the cumulative maintenance method for P controlling and delivered an experimental method, because when the OP level is less than 20, 20–40 or more than 40 mg kg^{-1}, the addition P amount should be 1.7 to 2 times of the amount of P absorbed by plants, the same to the amount of P absorbed by plants, or no addition, respectively. When the level of OP was more than 20 mg kg^{-1}, the amount of P applied by using our method and Li's method was equal to the absorption value of P by crops. When the OP level was 15.2–20 mg kg^{-1}, the amount of P applied was 1.7 times that of P uptake, in our study, and also the same in Li's method. When the OP level was less than 15.2 mg kg^{-1}, the P addition amount estimated by Li's method was 123.8 kg P ha^{-1}, which was also more than that value in our method as shown in (Table 7). From saving limited P resources, the application amount of P can be estimated by using our method, because this method is more accurate in determining the amount of P fertilizer application to obtain optimum productivity.

CONCLUSIONS

The mean agronomic critical value of OP for maize was 14.2 mg $kg^{-1}$and for wheat was 13.6 mg kg^{-1} when using the Linear- Linear and Mitscherlich model. The increase in soil OP in the 0–20 cm topsoil layer was 2.8 mg kg^{-1} for each 100 kg ha^{-1} P budget. P fertilizer application rate in the following five years was calculated based on the ACV of OP and the increase in soil OP due to P surplus. This information is useful for optimizing crop production, diminishing P accumulation in soil, and reducing the potential risks of water body contamination.

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Some physical and mechanical properties of wood of Fast-growing tree species eucalyptus (*Eucalyptus grandis*) and radiata pine (*Pinus radiata* D.Don)

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Abstract. Fast-growing imported plantation tree species have become an available wood resource for Europe's wood industry in the last decades. This sustainable alternative may reduce the gap between the increasing demand for and decreasing supply of the local tree species. The aim of the study was to determine and compare basic physical and mechanical properties of eucalyptus (*Eucalyptus grandis*) wood from Uruguay and radiata pine (*Pinus radiata* D.Don) wood from New Zealand as an alternative for Scots pine (*Pinus sylvestris* L.) from Latvia, to produce non-structural semi-finished glued laminated timber members for the manufacturing of windows. Such properties as density, swelling, bending strength, bending modulus of elasticity, compression strength and resistance to impact were determined according to ISO 13061 series standards test methods for small clear wood specimens. As the result of this study it was established that there is not significant difference between the majority of radiata pine and Scots pine properties, with the exception of resistance to impact and radial swelling where radiata pine shows significantly higher values. Not surprisingly all the properties of deciduous eucalyptus wood were significantly higher compared to both coniferous tree species. Higher swelling and density properties of eucalyptus compared to radiata pine and Scots pine should be taken into consideration for the design and production of wooden window elements.

Key words: *Eucalyptus grandis*, Fast-growing wood, mechanical properties, *Pinus radiata* D.Don, plantation wood.

INTRODUCTION

Fast-growing imported plantation tree species have become an available wood resource for the European wood industry. Two tree species eucalyptus (*Eucalyptus grandis*) and radiata pine (*Pinus radiata* D.Don) are at the top of interest. These sustainable alternatives may replace some of the local tree species for wooden window and structural element production (Liao et al., 2017).

Radiata pine (*Pinus radiata* D.Don*)* is the most widely spread commercial forestry Fast-growing tree species covering an estimated 1.8 million ha in New Zealand (Palmer et al., 2010) where its rotation period is 28 years for sawlog production and annual growth rate is evaluated as 17 m^3 ha^{-1} $year^{-1}$ (Cubbage et al., 2010). In total 13.7 million m^{-3} of radiata pine pulp wood and sawn timber was exported from New Zealand in 2011 (Ministry of Primary Industries, 2020).

Eucalyptus is one of the fastest growing tree species in the world and the most commonly planted forest species in Uruguay (over 0.25 million ha) (Rachid-Casnati et al., 2019). Eucalyptus rotation period is 16 years for saw log production and the annual growth rate is evaluated at 30 m^3 ha^{-1} $year^{-1}$ in Uruguay (Cubbage et al., 2010) and up till 50 m^3 ha^{-1} $year^{-1}$ in Turkey (Gürses et al., 1995). The availability of eucalyptus sawn timber is evaluated as 0.7 million m^{-3} according to Dieste et al. (2019).

In comparison the most widely spread tree species and typical raw material for wooden window and structural element production in Europe is Scots pine (*Pinus sylvestris* L.). It covers approximately 33% of the total forest stands with 0.85 million ha area in Latvia (Central Statistical Bureau of Latvia, 2019). For Scots pine the cutting age varies from 101 to 121 years for saw log production. The annual growth rate for Scots pine in Latvia has been evaluated as 6.8 m^3 ha^{-1} $year^{-1}$ and yield as 245 m^3 ha^{-1} on average (Lībiete, 2008). Approximately 4.3 million m^{-3} of Scots pine was harvested in Latvia in 2019 (Ministry of Agriculture, 2020).

The growth region, growth and climate conditions of trees have a great influence on both the forest yield and the quality of timber, thereof physical and mechanical properties of fast growing tree species vary significantly (Álvarez et al., 2013; Elzaki & Khider, 2013; Mederski et al., 2015). Even in one forest stand and within one timber trunk the dispersion of wood properties may vary within wide range (Aleinikovas & Grigaliūnas, 2006; Bal & Bektaş, 2012). The age of tree is one of the factors that affect the physical and mechanical properties of wood. According to Cown (1980) the density of radiata pine wood increases with the increasing the age of tree. The density of the wood in the trunk cross section increases in the direction from pith to bark. In the case of eucalyptus there is a weak but positive correlation between the tree age and density and bending strength, but modulus of elasticity was almost non-correlated with the age of a tree (Lemenih & Bekele, 2004). Wood genetics influence wood competence for growth and the physical and chemical nature of tree growing. There is a correlation between genetic factors and wood density and related physical and mechanical properties of wood. The combination of genetic and environmental conditions is the main factors influencing the changes in wood characteristics (Savidge, 2003; Rocha et al., 2020). Due to the presence of large volume of juvenile wood, the mechanical properties of eucalyptus and radiata pine may vary significantly and they are unstable (Wagenführ 1996; Kojima et al., 2009).

Differences in the macroscopic structure of conifers and deciduous trees most often explain the differences in wood density and mechanical parameters (Wheeler, 2001). In general, the coniferous species have a higher cellulose content (40–45%), higher lignin (26–34%), and lower pentosan (7–14%) content as compared to deciduous species (cellulose 38–49%, lignin 23–30%, and pentosans 19–26%). The angle of the cellulose microfibrils in relation to the fiber axis is one of the most important structural parameter determining the mechanical properties of wood. The reduced cellulose content and high microfibril angle may cause the reduction of mechanical properties of wood (Rowell et.al., 2012).

Radiata pine may be considered as medium density, good structural and general purpose wood according to Elzaki & Khider (2013). In comparison eucalyptus has relatively better properties: average density 650 kg m^{-3}; bending strength 103 MPa and modulus of elasticity in bending 15.2 GPa according to Paradis et al. (2011). These properties even exceed average European oak (*Quercus robur*) properties: average density 610 kg m^{-3}; bending strength 88 MPa and modulus of elasticity in bending 11.7 GPa (Wagenführ, 1996). Similar density values, comparable mechanical properties and high growth rate of Fast-growing plantation tree species eucalyptus and radiata pine make them an ideal choice for substitution of local wood species European oak and Scots pine in Europe.

Glued laminated timber for wooden window production is a complex process where the physical and mechanical properties of wood determine the main production parameters and end use characteristics of windows. Bending strength, compression strength parallel to the grain and thickness swelling of each individual wood species should be taken into account in self-supporting wood window frame and joint design. Meanwhile, the actual wood density and hardness have to be taken into account in the production process of wood window blanks, as they affect most of the wood processing processes, such as: wood drying, sawing and planing as well as gluing (Kurowska & Kozakiewicz, 2010; Konnerth et al., 2016). The determination of the properties of wood allows the manufacturer of wooden window blanks to declare the properties of the raw material to the end-user. Therefore, appropriate manufacturing regimes and bonding parameters should be established for Fast-growing tree species if different physical and mechanical properties would be observed comparison to conventional European wood species.

The physical and mechanical properties of wood of the same tree species can differ significantly in different growth regions and climate conditions according to literature. Therefore direct data from testing with well recognised international standard test procedures were used to compare wood properties of Fast-growing plantation tree eucalyptus and radiata pine from a particular region with the reference wood of Scots pine from Latvia.

The aim of this study was to determine and directly compare basic properties of plantation wood: eucalyptus (*Eucalyptus grandis*) from Uruguay and radiata pine (*Pinus radiata* D.Don) from New Zealand as an alternative for Scots pine (*Pinus sylvestris* L.) from Latvia to produce non-structural semi-finished glued laminated timber members for windows manufacturing.

MATERIALS AND METHODS

For this study wood of two Fast-growing plantation tree species eucalyptus (*Eucalyptus grandis*) from Uruguay ('Rivera' Department) and radiata pine (*Pinus radiata* D.Don) from New Zealand ('Taupo' region) were used. As a reference species Scots pine (*Pinus sylvestris* L.) from Latvia ('Vidzeme' region) was tested. The age of the trees at harvesting was following: 20–22 years for eucalyptus, 31–32 years for radiata pine and 112–118 years for Scots pine. The raw material was kiln dried sawn timber (moisture content 12 ± 3%) with nominal cross section dimensions of 35×150 mm and length of 4 m for all three species. Initial moisture content at the timber delivery was measured by electrical resistance moisture meter Brookhuis FMD6 and the average moisture content was as follows: 10.2% for eucalyptus; 13.8% for radiata pine and 14.3% for Scots pine. A total of 30 boards were randomly selected and cut into test specimens according to the standard ISO 3129 (ISO 3129, 2012) for each wood species. A set of 9 specimens was cut from a single board to determine the following physical and mechanical parameters: moisture content (ISO 13061-1, 2003), density (ISO 13061-2, 2014), swelling in radial and tangential directions (ISO 13061-15, 2017), bending strength and modulus of elasticity (ISO 13061-3, 2017 and ISO 13061-4, 2017), compression strength (ISO 13061-17, 2017) and resistance to impact in radial and tangential directions (ISO 13061-11, 2017). The set of the specimens were produced 300 mm from the sawn timber end and 50 mm from the edge. Smooth surfaces of specimens were obtained by planing operation. In total, 30 specimens were tested to obtain 30 valid test results for each physical and mechanical property of Fast-growing and reference wood species.

All parameters were determined after the conditioning of specimens to a constant mass in the standard climate (air temperature 20 ± 2 °C; air humidity 65 ± 2%). Radiata pine test specimens at the conditioning presented in the Fig. 1. In the same way eucalyptus and Scots pine specimens were produced and tested.

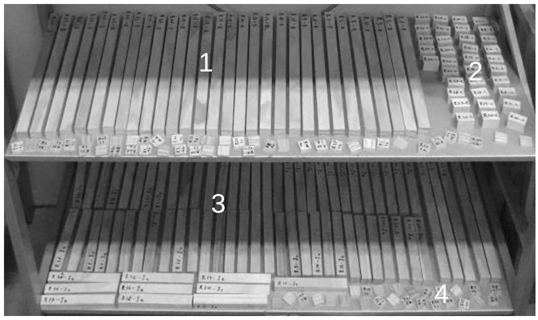

Figure 1. Example of radiata pine specimens: 1 – bending strength and modulus of elasticity specimens 2 – moisture content, density and compression strength specimens; 3 – resistance to impact specimens and 4 – swelling specimens.

The width and depth of all specimens was 20 mm. The length of specimens was: 5 mm for swelling specimens, 30 mm for moisture content, density and compression strength specimens; 150 mm for resistance to impact specimens and 320 mm for bending strength and modulus of elasticity specimens. All specimens were defect free without cracks, splits, knots, resin pockets, cross grain or other defects.

Three point bending strength and modulus of elasticity tests were carried out on the electromechanical testing device Zwick Z100, with a support span of 280 mm and a test speed 6 mm min^{-1}. Compression test was carried out with a test speed of 0.6 mm min^{-1}. Resistance of wood to impact was determined with steel ball (diameter 25 mm, density 7.8 g cm^{-3}) and dropping height of 500 mm. The diameter of indentation was measured by sliding calliper. Linear swelling was calculated from the measurement of dimensions of specimens at the absolutely dry condition and after fully saturated with water condition. Dimensions were measured by sliding calliper. For each wood species and each property the average value and standard deviation were calculated (ISO 3129, 2012). To compare the average values for all three species the Student's t-test with p-value method (α = 0.05) was used.

RESULTS AND DISCUSSION

The average physical and mechanical properties of Fast-growing eucalyptus and radiata pine plantation wood species in comparison to Scots pine characteristics are presented in Table 1.

Table 1. Eucalyptus and radiata pine physical and mechanical properties in comparison to Scots pine wood (average values and standard deviations)

Property		Wood species / Origin		
		Eucalyptus (*Eucalyptus grandis*)/ Uruguay	radiata pine (*Pinus radiata* D.Don)/ New Zealand	Scots pine (*Pinus sylvestris* L.)/ Latvia
Moisture content, (%)		12.0 (0.513)	12.7 (0.438)	12.8 (0.795)
Density, (kg m^{-3})		588 (97.2)	504 (37.2)	485 (68.3)
Bending strength, (MPa)		97.5 (16.7)	89.5 (10.9)	86.0 (11.8)
Modulus of elasticity in bending, (GPa)		11.9 (1.8)	10.8 (1.7)	10.3 (1.3)
Compression strength, (MPa)		54.1 (8.13)	42.8 (6.37)	43.8 (6.67)
Resistance to impact,(kJ m^{-2})	tangential	8.46 (1.30)	7.46 (1.22)	6.71 (0.794)
	radial	7.35 (1.08)	7.01 (0.599)	5.96 (0.928)
Swelling, (%)	tangential	11.7 (3.03)	8.17 (1.16)	8.43 (1.14)
	radial	6.34 (1.68)	4.39 (1.05)	3.57 (0.535)

The results of the study show that after conditioning of specimens in the standard climate the target equilibrium moisture content 12% (Forest Products Laboratory, 2010) was reached for all three wood species. The average moisture content of both coniferous wood species did not differ significantly ($p = 0.22$). Radiata pine obtained 12.7% and Scots pine 12.8% moisture content after conditioning of specimens in the standard climate. For the deciduous eucalyptus wood species the moisture content was 12.0% and it was significantly lower ($p < 0.05$) compared to both coniferous wood species. It can be explained by the differences in the initial moisture content and water sorption hysteresis effect in wood (Patera et al., 2016).

The average density of radiata pine varied from 436 to 568 kg m^{-3} (504 kg m^{-3} on average) but for Scots pine from 372 to 666 kg m^{-3} (485 kg m^{-3}). Four percent difference between the average density values of fast growing radiata pine and conventional Scots pine wood species was not significant ($p = 0.10$). In comparison 14 to 18% higher average density 588 kg m^{-3} was observed for deciduous eucalyptus wood within the range from 394 to 738 kg m^{-3}. The average density differences in both cases were significant ($p < 0.05$) compared to the above mentioned coniferous wood species. Since samples were randomly chosen from different boards, the considerable variability in density was not surprising.

The estimated bending strength and modulus of elasticity of radiata pine (89.5 MPa and 10.8 GPa) compared to Scots pine (86.0 MPa and 10.3 GPa) did not differ significantly ($p = 0.12$ and $p = 0.16$). As it was expected from literature (Paradis et al., 2011), the bending strength and modulus of elasticity of eucalyptus (97.5 MPa and 11.9 GPa) in both cases were significantly higher ($p < 0.05$).

A similar situation was observed with the compression strength parameters. The average compression strength values for radiata pine and Scots pine 42.8 MPa and

43.8 MPa did not differ significantly ($p = 0.28$). For eucalyptus grandis the compression strength value 54.1 MPa was evaluated as significantly higher ($p < 0.05$) compared to both coniferous wood species.

The parameters of the resistance to impact varied between wood species and loading directions. The highest resistance to impact values was observed in the tangential direction: 8.46 kJ m^{-2} for eucalyptus, 7.46 kJ m^{-2} for radiata pine and 6.71 kJ m^{-2} for Scots pine. For all three wood species the tangential direction provided significantly higher resistance to impact values compared to the radial direction ($p < 0.05$). In the radial direction the values were as follows: 7.35 kJ m^{-2} for eucalyptus; 7.01 kJ m^{-2} for radiata pine and 5.96 kJ m^{-2} for Scots pine. In both directions eucalyptus provided significantly higher resistance to the impact values compared to both coniferous wood species, with the exception of resistance to the impact of eucalyptus and radiata pine in the radial direction, where a significant difference was not observed ($p = 0.08$).

The thickness swelling parameter was evaluated and compared as the last parameter. The highest thickness swelling values were observed in the tangential direction: 11.7% for eucalyptus; 8.4% for Scots pine and 8.2% for radiata pine. For all three species the thickness swelling values in the tangential direction were approximately two times higher than in the radial direction and the difference was significant ($p < 0.05$). In the radial direction the swelling values were as follows: 6.3% for eucalyptus; 4.4% for radiata pine and 3.6% for Scots pine. For both directions eucalyptus provided significantly higher thickness swelling values compared to both coniferous wood species. The difference between radiata pine and Scots pine tangential thickness swelling was not significant ($p = 0.19$), but in the radial direction radiata pine provided significantly higher swelling ($p < 0.05$).

Differences in the macroscopic structure of conifers and deciduous trees most often explain the differences in wood density and mechanical parameters. Softwood species consists of single cell longitudinal tracheids, their arrangement is more regular with functions for both support and conduction. In comparison deciduous trees have a porous vascular system with large vessel elements for water conduction and fibers to provide most of the structural support. Wood that has a high proportion of thick–walled fibers is hard and heavy while wood with a high proportion of vessels are soft and light (Wheeler, 2001).

Physical and mechanical properties of Fast-growing wood species eucalyptus and radiata pine in comparison to Scots pine according to the literature are presented in Table 2. Only a small number of results representing different sites have been included in this comparison.

The data from the study showed that the average density for both coniferous wood species and deciduous wood species mostly corresponded to the data reported in the literature.

Eucalyptus from Uruguay showed slightly lower density 588 kg m^{-3} compared to the data from Australia 650 kg m^{-3} (Paradis et al., 2011), but slightly higher data compared to the data from Turkey, where density of 555 kg m^{-3} was evaluated (Bal & Bektaş, 2012). For comparison European oak density is 610 kg m^{-3} (Wagenführ, 1996). The bending strength 97.5 MPa and compression strength 54.1 MPa from the study were slightly lower compared to the corresponding literature data 103 MPa and 59 MPa (Paradis et al., 2011). The modulus of elasticity in bending 11.9 GPa for Uruguay were observed much lower compared to 15.2 GPa for Australia (Paradis et al., 2011). Average bending strength 88 MPa and average modulus of elasticity 11.7 GPa were reporteds by Wagenführ for European Oak (1996). No literature data were found about the resistance to impact for the assessed wood species.

Table 2. Eucalyptus and radiata pine properties compared to Scots pine parameters according to the literature

Properties		Wood species, literature source and timber origin								
		Eucalyptus (*Eucalyptus grandis*)		Radiata pine (*Pinus radiata* D.Don)				Scots pine (*Pinus sylvestris* L.)		
		Bal & Bektaş (2012) / Turkey	Paradis et al. (2011) /Australia	Wagenführ, (1996) / [1]	Cockrell (1959) / California, USA	versus Australia	Elzaki & Khider (2013) / Sudan	Wagenführ (1996) / [1]	Paradis et al. (2011) / Europe	Mederski et al. (2015)
Density (kg m^{-3})		555	650	450–580	490	450	529–600	510	550	455
Bending strength (MPa)		-	103	60–91	91	79	78–81	80	97	85–87
Modulus of elasticity in bending (GPa)		-	15.2	8.5–3.6	12.4	10.0	11.6–12.7	12.0	12.9	-
Compression strength (MPa)		-	59	36–65	51	39	42–45	55	50	41–46
Swelling (%)	Tangential	7.7	10		7.0	6.3	-	7.5–8.7	8.3	-
	Radial	4.4	5.8	_	5.2	4.0	_	3.3–4.5	5.2	_

[1] No data about the origin of the wood species origin were given.

[2] Resistance to impact data for the studied wood species were not found in the literature.

The tangential and radial swellings were observed as 11.7% and 6.34% in the study, and these values were slightly higher compared to the corresponding data reported by Paradis et al. (2011): 10.0% and 5.8%. The greatest thickness swelling differences were observed when the results were compared to the data reported by Bal & Bektaş (2012) 7.7% and 4.4%, where the origin of eucalyptus timber was Turkey.

The fast growing radiata pine wood from New Zealand showed the average density 504 kg m^{-3} while according to the literature this parameter may vary from 450 kg m^{-3} in Australia (Cockrell, 1959) to 600 kg m^{-3} in Sudan (Elzaki & Khider, 2013).

Radiata pine bending strength 89.5 MPa and compression strength 42.8 MPa from the study corresponded to the most values cited by the literature. According to Cockrell, radiata pine bending strength in Australia was 79 MPa and compression strength value was 39 MPa but in California (USA) the values were the following: 91 MPa and 51 MPa (Cockrell, 1959). The modulus of elasticity in bending 10.8 GPa for radiata pine from New Zealand was slightly lower compared to 12.4 GPa for California, USA (Cockrell, 1959) and 11.6–12.7 MPa for Sudan (Elzaki & Khider, 2013), but the result was comparable with 10.0 MPa for Australia (Cockrell, 1959).

The tangential swelling was observed as 8.2% for radiata pine in the study and this value was slightly higher compared to the corresponding data reported by Cockrell (1959):

7.0% (California, USA) and 6.3% (Australia). Radial swelling 4.4% was comparable with the average literature data: 5.2% (California, USA) and 4.0% (Australia) (Cockrell, 1959).

Local reference wood species Scots pine from Latvia showed slightly lower density value 485 kg m^{-3} compared to the general literature data 510 kg m^{-3} (Wagenführ, 1996) and 550 kg m^{-3} (Paradis et al., 2011), but higher value compared to 455 kg m^{-3} reported from Poland by Mederski et al. (2015). All other physical and mechanical properties of Scots pine from Latvia were slightly lower compared to general data (Wagenführ, 1996; Paradis et al., 2011), but they were comparable with the data reported from Poland by Mederski et al. (2015) according to Table 2. Genetic and environmental conditions are the main factors influencing the changes in wood characteristics within the same species from different origins (Savidge, 2003; Rocha et al., 2020).

CONCLUSIONS

Direct data from testing with well recognised international standard test procedures were used to compare wood properties of fast-growing plantation tree eucalyptus and radiata pine with the reference wood of Scots pine.

All determined properties of the fast-growing plantation wood species obtained in the study correspond to the certain range of properties cited in the literature.

As a result of the study it was established, that there was no significant difference between the majority of coniferous radiata pine (from New Zealand) and Scots pine properties (from Latvia), with the exception of the resistance to impact and radial swelling where radiata pine shows significantly higher values. Therefore, the use of radiata pine instead of Scots pine is highly potential.

All properties of the deciduous wood eucalyptus grandis (from Uruguay) were significantly higher compared to radiata pine and Scots pine properties. Higher swelling and density of eucalyptus should be taken into consideration for wood windows element design and production.

Due to the fast growth rate and similar physical and mechanical properties to European oak, eucalyptus has a great potential as a substitute for some deciduous wood species, while Scots pine can be substituted by radiata pine to manufacture wooden window and structural elements in Europe.

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Investigation into sugars accumulation in sweet cherry fruits under abiotic factors effects

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Abstract. The level of sugars content in sweet cherry fruit depends on many factors. However, the decisive influence of weather factors is noted. In view of this, the issue of predicting the sugars content in sweet cherry fruit depending on the share of weather factors is relevant for further improvement of transportation technology, storage and processing.
It has been established that according to sugars content and to the variability of their formation the most perspective from the technological point of view were varieties: Zabuta (12.47%), Dachnytsia (15.60%), Krupnoplidna (14.35%). Low and medium variabilities of the selected varieties according to the investigation years were established (Vp = 8.6%–13.0%). It has been shown that weather conditions had dominating effects on sugars accumulation for all varieties groups irrespective of the ripening period. According to the results of a two-factors dispersion analysis it is expedient to prognosticate the sugars content in sweet cherry fruits by medium values for a particular group of sorts but not for each pomological sort.
In the course of the work the average and strong correlation dependence between 14 weather factors (X_i, i = 1..14) and the sugars content for sweet cherries of early, medium, and late ripening period ($\left|r_{Y_j X_i}\right| \geq 0.55,\ i = 1..14,\ j = 1..3$) were defined. The ranges of share of weather factors participation that have the maximum influence on sugars fund formation in sweet cherry fruit are established (Δ_i 9.50% to 30.99%).
Based on regression analysis, it is substantiated that the sugars accumulation in sweet cherry fruit, regardless of the ripening period, is most influenced by weather conditions of the blossoming period, the last month of fruit formation and thermal parameters and humidity index at the stage of fruit picking.

Key words: fruits, sweet cherry tree, weather conditions, total sugar content, simple sugars, saccharose, connection, correlation.

INTRODUCTION

Fruits are of great importance in people's nutrition (Einhorn 2013; Khadivi-Khub, 2015). They are the sources of biologically active and mineral substances, enzymes, carbohydrates, organic acids which are supplied into the organism (Fallahi, 2017;

Serdyuk et al., 2020). The fruits chemical composition is not constant and has its accumulation peculiarities as to the sorts of various ripening periods. The chemical composition is constantly changing in the process of their growing, ripening and depends on a number of factors: variety, sort, rate of ripening, period of ripening, product treatment, conditions and period of storing etc (Koumanova et al., 2016; Dziedzic et al., 2017; Wenden et al., 2017; Lafuente et al., 2018). But it should be mentioned that a negative impact of abiotic factors has a great influence on the formation of the main components of chemical composition. Thus, it is necessary to study the accumulation of the basic biochemical parameters of sweet cherry fruits of different periods of ripening and to select the best sort-samples for further storing and processing; for studying the mechanism of formation of the basic components of fruits chemical composition under the influence of stress abiotic factors and a degree of their impact on the accumulation of fruits basic quality parameters. A considerable attention is paid by the scientists from many countries to the problems and prospects of growing cherries as well as improving the fruits quality of early, middle and late ripening varieties (Jänes et al., 2010; Kask et al., 2010; Sîrbu et al., 2012).

It is well known that the excellent gustatory qualities of sweet cherry fruit is directly correlated with a high content of dry soluble substances. Ranges of dry soluble substances according to Ukrainian and foreign researchers (Long et al., 2008; Basanta et al., 2014; Serdyuk et al., 2020) range from 12.1–19.9%. The main part of dry soluble substances are carbohydrates. They are the primary products of photosynthesis and the main derivatives of the biosynthesis of other substances in fruit crops. In particular, carbohydrates form cell membranes and other structures, take a reaction in the organism's protective reactions, and form immunity. It is carbohydrates that provide nutritional value and special gustatory qualities of the fruit. The level of carbohydrates accumulation in the dry substances can change under the influence of soil and weather conditions, and the degree of fruit ripeness (Slavin & Lloyd, 2012; Wang & Einhom, 2017; Yevlash et al., 2019). Carbohydrates are 70–80% formed by sugars, which are most often represented in fruit raw materials by monosaccharaides - fructose and glucose, disaccharide-sucrose. The complex of monosaccharaides in the fruit of stonecrops exceeds the content of other components in the dry soluble substances (Serdyuk & Stepanenko, 2015). It has been established that fruits sweetness depends on sort characteristics, ripening periods, place of growing etc. (Slavin & Lloyd, 2012; Basanta et al., 2014). The content of monosaccharaides and disaccharides depends on the growing area. As the culture progresses from the north to the south, the sugars content in the fruit of the same varieties usually increases. Thus, in the southern regions of the world, the fruit of the best varieties of sweet cherries contain dry soluble substances - 12.1–23.5%, total sugars - 12.4–17.7%. The content of sugars in sweet cherry fruit grown in the south of Ukraine ranges from 12.82 to 15.00% (Yevlash et al., 2019; Ivanova et al., 2019).

A number of authors have established that during the years with a maximal precipitation there were less dry substances in fruits, including those of sugars, but more acids. In dry years the total volume of nutrients in fruits was low, but the volume of sugars increased due to the decrease in humidity level (Bizjak et al., 2013).

Thus, the level of sugars content in sweet cherry fruits, their accumulation and further storing depend on many factors. That is why, the tasks which have not been solved within this problem are: for different regions, where fruits are grown, the degree of biochemical indices accumulation is different, the potential of sugars accumulation in varieties of different ripening periods and the impact degree of stress climatic factors are different as well. There are no data as to the degree of impact of stress weather factors on sugars formation in sweet cherry fruits in terms of different ripening periods and which were grown under conditions of a Southern Steppe Subzone of Ukraine. It has conditioned the purpose of our research. With this in mind, the problem of sugars content prognostication in sweet cherry fruits depending on the degree of weather factors impact by using a Method of Principal components (Kelechi, 2012) is topical for a further improving of the technology of fruits transportation, storing and processing.

MATERIALS AND METHODS

The goal of the research was to develop a mathematical model in order to improve the sugars content prognostication in sweet cherry fruits depending on weather factors. The received mathematical model can be used under the conditions of the regions with similar hydrometrical parameters of Southern Steppe Zone of Ukraine. The purpose of the research program was to select the sweet cherry varieties of three periods of ripening with high sugars index for maintaining the fruits quality and biological value.

The expedience of sugars content prognostication in sweet cherry fruits by using mean values for a separate varieties group was substantiated by conducting a two-factor dispersion analysis during the research, and the factor which maximally affects the sugars accumulation in sweet cherry fruits has been found out as well.

To realise the goal, it was necessary to solve the following problems: to analyse the weather conditions for fruits formation; to determine the mass fraction of sugars in sweet cherry fruits in the period of economic maturity as to each period of fruit ripening; to select the best varieties according to test-index; to determine the interconnections between the processes of sugars accumulation and stress weather factors; to develop he mathematical models of sugars content dependence on weather factors; to analyse the developed model in order to determine the degree of each factor's impact on sugars fund accumulation in sweet cherry fruits of three periods of ripening.

The research was conducted during 2008–2019. Daily meteorological data of Melitopol meteorological station, Ukraine, were used. Horticultural farms of the study region are located in the Southern steppe subzone of Ukraine. The landscape of the territory is flat with an Atlantic-continental climate and a high temperature regime. The range of average annual air temperature is 9.1–9.9 °C. The average monthly air temperature in the warmest months ranges from 20.5 to 23.1 °C. The sum of active temperatures above 10 °C from April to October is 3,316 °C. The average annual precipitation is 475 mm. The region in terms of precipitation belongs to the area with insufficient humidification. The average annual relative humidity is within 73%. The average annual wind speed is 3.7 m s^{-1}. The climate is arid with the dominance of dry east and northeast winds. The value of the hydrothermal coefficient is in the range of 0.22–0.77 (Serdyuk et al., 2020).

The agrophone at the experimental sites during all research years met the requirements of agricultural technology (Table 1). The accumulation of moisture in the soil occurs mainly in autumn, partly in winter and early spring. The soils of the experimental sites where the culture is grown are light loam black soil. Soil-forming solid is forest. This type of soil in terms of grain size composition has a high content of physical sand.

Table 1. Agrochemical characteristics of soil

Type of soil	Depth of arable layer, cm	Humus content, %	pH of salt extract	Nutrient content, mg kg^{-1} of soil		
				Easily hydrolyzed nitrogen (N)	Mobile phosphorus (P_2O_5)	Potassium exchange (K_2O)
Light loam black soil	40.0	1.38	6.9	27.0	90.0	154.0

Sweet cherry fruit of 33 varieties were selected for the study, which are divided into three groups according to the ripening period: Group 1 - 7 varieties of early ripening period - Sweet Erliz, Merchant, Bigaro Burlat, Rubinova Rannya, Valeriy Chkalov, Kazka, Zabuta; Group 2 - 13 varieties of medium ripening perio - Kordia, Octavia, Vinka, Pervistok, Temp, Uliublenytsia Turovtseva, Talisman, Dilemma, Melitopolska Chorna, Orion, Chervneva Rannia, Dachnytsia, Prostir; Group 3 - 13 varieties of late ripening period - Karina, Regina, Mirazh, Krupnoplidna, Udivitelna, Zodiak, Surpriz, Kolkhoznytsia, Kosmichna, Prazdnichna, Anons, Temporion, Meotyda.

To determine the sugars content, a sample was taken for each pomological variety from 100 fruit from 6 trees that came into full fruiting. Trees typical of a certain pomological variety, of the same age, with a medium intensity of fruiting were selected for the research. A threefold repeatability. The trees were planted in 2010 on the pattern 5×3, row spacing's were under black fallow Fruit were weighed and counted directly at harvest time (Serdyuk et al., 2020).

The sweet cherries fruits of each pomological sort were carefully picked on a stage of economic maturity when the fruit pulp is firm, but the flavor and the color are typical for a given pomological sort.

The fruits were picked from the trees from four different sides of a crown. The fruits from each pomological variety were of the first commercial sort and picked with fruit-stalks. The date of picking fruits will be established according to the following quality indices of sweet cherry fruits: visual appearance (fruits analysis in terms of form and colour which must be typical for a given pomological variety, fruit-stalk availability, determining the rate of mechanical damages of fruits, their infestation with pests and fungus diseases), the fruits size in cross-section diameter. The fruits were stored and transported to the laboratory, provided that the fruit in the period of the indicator determination had the appearance and taste inherent for the pomological variety.

The content of sugars mass concentration (S, %) was determined by ferricyanide method (Yevlash et al., 2019). The ferricyanide method for determining the mass fraction of sugars is based on the property of reducing monosaccharides to renew ferricyanide potassium - K3[Fe(CN)6] (potassium ferricyanide) to ferrocyanide potassium (ferrocyanide) potassium - K4[Fe(CN)6] (potassium ferrocyanide). Methylene blue is used as an indicatorin alkaline environment. When potassium ferricyanide is restored, the color changes from blue to colorless or light yellow. The amount of sucrose is determined by pre-converting it to invert sugar.

Models construction of sweet cherry sugars dependence on weather factors was carried out according to the following scheme (Ivanova et al., 2019):

1. Determination of sugars content by ferricyanide method.
2. Creation of an information system of weather and climatic factors indicators in the years of research and their analysis. At this stage, the following indicators were

selected: average minimum air temperatures, average air temperatures, average maximum air temperatures, absolute minimum air temperatures, absolute maximum air temperatures, precipitation, average relative air humidity, minimum relative air humidity, absolute minimum relative air humidity.

3. Based on the above-mentioned indicators, the following indicators were calculated: hydrothermal coefficient, temperature differences for certain periods, the sum of active temperatures, the sum of effective temperatures.

4. To select weather and climatic factors that are the most meaningful ones which influence the research indicator we used the test of statistical hypothesis about the meaningfulness of calculated coefficients of correlation between factors and sugars content indicator in sweet cherry fruit. We checked the statistical hypothesis according to Student's test.

5. The share and degree determination of each of the factors influence selected in item 4 by studying the regression model constructed on the basis of the principal components analysis.

Correlation analysis (calculation of paired correlation coefficients and verification of their significance) is the initial stage of the analysis, which allows to identify the most significant influencing effects. The analysis of correlation coefficients gives a basic picture and allows selecting those factors, which most strongly correlate with the studied indicator.

However, other, more accurate methods of analysis should be used to compare the degree of factors influence between themselves and rank them in order of influence.

On the other hand, when constructing a regression model by method of least squares, we encounter the problem of the limited number of experiments, which directly depends on the number of years of research.

Due to the fact that the number of studied factors significantly exceeds the number of years of research, it is not possible to apply the classical regression analysis scheme.

Another important reason that imposes restrictions on the possibility of using the classical approach of regression analysis is the strong correlation of factors between themselves, which leads to the effect of multicollinearity and violation of the premises of the Gauss-Markov theorem (Damodar, 2004).

Therefore, it is suggested to use the principal components analysis (PCA) for constructing a regression model under conditions of a significant excess of the number of independent variables over the number of experiments.

Based on the principal components analysis, fictitious factors (principal components ($PC_i,\ i = 1 \dots n$), which are a linear combination of the initial factors, are constructed. An important advantage of the principal components is the fact that they provide maximum variance and at the same time weakly correlate with each other (Kelechi, 2012; Chen & Ma, 2015).

Thus, the research is suggested to be carried out according to the following algorithm:

1. Based on the experimental data $x_{ij}, (i = 1 \dots n$ – is the number of the weather factor, $j = 1 \dots m$ is the number of the year of study), we constract the set of principal components($PC_i, i = 1 \dots n$) in the form

$$PC_i = \sum_{j=1}^{m} p_{ij} \cdot x_{ij},\ i = 1 \dots n, \tag{1}$$

2. We single out a set of principal components ($PC_i, i = 1 \dots k$), which provide a cumulative variance of 90%.

3. Using the method of least squares, we construct the equation of the test parameter Y dependence (sugars content in sweet cherry fruit) on the principal components in the form

$$\hat{Y} = b_0 + \sum_{i=1}^{k} b_i \cdot PC_i, \quad (2)$$

4. We carry out the transformation of the model by substituting the expression of the principal components into the equation (2), through the initial set of factors and obtain the equation of the sugars content dependence in sweet cherry fruit on the indicators of the initial weather and climate factors of the form:

$$\hat{Y} = a_0 + \sum_{j=1}^{n} a_j \cdot X_j, \quad (3)$$

where X_j – are factors; a_j – model parameters; $\hat{Y}$ – an indicator of sugars content in sweet cherries.

5. We carry out the analysis of the constructed regressions (3) in order to determine the influence degree of each of the factors on the resulting indicators. To estimate the share of the influence of individual factors in the total effect of all factors, we use the delta coefficients Δ_j.

The coefficients Δ_j are determined according to the formula:

$$\Delta_i = \left| \frac{\tilde{a}_i \cdot r_{yx_i}}{R^2} \right| \quad (4)$$

where $\tilde{a}_i$ – parameters of the regression model in standardized factors $\tilde{X}_i$; r_{yx_i} – pair correlation coefficients; R^2 – coefficient of determination.

To make the statistical analysis, we used means of up-to-date computer technologies DataMining – the softwear environment RStudio.

RESULTS AND DISCUSSION

The average sugars content in sweet cherry fruits of 33 varieties under study, grown under conditions of an analyzed region of Ukraine was on the level of 12.63% (Table 2). A maximal average sugars content on the level of 12.85% was registered in sweet cherry fruits of a varieties group of an early ripening period of Sweet Erliz sort. Among the varieties of two other groups the fruits of Talisman, Kordia, Oktavia, Krupnoplidna, Meotyda varieties were characterised by the highest average sugars content. They accumulated 14.59–13.78% of sugars. The results of the research point at a high variability of sugars content according to the years of study in a varieties group of an early period of ripening. The varieties Rubinova Rannia and Valeriy Chkalov were exposed to the greatest impact of abiotic factors (with variation coefficients 24.6 and 23.6% respectively) on the sugars content in the fruits of a given group. The most resistant as to sugars content are Zabuta, Merchant and Bigaro Burlat varieties. The variation coefficients are in the range of 13.0–15.6%. The variability of these varieties as to sugars content under the weather factors impact is considered to be medium. The variability of sugars content in sweet cherry fruits of varieties group of a medium period of ripening according to the years of study was high (Vp = 20.6%). The variability coefficient for the fruits of a late period of ripening as to sugars content was on a medium level (Vp = 19.0).

Table 2. Sugars content in sweet cherry fruits of three periods of ripening, % (2008–2019), $\bar{x} \pm s\bar{x}$, n = 5

Pomological variety	Average sugars content, %	Min sugars content, %	Max sugars content, %	Variation according to years, Vp, %
Early peirod of ripening				
Rubinova Rannia	12.35 ± 3.04	8.22	15.87	24.6
Valeriy Chkalov	12.55 ± 2.96	8.56	15.94	23.6
Sweet Erliz	12.85 ± 2.83	9.52	16.91	22.1
Merchant	10.55 ± 1.64	8.21	12.56	15.6
Kazka	11.63 ± 2.03	8.65	14.17	17.4
Bigaro Burlat	11.11 ± 1.68	8.15	13.05	15.1
Zabuta	12.47 ± 1.62	9.58	14.58	13.0
Average value	11.93 ± 2.39	8.15	16.91	20.0
LSD_{05}	0.764	–	–	–
Medium period of ripening				
Vynka	12.24 ± 2.42	8.35	16.39	19.8
Pervystok	12.40 ± 2.94	7.98	17.81	23.70
Temp	13.16 ± 3.00	7.55	17.51	22.8
Uliublenytsia Turovtseva	10.82 ± 1.36	8.78	13.65	12.6
Talisman	14.59 ± 1.59	12.61	17.91	10.9
Dilema	12.86 ± 1.99	9.02	15.21	15.5
Melitopolska Chorna	11.16 ± 2.76	9.00	16.81	24.7
Kordia	13.79 ± 2.37	9.56	17.21	17.2
Oktavia	13.78 ± 2.87	9.02	17.45	21.4
Orion	13.43 ± 2.09	9.65	16.88	15.5
Chervneva Rannia	11.00 ± 1.48	8.27	14.54	13.50
Dachnytsia	15.60 ± 1.35	13.53	17.67	8.6
Prostir	12.67 ± 3.36	7.98	17.84	26.6
Average value	12.85 ± 2.64	7.55	17.91	20.6
LSD_{05}	0.363	–	–	–
Late period of ripening				
Krupnoplidna	14.35 ± 1.77	10.20	16.51	12.3
Karina	12.53 ± 1.97	9.56	17.21	15.7
Regina	11.90 ± 2.05	9.06	15.22	17.2
Mirazh	13.89 ± 2.67	9.24	17.21	19.2
Udivitelna	13.03 ± 2.75	8.31	18.21	21.08
Zodiak	13.14 ± 2.48	9.12	16.56	18.8
Surpryz	13.43 ± 2.30	9.21	17.56	17.11
Kolhoznytsia	12.64 ± 2.67	8.51	17.21	21.0
Kosmichna	13.55 ± 2.81	8.11	16.98	20.72
Prazdnichna	12.73 ± 2.45	8.15	17.05	19.3
Anons	12.36 ± 2.62	7.86	17.42	21.2
Temporion	12.82 ± 2.95	8.96	17.95	23.0
Meotyda	14.05 ± 2.76	7.96	17.45	19.6
Average value	13.11 ± 2.50	7.86	18.21	19.0
LSD_{05}	0.532	–	–	–

Among the varieties group of a medium period of ripening the most stable sugars content was in the fruits of Dachnytsia variety (Vp = 8%), and the most variable one in Prostir variety (Vp = 26.6%). In the varieties group of a late period of ripening the highest variability in sugars content was registered in fruits of Temporion variety (Vp = 23.0%), and the lowest one in Krupnoplidna variety (Vp = 12.3%).

Thus, according to the sugars content and the variability of their formation under the weather factors impact under the analized region conditions, the most perspective from technological point of view were Zabuta variety (of an early period of ripening), Dachnytsia (of a medium period of ripening) and Krupnoplidna (of a late period of ripening). These varieties differed in high sugars content and in their low variability during the years of study.

A two-factors dispersion analysis (Table 3) was made in order to establish the degree of weather factors impact as well as of sort characteristics on sugars fund formation in sweet cherry fruits. As follows from the results of the research, weather conditions during the years of the study (Factor A) had a dominating impact on sugars formation for all varieties groups irrespective of a period of ripening. The degree of impact of (Factor A) for varieties groups under study equaled from 74.5% to 61.9%. The impact of sort characteristics (Factor B) was less significant. The degree of this factor's impact was from 3.1% to 12.4% for a group of varieties which were analized by M. Ye. Serdyuk, I. Ye. Ivanova et al. (2020).

Table 3. Results of two-factors dispersion analysis under sugars fund formation in sweet cherry fruits

Source of variation	Sum of squares	Degree of freedom	Dispersion	F_{fact}	$F_{table\ 095}$	Impact, %
Sweet cherry varieties group of an early period of ripening						
Factor A (year)	2,074.4	11	188.6	2,892.5	1.8	74.5
Factor B (variety)	346.2	6	57.7	885.0	2.2	12.4
Interaction AB	351.4	66	5.3	81.7	1.4	12.6
Sweet cherry varieties group of a medium period of ripening						
Factor A (year)	3,226.4	11	293.3	3,753.0	1.8	61.9
Factor B (variety)	409.7	12	34.1	436.9	1.8	7.9
Interaction AB	1550.5	132	11.8	150.3	1.3	29.7
Sweet cherry varieties group of a late period of ripening						
Factor A (year)	3,254.9	11	295.9	1,947.6	1.8	69.4
Factor B (variety)	144.6	12	12.0	79.3	1.8	3.1
Interaction AB	1237.2	132	9.4	61.7	1.3	26.4

Thus, according to the results which were received, the prognostication of sugars content in sweet cherry fruits by medium values for a particular varieties group but not for a separate pomological sort has been shown to be expedient.

Then, the presence of correlation connections between the sugars fund accumulation indicator in the sweet cherry fruit of early (Y_1), medium (Y_2), late (Y_3) ripening period, and a set of weather conditions (factors) - X_i was analyzed.

The most significant factors are selected based on the calculated pair correlation coefficients $r_{Y_1X_i}$, $r_{Y_2X_i}$, $r_{Y_3X_i}$. Then, we test the significance of these correlation coefficients by checking the statistical hypothesis $H_0\colon \rho = 0$ (where ρ is the correlation coefficient of the general totality) with the alternative hypothesis $H_1\colon \rho \neq 0$ at the significance level $\alpha = 0.05$. Verification of the statistical hypothesis is carried out using Student's test. As calculations showed, the significant correlation coefficients at a significance level of 0.05 and the number of degrees of freedom $k = 10$ are within the range [-0.55; 0.55]. As a result, there were selected 14 indicators of weather factors (X_i,) which during the indicated growing season can significantly affect the sugars accumulation in the sweet cherry fruit of early (Y_1), medium (Y_2), and late (Y_3) ripening periods (Table 4).

Table 4. Table of pair correlation coefficients between weather factors (X_i,) and sugars content in early ($r_{y_1x_i}$), medium($r_{y_2x_i}$), and late($r_{y_3x_i}$) ripening periods

Symbol of the (X_i,)	Factors	Paired correlation coefficients $r_{Y_jX_i}$ for group varieties		
		Early	Medium	Late
		$r_{Y_1X_i}$	$r_{Y_2X_i}$	$r_{Y_3X_i}$
X_1	The average temperature in June, °C	-0.534*	-0.664	-0.654
X_2	The difference between the average maximum and minimum temperatures in May, °C	0.520*	0.569	0.359*
X_3	Absolute minimum relative air humidity during blossoming, %	0.965	0.958	0.615
X_4	The absolute maximum temperature during the fruit picking, °C	0.401*	0.464*	0.864
X_5	The amount of precipitation during the blossoming period, %	-0.649	-0.626	-0.284*
X_6	The average temperature in April, °C	0.621	0.705	0.192*
X_7	The total number of days with precipitation in April, %	0.714	0.743	0.414*
X_8	The average maximum temperature during the fruit picking, °C	0.785	0.851	0.546*
X_9	The average minimum relative air humidity during blossoming, %	0.528*	0.637	0.512*
X_{10}	The difference between the average maximum and minimum temperatures in the period of fruit picking, °C	0.624	0.653	0.589
X_{11}	Average temperature per year, °C	-0.581	-0.638	-0.480*
X_{12}	The difference between the absolute maximum and minimum temperatures during the fruit picking, °C	-0.300*	-0.380*	-0.763
X_{13}	The average minimum temperature during the fruit picking, °C	-0.658	-0.720	-0.685
X_{14}	The average temperature in May, °C	-0.588	-0.637	-0.729

*insignificant paired correlation coefficients $\left|r_{Y_jX_i}\right| \leq 0.55$, $i = 1..25$, $j = 1..3$ | (according to the hypothesis of the correlation coefficients significance checking in terms of Student's test at the significance level of 0.05).

These are thermal indexes of air (°C): average temperature of April (X_6), May (X_{14}), June (X_1); the difference between the average maximum and minimum temperatures in May (X_2) and during the fruit harvest (X_{10}); average temperature for the year (X_{11}); absolute maximum temperature (X_4), average maximum temperature (X_8), average minimum temperature (X_{13}), the difference between the absolute maximum and minimum temperatures (X_{12}) during the harvest. Air humidity indexes (%) during the blossoming period are: absolute minimum relative air humidity (X_3), average minimum relative air humidity (X_9) and the amount of precipitation (X_5), as well as the total number of days with precipitation in April (X_7).

Thus, for early, medium, and late varieties, 14 weather factors were determined, for which the average and strong linear correlation dependence with the analyzed indicator - sugars - was established.

Further research is carried out according to the scheme given above.

The first step. Five principal components were identified by the principal components analysis ($PC_i, i = 1..5$). These five principal components provided more than 92.12% of the Cumulative Proportion of Variance.

The second step. Regression models of the sugars indicator dependence for each group of varieties on the principal components ($PC_i, i = 1..5$) of the kind (2) were constructed.

The regression equation for early varieties has the form:

$$\hat{Y}_1 = 11.9533 + 0.6637PC_1 - 0.2324PC_2 - 0.2822PC_3 - 0.3528PC_4 + 0.3165PC_5$$

The regression equation for medium varieties has the for

$$\hat{Y}_2 = 12.853 + 0.6316PC_1 - 0.1546PC_2 - 0.2603PC_3 - 0.1628PC_4 + 0.2776PC_5$$

The regression equation for late varieties has the form:

$$\hat{Y}_3 = 13.1095 + 0.5884PC_1 + 0.6733PC_2 - 0.5537PC_3 + 0.0574PC_4 + 0.0111PC_5$$

The values R – squared for early varieties are 0.9209, for medium varieties 0.8159, for late varieties 0.8588, which indicates a strong influence of independent variables on dependent variable. P – value is less than 0.05 for all regression models, which indicates the adequacy of the models based on the Fisher criterion at a significance level of 0.05.

Third step: After going over to the initial factors, we obtain a model of the form (3). This regression model characterizes the dependence of the sugars indicator (for $\hat{Y}_1, \hat{Y}_2, \hat{Y}_3$) on weather factors. The coefficients of the models (in standardized factors $\tilde{x}_i$) are given in Table 5.

Table 5. Regression Model Coefficients in Standardized Factors

	$\tilde{a}_1$	$\tilde{a}_2$	$\tilde{a}_3$	$\tilde{a}_4$	$\tilde{a}_5$	$\tilde{a}_6$	$\tilde{a}_7$
$\hat{Y}_1$	-0.2833	-0.0020	0.3623	0.0863	-0.5228	0.1512	0.2950
$\hat{Y}_2$	-0.2537	0.008732	0.296458	0.126151	-0.3590	0.1668	0.2449808
$\hat{Y}_3$	-0.4019	0.029582	0.116499	0.638081	-0.0636	-0.1358	-0.098782
	$\tilde{a}_8$	$\tilde{a}_9$	$\tilde{a}_{10}$	$\tilde{a}_{11}$	$\tilde{a}_{12}$	$\tilde{a}_{13}$	$\tilde{a}_{14}$
$\hat{Y}_1$	0.2930	0.1218	0.2810	0.0317	0.0065	-0.1114	-0.0593
$\hat{Y}_2$	0.253276	0.194473	0.288316	0.010818	-0.00616	-0.12481	-0.09123
$\hat{Y}_3$	-0.00138	0.186504	0.4421	-0.05426	-0.4061	-0.20678	-0.23116

Based on the constructed models for each factor, the coefficients $\Delta_i, i = 1..14$ are calculated according to the formula (4). Coefficients Δ_i determine the share of each factor in the total variance of the sugars content indicator in the sweet cherry fruit. Based on the calculated indexes $\Delta_i, i = 1..14$, we rank all the factors according to the degree of their influence, from the most significant (rank 1) to the weakest influence (rank 14). Table 6 shows the values of the index Δ_i, % and the rank of factors.

Table 6. Index of the share of factors Δ_i influence and their rank

Factor (X_i,)	Symbol of factors (X_i,)	Coefficients of the share of factors (Δ_i)influence and factors rank indexes for varieties of early, medium and late groups					
		Early		Medium		Late	
		rank	Δ_i, %	rank	Δ_i, %	rank	Δ_i, %
X_1	The average temperature in June, °C	6	8.50	6	9.47	3	14.77
X_2	The difference between the average maximum and minimum temperatures in May, °C	14	0.06	13	0.28	13	0.60
X_3	Absolute minimum relative air humidity during blossoming, %	1	19.66	1	15.97	8	4.03
X_4	The absolute maximum temperature during the fruit picking, °C	11	1.95	10	3.29	1	30.99
X_5	The amount of precipitation during the blossoming period, %	2	19.06	2	12.64	12	1.01
X_6	The average temperature in April, °C	7	5.28	8	6.61	11	1.46
X_7	The total number of days with precipitation in April, %	4	11.84	5	10.24	9	2.30
X_8	The average maximum temperature during the fruit picking, °C	3	12.94	3	12.11	14	0.04
X_9	The average minimum relative air humidity during blossoming, %	9	3.62	7	6.96	7	5.37
X_{10}	The difference between the average maximum and minimum temperatures in the period of fruit picking, °C	5	9.86	4	10.58	4	14.64
X_{11}	Average temperature per year, °C	12	1.04	12	0.39	10	1.46
X_{12}	The difference between the absolute maximum and minimum temperatures during the fruit picking, °C	13	0.11	14	0.13	2	17.41
X_{13}	The average minimum temperature during the fruit picking, °C	8	4.12	9	5.05	6	7.97
X_{14}	The average temperature in May, °C	10	1.96	11	3.27	5	9.48

For varieties of early and medium ripening periods $\Delta_i$varies within 0.12–16.06% (Table 6), for fruit of the group of late ripening period varieties - 0.84–13.98%.

For further analysis of the research results, the factors depending on the values of the coefficients Δ_i(i = 1…14) were divided into 3 groups. Where: Group 1 - factors that have a strong influence on the accumulation of the sugars fund ($\Delta_i \geq 9.50\%$) ; Group 2 - factors that have a medium impact (Δ_i *from* 2.00% to 9.49%); Group 3 - other factors that have a weak impact on the accumulation of the sugars fund ($\Delta_i \leq 2.00\%$). For 1 group of factors that have a strong influence on the sugars accumulation in the sweet cherry fruit of early and medium ripening period, 4 common factors were identified. They have a meaning in the range from Δ_i 9.50 to 19.66%. These are the thermal indexes during the period of fruit picking, namely the difference between the average maximum and minimum temperatures (X_{10}), the average maximum temperature (X_8). Humidity indexes during the blossoming period are the sum of precipitation (X_5), the absolute minimum relative air humidity (X_3), as well as the total number of days with precipitation in April (X_7). For fruit of late ripening period, factors with the range Δ_i from 14.64% to 30.99% are referred to group 1. Namely, these are the thermal indexes during the fruit picking period: the absolute maximum air temperature (X_4), the difference between the average maximum and minimum temperatures (X_{10}), the difference between the absolute maximum and minimum temperatures (X_{12}), and the average June temperature (X_1).

According to Table 4, the second group includes factors that have an average impact on the sugars accumulation in the sweet cherry fruit of early and medium ripening periods with a value of Δ_i from 3.29% to 9.48%. Four common weather factors for the fruit of early and the medium ripening periods were identified: the average temperature in April (X_6) and June (X_1), the average minimum relative air humidity during blossoming period (X_9), the average minimum temperature during fruit picking period (X_{13}). Also for the group of medium ripening period varieties to weather factors that have an average influence the sugars accumulation such indexes are identified: the absolute maximum temperature during the fruit-picking period (X_4) and the average air temperature in May (X_{14}). For sweet cherry fruit of late ripening period a share of weather factors influence(Δ_i),which have average influence on sugars accumulation are in the range of values of 2.30–7.97%. Namely: the average temperature in May (X_{14}); average minimum relative air humidity during blossoming period (X_9); average minimum temperature (X_{13}) during fruit picking period; the total number of days with precipitation in April (X_7) and the absolute minimum relative air humidity during blossoming period (X_3).

The third group includes other weather factors that have a weak effect on the sugars accumulation. According to Table 4, the values of Δ_i for early ripening period varieties are from 0.06 to 1.9%; for the group of medium ripening period Δ_i 0.13–0.39% for late ripening period 0.04–1.46%. The total percentage of the share of factors influence of this group for the group of varieties of early ripening period is 5.12% (for varieties of medium ripening period it is 0.80%, for the group of varieties of late ripening period it is 4.57%). For all groups of varieties 2 common weather factors that do not significantly affect the sugars fund accumulation of the three ripening periods are: the difference between the average maximum and minimum temperatures in May and the average air temperature per year.

Thus, the sugars accumulation in sweet cherry fruit, regardless of the ripening period, is most influenced by weather conditions of the blossoming period, the last month of fruit formation and thermal parameters and humidity indexes at the stage of fruit picking.

CONCLUSIONS

1. According to sugars content and to the variability of their formation under the conditions of Southern Steppe subzone of Ukraine the most perspective from the technological point of view were Zabuta variety - 12.47% (of an early period of ripening), Dachnytsia - 15.60% (of a medium period of ripening and Kripnoplidna - 14.35 (of a late period of ripening). These varieties differed in high sugars content and in their low and medium variability during the years of study (*Vp* = 8.6–13.0%).

A correlation analysis of weather factors influence on the sugars content in the sweet cherry fruit of early, medium and late ripening periods was performed. The average and strong correlation between 14 weather factors (Xi, i = 1..14) and the sugars content for sweet cherry fruit of early, medium and late ripening periods ($\left|r_{Y_jX_i}\right| \geq 0.55$, $i = 1..14,\ j = 1..3$) was determined.

2. Based on the principal components analysis and method of least squares, models of the dependence of sugars funds accumulation on the influence of weather factors for groups of varieties of early, medium and late ripening periods are constructed.

3. On the basis of the constructed regression models the analysis of a share of each of weather factors influence on the indicator of sugars content is executed. The calculated coefficients of relative factors influence Δ_i, % showed that the greatest influence was established for the group of temperature and humidity indexes with the maximum share of $\Delta_i \geq 9.50\%$ in the total influence of factors on the indicator of sugars content in sweet cherry fruit.

4. The ranges of the share of weather factors that have the maximum influence on the sugars formation fund in sweet cherry fruit (Δ_i 9.50% to 0.99%) were identified.

5. For varieties of three ripening periods, the weather parameters that have the maximum impact on the process of sugars accumulation in sweet cherry fruit were determined: for early and medium varieties it is the difference between average maximum and minimum temperatures, average maximum temperature, precipitation amount and absolute minimum relative air humidity during blossoming period, as well as the total number of days with precipitation in April; for late varieties it is the absolute maximum air temperature, the difference between the average maximum and minimum temperatures, the difference between the absolute maximum and minimum temperatures in the period of fruit picking and the average temperature in June.

6. Based on regression analysis, it is substantiated that sugars accumulation in sweet cherry fruit, regardless of the ripening period, is most influenced by weather conditions of the blossoming period, the last month of fruit formation and thermal parameters and humidity indexes at the stage of fruit picking.

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An investigation into the state of agricultural lands under water erosion conditions

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Abstract. Protecting agricultural land from erosion continues to be the most important task within the overall issue of the protection and rational use of land resources. That is why it is necessary to comprehensively study patterns of development in the erosion processes, and to assess the specific nature and features of their impact upon soil and vegetation, water resources, and landscapes in various natural conditions. The work is based on the results of many years of experimental research on problems that are related to soil erosion, and on the accumulation of slope sediments in catchments and in the valleys of small rivers, based on the use of landscape geography, soil morphology, and cartography methods of research. The research methodology that is included the collection covers the analysis of cartographic and experimental materials on the geo-ecological situation regarding an formation and manifestation of spatial and temporal erosion processes in the territory, and, the carrying out of research work which cover the soil washout and erosion processes in key areas of agricultural landscapes. The methodology also includes, the process of conducting a determination of the influence of natural and anthropogenic factors on the intensity of erosion processes, and the cameral processing of results that have been obtained in the field, all of which characterise the erosion and hydrology situation in the basins of small rivers. The paper presents the latest levels of research on the spatial and temporal variability of the features of erosion processes, depending upon their mechanisms of functioning, the complexity of the territory's geomorphological landscape conditions, and the intensity of anthropogenic load on the catchment areas of small rivers. The management of the migration of biogenic elements in agricultural landscapes can be improved if, on the one hand, the share of cultivated land is reduced and, on the other hand, the area of meadow land and small forest plots is increased, which significantly serve to slow down the flow of erosion products, including biogenic elements, into the hydrosphere. It has been established that, with an increase in the share of arable land, the removal of biogenic elements with runoff increases in direct proportion. Therefore, with up to 50% of the territory being cultivated, nitrogen removal was seven times higher - and phosphorus two times higher - than with the same territory under 20% of cultivation. With 80% cultivation, nitrogen removal increases two times and phosphorus and potassium removal increases four times when compared to 40–50% cultivation of the same territory in the catchments.

Key words: agricultural landscape, catchment, environmental monitoring, runoff, soil erosion.

INTRODUCTION

The planet's soils have been undergoing a process of created over millennia under conditions that have since disappeared. Soil destruction has occurred due to human error in just a few years. Over historical time, these losses have amounted to about twenty million square kilometres. For comparison, we note that the entire modern arable area in the world amounts to fifteen million square kilometres (Boardman et al., 1994; hazarika & Honda, 2001; Morgan, 2005, 2006).

Long-term irrational human activity has led to the fact that natural landscapes in a number of the world's regions have been practically destroyed, and the agricultural landscapes that have been created during this process are imperfect and unstable.

Both from an ecological and an economic point of view, it is much more expedient to prevent unfavourable changes in soil cover than to carry out expensive work to restore it or recreate those properties that are responsible for fertility having been lost (Kõlli & Tõnutare, 2020).

Most of the world's soil resources are either in a satisfactory, poor, or very poor condition. In particular, 33% of soil resources have been degraded to a moderate or high degree due to erosion, salinization, compaction, oxidation, and chemical contamination of those soils (Kurdyukova, 2020; Slyusar et al., 2020).

Amongst the most important problems, when it comes to providing a solution upon which the very existence of mankind depends, the erosion of soils and their degradation tops the list. In decisions that have been taken by the UN World Conferences on Environment and Development (Rio de Janeiro 1992; 2012; Johannesburg 2002; New York 2000; 2005; 2008; 2010; 2013; 2015), it was noted that the protection and rational use of soils should be the central theme of state policy, because it is the condition of soils that determines their ability to perform productive and ecological functions which are characteristic of the nature of human life, and any deterioration in that condition also affects the environment.

Soil washout from one shower of rain can affect as much as between 330–350 t ha^{-1} (or 2.5 cm of the upper layer). In this context, flushing away just 1 mm irrevocably removes 76 kg of nitrogen, up to 240 kg of phosphorus, and up to 80 kg of potassium from one hectare of land.

Researchers (Martin, 2002; Hines & Bishop, 2007; Cerdan et al., 2010; Panagos et al., 2014a, 2014b) have noted that, worldwide, more than three million hectares are irretrievably lost every day due to erosion, and in every minute that passes a total of forty-four hectares of land which is suitable for agriculture is taken out of agricultural use.

In the world's scientific community, great efforts are being made to study the processes of soil erosion and to develop effective measures to combat it (Kovář et al., 2012; Tarariko et al., 2017; Kachmar et al., 2018; Brychta & Janecek, 2019; Tarariko et al., 2019). Forecasting and controlling the hydrological erosion processes (HEP) in catchment areas should be based on knowledge of the surface runoff formation processes, and on an assessment of natural and anthropogenic factors and their interaction. Numerous bodies of data exist to show the role of various factors in terms of the formation of runoffs (Baboshkina et al., 2020).

Therefore a change in the structure of land use - that is the ratio between the areas of different categories of land and types of land - leads to the most significant levels of change in terms of the volume of sediments being transported down the slopes and entering the channel network of catchment areas. Therefore the share of agricultural development is one of the main factors involved in the modulus of suspended sediment runoff in lowland rivers (Litvin et al., 2010; Ran et al., 2020).

On slopes that are covered by natural herbaceous vegetation, the intensity of washout and runoff is lower than it is under agricultural crops (Ivanov & Nazarenko, 1998). It is believed that perennial grasses have the highest anti-erosion effect, while annual crops of continuous sowing are much weaker, and row crops are the worst of all. The washout from those areas that are occupied by perennial grasses is between 0.5–2.0 t ha^{-1}, while from plots that have been sown with cereal crops it is between 3–58 t ha^{-1}, from fields that are occupied by row crops it is between 4–264 t ha^{-1}, and from fallow plots it is between 10–475 t ha^{-1} (Ivanov, 2007).

Therefore the diversity of vegetative cover is a prerequisite for a wide range of influences on the processes of the water erosion of soil, while ploughed watersheds are an arena of its intensive development. Sown agricultural crops are significantly inferior to natural vegetation in their soil-protecting properties. In this regard, it is necessary to study natural vegetation as well as its transformed state under the influence of anthropogenic pressure. This information is important in the successful development of effective methods for protecting soil against erosion.

Several studies (Pimentel & Kounang, 1998; Misir, 2012; Ma et al., 2019) have established that woody vegetation is the most effective when it comes to protecting soil from water and wind erosion. The forest regulates snow melting and, therefore, moisture is fully absorbed into the soil, replenishing groundwater levels, while the forest underbrush almost completely prevents the surface runoff of melt water, which is the main reagent of the erosion processes.

The forest has an important regulatory effect on water flow, serving to improve its quality, transferring surface water to subsurface and underground water, smoothing out the peaks of spring floods and water spills after rainfall, and maintaining high water levels (Larocque, 2020). The felling of forests reduces the erosion resistance of landscapes and leads to the impoverishment of soil fertility. Deforestation by 50% of an area increases its annual runoff by between 40–50%, while soil washout increases between 1.2–1.8 times (Kastridis, 2020).

The intensity of the manifestation of soil erosion depends upon the relative degree of cultivation in the area; its share of arable land in the overall volume of agricultural land.

Within the context of the further intensification of agriculture, the question arises regarding the introduction of a system of anti-erosion measures in agricultural landscapes. It is possible to reduce runoff and soil washout from arable land by applying a deep loosening of the soil, leaving stubble on the surface of the field; in this case, the volume of surface runoff water is reduced by between 2.2–5.1 times when compared to the effect that is produced by ploughing (Lyakh et al., 2011). Winter ploughing across the slope with an additional slotting to a depth of between 40 to 50 cm, carried out at different times and depending upon the cultivated crop, also provides a noticeable reduction in surface runoff. The loosening and turning of the upper layer, the cutting of soil horizons by stalks, and the decompaction of the subsurface layer all serves to contribute to the active transfer of runoff water deep into the soil, into the low humidity zone (Belolyubtzev, 2009; de Menezes et al., 2020)

The patterns regarding the manifestation of accumulative erosion processes are largely determined by the features of the territory's relief.

A very important indicator which determines the risk of erosion is the slope's compass direction. In different climatic conditions, the influence of this factor on the development of erosion processes is different (Routschek et al., 2014). It has been proven that on northern slopes the snow reserves and the depth of soil freezing are greater than on southern ones. Therefore, here, as the snow melts, there is a gradual washout of soils, which in quantitative terms exceeds the washout on southern slopes.

Scientific studies have shown that southern, south-western, and south-eastern slopes are characterised by maximum intensity washout (ranging from significant to very strong). Northern slopes are less prone to erosion processes. On these slopes, intensity does not exceed 15 m^3 ha^{-1}.

In addition to the slope's compass direction, the slope's length is also one of those factors that influences the intensity of the erosion processes. It has been established that, with a twofold increase in slope length, soil washout can increase by between 1.4–1.5 times and, with a threefold increase in slope length, the washout rate increases by a factor of ten.

Slopes of up to 500 m in height are characterised by weak flushing intensity, while the maximum possible intensity of soil washout is typical for slopes of 1.5 km or more, where soil loss from a catchment area onto the lower part of the slopes increases significantly (Kovář et al., 2012). On steep, short, and high northern slopes, the intensity of accumulative erosion processes is higher than it is on longer and more gentle northern slopes.

It should also be noted that, in addition to the length, steepness, shape, and direction of slopes, the development of washout is also influenced by the shape of the catchment area, the density of the section that is vulnerable to erosion, the depth of local erosion bases, and so on.

Recently, scientists from various countries around the world have been engaged in the creation of models that will aid in calculating soil washout levels, both during snow melt and during rain showers. In the USA, a special organisation called WEPP (Becker, 1997) was created for such purposes. Japanese researchers have developed a model for determining soil loss depending upon the catchment area's size, and have determined a sediment movement index; scientists from Belgium have created a soil loss model for various landscapes; in Iraq they have prepared a universal equation in order to be able to predict potential annual soil loss rates (Hussein, 1998; Van Oost et al., 2000; Li et al., 2001).

Diagnosing and preventing soil erosion will be a key contribution to achieving the EU's goals.

Washed-off soils have impaired hydrological properties. In turn this affects, first and foremost, the ability of the soil to absorb and retain moisture. Water absorption on strongly washed-off soils is only 55% of the normal maximum when compared to non-washed-out analogues, and the maximum possible reserves of productive moisture are reduced by as much as 40%, which sharply increases surface runoff and impairs water supply to plants, as well as the chemical and physical properties of the soils themselves.

The researchers predict three scenarios that are also being used by the Intergovernmental Panel on Climate Change (IPCC). All of them, when including the impact of climate change and land use in their forecasts, predict increasing water erosion levels in most countries around the world, regardless of climatic conditions. The results that have been obtained indicate that climate change is the main factor that contributes towards the intensification of soil erosion.

Depending upon the simulation scenario being used, soil erosion is projected to increase significantly (from 30% to 66%) by 2070 when compared to the figures for 2015 (FAO, 2015).

Unfortunately, practically all soils can be subject to water erosion under certain conditions.

The optimisation of land use is one of the most important modern problems in terms of the sustainable development of agriculture. It is inextricably linked to the development of theoretical and methodological foundations for managing accumulative erosion processes in order to preserve soil fertility and a favourable ecological state of the environment. Significant advances have already been made in tackling this complex problem. On the basis of the theory of erosion processes, two strategies have been objectively formed when it comes to protecting soil from erosion, these being the balance-based strategy and the energy-based strategy. The balance-based strategy is aimed at achieving a level of permissible soil washout, the intensity of which does not exceed their self-recovery rate. An energy-based strategy for protecting soil against erosion aims to regulate the balance between the energy of water flows and the soil's erosion resistance. But it turns out that it is difficult to achieve 'normal erosion' or 'a non-erosive rate of water flows', because the problem of forecasting accumulative erosion processes with sufficient accuracy in order to be able to solve design decisions is one that remains unsolved.

Therefore there still exists a contradiction between the needs involved in managing accumulative erosion processes and the impossibility of their accurate prediction. This contradiction necessitates the further development of a solution to the problem at the theoretical and methodological levels of research.

The purpose of this paper was the study of regularities in the distribution of accumulative erosion processes on agricultural lands, and an assessment of their impact on the ecological state of the catchment systems of small rivers.

MATERIALS AND METHODS

The methodology being used in the analysis, assessment, and planning regarding the structure of agricultural land use is primarily one that concentrates on ecological land management. Generally accepted methods include experimental field observations and laboratory analyses, morphological comparisons and analogues, the use of ecological landscape axiomatics as a method of cognition, cartographic, balance-based, statistical economic, and computational constructive methods of landscape modelling, and the instrumental determination of the intensity of development of water-erosion processes.

The landscape unit that was chosen in order to determine the size and nature of the influence of agriculture on soil cover and on natural waters, and to study the incoming and outgoing flows of chemical elements, is that of the catchment area of a small river (Kovář et al., 2012).

The nature of conducting a study and the methods in which agricultural landscapes and farming systems can be optimised in the catchment areas of small rivers have been based on the application of a systematic approach to solving the problem, which in turn determined the methodological approach to the selection and determination of the main directions for research (Kurdyukova, 2020).

The first approach covers studies that are primarily aimed at the quantitative characteristics of the geomorphological, soil, and climatic conditions of the catchment area of a small river that is being studied, and its place in Ukraine's physical and geographical zoning, as well as its spatial location.

The second approach is to research the quantitative characteristics of anthropogenic factors, including the organisation of the land use area and the structure of sown areas and crop rotations.

The third approach characterises the complex influence of natural and anthropogenic factors (such as the structure of the agricultural landscape) on the state of agro-ecosystems in individual catchment areas, including the development of erosion processes.

The fourth approach consists of determining the quality indicators of groundwater and surface runoff in terms of their content of biogenic elements, depending upon the structure of agricultural landscapes and applied farming systems.

These four main directions are based on the fact that the study of interaction in a complex system such as the structure of the agro-landscape of a river's catchment area, and of the qualitative composition of water resources (in terms of nutrients), should be carried out, on the one hand, in a broad sense, ie. that it should cover the entire river catchment area and, on the other hand, as a deep and detailed analysis of the role of and interaction between individual natural and anthropogenic factors.

RESULTS AND DISCUSSION

Modern trends in the development of agriculture are associated with a change in the paradigm of nature management, and a gradual rejection of the anthropocentric approach to these, with an orientation towards nature conservation as a priority.

The scientific basis of modern agriculture is a deep knowledge of the ecological regularities of the landscape, and the relationship between agricultural ecosystems and natural ecosystems in the general natural-anthropogenic cycle.

It has already been proven that, at the current stage of the formation of the environmental paradigm in terms of agricultural land use, there is a need to improve existing measures and to develop new comprehensive measures in order to be able to protect soil from erosion, which will be aimed not only at diagnosing disruptions of the ecological state of erosion, but will also determine a differentiated approach to regulating the intensity of destructive processes in agricultural landscapes.

At the same time, the best soil-protective effect (and, in general, environmental effect) is achieved through the use not of separate anti-erosion (anti-deflationary) measures but in their interrelated and mutually coordinated complexes. It is precisely the creation and operation of effective complexes of anti-erosion (anti-deflationary) measures which will ensure the ecological sustainability of agricultural landscapes.

In order to plan and efficiently manage the land erosion control system, it is necessary to use all modern methods of forecasting the erosion processes. However, the large number of mathematical models that are currently available for forecasting and assessing accumulative erosion processes on slopes and in river beds indicates that they are somewhat limited, and that the problem in general remains unsolved. The desire to take into account a larger number of factors in order to improve the accuracy of forecasting erosion leads to a decrease in the credibility of the results that are obtained using the model. Each factor is taken into account with a certain level of probability, and the probability of a simultaneous combination of several independent events is equal to the product of the probabilities of these events.

This increases the relevance of the need for scientific research that is aimed at obtaining new areas of knowledge in terms of managing accumulative erosion processes within catchments along their entire length, from watersheds to channels, on the basis of the mathematical modelling of the structure of erosion geosystems, as a set of their system-forming connections.

We carry out constant monitoring of land use by agricultural enterprises through the means of cartographic and field surveys of the state of land plots, in order to assess the natural resource potential and the conditions for the formation and progressing of erosion processes.

Figure 1. The development of water erosion processes in the snowmelt period.

Figure 2. A full-scale study of soil washout products during a period of heavy rainfall.

The ratio of rain and melt washout changes significantly in terms of space.

The temperature regime and the depth of soil freezing have a significant impact upon the development of erosion processes during the spring snowmelt period (Fig. 1).

During the warm part of the year, the development of water erosion on slopes is determined first and foremost by the intensity and duration of rainfall. It is believed that runoff water is not formed when less than 10 mm of rain falls, since this quantity of rainwater is equal to the water-holding capacity of any soil which does not have vegetation.

The quantitative characteristics of the products of soil washout from agricultural land were measured by means of the volumetric water method (Fig. 2).

The basis for modelling accumulative erosion processes is that of a discrete model of the catchment structure, one which takes into account the spatial hierarchy of its elements and their functional interrelationships through the integral temporary or permanent water-soil flow of the main valley (Kachmar et al., 2018). In order to design environmentally sustainable agricultural landscapes, it is necessary to take into account the spatial structure of the surface runoff, which is the carrier for the system of forming links.

The catchment area is defined here not only as a fundamental geomorphological element, but also as a structural formation unit that reflects the specifics and patterns of the distribution of elementary catchment areas in the lower level; that is, the allocation of functionally integral systems, the elements of which are connected by unidirectional flows of matter and energy and which differ in their natural properties and the degree of their anthropogenic influence.

Small river basins are characterised by the greatest levels of response to anthropogenic impacts. Any change of the load in the natural structure of the basin affects the integral indicators of its functioning, which are liquid and solid flows. Therefore the composition of the water in small rivers is a reflection of the total of the surrounding natural processes and anthropogenic activities in the catchment areas of those rivers.

The research was carried out in the catchments of small rivers that were flowing into the River Dnieper at its midpoint.

Within the catchments of small rivers, elementary catchment areas have been identified which are of the second order and which come with depressions (Fig. 3, a). In order to detail the features of the functioning of geosystems at the local level, and the patterns of processes that occur between them as a result of the lateral movement of matter and moisture, a key site (a cross-section polygon) was set up in one of the micro-catchments (Fig. 3, b).

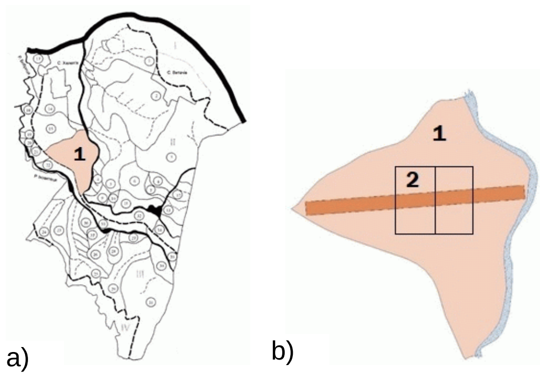


Figure 3. This is the block diagram for monitoring the accumulative erosion processes in small river basins: a) is the schematic map of micro-catchments for the small river basins; and b) is a diagram of the key site (the cross-section polygon).

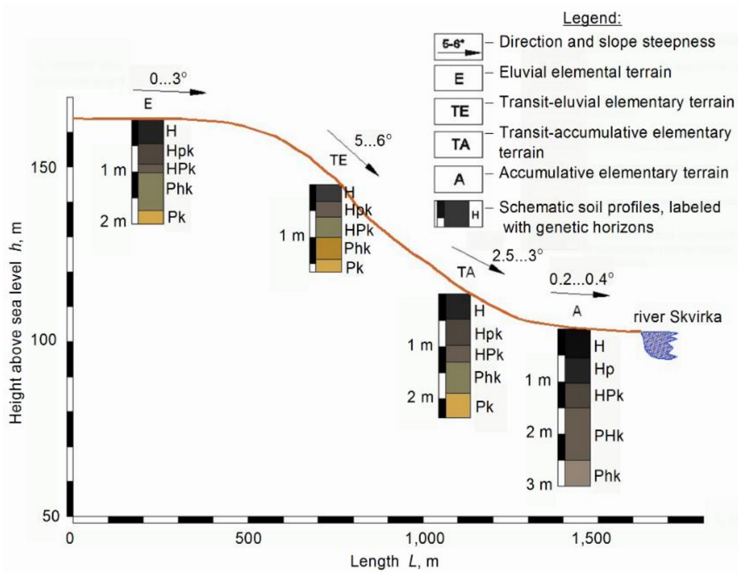


Figure 4. Monitoring platforms at reference sites.

Monitoring sites were laid at the reference plot points for the purpose of carrying out a spatial assessment of the erosion hazard in agricultural landscapes, while also establishing patterns of distribution, intensity, and trends in the development of accumulative erosion processes in the system of a ‘slope element - slope - primary catchment area - river catchment area - channel’ (Fig. 4).

The research solved the problems of a connected biogeochemical analysis of various geosystems of small river basins: soils, rocks, surface and ground water, precipitation, agricultural ecosystems and natural ecosystems, in order to be able to compile the initial ecological erosion characteristics of the territory and the trends regarding their change (Fig. 5).

Figure 5. The object of the research on a topographic base, in Ukraine’s Kiev region.

In order to be able to solve problems that are related to developing design solutions in models that cover the adaptive landscape arrangement of land that remains in use, taking into account their geomorphological features, the use was made of modern forms of technology for monitoring, modelling, analysis, and information processing.

When using the spatial interpolation method, a digital relief model was built of the research area. This relief model is the basis for compiling data on the steepness and exposure of the slopes, as well as the longitudinal and transverse curvature of the slopes, while also serving to make it possible to identify structural relief lines which include the lines of the erosion network and watersheds, while additionally serving to delineate catchment areas (Fig. 6).

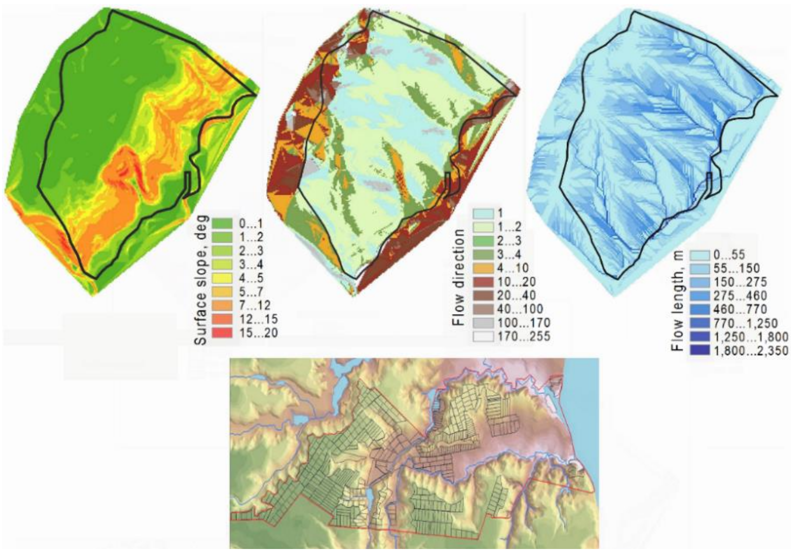


Figure 6. An element in the digital elevation model, by characteristics.

It was determined that the geomorphological characteristics of the terrain relief are necessary in order to be able to determine the elements, forms, and types of relief, the degree of its sectionality, length, steepness, and shape. A quantitative assessment was carried out of the relief of those agricultural landscapes which are at risk of erosion in order to develop a system of anti-erosion measures, while the relief serves as the basis for the rational allocation of agricultural land (Fig. 7). The degree of soil erosion is in direct proportion to the geomorphological characteristics of the territory, especially the steepness of the slopes (Shevchenko & Kolomiiets, 2014; Kaminskyi et al., 2018a, 2018b; Kolomiiets et al., 2019).

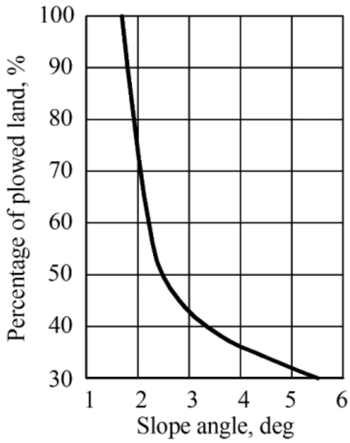


Figure 7. The dependence of the percentage of ploughing in catchment areas on slope steepness.

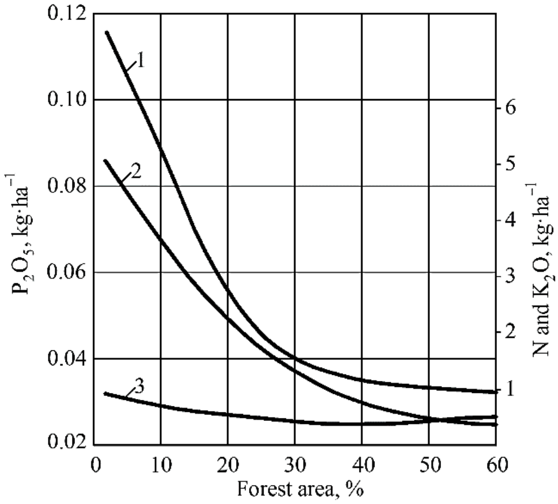
Eroded arable land is not just lost soil cover. It is also tons of lost crops, since the yields tend to be between 10–30% lower than before the soil cover was lost, and the cost of obtaining 1 Kcal of energy is between three and five times higher (Kutzenko, 2012). Therefore it is very important to ease the pressure of intensification on this type of land which provides such a vital means of livelihood.

In order to establish the relationship between the percentage of ploughed catchment area and trends in the development of accumulative erosion processes, low, medium, and high-intensity models were developed by type of anthropogenic load. Within the catchment models with their increased ploughing levels, the amount of soil washout changes during the spring snowmelt and summer rain periods as follows: on catchments with a slope of between 5–7 degrees, which are completely occupied by forest and meadow, the soil washout is insignificant at between 0.4–0.9 t ha^{-1} (Kutzenko, 2012). With a ploughing rate of between 10–20% and an average steepness of three degrees, soil washout increases to 3 t ha^{-1} (Kutzenko, 2012).

It has been established that, with an increase in the specific gravity of arable land, the removal of biogenic elements with runoff increases in direct proportion. So with a ploughing rate of up to 50%, nitrogen removal was seven times higher than with a 20% ploughing rate for the territory in question, while phosphorus removal was two times higher (Litvin et al., 2010). With an 80% ploughing rate, nitrogen removal increases by a factor of two when compared to a ploughing rate of the catchment area of between 40–50%, and phosphorus and potassium removal increases four times (Litvin et al., 2010).

The influence of forest planting on the removal of biogenic elements with surface runoff in catchment areas with between 2–62% forest cover was something that was also studied.

It was found that with a forestation level of only 2%, the removal of nitrogen with liquid runoff was at 5.0 kg ha^{-1}, phosphorus was at 0.12 kg ha^{-1}, and potassium was at 0.91 kg ha^{-1}. With an increase in forest cover to 27%, the removal of nitrogen, phosphorus, and potassium with liquid runoff decreased to 1.96 kg ha^{-1}, 0.04 kg ha^{-1}, and 0.43 kg ha^{-1}, respectively. With a forestation level of more than half the territory (57%), the removal of nitrogen, phosphorus, and potassium with liquid runoff decreased, respectively, to 0.10 kg ha^{-1}, 0.02 kg ha^{-1} and 0.22 kg ha^{-1} (Fig. 8).



a)

b)

Figure 8. The dependence of the removal of biogenic elements with surface runoff on the forestation of the catchment area (a) and a general view of the runoff (b): 1 – P_20_5; 2 – N; 3 – K_2O.

In this way, the integrated nature of information about space and time, and about the functioning of the erosion-denudation systems in general, greatly complicates the search for connections between factors and erosion processes.

However, it should be noted that natural factors (the hydrogeological conditions of the basin and the drainage capacity of the soils) serves to determine the situation regarding ecological erosion to a lesser extent; it depends more on economic activities in the river basin, and especially in the river valley.

CONCLUSIONS

1. The problems have been analysed in relation to the involvement of managing accumulative erosion processes in terms of agricultural lands, which largely determine the ecological state of the basin systems of small rivers.

2. The main methodological principles of geo-ecological landscape research have been studied and implemented at the regional level, which has made it possible to carry out a quantitative assessment of the factors involved in the spatial differentiation of accumulative erosion processes on the basis of the basin approach.

3. A comprehensive analysis has been carried out regarding the effect of erosion on the ecological state of the environment, and a territorial assessment has been provided of the danger of the pollution of ground and surface waters by means of biogenic elements due to soil erosion.

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Anatomical changes in the epidermis of winter pea stipules and their area under usage of herbicide, stimulator of plant growth and microbial preparation

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Abstract. The use and search for new methods and ways to reduce negative herbicidal effect on crops is a key factor in increasing the level of yield and quality in modern agricultural conditions, including cultivation of crops such as winter peas. One of the factors that reflects the depth of the effect of herbicides on the plant organism may be the anatomical structure of the leaf, thus, the aim of the research was to study the characteristics of epidermis and size of stipules of winter peas with the complex use of stimulator of plant growth and microbial preparation in herbicide cultivation technology.

To determine the optimal combination of preparations and rates of their introduction, a field experiment was established in the Department of Biology of Uman National University of Horticulture (2018–2019), which included options: without herbicide, stimulator of plant growth and pre-sowing seed treatment with microbial preparation (control); treatment of plants with MaxiMox herbicide during the growing season in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} separately and in mixtures with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} without and against the background of pre-sowing treatment of seeds with microbial preparation Optimize Pulse in the rate of 3.28 L t^{-1} (background). The experiment was repeated 3 times. Treatment of winter pea plants with preparations was carried out in the phase of 3–4 developed tendrils (BBCH 13–14). During the experiment it was found that treatment of winter pea plants with MaxiMox herbicide, especially with increasing level of the preparation to 1.1 L ha^{-1} led to anatomical and morphological changes in plant stipules and affected the stipule size of winter pea crops. The number of epidermal cells on average decreased by 14–53 pcs (6–22%) at LSD_{05} 9.8 pcs, but their size increased by 28.42–394.52 μm^2 (2–35%) at LSD_{05} 71.7 μm^2, while the size of the stipulate apparatus of crops increased on average by 2.7–4.6 thousand m^2 ha^{-1} (13–22%) at LSD_{05} 1.3 thousand m^2 ha^{-1}. The complex application of the herbicide with stimulator of plant growth, especially against the background of pre-sowing treatment of seeds with the microbial preparation Optimize Pulse in the rate of 3.28 L t^{-1}, caused a decrease in the number of epidermal cells per unit of stipules surface on average by 50–84 pcs (21–35%) at LSD_{05} 9.8 pcs and with an increase in the stipule size by 9–12 thousand m^2 ha^{-1} (44–59%) at LSD_{05} 1.3 thousand m^2 ha^{-1}, this may indicate the optimal effect of these mixtures of preparations on metabolic processes in plants against the background of reducing negative impact of the herbicide.

Key words: anatomy, epidermis, stipules, herbicide, winter pea (*Pisum sativum* L.), stimulator of plant growth, seed microbial preparation.

INTRODUCTION

The world area of pea crops is 8 million hectares with gross grain harvest - 16 million tons per year (FAOSTAT, 2019). Pea grain has a high content of protein, minerals, carbohydrates, fiber (Tosh et al., 2013) and is characterized by a complete absence of gluten proteins in beans, which is very important in meeting the needs of gluten-free diets for people suffering from celiac disease (Singh et al., 2017). Winter pea (*Pisum sativum* L.) has a number of advantages over spring forms: a longer and more effective period of symbiotic relationships with *Rhizobia leguminosarum* at the minimum expense of energy resources, low emissions of greenhouse gases into the atmosphere during growing season, and due to the formation of plants with high immunity - interrupting life cycle of many diseases and pests. Higher yields of winter peas compared to spring forms make it more cost-effective (McGee et al., 2017). Among negative factors that dramatically reduce the yield of peas is the presence of a weed component in crops that can reduce yields by 64.4%. The herbocritical period in peas is from 45 to 50 days, after which the crop can compete with weeds due to the growth of vegetative mass. A wide range of herbicides has now been developed to kill weeds at minimal cost compared to mechanical and other control methods (Shalini et al., 2017). However, their phytotoxic effects on pea plants are no exception, which is expressed in morphological, anatomical, physiological and biochemical changes that occur in plants. Herbicides affect the number of chloroplasts in assimilation tissues (Jung et al., 2008), disrupt the direction of biochemical and physiological processes (Warabi et al., 2001; Ha et al., 2003; Jung et al., 2004; Yang et al., 2006; Karpenko et al., 2019), causing primary anatomical and morphological changes (Guh & Kuk, 1997; Kamble, 2007a, 2007b), which generally cause growth inhibition or plant death. Thus, according to the data of Silva et al. (2017), in cassava plants (*Manihot esculenta Crantz.* cv. IAC-12) under the action of herbicides based on nicosulfuron (60 g a.i ha^{-1}), fluazifop (250 g a.i ha^{-1}), fomesafen (250 g a.i ha^{-1}), metribuzin (480 g a.i ha^{-1}), oxyfluorfen (720 g a.i ha^{-1}) and the mixture fluazifop + fomesafen (200 + 250 g a.i ha^{-1}) no visual signs of damage were observed, but there were changes in the anatomical structure of the leaf apparatus. In particular, in the treated cassava plants with a mixture of herbicides fluazifop + fomesafen (200 + 250 g a.i ha^{-1}), nicosulfuron (60 g. a.i ha^{-1}), fluazifop (250 g. a.i ha^{-1}) compared to the control, the thickness of the adaxial wall of the epidermis decreased by 75%, 60% and 50%. Treatment of pea plants with 0.2 mM aqueous solution of paraquate (Moskova et al., 2011) disrupted the cellular organization of the leaf - decreased intercellular space and cell size, including epidermal.

The results of the research by Cabral et al. (2017) showed that in plants *Inga marginata, Handroanthus serratifolius, Cedrela fissilis, Calophyllum brasiliense, Psidium myrsinoides, Tibouchina granulosa, Caesalpinia ferrea, Caesalpinia pluviosa, Terminalia argentea, Schizolobium parahyba* with two treatments on 60 and 80 days after planting with Gamit® 36CS (FMC, 360 g L clomazone) herbicide in the rate of

2 L ha^{-1}, there was a decrease in the thickness of the spongy parenchyma (SP) on average by 16.3% - after the first application and 17.9% - after the second application, while palisade parenchyma (PP), upper (adaxial, AET) and lower (abaxial, ABE) epidermis, decreased on average by 13.1, 8.22 and 7.73%, respectively, with double application of the herbicide. Pereira et al. (2017) reported that when the herbicide Sethoxydium® SPC (Nufarm, 2-[1-(ethoxyimino)butyl]-5-[ethythio)propyl]-3-hydroxy-2-cyclohexen-1-one - 13.0%; other ingredients: 87.0%) in the rate of 184 g a. i. ha^{-1} + Assist mineral oil was applied to Urochloa decumbens plants, restriction of epidermal cell growth, bulimorphic cells and total leaf thickness was observed in the leaves. Batistão et al. (2018) note that in tomato plants, treated with different concentrations of herbicide Tordon® 22K (Corteva agriscience, 4-amino-3, 5, 6-trichloropicolinic acid, potassium salt - 24.4%; other ingredients - 75.6%) herbicide in the rates of 25% (1.0 L ha^{-1}), 50% (2.0 L ha^{-1}), 75% (3.0 L ha^{-1}) and 100% (4.0 L ha^{-1}), the thickness of the leaf blade increased with growing concentration of herbicide by 184%, however, there was a disorganization of leaf tissues: cells of irregular shape were formed.

Studies by Anastasov (2010a) showed a negative effect of the herbicide based on imazamox on certain anatomical parameters of sunflower leaves, which was accompanied by an increase in the size of the assimilation parenchyma, a decrease in the number of stomata and their atrophy, which caused their inefficient functioning. Inefficient functioning of stomata along with a significant increase in the size of the assimilation parenchyma and a decrease in the number of stomata per unit of leaf area, led to disruption of photosynthetic, transpiration and gas exchange processes, expressed by visible signs of phytotoxicity (deformation of leaves and tops of plants, inhibition of plant growth, chlorosis and subsequent formation of necrotic spots on sunflower leaves). According to Asharaf & Murtaza (2016), 0.04 M concentration of the herbicide 2,4-D caused a decrease in the number of stomata on the surface of a wheat leaf, as reported in other crops and other researchers (Anastasov, 2010b; Semerdjieva et al., 2015; Kamble, 2013).

Anatomical structure of the leaf may change due to the action of plant growth regulators. Thus, in the treated Heteroauxin bean plants (0.2 g L^{-1}), leaf thickness increased (due to the increase in assimilation tissue), the number of epidermal cells increased, the number of stomata and their area due to the effect of Heteroauxin on meristematic tissues at the stage of division, stretching of cells and formation of their sizes (Shevchuk et al., 2019). Studies of the anatomical structure of soybean leaves showed that complex application of the antihyberellin preparation Chlormequat Chloride at 0.5, 0.75 and 1.0% concentration with pre-sowing inoculation of seeds with the microbial preparation Optimize Pulse (2.8 L t^{-1}) increased the cell area of spongy parenchyma, columnar parenchyma cell volume and the number of chloroplasts (Chorna, 2016). According to Hrytsayenko & Ivasiuk (2014), the application of Fabian herbicide in the rate of 90 g ha^{-1} in combination with the plant growth regulator Rehoplant in the rate of 50 mL t^{-1} on the background of pre-sowing treatment of seeds with the microbial preparation Ryzobofit in the rate of 100 mL t^{-1} with Rehoplant in the rate of 100 mL t^{-1}, contributed to the formation of mesomorphic features of soybean leaf apparatus: the size of epidermal cells and the total leaf area of crops increased.

The main indicator that determines potential productivity of crops is leaf surface area (Nichiporovich, 1971), the value of which can vary significantly depending on the varietal and climatic characteristics of the area, place of cultivation, as well as used preparations, including herbicides and plant growth regulators. According to Sivchev

(1973), herbicides are able to inhibit the formation of leaves. Therefore, complex application of chemical and biological preparation can have a significant impact on the formation and size of photosynthetic apparatus of plants (Hoysyuk, 2003).

The results, obtained by Datsenko (2016), give reason to claim that application of the microbial preparation Diazobakterin in the rates of 150; 175 and 200 mL t^{-1} in the mixture with plant growth regulator Radostim 250 mL t^{-1} for pre-sowing treatment and treatment during growing season of buckwheat with the same plant growth regulator Radostim in the rate of 50 mL ha^{-1} caused the activation of growth processes of individual tissues and organs, which was expressed in increase in leaf area by an average of 20–30%. Double spraying of pea plants during the growing season with plant growth regulators Gumaksid in the rate 0.6 L ha^{-1} and AKM 0.5 L ha^{-1}, caused an increase in the area of stipules by 30–43% after the first treatment (phase 3–4 stipules) and by 15–18% after the second (budding phase) compared with untreated plants (Kalitka et al., 2015). The study by Pylypenko et al. (2016) showed that the application of suspension microbial preparation Rhizohumin in the rate of 150 mL t^{-1} and complex application of $N_{10–90}P_{10–90}K_{60}$ under the main tillage, contributed to the formation of pea plants in the flowering phase of the largest assimilation surface of leaves (543.5–555.5 cm^2 per plant) and the content of pigments in them compared to the control version.

Unfortunately, studies on complex effects of herbicides, plant growth regulators and microbial preparations on anatomical changes in the stipulate apparatus of winter peas and their formation area in the scientific literature are hardly given, which determined the relevance and purpose of our studies.

MATERIALS AND METHODS

Field experiments were established at the experimental sites of the Department of Biology of Uman National University of Horticulture in 2018‒2019. The soil of the experimental plots is low-humic heavy loam podzolized chernozem on the loess with a content of humus in the arable layer of humus 3.20–3.31%.

Weather conditions in the years of research on temperature and water regime were typical for this region, in particular the average temperature during the spring and summer vegetation (March-June) of the crop was + 13.0 °C, precipitation – 40.9 mm with their long-term average annual indicators of + 10.2 °C and 57.2 mm respectively.

The objects of the study were winter pea plants *Pisum sativum* L. (NS Moroz variety) - the originator NS Seme (Serbia) having aphid-type leaf (tendril), herbicide MaxiMox s.c. (Bayer, active substance imazamox 40 g L^{-1}), stimulator of plant growth Agriflex Amino (Agrisol, a complex of 18 types of free L-amino acids (not less than 50%) of plant origin), microbial preparation Optimize Pulse (Monsanto BioAg, bacterial strain *Rhizobium leguminosarum*, minimum 2×10^9 live cells mL^{-1} + lipohitooligosaccharide in water solution).

The area of plots in the experiment was 100 m^2 with three repetitions. The sowing rate of winter pea seeds of NS Moroz winter variety was 1.1 million seeds per hectare. Treatment of winter pea seeds with the microbial preparation was carried out according to the rate, calculated on the weight of seeds on the day of sowing. Spraying of vegetative plants with herbicide and their tank mixture with stimulator of plant growth was carried out in the phase of 3–4 developed tendrils (BBCH 13-14) of the culture using a cordless sprayer Forte CL-18A, with a consumption rate of 300 L ha^{-1}.

A mixed type of weeding dominated in the experimental fields with a predominance of dicotyledonous weeds: *Fumaria officinalis* L., *Galium aparine* L., *Tripleurospermum inodorum* L., *Viola arvensis* L., *Papaver rhoeas* L., *Thlaspi arvense* L., *Capsella bursa- pastoris* L., *Euphorbia agraria* L., *Descurainia Sophia* L., *Setaria glauca* L., *Setaria viridis* L.. The level of weediness of crops before the use of microbial preparations ranged from 118 to 182 pcs per m^2.

The scheme of the experiment included options: 1 – without the application of herbicide, stimulator of plant growth and pre-sowing treatment of seeds with the microbial preparation (control); 2, 3, 4, and 5 – treatment of plants with MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} (M0.8, 0.9, 1.0, 1.1); 6 – treatment of plants with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} (A1.0.); 7, 8, 9 and 10 – treatment of plants with MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} in a tank mixture with the stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} (M0.8, 0.9, 1.0, 1.1 + A1.0.); 11 – pre-sowing treatment of seeds with the microbial preparation Optimize Pulse in the rate of 3.28 L t^{-1} (M0.8, 0.9, 1.0, 1.1 + O3.28); 12, 13, 14 and 15 – treatment of plants with herbicide MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} on the background of pre-sowing seed treatment with the microbial preparation Optimize Pulse in the rate of 3.28 L t^{-1} (M0.8, 0.9, 1.0, 1.1 + O3.28); 16 – treatment of plants with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} on the background of pre-sowing seed treatment with the microbial preparation Optimize Pulse in the rate of 3.28 L t^{-1} (A1.0 + O3.28); 17, 18, 19 and 20 – treatment of plants with MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} in a tank mixture with the stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} on the background of pre-sowing seed treatment with Optimize Pulse normal 3.28 L t^{-1} (M0.8, 0.9, 1.0, 1.1 + A1.0 + O3.28).

Anatomical studies of the pea stipule epidermis were performed on a LEICA-295 system microscope using a MOV-1-15 eyepiece micrometer and a SHO-2 object micrometer scale. Sampling for anatomical studies was performed from the middle part of the stem, from 20 typical plants in each variant of the experiment in the budding-flowering phase (BBCH 51-60), according to the methodology proposed by Hrytsaenko et al. (2003): after that, cuts of the stipules were made with a cork drill No. 3.

Discoloration and clarification of the selected cuts were performed in javelin water (KOCl + KCl) for 3 days, then they were washed (with four changes) in distilled water, epidermis was removed and stained with a solution of crystal-violet stainer (mixture 1 mL^{-1} 5% H_2SO_4 and 1 mL^{-1} 1% of water stainer solution) for 10 minutes followed by washing in distilled water. The preparations prepared in this way were fixed under coverslips in glycerin (30 preparations were used). In particular, the number of epidermal cells by counting in the field of view of a microscope with the subsequent recalculation on 1 mm^2 of a stipule, the sizes - measurements of length and width by an eyepiece micrometer. The area of the stipules was determined by calculation using cuts (Hrytsaenko et al., 2003). The morphostructure coefficient (Km) was calculated according to the methodology of Karpenko et al. (2012), as the ratio of the number of epidermal cells per unit surface area of the stipule under the action of the preparation (or other factor) to the number of epidermal cells in the variant where the action of the preparation (factor) was excluded (control) (Karpenko, 2008; Karpenko et al., 2012).

The accuracy of the experiment and the significance of the difference between the indicators (*LSD*) in the study were evaluated by the results of analysis of variance (Ehrmantraut et al., 2000) using Microsoft Excel. The sampling error did not exceed 5% of the mean values.

RESULTS AND DISCUSSION

During the experiment it was found that anatomical structure of epidermis of the stipulate plate of winter peas varied depending on the application of the herbicide alone and in combination with a stimulator of plant growth and a microbial preparation (Table 1). In particular, under the application of MaxiMox herbicide alone in crops in the rates of 0.8, 0.9, 1.0 and 1.1. L ha^{-1} the number of epidermal cells per 1 mm^2 of pea stipules on average in 2018–2019 in the budding-flowering phase decreased relatively to control by 32, 53, 44 and 14 pieces that at LSD_{05} 9.8 pcs. mm^2 was reliable, but there was an increase in their size (width and length) and, respectively, the area, which increased relatively to control by 200.12, 394.52, 132.12 and 28.42 μm^2 (18, 35, 12 and 2%), that at LSD_{05} 71.7 μm^2 was essential for variants with MaxiMox 0.8, 0.9, 1.0 L ha^{-1}. Changes in the number and area of epidermal cells, on the one hand, may be a response of winter pea plants to reduced competition from segetal vegetation in crops (from 42 pcs m^{-2} in the rate of 0.8 L ha^{-1} to 13 pcs m^{-2} - in the rate of 1.1 L ha^{-1}), and, as a consequence, improved lighting, moisture and mineral nutrition of cultivated plants. However, it is obvious that the maximum rate of herbicide (1.1 L ha^{-1}) showed some phytotoxic effect, which may indicate a decrease in cell area (compared to other rates) at the lowest level of weed infestation.

Treatment of plants with herbicide MaxiMox in the same rates in the mixture with the stimulator of plant growth Agriflex Amino 1 kg ha^{-1} also caused a decrease in the number of cells both relatively to control and to options with self-application of the herbicide MaxiMox. However, in comparison with the variants with self-application of the herbicide, the area of epidermal cells increased by 52, 90, 273 and 188, respectively, that at LSD_{05} 71.7 μm^2 was essential for the variants with MaxiMox 0.9, 1.0, 1.1 L ha^{-1} + Agriflex Amino 1 kg ha^{-1}.

A similar tendency was observed in the variants with the application of herbicide MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} on the background of treatment of seeds with microbial preparation Optimize Pulse 3.28 L t^{-1}, where the area of epidermal cells relatively to the control variant increased by 312, 624, 421, 248 μm^2 and by 112, 129, 289 and 220 μm^2 relatively variants with self-application of the herbicide, that at LSD_{05} 71.7 μm^2 was essential. Obviously, the reduction of herbocritical pressure on winter peas and the intensification of the bean-rhizobial apparatus contributed to the stabilization of the basic physiological and biochemical processes in plants, which reduced the phytotoxic effect of MaxiMox herbicide on plants and generally increased epidermal cell area.

Under the combined application of the studied preparations (MaxiMox 0.8–1.1 L ha^{-1} + Agriflex Amino 1 kg ha^{-1} + Optimize Pulse 3.28 L t^{-1} (pre-sowing treatment seeds, background)) the number of cells per 1 mm^2 of stipulate epidermis decreased relatively to control by 50–84 pcs., by 25–36 pcs. - before variants with self-application of the herbicide MaxiMox and by 14–21 pcs. - before variants with MaxiMox + Agriflex Amino. Herewith, in these variants an increase in the area of cells relatively to control was observed by an average of 27–52%, that at LSD_{05} 9.8 pcs. mm^2 was essential.

Table 1. Anatomical characteristics of epidermal cells of winter pea stipules under the effect of herbicide MaxiMox, stimulator of plant growth Agriflex Amino and microbial preparation Optimize Pulse (budding-flowering phase, average values for 2018–2019)

Experimental variants	The number of cells per 1 mm^2, pcs.	Dimensions of one cell, μm		Area of one cell, $μm^2$	K_M
		Length	Width		
Control (without application of preparations)	240	91.4	12.2	1,115.08	1
M0.8	208	100.4	13.1	1,315.2	0.86
M0.9	187	111.0	13.6	1,509.6	0.77
M1.0	196	101.4	12.3	1,247.2	0.81
M1.1	226	94.9	12.05	1,143.5	0.94
A1.0	219	100.9	13.2	1,331.8	0.91
M0.8 + A1.0	203	100.5	13.6	1,366.8	0.84
M0.9 + A1.0	172	111.1	14.4	1,599.8	0.71
M1.0 + A1.0	181	110.2	13.8	1,520.7	0.75
M1.1 + A1.0	211	102.0	13.05	1,331.1	0.87
O3.28 (pre-sowing treatment of seeds, background)	217	101.1	13.7	1,385.07	0.90
M0.8 + O3.28	196	101.2	14.1	1,426.9	0.81
M0.9 + O3.28	166	111.5	14.7	1,639.05	0.69
M1.0 + O3.28	181	110.5	13.9	1,535.9	0.75
M1.1 + O3.28	201	102.5	13.3	1,363.2	0.83
A1.0 + O3.28	206	101.8	13.9	1,415.02	0.85
M0.8 + A1.0 + O3.28	183	101.6	14.6	1,483.3	0.76
M0.9 + A1.0 + O3.28	156	112.4	15.1	1,697.2	0.65
M1.0 + A1.0 + O3.28	167	109.9	14.4	1,582.5	0.69
M1.1 + A1.0 + O3.28	190	103.5	13.7	1,417.9	0.79
LSD_{05}	9.8	5.2	0.68	71.7	

According to statistical analysis of quantitative variability, the studied indicators of the anatomical structure of the epidermis of pea stipules varied slightly and moderately. In particular, the indicators of the number of epidermal cells per 1 mm^2 and the area of cells $μm^2$ varied moderately - the coefficient of variation was V = 11%, while the indicators of width and length of cells varied slightly (V = 6%).

The obtained data give grounds to claim that the complex application of the studied preparations demonstrates a positive effect on growth processes in pea plants, due to the reduction of phytocenotic factor in the form of weed component (herbicide-destroying effect), stimulating effect of stimulator of plant growth (growth activation) and improved conditions of nitrogen nutrition (activation of symbiotic relations peas (*Pisum sativum* L.) - *Rhizobium leguminosarum* under the action of the microbial preparation), which is consistent with the studies of other scientists (Hrytsayenko & Ivasiuk, 2014; Karpenko et al., 2012; Viniukov et al., 2013). At the same time, the separate action of MaxiMox herbicide in our research, especially in the rate of 1.1 L ha^{-1}, showed a decrease in epidermal cells compared to other rates, despite the minimum weediness of crops, which is also consistent with the data of Moskova et al., 2011.

Studies have shown that the size of winter pea stipules varied depending on the combination of the studied preparations and correlated with the area of epidermal cells (generalized correlation coefficient was $r = 0.78$). Thus, in the budding-flowering phase (Fig. 1) in control (without application preparations) the total area of stipules was 20.5 thousand m^2 ha^{-1}. Application of MaxiMox in the rates of 0.8, 0.9, 1.0 and 1.1. L ha^{-1} led to an increase in the size of stipules relatively to control by 3.9, 4.6, 4.2 and 2.7 thousand m^2 ha^{-1} (19, 22, 20 and 13%) respectively, that at LSD_{05} 1.3 thousand m^2 ha^{-1} was statistically reliable. During the treatment of winter pea plants with Agriflex Amino stimulator of plant growth in the rate of 1.0 kg ha^{-1}, stipules area increased relatively to control by 0.8 thousand m^2 ha^{-1} (4%), that at LSD_{05} 1.3 thousand m^2 ha^{-1} was not significant.

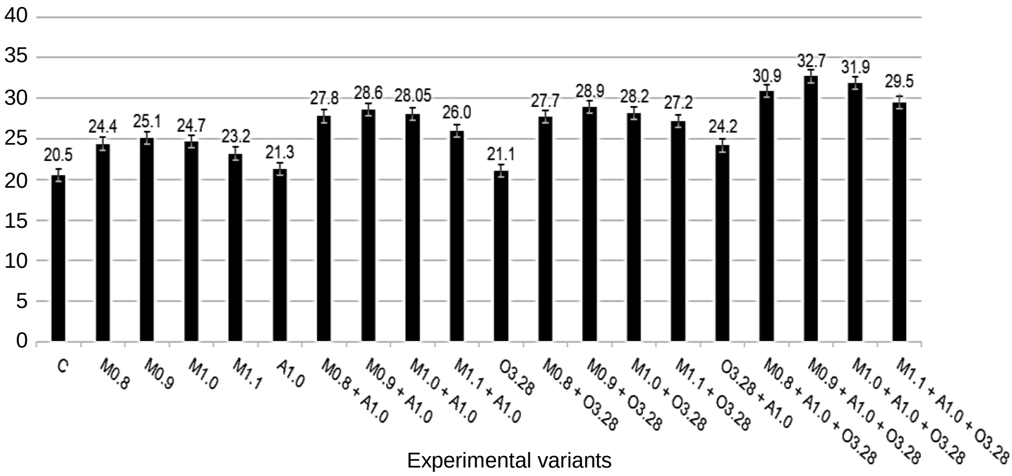

Figure 1. Area (thousand m^2 per ha) of winter pea stipules under the action of MaxiMox herbicide, stimulator of plant growth Agriflex Amino and Optimize Pulse microbial preparation (budding-flowering phase, average value for 2018–2019) (*LSD*05: 1.3).

C – without application preparations (control); M0.8, M0.9, M1.0, M1.1 – MaxiMox 0.8, 0.9, 1.0, 1.1 L ha^{-1}; A1.0 – Agriflex Amino 1.0 kg ha^{-1}; M0.8, M0.9, M1.0, M1.1 + A1.0 – MaxiMox 0.8, 0.9, 1.0, 1.1 L ha^{-1} + Agriflex Amino 1.0 kg ha^{-1}; O3.28 – Optimize Pulse 3.28 L t^{-1} (pre-sowing treatment seeds, background); M0.9, M1.0, M1.1 + O3.28 – MaxiMox 0.8, 0.9, 1.0, 1.1 L ha^{-1} + Optimize Pulse 3.28 L t^{-1} (pre-sowing treatment seeds, background); O3.28 + A1.0 – Optimize Pulse 3.28 L t^{-1} (pre-sowing treatment seeds, background) + Agriflex Amino 1.0 kg ha^{-1}; M0.8, M0.9, M1.0, M1.1 + A1.0 + O3.28 – MaxiMox 0.8, 0.9, 1.0, 1.1 L ha^{-1} + Agriflex Amino 1.0 kg ha^{-1} + Optimize Pulse 3.28 L t^{-1} (pre-sowing treatment seeds, background).

The application of MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1 L ha^{-1} in tank mixtures with stimulator of plant growth Agriflex Amino 1.0 kg ha^{-1} provided an increase in the area of stipules of winter peas by 7.3, 8.1, 7,55 and 5.5 thousand m^2 per ha (36, 39, 37 and 27%) to control, that at LSD_{05} 1.3 thousand m^2 per ha was statistically significant.

A similar dependence of the formation of the area of winter pea stipules was observed in variants using the herbicide MaxiMox in the rates of 0.8, 0.9, 1.0 and

1.1 L ha^{-1} on the background of seed treatment with the microbial preparation Optimize Pulse in the rate of 3.28 L t^{-1}, that is consistent with the data obtained by Zabolotniy et al., 2019. Under postemergence treatment with stimulator of plant growth Agriflex Amino in the rate of 1.0 kg ha^{-1} on the background of seed treatment with the microbial preparation Optimize Pulse 3.28 L t^{-1}, the total stipulate area exceeded the control by 3.7 thousand m^2 per ha (18%) respectively, that at LSD_{05} 1.3 thousand m^2 per ha was essential.

Application of MaxiMox herbicide in the rates of 0.8, 0.9, 1.0 and 1.1. L ha^{-1} in a tank mixture with stimulator of plant growth Agriflex Amino 1.0 kg ha^{-1} on the background of seed treatment with the microbial preparation Optimize Pulse 3.28 L t^{-1} provided the formation of stipules by 12.2–9 thousand m^2 per ha (59–44%) larger than control that at LSD_{05} 1.3 thousand m^2 per ha was statistically significant.

It is obvious that the complex application of a mixture of herbicide MaxiMox with the stimulator of plant growth Agriflex Amino on the background of seed treatment with the microbial preparation Optimize Pulse had a total positive effect on winter pea plants, which manifested in increased growth of stipules under several factors: phytocenotic (reduced effect of weeds on crops due to the herbicide) and physiological and biochemical (action of biologically active components of the plant growth regulator against the background of improving nitrogen nutrition due to active work of legume-rhizobial apparatus) as indicated in the studies by Ivasiuk (2017); Pidan (2017); Korobko (2019).

To find out the peculiarities of the formation of the stipular apparatus of peas, we calculated the coefficient of morphostructure (K_M), which characterizes the direction of passage of morpho-physiological processes in plants under the action of biologically active compounds: an increase of K_M to one or more indicates the formation of signs of xeromorphism in the leaf apparatus, and a significant decrease in K_M index indicates the formation of a mesomorphic type in the leaf apparatus of plants. Analyzing the obtained data (Table 1), the lowest K_M values were recorded in variants with the complex application of preparations (MaxiMox 0.8–1.1 L ha^{-1} + Agriflex Amino 1 kg ha^{-1} + Optimize Pulse 3.28 L t^{-1}), where K_M fluctuated in the range of 0.69–0.79, while the highest K_M was observed in variants with independent application of the herbicide MaxiMox (close to 1.0).

It is safe to assume that the obtained K_M data indicate that the complex use of the studied preparations is one of the indirect factors in the formation of the stiff apparatus of the mesomorphic type in peas, which is typical for highly productive crops (Karpenko et al., 2018). Calculation of correlation dependence between generalized values ‘total area of stipules’ ↔ ‘area of cells’ demonstrates close correlation (r = 0.78), indicating a direct dependence of the formation of the area of the stipules on the peculiarities of the anatomical structure of cells of epidermis.

CONCLUSIONS

The obtained results give grounds to draw the following conclusions:

Application of MaxiMox herbicide 0.8–1.0 L ha^{-1} leads to an increase in the area of epidermal cells additionally reducing their number, while increasing the rate of MaxiMox to 1.1 L ha^{-1} slightly reduced the size of epidermal cells and their area. The most optimal effect on winter pea stipules was the complex application of herbicide

MaxiMox 0.8–1.1 L ha^{-1} with the stimulator of plant growth Agriflex Amino 1.0 kg ha^{-1} on the background of pre-sowing bacterization of seeds with microbial preparation Optimize Pulse 3.28 L t^{-1}, where a decrease in the number of epidermal cells per unit area of stipules with a simultaneous increase in their size was observed: the area increased by 27–52% with morphostructure coefficient 0.69–0.79, photosynthetic area of stipules increased by 44–59%.

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Activation effect of β-alanine and chitosan derivative on *A. glycyphyllos* and *A. membranaceus* seed germination and seedling growth and development

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Abstract. Agricultural cultivation of astragalus is fraught with a number of difficulties caused by both certain requirements for climatic conditions and individual characteristics of plants of this genus. In this study, carboxyalkylated derivative of chitosan was first proposed to use for improvement of astragalus propagation. Effects of N-(2-carboxyethyl)chitosan on *in vitro A. glycyphyllos* and *A. membranaceus* seed germination and seedling growth and development in comparing with β-alanine and chitosan acetate were detected. Carboxyethylation of chitosan leads to an increase in hydrophilic properties of the molecule, which enhances a penetration of nutrients inside the plant owing to improved solvating effect and bioadhesive activity. Seed germination assay were performed on Murashige-Skoog growth medium with or without tested compounds. N-2-Carboxyethylated derivative of chitosan was found to demonstrate active stimulating effect on the plant growth and development, contrary to the effect of acetate chitosan, but not to cause an activating effect on seed germination, while β-alanine does.

Key words: agricultural benefits, bioactivity, functional derivatives, morphological measurements, plant grow regulators, polysaccharides, statistical Friedman test, stimulating effect.

INTRODUCTION

Astragalus glycyphyllos L. (Bunge) and *Astragalus membranaceus L.* are nemoral plants, belonging to the *Fabaceae* family, which grow in a broad-leaved forest of the temperate zone. Astragalus is usually found in sparse oak, pine, deciduous and birch forests, steppificated meadow, in a steppe, on rocky hills and on sandy banks of rivers. These amnicolous plants grow in Europe, East Asia (Northern Mongolia (*A. membranaceus*), Korea, Northeast, North and Northwest China), Western (*A. glycyphyllos*) and Eastern (*A. membranaceus*) Siberia, the Far East (World Health

Organization. Regional Office for the Western Pacific, 1998; Podlech, 2008; Podlech & Zarre, 2013). Sub-acid to alkaline, loam and sandy-loam soils favor the growth of them (Liu et al., 2016; Yang et al., 2020). The loss, alteration, and degradation of habitats from recreational load, livestock grazing and trampling, residential, commercial, and industrial development, forest fires and colonization by trees and shrubs are limiting factors contributing to threatened status of *A. glycyphyllos* species. The potential danger to *A. membranaceus* is low temperatures in the range of 0–10 °C, which have negative effects on the development of the plants in China and Europe and make population to decrease in size and density (Liu et al., 2019). At the same time, high temperatures (30–40 °C) cause oxidative stress, accumulation of reactive oxygen species, photosynthetic disorders and therefore limit the growth of seedlings (Zhou et al., 2012; Chopra et al., 2017; Laxa et al., 2019). Finally, human intervention transforms the established evolutionary relationships among species and results in their degradation (Zhuravlev et al., 1999).

Biologically active components of *A. glycyphyllos* and *A. membranaceus* species are of great importance for prophylaxis and therapy, and their efficient extraction and alternative production are important biotechnological task due to the high harvest pressure on wild populations (Bratkov et al., 2016). Extracts of leaves and roots are used in the treatment of neurological disorders, dermatitis, rheumatism, dysentery, venereal, gastrointestinal and acute respiratory diseases (Godevac et al., 2008), as well as in body weight reduction due to their antidiabetic properties (Shen et al., 2006; Ng et al., 2014). Diuretic properties make their application in kidney disease and gout possible (Guarino, De Simone & Santoro, 2008).

Secondary metabolites of the flavonoid class, especially formononetin and calicosin, exhibit strong antioxidant properties owing to the presence of hydroxyl groups on the polyphenol ring (Shirataki et al., 1997; Toda & Shirataki, 1999; Bratkov et al., 2016). At the same time, polyphenols have a detrimental effect on tumor cells, acting as initiators of caspase-dependent apoptosis and inhibitors of anti-apoptotic proteins of the mitochondrial membrane (Auyeung & Ko, 2010). Along with that, formononetin and calycosin suppress the growth of human pathogenic intestinal bacteria (Zhang et al., 2014), and also exhibit antifibrotic, anti-inflammatory and hepatoprotective properties (Wang et al., 2001; Huh et al., 2010). It is significant that, the only flavone apigenin is contained in extracts of many plant species of the genus *Astragalus* (Pistelli, 2002) and possesses the entire spectrum of bioactive properties of individual flavonoids (Salehi et al., 2019).

Carbohydrates such as α-1,4(1,6)-glucan, arabinose-galactose, rhamnose-galacturonic acid polysaccharide and arabinose-galactoprotein one isolated from *A. glycyphyllos* and *A. membranaceus* are used not only in chemical, but also in supportive anticancer therapy owing to reduction the negative effect of radiation therapy (Wang et al., 2018). The immunoregulatory properties of the polysaccharides are due to the increased functioning of immunocompetent organs and cells and the associated effects include but not limited to antitumor, anti-inflammatory and antiviral ones (Jin et al., 2014; Wang et al., 2018).

Triterpenoid sapogenins, triterpenes and sterols are constituents of various modern drugs (Chandler, 1985; Badam, 1997; Pistelli, 2002; Salari et al., 2003; Yu et al., 2013). A distinctive feature of phytosterols is the ability to act as precursors of hormones (sex and corticoid) (Sundararaman & Djerassi, 1977) and vitamins (for example, D3)

(Kametani & Furuyama, 1987), as well as to reduce cholesterol levels (Choudhary & Tran, 2011). Although against all the above mentioned, one of the most significant substances of plants of the genus *Astragalus* remains the triterpenoid saponin, astragaloside IV (Zhang et al., 2006, 2020; Liu et al., 2017), since its derivative cycloastragenol is an effective geroprotector which activates the telomerase enzyme (de Jesus et al., 2011; Ip et al., 2014).

The polyaminosaccharide chitosan and its derivatives are widely studied for their application in plant growing (El Hadrami et al., 2010; Ferri & Tassoni, 2011; du Jardin, 2015; Xing et al., 2015; Malerba & Cerana, 2016; Rafiee et al., 2016). In addition to regulation of growth and development, their use, both individually and in combination with other preparations, leads to the improvement of plant resistance to pathogens and abiotic stress conditions (du Jardin, 2015; Faoro & Gozzo, 2015; Malerba & Cerana, 2016; Rafiee et al., 2016; Dawood, 2018; Sailo et al., 2018; Kolesnikov et al., 2020). The nature of these defence responses depends on the plant hosts, the stage of growth and development, the physicochemical properties, the composition and structure of applied chitosans (Faoro & Gozzo, 2015). Despite chitosan is a well-known biostimulator used to wide variety of food crops there are only a few publications dealing with medicinal plants, to our knowledge. To the best of our knowledge, there is only one publication on an elicitation practice using chitosan to *Astragalus* plants, namely, elicitation protocol for the production of the *A. membranaceus* hairy root cultures with enhanced level of formononetin and calycosin (Gai et al., 2019). Chitosan derivatives in this regard have not been previously studied.

When added in the composition of growth media, amino acids have been known to initiate and accelerate seedling growth. Thus, for example, the sprout formation processes are known to be accelerated in the meristematic zone when growing the seedlings of *Peltogyne purpurea* with the use of L-cysteine (Esteban et al., 2015).

Considering the abovementioned and having regard to the increased solubility of carboxyalkyl chitosans compared with the native chitosan (Bratskaya et al., 2009), we combined the chitosan core and the amino acid (β-alanine) moiety in one molecule. Such a modification of chitosan leads not only to an improvement in solubility, but also allows us to expect an increase in bioavailability, hydrophilic properties and, as a consequence, in solvating effect and bioadhesive activity, which promotes the conversion of bio-unavailable nutrients to the more accessible forms, suitable for the involvement of such compounds in metabolism. This properties are favourable to plant growth and development biostimulation. In this work, comparative study of N-(2-carboxyethyl)chitosan, β-alanine and chitosan acetate effects on *A. glycyphyllos* and *A. membranaceus* seed germination and seedling growth and development were performed *in vitro*.

MATERIALS AND METHODS

Plant materials

Mature *A. glycyphyllos* and *A. membranaceus* seeds, showing a germination percentage of 98% in the laboratory conditions, were used for studying the biological activity. All the seeds were harvested from wild habitats and stored at 4 °C prior to the experiments.

Feedstock and chemicals

N-(2-carboxyethyl)chitosan with the degree of substitution 1 (N-CECS), chitosan acetate (CA), and β-alanine (BA) were used for bioactivity evaluation. N-CECS was synthesized according to the previously described procedure using the 'synthesis in gel' method (Pestov, Zhuravlev & Yatluk, 2007). Physicochemical characteristics were found to be in accordance with the data published previously.

Chitosan were purchased from Bioprogress and used as starting material in the synthesis route. Chitosan acetate was synthesized (the degree of acetylation (DA) 0.18; 500 kDa; the ash content 0.16%) by dissolving the chitosan in the equivalent amount of 3% aqueous acetic acid solution with subsequent precipitation, filtration and drying at 25 °C. β-Alanine (98%) produced by AlfaAesar was used with no additional purification required. Premixed medium powder containing basal salts and vitamins of Murashige-Skoog medium were purchased from BioloT.

Seed germination

The seeds (30 seeds for each replicate) were sterilized in the following steps: 1. dipping in ethanol solution (70%) for two minutes, 2. immersion in a freshly prepared sodium hypochlorite (2.5%) at room temperature for 15 minutes, 3. rinsing with sterile distilled water three times for 10 minutes. The seeds were mechanically scarified using a sterile dissecting needle to break the exogenous physical dormancy. The seeds were sown on Murashige-Skoog growth medium (Murashige & Skoog, 1962) (1 × MS salts, 30 g L^{-1} sucrose, and 10 g L^{-1} agar, pH 5.7) with or without various tested compounds and then incubated in a growth chamber with a 16 h light photoperiod at 23 ± 2 °C. The studied compounds were added to the growth medium at a concentration of 100 mL g^{-1}. The medium without growth regulators served as control. The number of germinated seeds was recorded 5, 10 and 15 days after being sown on the medium. Duration of passages varied within 4–8 weeks. Radicle emergence of > 1 mm indicated seed germination.

Statistical analysis

Three replicates were used for each treatment and results of morphological measurements are presented as the mean values together with the standard errors. A p value of less than 0.05 was considered to show a statistically significant result. As the data distribution was not normal, the non-parametric statistical Friedman test (Friedman, 1937) was used for assessment the statistical significance of the effects caused by adding the tested compounds to the growth medium. For statistical processing of the qualitative data obtained, we introduced the following simple rating scale (Table 1). Statistical analyses were done using Statistica 10.0 portable software.

Table 1. Evaluation scale of the plant growth and development

Score	Development stage
1	seed germination start
2	first true leaf stage
3	apparent isolation of taproots
4	appearance of lateral roots
5	formation of a mature plant in the vegetative phase

RESULTS AND DISCUSSION

Germination, growth and development. The efficiency of chitosan application has been demonstrated for a variety of plants, including species of the genus *Astragalus* (El Hadrami et al., 2010; Ferri & Tassoni, 2011; Farouk & Amany, 2012; Mondal et al., 2012, 2013; Yin et al., 2012; Xing et al., 2015; Malerba & Cerana, 2016; Phothi & Theerakarunwong, 2017). Besides, the use of chitosan has been reported to promote accumulation of phytoalexins in *A. membranaceus* (Gai et al., 2019). Considering the known literature data, germination of the *Astragalus* seeds in the present study was initially carried out in growth media containing N-(2-carboxyethyl)chitosan or chitosan acetate or β-alanine.

Assessment of germination was commenced five days after being sown on the medium. A seed was considered germinated when the emerged radicle had reached a length greater than two length of the seed.

The total germination and seedling growth rate of *A. glycyphyllos* were considerably greater than those of *A. membranaceus,* which is probably associated with the seed storage conditions of the latter. According to the literature data, contamination of the seeds with mold fungi and putrefaction bacteria results in decreased germination energy and lowered germinability of *Astragalus* spp. (Galtsova & Silantieva, 2015). Most *Fabaceae* species are known for their hardseededness which is the cause of seed dormancy. So, germinating ability is usually low without scarification (Long et al., 2012; Statwick, 2016) and such treatment is used for overcoming the hardseededness and significant improving of the germination rate of *Astragalus* seeds (by 17–52%).

The seed germination start for *A. glycyphyllos* was observed on day 8 in the three different media (germination percentage lies in the range 9.09 to 16.7%, Table 2). Seedlings in the control line showed radicles much longer than those in the other ones.

Table 2. Germination percentage of *A. glycyphyllos* (*A. g.*) and *A. membranaceus* (*A. m.*) seeds in the growth media upon addition of various compounds

Days	Germination percentage							
	N-CECS		CA		Control		BA	
	A. m.	*A. g.*	*A. m.*	*A. g.*	*A. m.*	*A. g.*	*A. m.*	*A. g.*
5	0	0	0	0	0	0	0	0
8	0	9.1 ± 2.7	0	0	25.0 ± 4.4	12.5 ± 3.2	0	16.7 ± 4.6
12	0	27.3 ± 3.4	0	12.5 ± 3.6	25.0 ± 4.4	37.5 ± 4.1	0	25.0 ± 5.9
15	0	45.5 ± 7.5	0	37.5 ± 7.1	25.0 ± 4.4	75.0 ± 10.5	0	25.0 ± 5.9
22	0	54.6 ± 9.2	0	37.5 ± 7.1	50.0 ± 6.2	75.0 ± 10.5	0	25.0 ± 5.9
26	25.0 ± 5.3	54.6 ± 9.2	0	37.5 ± 7.1	50.0 ± 6.2	75.0 ± 10.5	0	33.3 ± 6.3
35	25.0 ± 5.3	54.6 ± 9.2	0	50.0 ± 8.9	50.0 ± 6.2	100.0 ± 11.2	0	33.3 ± 6.3
47	25.0 ± 5.3	63.7 ± 9.8	0	50.0 ± 8.9	50.0 ± 6.2	100.0 ± 11.2	0	33.3 ± 6.3

By day 12, the control seedlings had the first true leaf appeared, and the main root system was observed to be coming to the branching stage. To our knowledge, during the *A. glycyphyllos* ontogenesis process, radicle shows elongation on day 8 or 9, and the first true leaf develops 17–20 days after being planted. In the other media, cotyledons were observed to have appeared. In our experiments, the worst growth scenario was realized for the medium with CA added.

On day 15 of the experiment, the growing processes were observed to accelerate in case of the N-CECS-containing medium, where lateral roots appeared. Whereas the seedlings in the BA and CA-containing media continued to be inferior to the N-CECS-containing one in their growth and development rates.

By day 22, seedlings in the BA line showed considerable growth of the vegetative part of the plants with retarded growth of the root system; and the total lack of root development was observed in a number of cases. All of the plants in the CA line manifested the signs of considerable growth and development inhibition: the colorlessness (lack of photosynthetic chloroplasts), the lack of root system development, and the emerged cotyledons not taking the vertical position but lying down on the medium. This seems contrary to the literature data on a positive effect of chitosan on the growth of hairy root cultures of *A. membranaceus* and phytoalexins accumulation, which has been observed to be especially pronounced 18–24 hours after treatment depending on chitosan concentration (Gai et al., 2019). Good growth and development of the plants were observed in two media: the control medium and the N-CECS-containing one. Plants in the N-CECS was characterized by a significant both stems and petioles elongation.

By day 26, the young plants had been keeping the growth rate to be a constant in two line: the control and N-CECS-containing media. In the cases of CA and BA-containing media, the tested compounds apparently inhibited the growth processes: development of the root system was either not observed or seen with abnormalities; true leaves were either not developed or their growth was considerably retarded.

On day 35, grown in the N-CECS-containing and control media, the young plants were found to have been formed completely, i.e., they exhibited well-branched and proportionally developed root systems and clearly seen short shoots with four to five true leaves. In the case of BA-containing medium, the young plants were characterized by formation of short shoots having fewer than or equal to three leaves, and development of the root system was still retarded considerably. In the CA-containing medium, the root system failed to develop, a portion of the seedlings showed true leaves, and such plants demonstrated vertical position.

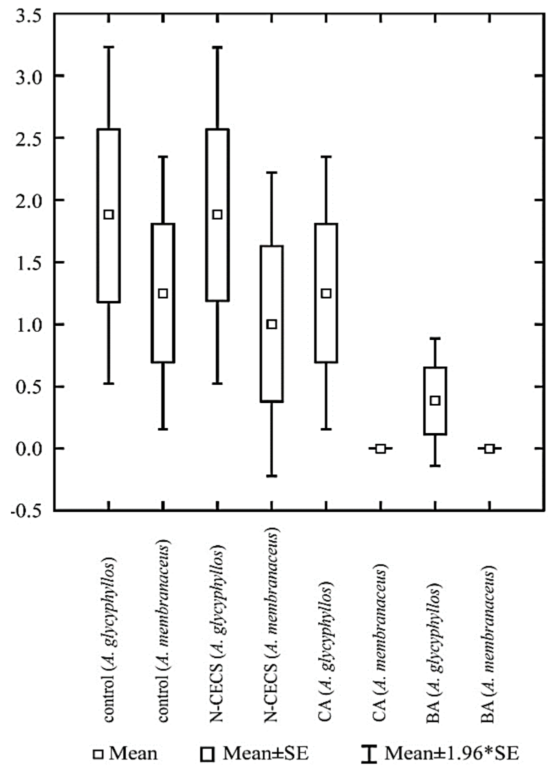


Figure 1. Average growth patterns for *A. glycyphyllos* and *A. membranaceus* plants. The growth patterns were obtained on the basis of the rating scale for the major qualitative growth parameters.

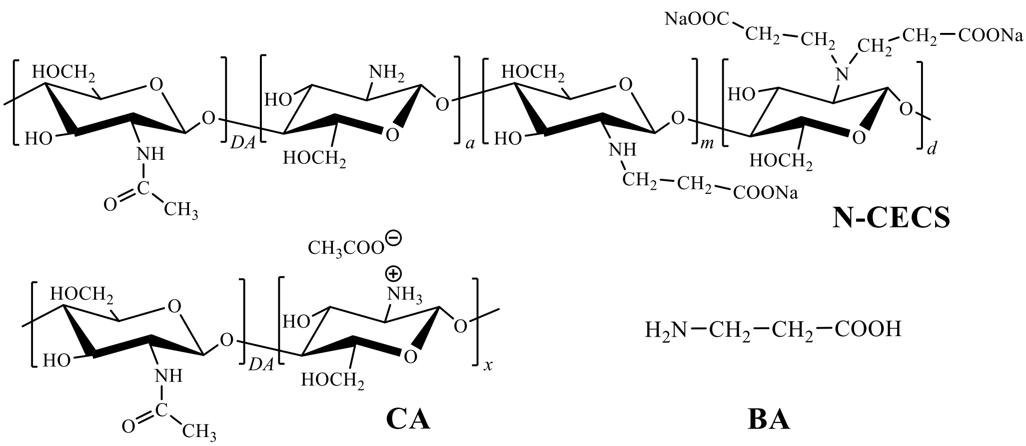
At the initial stages of germination, a 3 days delay observed in the control medium for *A. glycyphyllos* and a 21 days delay observed in the N-CECS-containing medium for *A. membranaceus* were considered as the most notable differences in the comparative analysis of the germination dynamic of the seeds.

The maximum germination rate was observed for the *A. glycyphyllos* seeds in the control medium. High germination rates were also noted for the mentioned seeds planted in the growth medium containing N-CECS; however, by day 47, the germination percentage there was found to be somewhat lower in comparing with the control medium.

The worst germination percentage (33.3%) of the *A. glycyphyllos* seeds were pointed out in the BA-containing medium, although, by day 5, the corresponding value had been the highest (16.7%) among all the media studied.

The statistical analysis of the obtained data was performed using the Friedman test and demonstrated that all the tested compounds cause a significant effect on the growth activation and all other factors and processes involved in mature plant formation (growth rate, development of vegetative and root systems). A growth pattern closest to the control was displayed by *A. glycyphyllos* plants exposed to N-CECS stimulation (Fig. 1). The patterns of *A. membranaceus* plants differ quite significantly between the lines; however, the general positive dynamics of stimulation are observed in the N-CECS and the control lines.

Evaluation the effect caused by the compounds. Contrary to the known efficacy of using chitosan to a variety of living organisms from single-celled to many-celled ones, including *Astragalus* plants (El Hadrami et al., 2010; Ferri & Tassoni, 2011; Farouk & Amany, 2012; Mondal et al., 2012, 2013; Yin et al., 2012; Xing et al., 2015; Malerba & Cerana, 2016; Phothi & Theerakarunwong, 2017), the present study demonstrates water soluble chitosan acetate to inhibit the growth and development of the plants, while its carboxyethylated derivative is shown to activate the growth processes. Considering N-CECS as N-derivative of β-alanine (Scheme 1), this amino acid was used as an additional reference compound and demonstrated a somewhat activating effect but only during the first eight days of the experiment.



Scheme 1. Structure of N-(2-carboxyethyl)chitosan with the degree of substitution 1 (N-CECS), of chitosan acetate (CA) and β-alanine (BA).

Generally, it should be suggested that β-alanine, being a low-molecular amino acid, causes an activating effect due to its ability penetrate the seed tissue through the seed coat. Despite the presence of the β-alanine fragment in the N-CECS structure, the latter does not cause such an activating effect because of inability to penetrate the tissues of germinated seeds. Therefore, the mechanism underlying the growth-regulating effect of chitosan and N-CECS is caused exclusively by an external interaction between macromolecules and tissues of a developing plant. The fundamentally different activities of acetate chitosan and N-CECS revealed in this study could result from different values of the hydrophilic-lipophilic balance. The presence of carboxyl groups in the molecule of N-CECS provides the greater hydration degree and, as a consequence, the greater solvating effect and bioadhesive activity as compared to CA.

CONCLUSIONS

Search and development of new fertilizers, sbiostimulators and plant protection agents based on safe and non-toxic organic compounds is agrochemistry frontiers in agricultural sustainability. Organic design and synthesis feasibilities allows for obtaining such compounds. Bio-based molecules and polysaccharides primarily have great advantages stemming from huge availability of feedstocks from large-scale synthesis wastes. Chitosan, being polymer of natural origin with increased reactivity in comparing with cellulose or starch, is currently commercially-available polysaccharide with boundless opportunities for development of new eco-friendly agrochemicals. Its synthetic flexibility offers the prospect of the design and construction of new plant protection agents and growth regulators with agricultural benefits.

The present work is focused on the mentioned research trend in so far as native chitosan is limited in use to agriculture due to, among other things, its limited solubility and a narrow spectrum of bioactivity. At the same time functional derivatives of chitosan expand the spectrum of both compounds/materials and biological effects. Using *A. glycyphyllos* and *A. membranaceus* as examples, it was found that N-2-carboxyethylated derivative of chitosan do demonstrate active stimulating effect on the plant growth and development, contrary to the acetate chitosan. Despite the presence of the β-alanine fragment in the N-CECS structure, the latter does not cause an activating effect on seed germination, while β-alanine does.

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The effect of succinic acid on the productivity of *Lactuca sativa* L. in artificial agroecosystems

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Abstract. The research of the effect of the biostimulant on the growth and development of plants of the 'Robin' lettuce variety was carried out in 2019–2020 in the department of closed artificial agroecosystems for crop production on the basis of the Federal State Budgetary Scientific Institution «Federal Scientific Agroengineering Center VIM», Moscow (VIM, Russia). Succinic acid was used at the stage of inoculation of seed and with constant drip irrigation of plants throughout the growing period. Lettuce plants were grown using a low-volume technology in a climatic chamber produced by VIM (Russia). According to the studies carried out, inoculation of seeds stimulates the development of plants at the initial stages, reducing the period from sowing to germination and increasing the rate of plant growth. As the lettuce plants grew, the leaf surface area increased from 221.0 to 1511.9 cm² with the addition of succinic acid to the hydroponic nutrient solution. The use of succinic acid under controlled growing conditions of 'Robin' lettuce plants allowed to increase the performance of the leaf apparatus in relation to the control. It was experimentally established that productivity directly depended on the value of photosynthetic potential and net productivity of photosynthesis, which is confirmed by a strong degree of dependence with a correlation coefficient of 0.98 and 0.77, respectively. Seed treatment and adding succinic acid to the hydroponic solution increases the accumulation of dry matter in lettuce plants. With this method of using succinic acid, significant differences in the accumulation of dry mass of plants were established. The share of the effect of the factor of inoculation of seeds with succinic acid was 17.5%, the addition of hydroponics to the nutrient solution was 50.1%. The use of succinic acid increases the accumulation of plant biomass, increases the total leaf surface area, and also contributes to an increase in the parameters of photosynthetic activity of the leaf apparatus of the 'Robin' lettuce plants under controlled conditions of the climatic chamber.

Key words: salad, biostimulant, succinic acid, climatic chamber.

INTRODUCTION

The development of the agricultural sector is an important task for the world economy in many countries. Thus, according to FAO, around 820 million people suffer from hunger and malnourishment in the world, part of the population is affected by the problem of a lack of certain substances in food or an unbalanced diet that causes obesity and other diseases. According to the agricultural forecast for 2020–2029, about 85% of global growth in crop production over the next years is associated with higher yields as a result of more intensive use of resources, investments in production technologies and improved cultivation methods (OECD-FAO, 2020). As a result, agriculture requires a dynamic interaction of science and technology to address the global challenges of climate change, land degradation, and next-generation food for the world's growing population (Rangga et al., 2021).

In last year huge attention is devoted to researches and developments in the field of urban agriculture with a completely closed and artificially controlled agroecosystems, which aimed at the safe and constant production of high-quality and functional nutrition (Nguyen et al., 2019; Kumar et al., 2020a).

The development of resource-saving, environmentally friendly technologies for cultivation in greenhouses of promising varieties and hybrids of vegetable crops is possible thanks to the development of new cultivation systems. The Federal Scientific Agroengineering Center VIM develops climatic chambers of various modifications, with fully automatic control of the parameters of plant growth and development in the dynamics of ontogenesis (Izmailov et al., 2020). Climate chambers are used in biological and agro-engineering research, as well as for food production (Dost & Ferkl, 2014; Nishida & Okada, 2019).

The complex use of agrochemicals in the production of crop products is an integral part of the modern level of intensive production. The intensification of the cultivation of seed material of different crops is a perspective direction for improving agrobiotechnology (Coomes et al., 2019; McDougall et al., 2019). The use of physiologically active substances (biostimulants) for plant growth and development makes it possible to mobilize the genetic potential of crops depending on their phenology (Colla & Rouphael, 2015).

Italy is the European leader in leafy herbs. Over the past two decades, interest in *Diplotaxis tenuifolia* L. from the *Brassicaceae* family has increased due to the high content of useful elements and the creation of functional foods. Scientists Giordano, M., El-Nakhel, C., Caruso, G. and others from the University of Naples Federico II (2020) conducted an experiment on the effect of biostimulants based on extracts of tropical plants containing active compounds of aspartic acid. The results showed that using of Auxym (PE) and Trainer (PH) has increased the yield and total dry biomass of greenhouse plants of *Diplotaxis tenuifolia* by an average of 48% and 37% compared to untreated plants (Giordano et al., 2020).

The exogenous addition of plant growth regulators (gibberellic acid) to the mineral base of the hydroponic nutrient solution promoted biomass accumulation, increasing leaf area and efficient using of water and nitrogen in *Lactuca sativa* L. and *Eruca sativa* L. plants grown in a floating system (Vetrano et al., 2020).

The use of salicylic acid contributed to an increase in yield of *Solanum melongena* L. eggplants by 7.3–22.7% (Wakchaure et al., 2020), an increase in plant height and the weight of 1,000 Plantago ovata Forsk grains (Roumani et al., 2019). Potato tubers had treated with a growth stimulant from hydrolyzed collagen with an increased dose of glycine and they had an increased content of biologically active compounds from 15 to 50% (Murashev et al., 2020).

Amino acid solution (salicylic, gibberellic and ascorbic acids) improve the antioxidant state, increase the accumulation of pigments (2.6–10.8%) and dry matter (1.4–4.0%) in the treated plants of *Allium sativum* L. (Ulianych et al., 2020a). It was found that the use of the 'MaxiMarin' growth regulator in greenhouses when treating seeds of *Spinacia oleracea* L. increased the profitability of the crop by 83–102% (Ulianych et al., 2020b).

The purposes of the research were evaluating the effect of biostimulant (succinic acid) on productivity and developing technological methods for producing high-quality commercial products *Lactuca sativa* L., which has been grown in the substrate hydroponics of the climatic chamber.

MATERIALS AND METHODS

The research of the biostimulant influence on the growth and development of plants of the 'Robin' lettuce variety was carried out in 2019–2020 in the department of closed artificial agroecosystems for crop production on the basis of the Federal State Budgetary Scientific Institution "Federal Scientific Agroengineering Center VIM", Moscow, Russia (VIM).

Succinic acid was used at the stage of seeds inoculation (factor A) and with constant drip irrigation of plants (factor B) throughout all the vegetative period - 40 whole days. Lettuce seeds were treated (soaked) with an aqueous solution of succinic acid with a concentration of 2.5 mM for 3 hours, and also the studied biostimulator was added to the nutrient hydroponics solution. Seeds soaked in water with the same exposure and without adding amber acid to the nutrient solution served as control. The experiment included 4 variants, each variant with 25 plants in five replicates.

This experiment included the following variants:

1 Control - without inoculation (seeds soaked in water);

2 Seeds without inoculation but with the introduction of succinic acid into a hydroponic solution;

3 Inoculation of seeds with Succinic acid;

4 Inoculation of seeds by the Succinic acid with and also the introduction of succinic acid into a hydroponic solution.

After treatment, the seeds were sown in a substrate soaked in a nutrient solution. As a substrate, we used mineral wool mats of the SPELAND VEGA brand (Russia) with a horizontal structure of fibers that ensure optimal uniform distribution of water and necessary nutrients along the entire length of the mat. This substrate is close to soil in terms of chemical composition, the main component of which is silica (silicon dioxide).

Biometric parameters (number of leaves and leaf area) were measured using a LI-COR-LI-3100C device (USA). The main indicators of photosynthesis of lettuce plants were calculated twice: on the 20^{th} and 40^{th} days of cultivation. Photosynthetic potential was determined as the product of the average area of lettuce leaves by the length of the growing season. Net productivity of photosynthesis is the ratio of the average daily growth of dry biomass of lettuce to the average leaf area for a specified period of time (20 and 40 days). The determination of the crude mass fraction substance was carried out on an LA 230S analytical balance (Germany). Determination of dry matter was made by drying a sample to constant weight in a Memmert UN-450 drying chamber (Germany) in accordance with GOST 31640-2012. The quantitative content of the main pigments (chlorophyll a, b and carotenoids) was determined on a spectrophotometer (Speks SSP-705M, Russia) in the lettuce leafs. The pigment concentration was calculated for 100% acetone using the Holm-Wettschnein equation (Lichtenthaler, 1987; Tretyakov, 1990). The content of chlorophylls, carotenoids were determined twice during the growing period, as well as indicators of the vegetative mass in the lettuce on the 20 and 40 day of the observation.

Plant material

The research material was the seeds of the leaf lettuce (*Lactuca sativa* L.) of the ‘Robin’ variety, included in the State Register of Breeding Achievements, approved for use on the territory of the Russian Federation. The 'Robin' variety is a late ripening, leafy lettuce, bred by the Czech company Agrofirma moravoseed (Registry, 2021).

Mineral nutrition

The plants were feed with Flora Series mineral fertilizers from General Hydroponics Europe in accordance with the regulations for growing lettuce plants in hydroponic systems from William Texier. The EC of the solution was maintained within 0.9–1.2 mS cm^{-1}, pH 5.8–6.0. The mineral base of the nutrient solution remained the same throughout the growing period of the plants (General Hydroponics Europe, 2018).

Experimental conditions

Lettuce plants were growing using a low-volume technology in a climatic chamber produced by VIM (Fig. 1).

Figure 1. Climatic chamber VIM.

A chamber are used specially for growing crops up to 1.5 m on an area of 3.8 m^2. The chamber is equipped with a control unit with a touch panel for the operator, through which information is collected from sensors with a configurable interval from 1 to 30 minutes with data saving to a removable flash drive.

Control is carried out using the operator's touch panel. The display shows the current parameters and operating modes, with the ability to enter data. The chamber is equipped with a forced air circulation system. Plant nutrition is carried out independently through two channels from two containers with a volume of 100 liters by means of a drip irrigation system. During the experiment, the following conditions were maintained in the climatic chamber (Table 1).

The climatic chamber is equipped with LED irradiators manufactured by VIM (Russia) with a customizable spectral composition and a function of dimming the radiation intensity, as well as the ability to adjust the lighting operation according to a given program and simulate the day-night function (Smirnov et al., 2018).

Table 1. Characteristics of the main parameters of the climatic chamber during the experiment

Parameter	Set values
Duration of daylight hours, h	14
Air temperature, °C	26
Relative humidity, %	70
Watering interval for plants, min	2
Adjustable pause between waterings, min	286

At the stage of germination of lettuce seeds, light with the presence of a far red range was chosen to create stress (700–780 nm - 600 mW m^{-2}, 122 mmol m^{-2} s) and then switch to red to provoke active growth and induce a reverse reaction of phytochrome. At the stage of vegetative mass formation, the illumination spectrum presented in Fig. 2 was used, with illumination at the plant level equal to 8,900 lux and total PAR - 232 mmol m^{-2} s: blue - 39 mmol m^2 s; green - 50 mmol m^2s; red - 137 mmol m^2s; far red - 5 mmol m^{-2} s (Proportions B: G: R ~ 13:22:65).

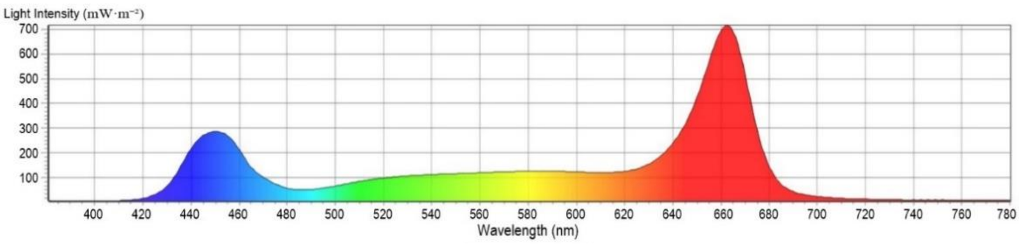

Figure 2. Spectral composition of optical radiation at the stage of vegetative mass formation.

Statistical analysis

Data analysis was evaluated by methods of variance and correlation analyzes using the STADIA 8.0 software. Least significant difference (LSD) was used to check the significance of the data obtained at a probability level of $p < 0.05$.

RESULTS

According to the research data, inoculation of the seeds stimulated the development of plants at the initial stages, reducing the period from sowing to germination and increasing the rate of plant growth. In the variants of the experiment with the seeds inoculation with an aqueous solution of amber acid, germination was observed 2 days earlier, on the 4th day, than in the other variants (on the 6th day) without the effect of the growth regulator on the seeds. Besides, the biostimulant promoted a more intensive formation of the plants vegetative mass and the formation of a rosette of 5–6 leaves in comparison with the control variant - 3–4 leaves. The additional supply of amber acid to the nutrient solution of hydroponics allowed plants to assimilate nutrients more actively throughout the growing season, which ultimately makes it possible to obtain high-quality and environmentally friendly products with greater productivity.

As it can be seen from Table 2 in all variants of the experiment, the biometric indicators of lettuce plants exceeded the control variant. The greatest indicator of the leaf surface area on the 20th day of cultivation was noted in variants 3 and 4 with inoculation of the seeds - 205.4 and 221.0 cm², exceeding the control variant of the experiment by 1.5 times.

Table 2. Biometric indicators of the assimilation apparatus of *Lactuca sativa* variety 'Robin'

Varient of experiment	Growing period			
	20 day of the observation		40 day of the observation	
	Number of leaves, pcs.	Leaf area, cm² plant^{-1}	Number of leaves, pcs.	Leaf area, cm² plant^{-1}
1	7.0 ± 0.4	137.0	16.2 ± 0.7	888.8
2	7.0 ± 0.5	178.6	17.8 ± 0.5	1399.7
3	8.4 ± 0.7	205.4	17.6 ± 0.5	1,266.4
4	7.2 ± 0.5	221.0	19.6 ± 0.9	1,511.9
	$LSD_{0.5}$ ob	36.20	-	1,71.48
	$LSD_{0.5}$ A	25.60	-	1,21.25
	$LSD_{0.5}$ B	25.60	-	1,21.25

During growing of the lettuce plants, the leaf surface area was increasing to 1,399.7–1,511.9 cm² in options 2 and 4 with the addition of succinic acid to the nutrient solution by the end of the vegetation period. Throughout the entire growing season, the number of leaves changed slightly as a result of the operation of the biostimulator in relation to the control variant. According to the analysis of variance calculation, it was noted that on the 20th day of cultivation, the share of the influence of factor A on the leaf area was 46.9%, the addition to the nutrient solution of hydroponics was 12.4%, on the 40th day of observation were 22.8 and 54.4%, respectively.

The efficiency of the photosynthetic activity of lettuce plants was estimated by the volume of work of leaves (photosynthetic potential) and the increase in dry matter per 1m² per day (net productivity of photosynthesis). As a result of the researches, it was found that the confirmed productivity directly depends on the value of photosynthetic potential and net productivity of photosynthesis which is confirmed by a strong degree of dependence with correlation coefficients of 0.98 and 0.77, respectively. The use of succinic acid under controlled growing conditions of lettuce varieties 'Robin' allowed to increase the performance of the leaf apparatus in relation to the control: photosynthetic potential from 17.13 (control) to 216.60 m² m^{-2} days (variant 4), net productivity of photosynthesis - from 1.66–2.30 g m^{-2} days (Fig. 3).

The optimal way to grow 'Robin' lettuce is the complex application of succinic acid at the stage of pre-sowing seed treatment in the climatic chamber with constant watering. A significant increase in the aboveground mass was noted both at the initial stages of growth (50.6%) and during further development of plants (52.6%) (Fig. 4).

There was a significant increasing in aboveground mass both in the initial stages of growth with an weight gain of 50.6%, and in the further development of plants with an weight gain of 52.6% (Fig. 4).

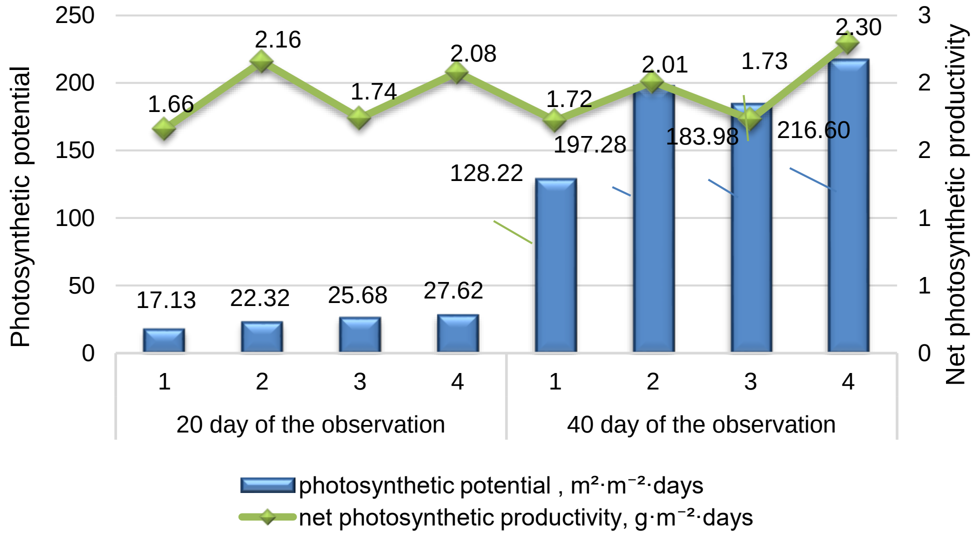

Figure 3. Indicators of photosynthetic activity of the leaf apparatus of *Lactuca sativa* variety 'Robin'.

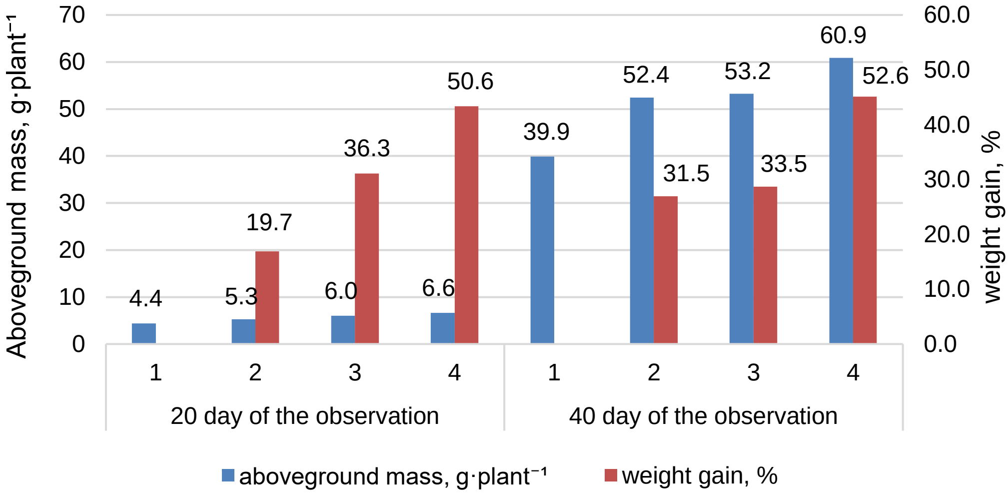

Figure 4. Growth rates of the aboveground mass of *Lactuca sativa* variety 'Robin'.

Seed treatment and adding succinic acid to the solution increases the accumulation of dry matter in lettuce plants. The dry mass from sprouting to the receipt of finished marketable products continuously increased. The most intense increase in dry matter mass was noted in variant 4–0.45 g plant^{-1} (on day 20 of observation) and 4.42 g plant^{-1} (on day 40 of observation) (Fig. 5). With this method of using succinic acid, significant differences in the accumulation of dry mass of plants were established. Analysis of variance showed that the use of succinic amber acid reliably affects the accumulation of dry plant biomass ($Ff > F_{0.5}$). The share of the effect of the factor of A seeds inoculation of amber acid was 17.5%, the addition of hydroponics to the nutrient liquor was 50.1%.

Lettuce 'Robin' variety, grown under controlled conditions of climatic chamber, corresponded to the quality of commercial products on 40 th day of the observation in accordance with GOST 33985-2016.

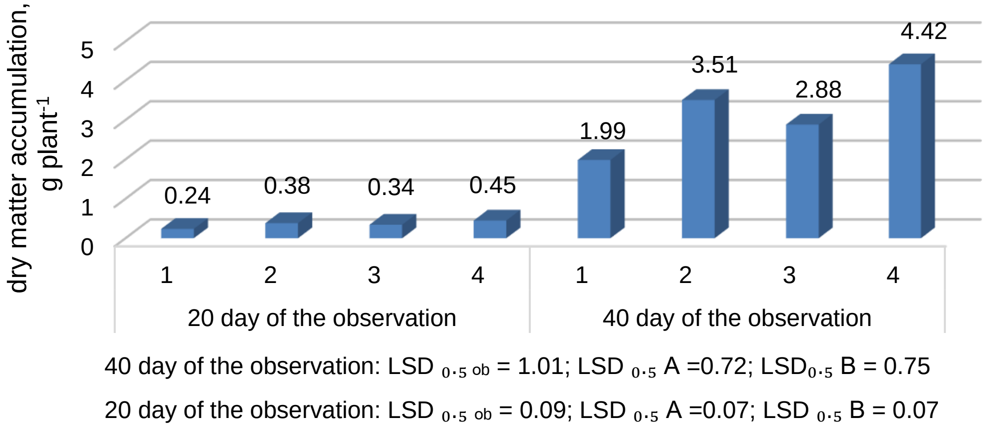

Figure 5. Dynamics of dry matter accumulation in *Lactuca sativa* variety 'Robin', g plant^{-1}.

When we are studying the quantitative content of pigments, it was found that the pre-sowing treatment of seeds and the addition of succinic acid to the nutrient solution had little effect on the accumulation of total chlorophyll and carotenoids in lettuce leaves (Table 3).

Table 3. The content of the main pigments in plants of *Lactuca sativa* variety 'Robin', mg g^{-1}

Varient of experiment	Growing period							
	20 day of the observation				40 day of the observation			
	ch. a[1]	ch. b[2]	ch.(a+b)[3]	car.[4]	ch. a	ch. b	ch. (a+b)	car.
1	2.1 ± 0.3	0.7 ± 0.2	2.5 ± 0.2	0.7 ± 0.1	1.7 ± 0.1	0.7 ± 0.1	2.5 ± 0.1	0.6 ± 0.1
2	2.6 ± 0.1	1.2 ± 0.2	3.8 ± 0.3	0.9 ± 0.1	2.4 ± 0.2	1.0 ± 0.1	3.3 ± 0.3	0.8 ± 0.1
3	2.2 ± 0.1	0.9 ± 0.1	3.3 ± 0.2	0.7 ± 0.1	2.0 ± 0.2	0.8 ± 0.1	2.8 ± 0.3	0.7 ± 0.1
4	2.2 ± 0.1	0.9 ± 0.1	3.1 ± 0.2	0.8 ± 0.1	2.1 ± 0.2	0.8 ± 0.1	2.8 ± 0.3	0.7 ± 0.1

[1]chlorophylls a, [2]chlorophylls b, [3]chlorophylls (a+b), [4]carotenoids.

The average, accumulation of the main pigments of chlorophylls a and b increased to 41.2% and 42.9%, respectively, and carotenoids increased to 33.3% under the influence of the biostimulant succinic acid in lettuce leaves by the 40th day of growing.

The use of succinic acid increases the accumulation of plant biomass, the total leaf surface area, as well as the accumulation of the main photosynthetic pigments in plants of 'Robin' lettuce throughout the growing season in the VIM climatic chamber.

DISCUSSION

According to the researches l^{ts}, it was found that the use of various options for treating lettuce plants with a solution of succinic acid made it possible to improve the morphometric characteristics and quality of marketable products in comparison with the control variant.

Recently, succinic acid has been produced from wastes of fruit and vegetable products (Jiang et al., 2017; Saxena et al., 2017; Kumar et al., 2020b). When inoculating seeds and during the growing season of crops, aqueous solutions of succinic acid are used at ultra-low concentrations (Vereshchagin & Kropotkin, 2010; Cao et al., 2018).

Previous researches have shown that growing plants using succinic acid is an environmentally friendly and effective way to increase the productivity of most crops (Marenych et al., 2019; Kovaliova et al., 2020; Pivovarov et al., 2020). At the stage of pre-sowing seed treatment, succinic acid activates the nutrients contained in the seeds, thereby contributing to an increase in productivity throughout the development of plants. It was found that the use of succinic acid with a solution concentration of 10^{-3} M increases the leaf surface area of wheat by 17–20%, the mass fraction of dry matter by 33%, the photosynthetic potential by 17–20% in comparison with the option without pre-sowing treatment (Tsyganova et al., 2019). Similar results on the stimulating effect of succinic acid on seeds of other crops were obtained by scientists Buntsevich et al. (2015) Oliva, et al. (2018) and Chursinov & Kovaleva (2019). The presented results indicate the effectiveness of the use of succinic acid at the stage of pre-sowing treatment of seeds of various crops. Our results also support research carried out by numerous scientists.

CONCLUSIONS

As a result of evaluating the efficiency of using succinic acid in order to increase the productivity of 'Robin' lettuce in artificial agroecosystems was identified technological method of using a biostimulant as a pre-sowing treatment of seeds and adding it to a nutrient solution (variant 4). The use of succinic acid allows increasing the crop yield from 4.9 (control) to 7.6 kg m^{-2} (variant 4) in the growing lettuce under controlled conditions of a climatic chamber. In this variant, a significant increase in plant biomass was noted by 52.6% (wet weight), 122.1% (dry weight) and the total leaf area by 70.1% compared to the control variant. The use of succinic acid is very perspectivly for growing 'Robin' lettuce plants in artificial agroecosystems.

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Apple scab detection using CNN and Transfer Learning

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Abstract. The goal of smart and precise horticulture is to increase yield and product quality by simultaneous reduction of pesticide application, thereby promoting the improvement of food security. The scope of this research is apple scab detection in the early stage of development using mobile phones and artificial intelligence based on convolutional neural network (CNN) applications. The research considers data acquisition and CNN training. Two datasets were collected - with images of scab infected fruits and leaves of an apple tree. However, data acquisition is a time-consuming process and scab appearance has a probability factor. Therefore, transfer learning is an appropriate training methodology. The goal of this research was to select the most suitable dataset for transfer learning for the apple scab detection domain and to evaluate the transfer learning impact comparing it with learning from scratch. The statistical analysis confirmed the positive effect of transfer learning on CNN performance with significance level 0.05.

Key words: agriculture, artificial intelligence, deep learning, fungus, machine learning, Malus, pathogen, precise horticulture, Venturia.

INTRODUCTION

Fruit growing has high profitability and potential for growth to provide the market with diverse local foods, and it occupies an important niche in the overall structure of agriculture. Apples are among the most widely grown and economically important fruit species worldwide and in Baltics (Kaufmane et al., 2017). In turn, scab disease caused by the ascomycetous fungi *Venturia inaequalis* (Cooke) G. Winter is economically the most important disease worldwide for apples (Tiirmaa et al., 2006; Belete & Boyraz, 2017). Currently, scab control heavily relies on fungicide applications. Due to environmental and food safety concerns, high adaptation ability of pathogens to applied fungicides as well as cost-effectiveness requirements, the need for changes in growing strategies have been highlighted during the last decade by apple scab research community and society. In cases where the use of pesticides can not be avoided, their applications should be more precise, more targeted and reduced substantially. One way to solve it is smart farming and precision horticulture that can greatly increase the effectiveness of pesticide applications and use them more selectively. In practice, smart farming and precision horticulture typically rely heavily on new technologies and digitalization, including early identification of diseases by image acquisition, recognition and severity analysis (Pujari et al., 2015).

With a goal to develop CNN model for embedded devices, which is possible to recognize apple scab in the early stage, our project group collected two datasets of images with a detected apple scab. Both datasets are accessible in *Kaggle* repository under *CC BY-NC-ND 4.0* license: *AppleScabFDs* (Web, 2021a) and *AppleScabLDs* (Web, 2021b). However, the collected datasets are imbalanced and are not sufficiently large. Meanwhile, CNN accuracy strongly depends on the size and quality of datasets (Soekhoe et al., 2016). One approach to overcome this problem is to use transfer learning. The learning techniques, which can learn from small datasets, are formally called few-shot learning (FSL). And there is a variety of FSL learning approaches that can be organized into four main categories: metalearning, metric learning, data augmentation, and transfer learning (Afifi et al., 2020). According to Weiss et al. (2016): 'The subject of transfer learning is a well-researched area as evidenced by more than 700 academic papers addressing the topic in the last 5 years'. Analyzing transfer learning impact using *ImageNet* datasets, Kornblith et al. (2019) found that, when networks are used as fixed feature extractors or fine-tuned, there is a strong correlation between *ImageNet* accuracy and transfer accuracy (r = 0.99 and 0.96, respectively). However, Ngiam et al. (2018) identify other important features for transfer learning - it is the usage of close categories. Cui et al. (2018) mention about the importance of domain similarity too and propose the methodology, which can be applied to measure a distance between datasets.

Generalizing, it is important to select an appropriate transfer learning dataset to train CNN for apple scab detection based on the collected *AppleScabFDs* and *AppleScabLDs* datasets. The methodology of Cui et al. (2018) can be applied for this task. However, according to the Cui et al. (2018), this methodology does not guarantee the optimal selection of the dataset for transfer learning, but they experimentally prove that this simple strategy works well in practice using different architectures of neural networks. Therefore, the accuracy of CNN model still must be tested. For example, *AlexNet* architecture can be applied as a benchmark to measure dataset similarity that will be additionally interesting, because it is a tradition to compare accuracy results using this architecture among deep learning scholars. Meanwhile, a feature extractor pretrained on *ImageNet* can be applied for measurement of domain similarity, as it is de-facto standard dataset for machine learning. However, it must be removed from source datasets for experiment clearance to avoid some possible feature extractor preferences.

The **goal** of our research is to select the most suitable dataset for transfer learning for the apple scab detection domain and to evaluate the transfer learning impact comparing it with learning from scratch.

The **objectives** of research:

- to collect the natural images of apple scab;
- to measure domain distance among datasets;
- to evaluate transfer learning impact on CNN accuracy.

The analysis of training results showed that transfer learning positively impacts CNN performance. The difference among the neural network models was statistically confirmed by Mann-Whitney-Wilcoxon test with significance level 0.05. *iFood251X* provided the best accuracy improvement compared with another datasets applied in the experiment: *CIFAR-10*, *CIFAR-100* and *PlantVillage*.

LITERATURE REVIEW

Lately, plant pathogen detection is a topical theme of research direction related to convolutional neural network (CNN) application in smart farming and horticulture. There can be mentioned that many modern researches, which are completed under smart farming trend, traditionally they are directed to develop a solution to identify specific pathogens of some plant or based on open datasets like *PlantVillage*. For example, Adhikari et al. (2018), Rangarajan et al. (2018) and Salih et al. (2020) presented solutions for tomato disease detection, which were based on CNN application. Meanwhile, Afifi et al. (2021) and Esgario et al. (2020) used deep learning for coffee pathogen detection, but Muhammad et al. (2021) - for disease of *Aloe vera*.

Describing the detection of apple pathogens, Liu et al. (2018) applied *AlexNet* for detection of following apple pathogens: mosaic virus, brown spot, rust and *Alternaria* leaf spot. Baranwal et al. (2019) applied augmentation and *LeNet-5* architecture design for apple subgroup classification extracted from *PlantVillage* dataset.

Considering the object of research - transfer learning, the related experiments, when transfer learning was applied for apple scab detection, were already completed. For example, Yan et al. (2020) and Khan et al. (2020) experimented with *VGG and AlexNet* architectures pretrained on *ImageNet*. May be Afifi et al. (2021) experiments are not directly related to apple scab monitoring, but they provide comprehensive study of FSL techniques based on *PlantVillage* dataset.

Touching on the topic of embedded devices. Petrellis, N. (2017) provided review of existing solutions, as well as, proposed own mobile application based on spectral analysis. However, Picon et al. (2019) proposed early identification application of three relevant European endemic wheat diseases.

Other active research direction is IoT application in the food industry. For example, Nasir et al. (2020) and Xenakis et al. (2020) proposed CNN solutions for IoT to detect plant diseases.

The current limitations of deep learning related to plant disease diagnostic are discussed in review of Arsenovic et al. (2019), where authors mention the limitation that currently available datasets do not contain images gathered and labeled from real-life situations. A more wide review of challenges is provided by Hasan et al. (2020), who underline the importance of new datasets with natural images and CNN solutions with smaller computation time for embedded solutions. That depicts the importance and **originality** of the current research, which considered dataset collection with natural images to develop an embedded CNN solution for mobile applications.

MATERIALS AND METHODS

The collection of digital images were carried out in different locations of Latvia (Fig. 1). Digital images with characteristic scab symptoms on leaves and fruits were collected from the Institute of Horticulture (LatHort) apple collection (Krimūnu parish, Dobeles district, two locations: 56.612338, 23.305949; 56.610936, 23.298447), commercial orchard (Skaistkalnes parish, Vecumnieku district 56.3652416, 24.6025912), and home gardens (Valgundes parish: 56.695785, 23.718894; Ozolnieku parish: 56.684399, 23.834508; Sējas parish: 57.285628, 24.479879). Data collection was done during the apple growing season, from the beginning of June 2020, when the first signs of apple scab infection began to appear on the leaves, until the end of September 2020, when both leaves and fruits showed other signs of damage preventing distinguishing from apple scab and were at the end of the growing season.

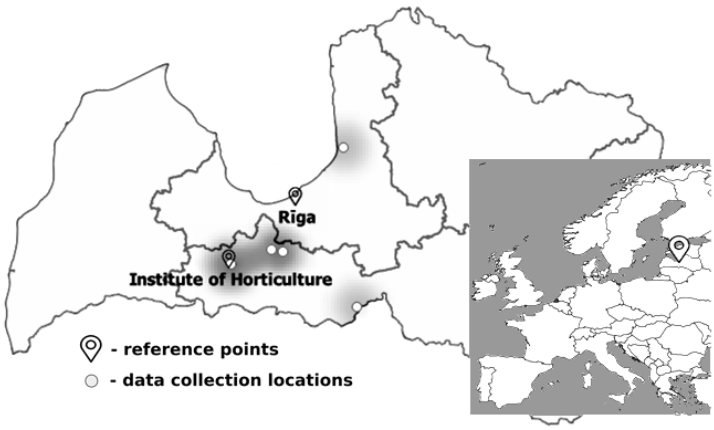

Figure 1. Locations of image acquisition for datasets *AppleScabDLs* and *AppleScabDFs*.

The collection of digital images was carried out using two types of devices with different camera resolutions - smartphone cameras (12 MP, 13 MP, 48 MP) and a digital compact camera (10 MP).

Apple leaves and fruits were photographed at different development stages and with different signs of damage.

The collection of images was carried out in field conditions, in orchards. Apple leaves and fruits were photographed as separate objects. The images were taken at three different stages of the day - in the morning (9:00–10:00), around noon (12:00–14:00), as well as in the evening (16:00–17:00) to provide a variety of natural light conditions. The images were also taken on both sunny days and overcast days to provide different types of light (soft light and hard light).

The leaves and fruits were framed so that they occupied the image area as much as possible and were in the center of the image, and the focal point was on the object. The object may have had other leaves or fruits in the background. The same object was photographed from multiple viewpoints.

For each subject, leaf or fruits, the signs of apple scab expression caused by *V. inaequalis* had been documented by imaging. Images of apple leaves and fruits without visual damage and with visual noise like drops of water, insects, shadows, mechanical or biological damage were obtained in parallel with sick leaves and fruits.

The resulting images were manually reviewed and grouped into two datasets called *AppleScabFDs* (images with apple fruits) and *AppleScabLDs* (images with apple leaves). Where in turn the images of leaves and fruits were grouped into two data subsets - images with scab symptoms and images without scab symptoms. The examples of dataset photos are provided in Fig. 2.

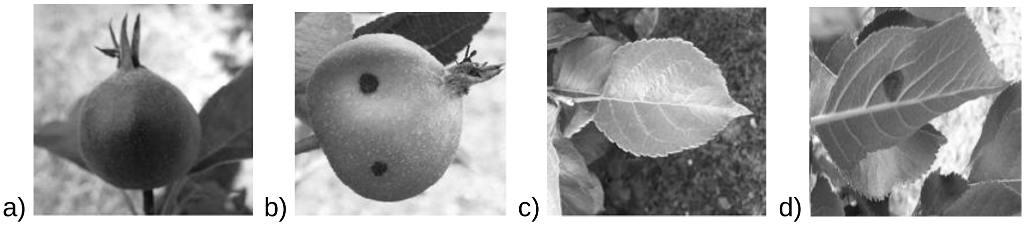

Figure 2. Photo examples from collected datasets: a) healthy apple fruit; b) apple fruit infected by scab; c) healthy apple leaf; d) leaf infected by apple scab.

The experiment consists of five stages (the activity diagram of the experiment is provided in Fig. 3):

1) Measurement of distance between image datasets is completed considering the methodology described in the scientific article of Cui et al. (2018).

2) *AlexNet* model is trained using transfer learning methodology, where the fine-tuning approach is applied for retraining CNN with a new dataset.

3) Investigation of CNN accuracy is completed. Training accuracy, validation accuracy and Cohen Kappa are measured.

4) Statistical analysis were performed to identify transfer learning impact neural network accuracy.

5) The relationship between EMD and obtained accuracy is analyzed using line diagrams.

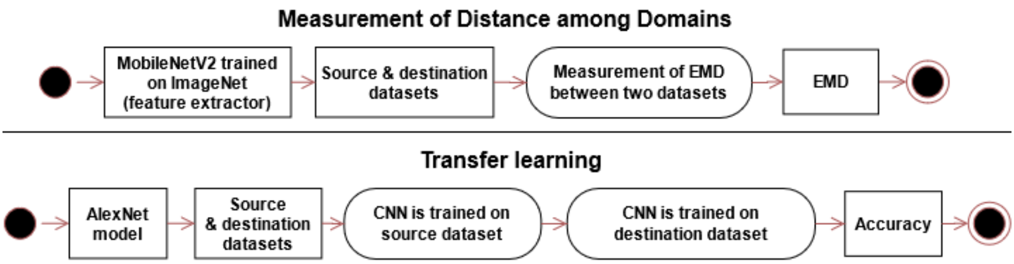

Figure 3. Activity diagram of experiment.

Cui et al. (2018) methodology is based on Earth Mover's Distance and feature extractor application, for example, CNN trained on a large dataset like *ImageNet*.

If S is the source dataset, but T – the target dataset, then each category can be represented as $S = \{(s_i, w_i)\}_{i=1}^{m}$ and $T = \{(t_j, w_j)\}_{j=1}^{n}$, where s_i is the i-th category of dataset S, but weight w_i are normalized numbers of images in datasets (1):

$$\sum w_i = \sum w_j = 1. \quad (1)$$

Using feature extractor, each image can be transformed into feature vectors $g(s_i)$ and $g(t_j)$ respectively. To calculate the distance between two categories, the mean vectors of each category is applied (2):

$$d_{i,j} = \|\bar{g}(s_i) - \bar{g}(t_j)\|. \quad (2)$$

Cui et al. (2018) propose Euclidean metric to calculate distance $d_{i,j}$ as the cost of flow from category i to j. Then linear programming algorithms are applied to search optimal flow $f_{i,j}$. In results, the Earth Mover's Distance is calculated using Eq. 3:

$$d(S,T) = EMD(S,T) = \frac{\sum_{i=1,j=1}^{m,n} f_{i,j} \cdot d_{i,j}}{\sum_{i=1,j=1}^{m,n} f_{i,j}}. \quad (3)$$

Considering domain similarity, Cui et al. (2018) propose next equition (4):

$$sim(S,T) = e^{-\gamma \cdot d(S,T)}, \quad (4)$$

where the coefficient $\gamma = 0.001$.

Python 3.6, *Keras* and *Jupyter Notebook* were applied to write scripts for experiments to measure Earth Mover's distances between datasets and to train convolutional neural networks. The experiment was completed using computers equipped with *NVIDIA GTX 1050* and *RTX 2070*.

Three destination datasets are investigated in the experiment: two our datasets (*AppleScabFDs* and *AppleScabLDs*) and *Fruits360* dataset with 3 categories ('apples', 'pears' and 'others'), which will be abbreviated as *Fruits360-3*. *Fruits360-3* dataset is selected due to our previous experiment (Kodors et al., 2020). This data provides the possibility to compare the previous results of learning from scratch with the results of transfer learning. Next, five source datasets were selected for transfer learning: *Fruits-360* (Mureşan & Oltean, 2018), *CIFAR-10* (Krizhevsky, 2009), *CIFAR-100* (Krizhevsky, 2009), *iFood251X* (Kaur et al., 2019) and *PlantVillage* (Hughes & Salathé, 2015). Some examples of images are provided in Fig. 4 to make a more intuitive understanding of the dataset content.

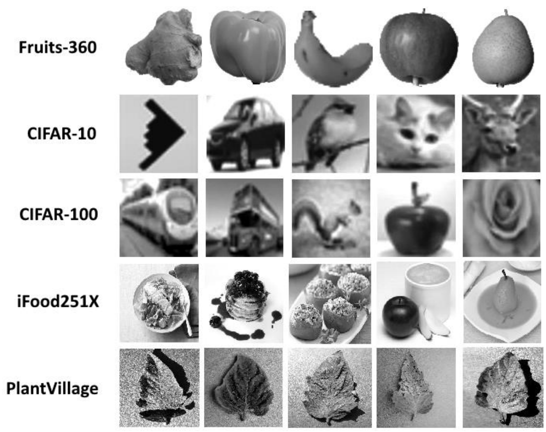

Figure 4. Image examples of datasets.

The domain similarity was calculated by the Earth Mover's distance (EMD) and the method described in the article of Cui et al. (2018) measuring EMD using *MobileNetV2* CNN trained on *ImageNet* dataset as a feature extractor. Therefore, *ImageNet* is not included in the comparison, because it is applied by the feature extractor - as benchmark.

AlexNet architecture is a benchmark to compare deep learning results. Additionally, *AlexNet* architecture was selected due to its weak classification results in our previous experiment (Kodors et al., 2020). Therefore, the transfer learning impact can be more easily detected. The *AlexNet* model of previous research (Kodors et al., 2020) is applied in the experiment to obtain comparable data (Fig. 5).

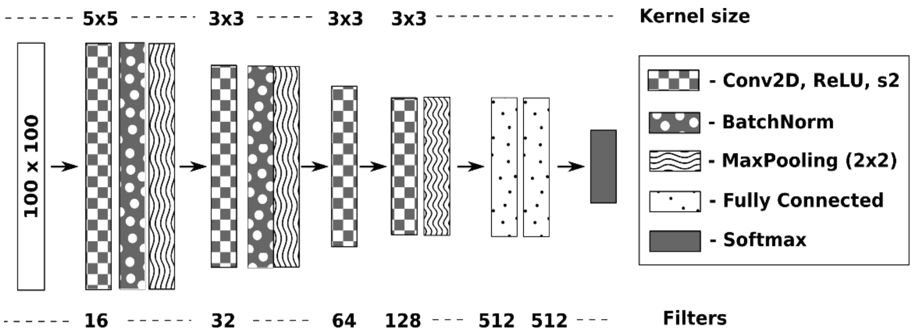

Figure 5. Structure of *AlexNet* model applied in the experiment.

The Mann-Whitney-Wilcoxon test with significance level 0.05 is applied to identify differences among the models trained on *Fruits360-3*, obtained during this experiment and in the previous our experiment (Kodors et al., 2020). The relationship between EMD and the obtained neural network accuracy, as well as, the most appropriate dataset for pretraining are analyzed using line diagrams constructed by the approach presented in the article of Cui et al. (2018).

RESULTS AND DISCUSSION

Two datasets with images of apple scab symptoms were collected (Fig. 2). One dataset contains examples of scab symptoms on leaves, another - on apple fruits. From the perspective of artificial intelligence engineering, it must be mentioned that both datasets are imbalanced (see Fig. 6), because it strongly impacts CNN training results.

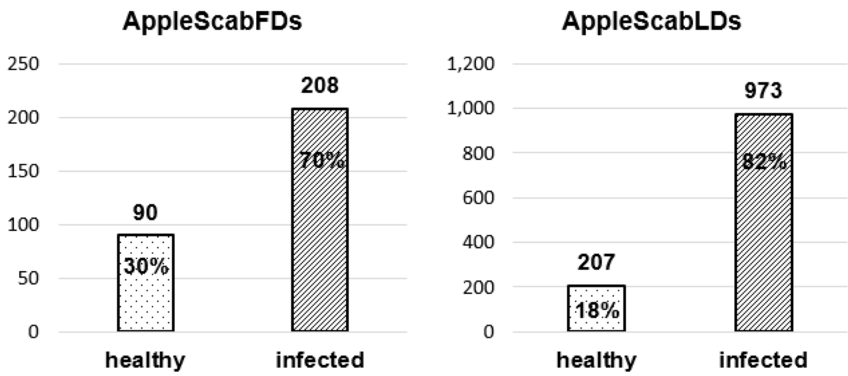

Figure 6. Proportion of the number of cases in different classes.

All calculated EMDs across the domains are presented in Table 1. *iFood251X* dataset showed the closest EMDs to all destination datasets of experiment. Meanwhile, *CIFAR10* is the furthest from the collected scab datasets, *AppleScabFDs* and *AppleScabLDs*. It must be noted that *CIFAR10* and *CIFAR100* have simple content images including apples and pears, but *PlantVillage* has images of healthy and infected leaves, however, they are not natural images and photographed in laboratory conditions.

At the same time, *iFood251X* contains natural images with complex content. Therefore, the closeness of *iFood251X* can be explained by the wealth of feature vocabulary. It means that datasets with inatural images are not effective for CNN pretraining, which will analyze natural images.

Table 1. Earth mover's distance between datasets

Datasets		Destination		
		Fruits360-3	*AppleScabFDs*	*AppleScabLDs*
Source	*CIFAR10*	0.79308	0.80581	0.79781
	CIFAR100	0.80161	0.81133	0.80229
	iFood251X [1]	0.80865	0.85017	0.83711
	PlantVillage	0.78553	0.82449	0.83655

[1] Source dataset, which is the closest to all destination datasets.

The Mann-Whitney-Wilcoxon test was completed to identify statistically reliable differences between training from scratch and transfer learning. *Fruits360-3* dataset was selected for comparison. The calculated probability values (*p*-values) are provided in Table 2.

Table 2. Results of Mann-Whitney-Wilcoxon test (*p*-values)

From Scratch	*Internal TL*	*CIFAR10*	*CIFAR100*	*iFood251X*	*PlantVillage*
1.00000	0.00912	0.00221	0.00221	0.00221	0.00459

However, average accuracies are depicted in the box diagram (see Fig. 7). Thus, it is important to mention that internal transfer learning provided performance improvement, when CNN was initially trained on the original *Fruits360* dataset with 120 classes and then retrained on the same dataset with 3 classes (*Fruits360-3*). These results agree with Ngiam, et al. (2018) conclusions that features learned on coarse-grained classes do not provide significant benefits transferred to fine-grained datasets, but transfer learning using close categories is preferable than usage of entire dataset (Barman et al., 2019). At the same time, *CIFAR* and *iFood251X* provided significant improvements. However, *CIFAR-100* dataset was better than *iFood251X* despite the higher EMD. According to Ngiam et al. (2018), transfer learning using close categories is preferable than usage of the entire dataset that can explain better results of CIFAR-100, because it contains apple and pear images. Meanwhile, *PlantVillage* provided the smallest EMD and decreased CNN accuracy. Authors of EMD method mention that this greedy way of selection has no guarantee on the optimality of the selected subset in terms of domain similarity, but this simple

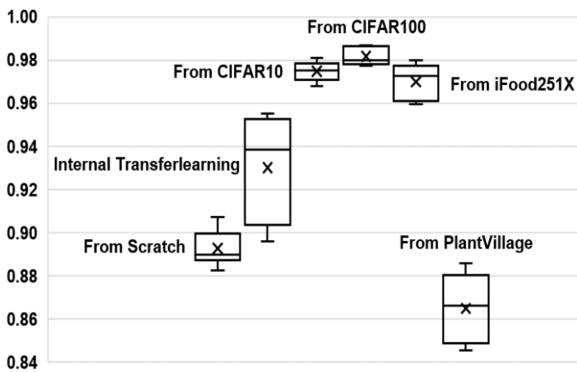

Figure 7. *Fruits360-3* trained using different approaches.

strategy works well in practice (Cui et al., 2018). Therefore, maybe it does not guarantee the optimality of the selected subset, but it can be an effective method to choose datasets for experiments with transfer learning.

The constructed line diagrams (Figs 8–10), which depict the relationship between EMD and neural network accuracy, showed that performance of the models trained on balanced dataset *Fruits360-3* was improved using transfer learning. These results coincide with the investigations of Cui et al. (2018). However, imbalanced datasets, *AppleScabFDs* and *AppleScabLDs*, must be analysed independently, because they provide other shapes of performance lines.

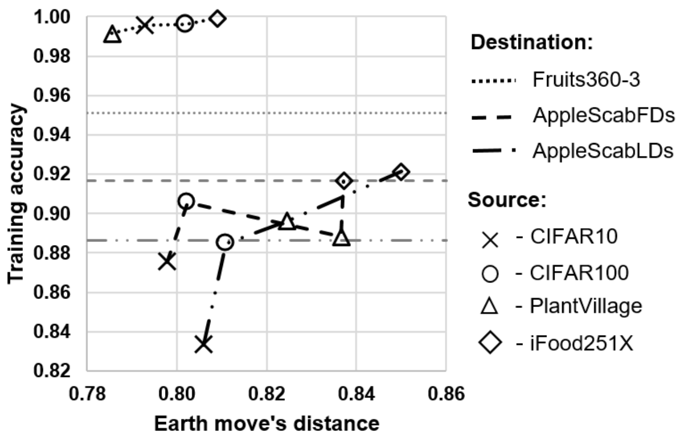

Figure 8. Relationship among EMD, transfer learning and training accuracy: horizontal lines - from scratch.

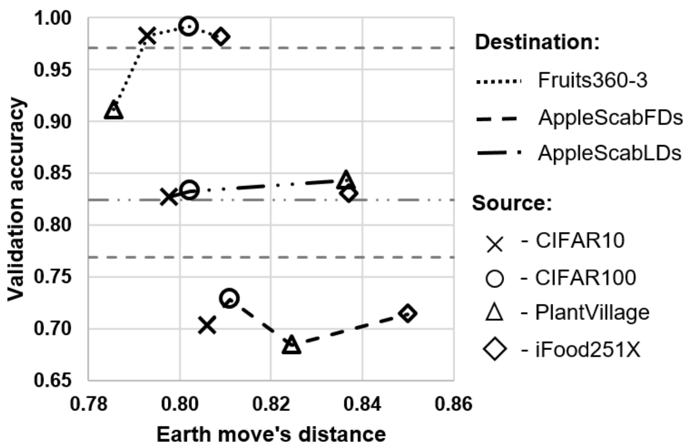

Figure 9. Relationship among EMD, transfer learning and validation accuracy: horizontal lines - from scratch.

AppleScabFDs and *AppleScabLDs* datasets obtained different performance results among themselves. Based on Cohen Kappa analysis and comparing it with training from scratch (see Fig. 10), transfer learning provided a stronger impact on the smallest dataset - *AppleScabFDs* (see Fig. 5), it can be explained by Weiss et al., 2016, and Barman et al., 2019, investigations, that transfer learning provides a strong positive impact on small dataset. It must be mentioned, that the performance lines are like the trends of lines provided in the article of Cui et al. (2018). Meanwhile, the systematic study of Buda et al. (2018) showed that the effect of class imbalance on classification performance is detrimental and recommend the application of oversampling to solve a problem with imbalanced datasets. However, Wang et al. (2014) showed that oversampling can call overfitting. *AppleScabLDs* is strongly imbalanced and large, therefore it provides such a dramatic effect on transfer learning comparing with training from scratch.

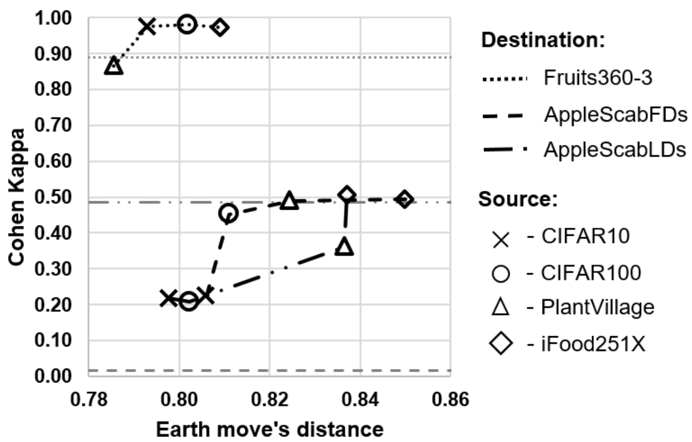

Figure 10. Relationship among EMD, transfer learning and Cohen Kappa: horizontal lines - from scratch.

CIFAR100 sufficiently well improved the classification accuracy for *Fruits360-3* dataset comparing with the closest dataset - *iFood251X* (see Table 1 and Figs 7–10), because they contained similar images. For the same reason, *PlantVillage* improved recognition accuracy for *AppleScabLDs* (see Figs 9–10). These results provide the similar conclusion to Huh et al. (2016) - subclasses which share a common visual structure allow the CNN to learn features that are more generalizable.

CONCLUSIONS

The resulting images were manually reviewed and grouped into two datasets called *AppleScabFDs* (images with apple fruits) and *AppleScabLDs* (images with apple leaves). Where in turn the images of leaves and fruits were grouped into two data subsets - images with scab symptoms and images without scab symptoms. Collected datasets are accessible in *Kaggle* repository (Web, 2021a, 2001b). It should be mentioned that both datasets are imbalanced (Fig. 6), that prevents CNN to get high accuracy.

The experiment showed that transfer learning positively affects CNN accuracy. However, it is important to measure Earth Mover's distance selecting dataset for pretraining, since an inappropriate dataset can otherwise provide a negative effect. Additionally, it is important to select datasets with similar categories considering data collection and usage principles. Therefore, if there are plans for the use of neural networks in natural conditions, source datasets must be collected in natural conditions too. As well as, the size of the dataset plays an essential role: transfer learning strongly improves the results of small imbalanced dataset, but a large imbalanced dataset is slowly compensated by transfer learning and may be the training from scratch is more effective for it.

Obtained results showed that the most important task for further data collection is to improve the datasets, *AppleScabFDs* and *AppleScabLDs*, by collecting more photos for minimal classes. The dataset should be more balanced and the image acquisition process should be monitored in a way to collect an equal number of images of all classes, since the plant disease experts are more concentrated during data collection on anomalies and manifestations of diseases than intact parts of plants. Comparing the results with related researches, it can be concluded: if the problem with imbalanced data is solved, the application accuracy will be obtained.

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Influence of humic acids, irrigation and fertilization on potato yielding in organic production

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Abstract. The study aimed at determining the impact of organic fertilization, humic acids and irrigation on potato yielding in organic production system. Fertilization variants included: Humac Agro; manure; vermicompost; Fertil Bioilsa C-N 40-12.5; manure + Humac Agro; vermicompost + Humac Agro; Fertil Bioilsa C-N 40-12.5 + Humac Agro. Irrigation was carried out using drip lines. The highest tuber yield was determined on treatments fertilized with vermicompost. The combined application of organic fertilizers and humic acids resulted in increased total yield between 6 and 9%, whereas commercial yield from 5 to 10%. Application of fertilization resulted in increased total yield of tubers in individual fertilizer variants from 1.9 to 10.8 t ha^{-1}, and commercial yield from 1.6 to 12.3 t ha^{-1}. Water-use efficiency remained in the range from 35.2 to 113.1 kg mm^{-1}, whereas irrigation water-use efficiency from 9.9 to 166.3 kg mm^{-1}. Humic acids used in the study enhanced fertilizer and water-use efficiency.

Key words: *Solanum tuberosum*, water–use efficiency, tuber yield and its structure.

INTRODUCTION

Intensive agricultural production systems ensuring high efficiency and quality of yields are among the most destructive practices in the field of the Earth's resources, but they are justified by economic requirements and the need to provide food for the growing population. Excessive use of artificial fertilizers and pesticides leads to deteriorating condition of soil, water resources and environmental quality. Modern trends in agriculture reflect the growing interest in the shift from conventional agriculture to organic farming, particularly in production of vegetables consumed by humans.

Potato is one of the most popular and nutritious vegetable plants in the world. It is cultivated in 164 countries, and the tubers are consumed by close to one billion people on an almost daily basis. Development of a thriving, profitable and sustainable sector of organic potatoes depends largely on the soil welfare and biological control of diseases and pests. Soil welfare can be obtained by using a multi–annual crop rotation and appropriate agrotechnical practices, including correct nutrient management.

Soil property enhancers, containing i.a. humic acids (HA) have a positive impact on the state of biological balance and soil welfare. Humic substances area among the most complex and biologically active organic compounds found in soil. They exhibit positive effect on physicochemical properties of soil, but also on the structure and activity of microorganisms, which ensures better supply of nutrients to plants (Canellas & Olivares, 2014). Research has show positive impact of HA on the growth and development of numerous plant species, i.a. potato, sugar beet, tomato, corn and berry plants (Canellas et al., 2013; Suh et al., 2014; Olivares et al., 2015; Schoebitz et al., 2016; Wilczewski et al., 2018; Marenych et al., 2019). According to Nardi et al. (2002), humic substances have a more pronounced impact on root growth than on the above-ground parts of plants, which points to their special significance in the cultivation of root vegetables. In the study of Sanli et al. (2013), the use of leonardite at the amount between 200–600 kg ha^{-1} resulted in increased total yield of potato tubers in the range from 6 to 25%.

All production practices that enhance nutrient use are of interest for farmers, particularly in the context of nitrogen directive, which accepts the use of up to 170 kg N ha^{-1} $year^{-1}$ in natural fertilizers. One such activity is vermicomposting, which according to Jjagwe et al. (2019) constitutes an organically sustainable technology of manure management. Many studies show that vermicompost is more productive than manure and mineral fertilizers used individually or proves the synergy and complementarity of vermicompost with mineral fertilizers (Singh & Chauhan, 2009; Meenakumari & Shehkar, 2012).

The factor that significantly restricts fertilization efficiency is the increasingly common rainfall shortage and its unequal distribution in the potato vegetation period. Total yield is low in drought conditions, and the bulk of the mass comprises of small and medium sized tubers. What is more, the share of deformed, green, infected with common scab, as well as nitrate accumulation in tubers (Simson et al., 2016). Reduced tuber yield or deterioration of their quality forces the need for irrigation. The irrigation technique is highly important. Drip irrigation is believed to be most precise, as it ensures water saving, reduces ridge loosening, increases the total length of the roots and it does not affect the moisture level of air in the crop field (Svoboda et al., 2020).

The aim of the research was to determine the influence of variable organic fertilization, application of humic acids and supplementary drip irrigation on the size and structure of potato tuber yield in organic production.

MATERIAL AND METHODS

Field experimental design

The research was carrierd in the period 2017–2019 on a farm located in Raszków (50°58' N, 19°95' E, 283 m a.s.l.) Poland. The field experiment was established in the split–plot system in 3 replications. The experimental factors were: organic fertilization and irrigation. The fertilization variants included: control (without fertilization) - C; Humac Agro (400 kg ha^{-1}) - HA; manure (30 t ha^{-1}) - FYM; vermicompost (10 t ha^{-1}) - VC; Fertil Bioilsa C-N 40-12.5 (600 kg ha^{-1}) - FCN; manure + Humac Agro (30 t ha^{-1} + 400 kg ha^{-1}) - FYM + HA; vermicompost + Humac Agro (10 t ha^{-1} + 400 kg ha^{-1}) - VC + HA; Fertil Bioilsa C-N 40-12.5 + Humac Agro (600 kg ha^{-1} + 400 kg ha^{-1}) - FCN + HA. Humac Agro contains 79% of organic substance, 62% humic acids, 16.8 g Fe kg^{-1}, 15.7 g Na kg^{-1}, 15.1 g Ca kg^{-1}, 1.2 g K kg^{-1}, 77 mg B kg^{-1}, 64 mg Zn kg^{-1}, 19 mg Cu kg^{-1}. The characteristics of the remaining fertilizers is presented in Table 1.

Table 1. Physio–chemical parameters of organic fertilizers

Parameter	Farmyard manure	Vermicompost	Fertil Bioilsa C-N 40-12.5
pH	7.4 ± 0.1	7.4 ± 0.2	4.8
dry matter (g kg^{-1})	270.1 ± 12.6	302.0 ± 15.5	954.0
Total N (g kg^{-1})	19.6 ± 1.4	30.2 ± 1.7	115,2
Total C (g kg^{-1})	413.2 ± 9.3	293.1 ± 8.9	427.4
C/N	21.1 ± 1.0	9.7 ± 0.3	3.7
Total P (g kg^{-1})	5.9 ± 0.4	11.8 ± 0.7	10.5
Total K (g kg^{-1})	22.8 ± 1.5	36.3 ± 1.8	2.1
Total Mg (g kg^{-1})	3.7 ± 0.3	9.3 ± 0.6	1,3
Total Ca (g kg^{-1})	12.4 ± 0.8	16.2 ± 1.1	62.3

Irrigation was carried out with the use of subsurface (5 cm) system of T-Tape drip lines. Drip lines with water emitters distributed every 20 cm, spread on the top of the ridges were set at the consumption of 5 dm^3 h^{-1} running $meter^{-1}$. A subsurface drip system was used in the research as it ensures that the water used becomes available to a large part of the plant's root system while keeping the soil surface relatively dry, thus reducing evaporative water loss compared to other irrigation methods. In addition, subsurface drip application offers many other advantages for crop production, including less nutrient leaching compared to surface irrigation, higher yields, a dry soil surface for improved weed control and crop health, which is especially important in organic potato production (Selim et al., 2009; Badr et al., 2010). Irrigation dates were determined based on soil humidity with the use of tensiometers. Irrigation procedures were commenced when the water potential in soil reached - 40 kPa (Bailey, 2000). The total amount of water used for irrigation was 98 mm in 2017, 67 mm in 2018 and 145 mm in 2019, respectively. The tubers of early potato cultivar Vineta were planted at a spacing of 67.5×29 cm in the first decade of April, whereas the harvest was carried out in the first decade of August. The size of the field intended for harvest was 27 m^2. In order to protect potatoes against infection Copper Max New 50 WP (2 kg ha^{-1}) was used twice, whereas SpinTor 240 SC (0.15 L ha^{-1}) was used against potato beetle.

Soil and meteorological conditions

The field experiment was located on a typical brown soil (*BEt*) (*Haplic Cambisol Eutric*) with granulometric composition of sandy loam. The arable layer of the soil (0–25 cm) was characterized by: moderate P abundance (45.1–50.5 mg kg^{-1}) and K abundance (98.5–103.8 mg kg^{-1}); high Mg abundance (56.0–67.0 mg kg^{-1}); acidic to slightly acidic pH value (pH_{KCl} 5.4–6.0); sand content 570–610 g kg^{-1}; silt 240–340 g kg^{-1}; clay 90–150 g kg^{-1}.

Fig. 1 presents characteristic of precipitation and thermal conditions. The total rainfall for 2017 and 2019 between April and August was close to long-term average, but in 2018 it was lower by 68 mm (19%). An unequal distribution of precipitation in all years of the experiment was determined. Rainfall deficiencies occurred in June 2017, April and May 2018 and in June and July of 2019. On the other hand, excessive rainfall was recorded in April 2017, July 2018 and in May and August 2019 r. Low temperatures in April and May 2017 and 2019 combined with high levels of precipitation delayed potato emergence. Particularly unfavorable humidity and thermal conditions prevailed in June 2019 due to the very low amount of precipitation and air temperature higher than average by 4.5 °C.

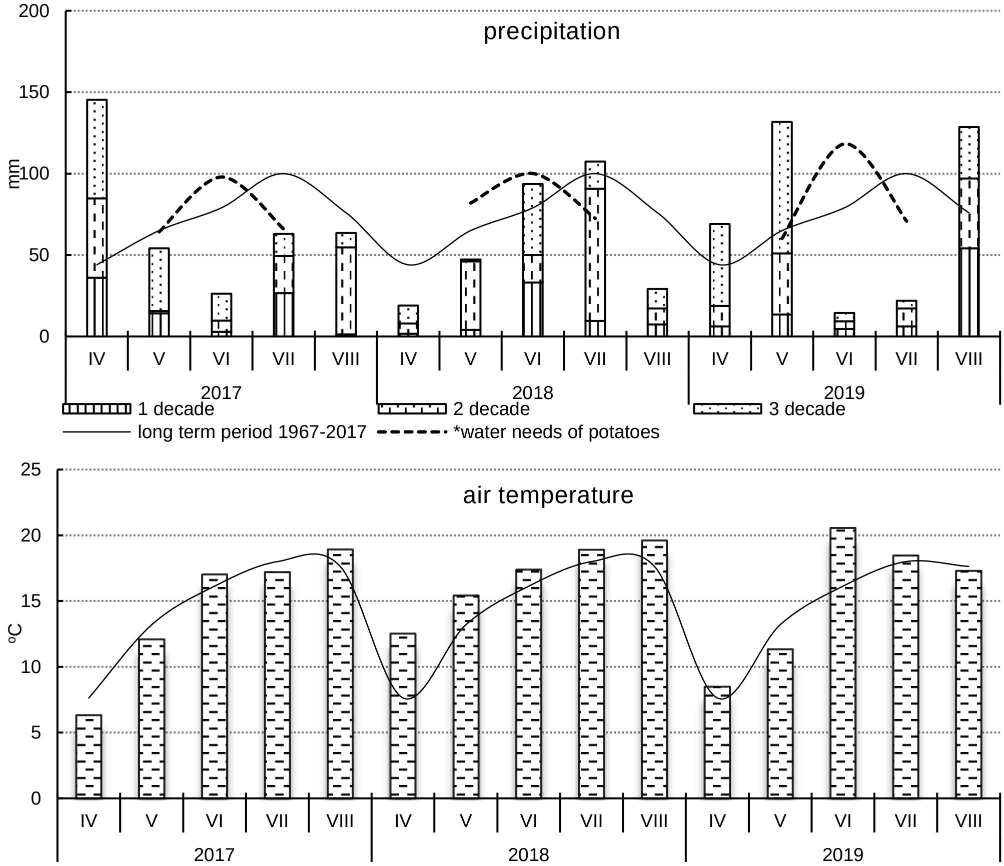

Figure 1. Characteristics of weather conditions, *water needs according to Klatt, citation for Nyc (citation for Nyc, 2006).

Assessment of the size and structure of tuber yield and irrigation efficiency

Before the harvest, tuber samples were collected from 10 plants per each field in order to assess the number of tubers per plant and the share of marketable and large tubers (with respective transverse diameter of > 35 and > 50 mm) in the yield. During the harvest, total tuber yield size was determined, whereas the commercial yield was estimated based on the share of marketable fraction, by separating green and deformed tubers.

Water use efficiency (*WUE*) and irrigation water use efficiency (*IWUE*) were calculated according to the equations of Kirda (2002) and Howell et al. (1990). Water use efficiency (*WUE*) for each treatment was calculated as tuber yield divided by seasonal evapotranspiration (*ETa*):

$$ETa = P + I - D \pm \Delta W \quad (1)$$

where *P* is the raifall (mm); *I* is the irrigation applied to individual plots (mm); *D* is the deep percolation; ΔW is the change in the water storage of hte soil profile (mm). Since the amount of irrigation water was only sufficient to bring the water deficit to field capacity, deep percolation was ignored.

Irrigation water use efficiency (*IWUE*) was determined as:

$$IWUE = \frac{(Y_I - Y_{NI})}{I} \quad (2)$$

where Y_I is the tuber yield of irrigation treatments (kg ha^{-1}); Y_{NI} is the tuber yield of non-irrigation treatment (kg ha^{-1}); I is the amount of irrigation water (mm).

Statistical analysis

The results were subjected to statistical evaluation using an analysis of variance. Highly significant differences (*HSD*) for the investigated features were verified using Tukey's test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Total yield of non-irrigated potato tubers was on average 24.2 t ha^{-1}, whereas commercial yield 19.0 t ha^{-1} (Table 2). Application of the supplementary drip irrigation resulted in an increase of total tuber yield in individual fertilizer variants in the range from 1.9 t ha^{-1} (13%) to 10.8 t ha^{-1} (44%), and commercial from 1.6 t ha^{-1} (18%) to 12.3 t ha^{-1} (63%). The greatest increase in yield was observed on the treatments fertilized with vermicompost, and the lowest on the control. The yield forming effect of vermicompost results from its properties. As shown by Manivannan et al. (2009) and Demir (2019), application of this fertilizer considerably improves soil properties by expanding the pore spaces, water retention and cation exchange capacity, availability of micro- and macronutrients and microbial activity.

Among the investigated organic fertilizers, Fertil Bioilsa C-N 40-12.5 was characterized by the highest productivity, and the lowest was exhibited by manure. The total yield of potato fertilized with Fertil Bioilsa C-N 40-12.5 in the amount of 600 kg ha^{-1} (66 kg N ha^{-1}) was 34.3 t ha^{-1} and was higher by 4.4 t ha^{-1} than on the treatments fertilized with vermicompost in the amount of 10 t ha^{-1} (91 kg N ha^{-1}) and by 6.9 t ha^{-1} more than after the application of manure at the dose of 30 t ha^{-1} (159 kg N ha^{-1}). These results show a better efficiency of nitrogen used in processed organic fertilizers than in unprocessed fertilizers. In all fertilizer variants, positive effect of humic acids on potato yielding was determined. Application of HA resulted in increased total yield of tubers relative to the control by 5%, and commercial yield by 8%. The total application of organic fertilizers and humic acids resulted in increased total yield between 6 and 9%, whereas commercial yield from 5 to 10%. Similar results were obtained by Selim et al. (2010), who administered humic substances by means of drip irrigation system. A considerably higher efficiency of humic acids was demonstrated by Ekin (2019). This author utilized 400 kg ha ha^{-1} and obtained tuber yield corresponding to mineral fertilization of 100 + 50 + 50 kg NPK ha^{-1} and 44% higher than on control facility. In the three-year experimental period highest tuber yields were obtained in 2017 and 2018. Lowest yield was recorded in 2019, mainly due to the low amount of precipitation and high air temperature in the tuber formation period. Potato yields on irrigated treatments in 2019 were at the level of yields harvested in 2017 and 2018 from non-irrigated treatments (Fig. 2).

Table 2. Potato tuber yields (t ha^{-1})

Fertilization	Irrigation		Year			Mean
	I$_0$**	I$_1$	2017	2018	2019	
	total tuber yield					
C*	14.4^{e}	16.3^{f}	13.5^{f}	16.4^{e}	16.1^{d}	15.3^{F}
HA	14.8^{e}	17.2^{f}	14.4^{f}	16.9^{e}	16.8^{d}	16.0^{F}
FYM	24.2^{d}	30.6^{e}	29.4^{e}	30.2^{d}	22.6^{c}	27.4^{E}
FYM+HA	26.2^{cd}	33.4^{d}	32.2^{d}	31.6^{cd}	25.5^{b}	29.8^{D}
VC	24.5^{cd}	35.3^{c}	33.7^{d}	33.1^{c}	22.8^{bc}	29.9^{D}
VC+HA	27.2^{c}	36.2^{c}	36.2^{c}	35.5^{b}	23.6^{bc}	31.7^{C}
FCN	30.2^{b}	38.4^{b}	39.7^{b}	36.2^{ab}	27.0^{ab}	34.3^{B}
FNC+HA	31.9^{a}	41.7^{a}	43.8^{a}	38.0^{a}	28.7^{a}	36.8^{A}
Mean	24.2^{B}	31.1^{A}	30.4^{A}	29.7^{A}	22.9^{B}	
	marketable tuber yield					
C	8.3^{d}	9.8^{f}	8.0^{f}	9.6^{e}	9.5^{d}	9.1^{F}
HA	8.7^{d}	10.8^{f}	8.7^{f}	10.2^{e}	10.5^{d}	9.8^{F}
FYM	18.5^{c}	26.7^{e}	26.3^{e}	24.7^{d}	16.8^{c}	22.6^{E}
FYM+HA	20.7^{bc}	29.2^{d}	28.4^{de}	27.4^{c}	19.1^{c}	24.9^{D}
VC	19.4^{c}	31.7^{c}	30.7^{cd}	29.0^{c}	17.0^{c}	25.6^{D}
VC+HA	22.2^{b}	31.9^{c}	32.0^{c}	31.6^{b}	17.5^{c}	27.0^{C}
FCN	26.1^{a}	34.8^{b}	36.1^{b}	32.6^{ab}	22.6^{b}	30.4^{B}
FNC+HA	27.8^{a}	37.5^{a}	38.6^{a}	34.3^{a}	25.1^{a}	32.7^{A}
Mean	19.0^{B}	26.5^{A}	26.1^{A}	24.9^{B}	17.3^{C}	

*C – control; HA – humic acid; FYM – farmyard manure; FYM+HA – farmyard manure + humic acid; VC – vermicompost; VC+HA – vermicompost + humic acid; FNC – Fertil Bioilsa C-N 40-12.5; FNC+HA – Fertil Bioilsa C-N 40-12.5 + humic acid; **I$_0$ – without irrigation; I$_1$ – drip irrigation; Different letters in the kolumns mean a honestly significant difference (HSD) at $P < 0.05$.

The excessive air temperature in June was likely the factor that restricted potato yielding on facilities irrigated in 2019. Similar relationship was observed earlier in a very early cultivar by Elzner et al. (2018). According to Burton (1981), the optimum temperature for the photosynthesis of European potato cultivars is approx. 20 °C, and each 5 °C leaf temperature increment reduces the photosynthesis rate by 25%. Rykaczewska (2015) demonstrated that the combined thermal and drought stress maintaining for two weeks in potato flowering period may reduce the yield of sensitive cultivars by 50%, and of tolerant cultivars by 25%. The number of initiated tubers ranged between 6.1 to 8.0 and depended on fertilization, irrigation, year of study and interaction of these factors (Table 3). On average, potato plants initiated 5.6% more tubers on irrigated fields than on non-irrigated ones. In the study of Mokh et al. (2015), the highest number of potato tubers were initiated under the conditions of field water capacity, whereas the number of tubers at deficit irrigation was

Table 3. Number of tubers per plant

Fertilization	Irrigation		Year			Mean
	I$_0$**	I$_1$	2017	2019	2019	
C*	6.3^{b}	6.6^{c}	6.1^{c}	6.5^{c}	6.8^{b}	6.4^{B}
HA	6.3^{b}	6.7^{c}	6.2^{c}	6.6^{c}	6.8^{b}	6.5^{B}
FYM	7.1^{a}	7.4^{b}	7.0^{b}	7.3^{b}	7.5^{a}	7.3^{A}
FYM+HA	7.4^{a}	7.7^{ab}	7.2^{ab}	7.8^{a}	7.7^{a}	7.6^{A}
VC	7.3^{a}	7.8^{ab}	7.2^{ab}	8.0^{a}	7.5^{a}	7.6^{A}
VC+HA	7.4^{a}	7.9^{a}	7.4^{a}	7.9^{a}	7.6^{a}	7.6^{A}
FCN	7.3^{a}	8.0^{a}	7.4^{a}	7.7^{ab}	7.7^{a}	7.6^{A}
FNC+HA	7.4^{a}	8.0^{a}	7.6^{a}	7.8^{ab}	7.8^{a}	7.7^{A}
Mean	7.1^{B}	7.5^{A}	7.0^{B}	7.4^{A}	7.4^{A}	

*, ** See Table 2.

considerably lower. The highest increase of the number of tubers per plant under the influence of irrigation was determined after the application of Fertil Bioilsa C-N 40-12.5 (9.6%) fertilizers, and lowest on the fields fertilized with manure (4.1%). The number of tubers initiated on fertilizer fields (treatments 3–8) remained at an equal level from 7.3 to 7.7, but it was significantly greater than on the control field (6.4). HA administration did not affect the number of tubers per plant. The highest number of initiated tubers was determined in 2018 and 2019, which were characterized by higher air temperature in June and July than in 2017. Similar phenomenon was observed by Rykaczewska (2015), who revealed that high temperature during potato flowering and tuber development (BBCH 65–79) may result in a secondary tuberization in certain cultivars.

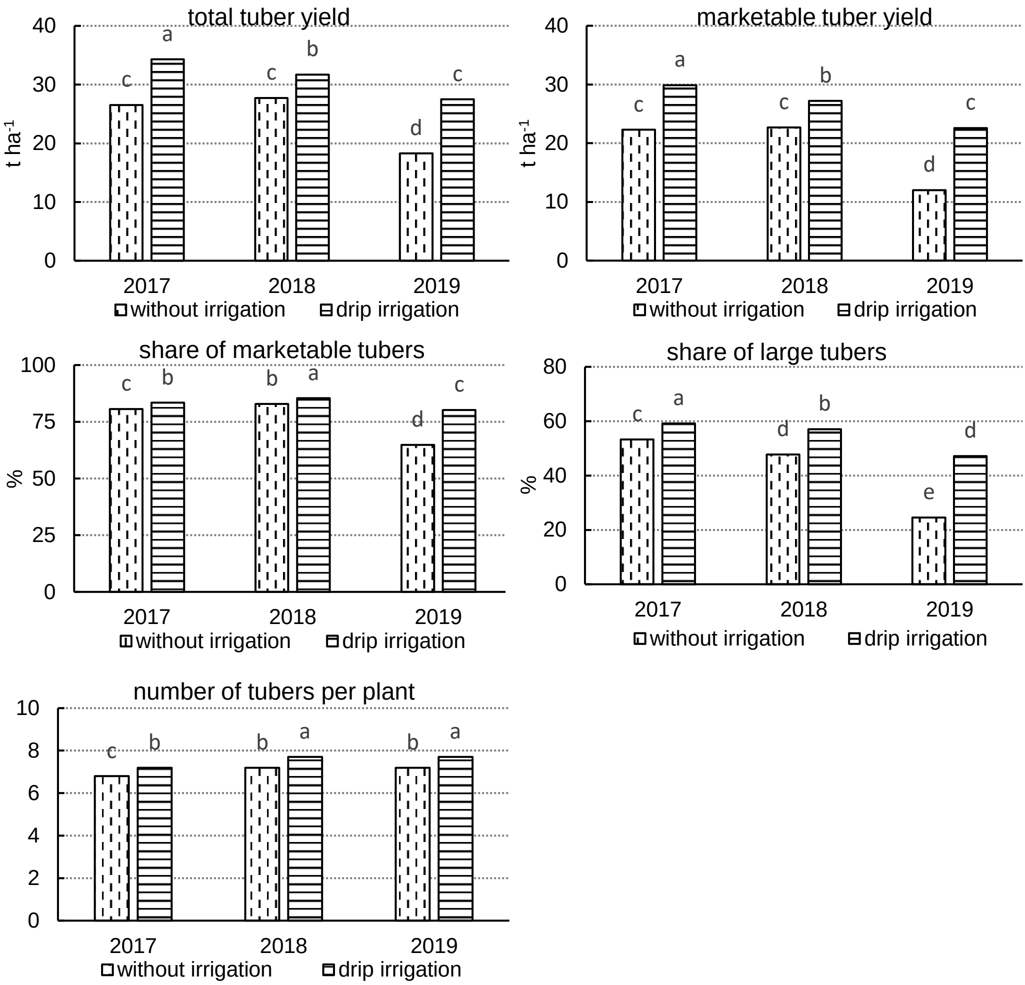

Figure 2. Effect of drip irrigation on tuber yield and its structure.

The share of marketable tubers in the total yield on non-irrigated fields was on average 76.1%, whereas large tubers 41.9% (Table 4). The applied irrigation resulted in increased share of these tuber fractions in yield by 2.8 and 5.9% in 2017, 2.6 and 9.3%

in 2018 and 15.4 and 22.6% in 2019, respectively (Fig. 2). The highest increase in the share of marketable fraction under the influence of fertilization was observed on the fields fertilized with vermicompost (11.9%), whilst the large tuber fraction after the administration of vermicompost and humic acids (17.4%). In general, the highest amount of marketable and large tubers were collected on the fields fertilized with Fertil Bioilsa C-N 40-12.5, significantly lower amounts on the fields fertilized with manure and vermicompost, and the lowest amount on the control field and after the application of HA.

Table 4. Share of marketable and large tubers (%)

Fertilization	Irrigation		Year			Mean
	I_0**	I_1	2017	2018	2019	
	marketable tubers					
C	59.1^{c}	60.8^{d}	59.6^{d}	61.5^{c}	58.9^{c}	60.0^{D}
HA	60.2^{c}	63.5^{d}	61.1^{d}	62.0^{c}	62.4^{c}	61.8^{D}
FYM	76.8^{b}	87.6^{c}	83.4^{c}	91.0^{ab}	72.2^{b}	82.2^{C}
FYM+HA	78.1^{b}	88.2^{c}	88.2^{b}	89.6^{b}	71.7^{b}	83.2^{BC}
VC	78.3^{b}	90.2^{abc}	88.7^{ab}	90.8^{ab}	73.2^{b}	84.2^{BC}
VC+HA	79.7^{b}	89.2^{bc}	90.8^{a}	90.4^{ab}	72.1^{b}	84.5^{B}
FCN	87.8^{a}	92.1^{ab}	92.1^{a}	94.1^{a}	83.6^{a}	89.9^{A}
FNC+HA	88.8^{a}	92.5^{a}	92.4^{a}	93.4^{ab}	86.1^{a}	90.7^{A}
Mean	76.1^{B}	83.0^{A}	82.0^{B}	84.1^{A}	72.5^{C}	
	large tubers					
C	20.1^{c}	24.7^{c}	23.0^{b}	23.2^{d}	21.0^{c}	22.4^{C}
HA	21.8^{c}	26.8^{c}	24.3^{b}	23.3^{d}	25.2^{c}	24.3^{C}
FYM	46.1^{b}	62.0^{b}	65.6^{a}	60.6^{b}	35.9^{b}	54.0^{B}
FYM+HA	46.7^{b}	61.5^{b}	65.7^{a}	56.6^{bc}	40.0^{b}	54.1^{B}
VC	46.2^{b}	62.6^{b}	66.0^{a}	59.5^{b}	37.7^{b}	54.4^{B}
VC+HA	45.1^{b}	62.5^{b}	68.9^{a}	54.3^{c}	38.3^{b}	53.8^{B}
FCN	54.4^{a}	67.8^{a}	69.0^{a}	69.9^{a}	44.3^{ab}	61.1^{A}
FNC+HA	55.0^{a}	68.1^{a}	67.4^{a}	72.3^{a}	44.9^{a}	61.5^{A}
Mean	41.9^{B}	54.5^{A}	56.2^{A}	52.5^{B}	35.9^{C}	

*, ** See Table 2.

Water use efficiency (WUE) was on average 66.6 kg mm^{-1} and it remained in the range from 35.2 to 113.1 kg mm^{-1} (Table 5). The highest WUE value was found for the fertilized field FNC+HA, and lowest on the control field. Humic acids significantly increased the efficiency of water use in all fertilizer facilities (treatments 3–8). In the three-year period of study, the highest water productivity was obtained in 2017 (78.4 kg mm^{-1}), and lowest in 2019 (49.7 kg mm^{-1}). The low water use efficiency in 2019 was linked to the highest evapotranspiration (Et_a) in the entire study cycle. Irrigation water use efficiency (IWUE) was in a very broad range of 9.9 to 166.3 kg mm^{-1} and on average it amounted to 67.2 kg mm^{-1}. The conducted study presents similar mean WUE and IWUE values. Ati et al. (2012) and Mokh et al. (2015) demonstrated a markedly higher IWUE value (vs. WUE), whereas in the study of Eissa (2018) the WUE index attained higher value than IWUE. The greatest water use efficiency was determined on the field fertilized with vermicompost, and lowest on the control field. Humic acids increased irrigation water use efficiency (IWUE) only on the fields fertilized with manure and Fertil NC. IWUE was significantly affected by the weather conditions in the individual years of study. The highest irrigation water use efficiency, with a mean of 79.1 kg mm^{-1} was determined in 2017, which was characterized by the lowest total precipitation in the period from May to July observed throughout the study.

Table 5. Water productivity (kg mm^{-1})

Fertilization	WUE				IWUE			
	Year			Mean	Year			Mean
	2017	2018	2019		2017	2018	2019	
C*	35.2^{f}	39.7^{e}	35.5^{d}	36.8^{F}	18.3^{e}	9.9^{d}	22.0^{d}	16,7^{F}
HA	37.5^{f}	40.8^{e}	37.0^{d}	38.4^{F}	23.5^{e}	16.4^{d}	28.1^{d}	22.7^{F}
FYM	76.5^{e}	72.7^{d}	48.9^{c}	66.0^{E}	47.5^{d}	54.7^{c}	74.5^{bc}	58.9^{E}
FYM+HA	83.8^{d}	76.2^{cd}	54.9^{bc}	71.6^{D}	40.9^{d}	65.6^{bc}	91.7^{a}	66.1^{D}
VC	85.8^{d}	79.5^{c}	49.6^{bc}	71.6^{D}	166.3^{a}	101.0^{a}	63.4^{c}	110.2^{A}
VC+HA	93.0^{c}	85.2^{b}	51.2^{bc}	76.5^{C}	114.7^{b}	88.1^{a}	68.6^{c}	90.4^{B}
FCN	102.6^{b}	87.1^{ab}	58.8^{ab}	82.8^{B}	100.1^{c}	66.8^{bc}	70.3^{c}	79.0^{C}
FNC+HA	113.1^{a}	91.4^{a}	62.1^{a}	88.9^{A}	121.6^{b}	71.6^{b}	87.3^{ab}	93.5^{B}
Mean	78.4^{A}	71.6^{B}	49.7^{C}		79.1^{A}	59.3^{C}	63.2^{B}	

* See Table 2.

CONCLUSIONS

The results of this study confirm the significant effect of drip irrigation on the increase in the yield of potato tubers by increasing the number of tubers and the share of marketable and large tubers in the total yield. The use of humic acids increases the yield of tubers, especially in the case of their combined application with organic fertilizers, and additionally increases of the water use efficiency. Processed natural fertilizers such as Fertil Bioilsa N-C 40-12.5 and vermicompost show higher yield forming efficiency in potato cultivation than manure.

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Comparison of the effect of ultraviolet light, ozone and heat treatment on muesli quality

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Abstract. Various muesli processing technologies can be used to lower microbiological parameters. It is necessary to find the best treatment technology to ensure that the product can meet various regulatory limits and to increase the shelf life of the product. The aim of this study was to compare the effect of ultraviolet light, ozone treatment and sterilisation on muesli quality. Muesli samples with dried fruits were tested, comparing the change in total plate count, yeast count and mould count. Short-wave ultraviolet (UV-C) light with a wavelength of 254 nm was used for ultraviolet light treatment, and the product was treated for 1, 2, and 5 minutes. As for ozone treatment, the samples were treated with an ozone concentration of 35 ppm for 30 minutes. Heat sterilisation was performed using sterilisation mode 25-30-50 (heating, holding, cooling), 119 °C, 2.2×10^5 Pa. Ultraviolet light and ozone treatment did not have a significant impact on total plate count, yeast count and mould count. Heat sterilisation had the most significant effect on muesli sample microorganism level, total plate count, yeast count and mould count level were 10 log cfu g^{-1}.

Key words: muesli, microbiology, heat treatment, ozone, UV.

INTRODUCTION

Muesli is a mixture of different raw materials such as cereal flakes, dried fruits, seeds, and nuts. Usually, all raw materials have different microbiological parameters, moreover not all of them are microbiologically safe. Globalization is increasing and products are shipped to different countries with different legislations and limits. In order to lower the microbiological parameters and extend the product shelf life, different muesli processing technologies can be used.

The wavelength of ultraviolet (UV) light ranges from 100 to 400 nm in the electromagnetic spectrum. UV radiation is divided into four segments, vacuum UV from 100 to 200 nm, short-wave ultraviolet (UV-C) from 200 to 280 nm, medium-wave (UV-B) from 280 to 320 nm, and long-wave UV (UV-A) from 320 to 400 nm. The highest germicidal efficacy is shown in the UV-C range (Umagiliyage & Choudhary, 2018). The basic principle is that UV at certain wavelengths destroys and damages the DNA of various types of microbes, rendering them inactive so that they cannot reproduce and multiply (Vasuja & Kumar, 2018). In the food industry, ultraviolet light is mostly used to disinfect equipment surfaces and decontaminate conveyor surfaces and packaging containers. Despite the effectiveness of ultraviolet light for disinfecting smooth surfaces, there are relatively few applications of this technology in the food processing industry (Koutchma, 2008). The advantages of ultraviolet light treatment over other disinfection methods are obvious: no chemicals are used, it is a heat-free process, changes in colour, taste, odour, and pH are minimized (Sorour et al., 2014)

Ozone treatment is a chemical method of decontaminating food by exposing the food to ozone in aqueous and/or gaseous phase. The bactericidal effect of ozone has been confirmed for a wide range of microorganisms, including Gram-positive and Gram-negative, as well as bacterial spores (Brodowska et al., 2017). Ozone is the attractive choice for food processing and preservation industries to ensure microbial food safety because it is fast acting and has strong oxidative properties (Pandiselvam et al., 2017). This is the reason why the application of ozone both improves the microbiological safety of foods and extends their shelf life without significantly altering their nutritional, chemical, and physical properties. Ozone can be generated by the exposure of air or another gas mixture that contains oxygen to a source of energy such as high-energy electrical field (corona discharge method), ultraviolet radiation (phytochemical method), or conversion of oxygen molecules to ozone (chemical method). Mostly only two methods are used in practice: photochemical (UV) and corona discharge (CD). First one seems to be the most applicable in the food industry (Brodowska et al., 2017). CD discharge generators require an air preparation prior to ozone generation. The two common types of gas to feed CD ozone generators are oxygen and dry air. Product is treated in treatment chamber. It is collector where ozone enters from the top and returns though the bottom outlet after completing the exposure time. There are two electrodes in corona discharge, the high tension and lows-tension electrode, separated by a dielectric medium in a narrow discharge gap. When electrons have sufficient energy to dissociate the oxygen molecule, a certain fraction of these collisions occur, and a molecule of ozone can be formed from each oxygen atom. The outlet is connected to ozone analyser and then to ozone destructor. Ozone analyser is a device that is used for measuring the ozone concentration. At the outlet of the ozone destructor, excess ozone is destroyed, and the cleaned and decontaminated air is re-circulated to its intended enclosure or discharged to the ambient atmosphere (Prabha et al., 2015).

Sterilisation is the complete destruction or elimination of all viable organisms in/on an object to be sterilised. Sterilisation destroys yeasts, moulds, vegetative bacteria, and spore-forming organisms and allows food processors to store and distribute products at ambient temperatures, thereby extending shelf life. The sterilisation process includes four distinct stages. First, the product is heated to 110 to 125 °C to ensure sterilisation. Then, the product takes a few minutes to equilibrate as the surface is hotter and the middle part of the container is still cool. The equilibration phase allows the temperature gradient to be reduced. Then, the product must be kept at this temperature for a certain time to ensure a predetermined sterilisation value, which is determined by the F0 value. At the end, the product must be cooled, mainly to prevent further heat treatment and to avoid bursting of the container in hot conditions (Ramesh, 2003).

The microflora of cereals and cereal products is varied and includes moulds, yeasts, bacteria (psychotropic, mesophilic, and thermophilic), lactic acid bacteria, rope forming bacteria (*Bacillus* spp.), bacteria pathogens, coliforms, and Enterococci (Bullerman & Bianchini, 2008). During previous tests it is observed that in similar muesli samples no pathogen bacteria has been detected also coliform and Enterococci amount is predictable. Most changing is total plate count, mould count and yeast count. There are basically no studies about different muesli treatments to reduce and control their microbial level. Most studies are concerned with one raw material treatment. Muesli is a mixture of different raw materials such as cereal flakes, dried fruits, seeds, and nuts. It is a non-homogenous product and usually, all raw materials have different microbiological parameters and irregular surface. The aim of this study was to compare the effects of ultraviolet light, ozone treatment and sterilisation on muesli quality. It is necessary to find the best treatment technology to ensure that the product can meet various regulatory limits and to increase the shelf life of the product. During this study, the change in total plate count, yeast count and mould count were compared. To compare the results obtained, European Union and Chinese legislation for microbiological limits were used.

MATERIALS AND METHODS

All muesli samples were prepared and supplied by Felici LLC. For this study, muesli samples were prepared with 79% cereal flakes and 21% dried fruits and vegetables. The recipe contained various ingredients such as dried date pieces, goji berries, carrot powder, mulberries, apple pieces, and raspberries. Muesli samples for each treatment experiment were prepared at different times. Before each experiment, the total plate count of the muesli, mould count, and yeast count were analysed. The total plate count was performed according to the method LVS EN ISO 4833-1:2014, while the mould and yeast counts were performed according to the method LVS ISO 21527-2:2008.

Ultraviolet light

Ultraviolet light Bactostop 30 lamp (Bactoscop, Poland), power 30 W, was added to the horizontal mixer. A total of 80 ± 0.1 kg of muesli sample was prepared using Matas (KEMEK Engineering, Lithuania) balances and added to the horizontal mixer. Short wavelength ultraviolet (UV-C) light with a wavelength of 254 nm was used. Muesli samples were mixed and irradiated with ultraviolet light during mixing (Fig. 1). The samples were mixed for one, two and five minutes. Ultraviolet light treatment was performed twice. After each treatment, muesli sample was taken to analyse the total plate count, mould count and yeast count. Muesli samples were mixed and treated according to the muesli production technological process to see if ultraviolet light treatment can be used during muesli production.

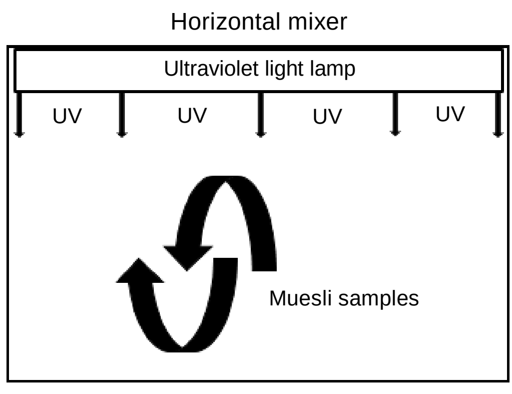

Figure 1. Designed scheme of ultraviolet light treatment.

Ozone treatment

Corona discharge ozone generator DNA-20G (Dino purification, China) was used for ozone treatment, capacity 20 g h^{-1}. Ozone was generated from ambient air. Ozone concentration was measured by ozone device QL -800-Q3 (QLOZONE, China). For ozone treatment, 100 ± 0.01 g of the sample was weighed (KERN & Sohn GmbH, Germany). Muesli samples were treated in the manufacturer laboratory, samples were treated in ozone concentration of 35 ppm for 30 minutes (Fig. 2) according to the manufacturer recommendation, based on previous tests for cereal flakes. After passing ozone through the product, the gas was directed to thermal ozone destructor. Clean and decontaminated air was discharged to the ambient atmosphere. Ozone treatment was performed twice.

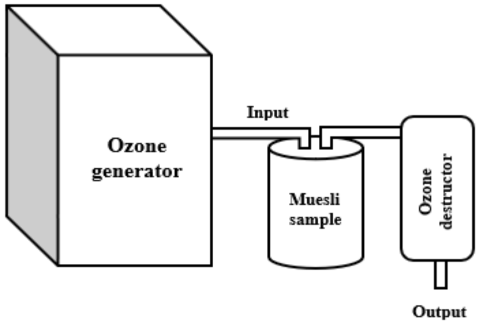

Figure 2. Designed scheme of ozone treatment.

Heat sterilisation

First, 100 ± 0.01 g of the sample was weighed (KERN &Sohn GmbH, Germany) and poured into a glass jar. The filled glass jars containing the muesli samples were sterilised using Steriflow device (Steriflow, France). Sterilisation mode 25-30-50 (heating, holding, cooling), 119 °C, 2.2×105 Pa (Fig. 3). The maximum temperature in the muesli sample during sterilisation was 112 °C. Sterilisation treatment was performed twice.

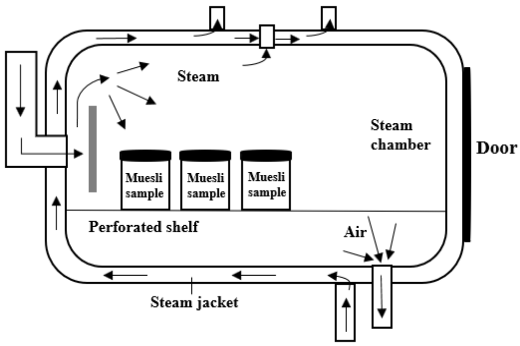

Figure 3. Designed scheme of sterilisation treatment.

Data processing

MS Excel 2016 was used to analyse the data obtained. ANOVA analysis was done to determine the difference between the samples. Samples were tested in triplicate. Factors were defined as significant if the p-value was less than 0.05.

RESULTS AND DISCUSSION

Table 1 shows the content of microorganisms in the commodities according to their specifications. It shows that the total microbial count varies from 10^4 to 10^5 cfu g^{-1}, while the mould and yeast count vary from 10^2 to 10^4 cfu g^{-1}. The highest microbiological counts are found in dried fruits and vegetable powders. As with cereals, microbial growth can occur during the wet phase, but further processing involves heat treatments that reduce the microbial load (ICMSF, 2005). Breakfast cereals processed in this manner under good sanitary conditions typically have aerobic plate counts of 1,000 cfu g^{-1}

(Deibel & Swanson, 2001). In a study of the microbial flora of whole grain cereal products in Germany, the aerobic plate count was about 10^6 cfu g^{-1}, but the fungal count was up to 2.0×10^5 cfu g^{-1}.

Table 1. Microorganism levels in different raw materials according to their specifications

Raw material	Total plate count, cfu g^{-1}	Mould count, cfu g^{-1}	Yeast count, cfu g^{-1}
Grain flakes	$\leq 1.0\times10^4$	$\leq 1.0\times10^2$	$\leq 1.0\times10^2$
Dried date pieces	$< 5.0\times10^4$	$< 1.0\times10^4$	$< 1.5\times10^3$
Dried goji berries	$< 1.0\times10^5$	$< 1.0\times10^4$	$< 1.0\times10^4$
Dried carrot powder	$\leq 1.0\times10^5$	$\leq 5.0\times10^2$	$\leq 5.0\times10^2$
Dried mulberries	$< 7.5\times10^4$	$< 1.0\times10^4$	$< 1.0\times10^4$
Dried apple pieces	$< 1.0\times10^4$	$< 1.0\times10^3$	$< 1.0\times10^3$
Freeze dried raspberry	$< 5.0\times10^4$	$< 1.0\times10^3$	$< 1.0\times10^3$

Although bacterial and mould counts were high, pathogen counts were low. Nevertheless, the mycoflora of cereal products can be very diverse. Microbial spoilage of breakfast cereals is rare because they have a low water activity of less than 0.50. The results of Kince et al. (2018) study on water activity varied from 0.108 to 0.494. Outbreaks of foodborne diseases associated with cereals are not common. As for spoilage, low water activity of the product prevents microbial growth (ICMSF, 2005) because water activity affects various biochemical reactions and microbial growth in food (Syamaladevi et al., 2016). Unsulphured fruits were used for this formulation. The microbiology involved in the processing of these fruits is described below. Mechanical dehydration reduces the overall microbial load, but the extent of reduction depends on both the type of fruit and the severity of the process. Poor sanitary conditions in the factory can lead to contamination of dried fruit during packing. Thus, the microbial population varies according to the type of fruit and the conditions of cultivation and processing (ICMSF, 2005).

Guirguis (2018) reported aerobic mesophilic bacteria in the range of 10^2 to 10^3 cfu g^{-1} and moulds and yeasts in the range of 10^3 to 10^4 cfu g^{-1}. For dried vegetables, the flora depends to a considerable extent on whether the flora of the raw, cleaned product has been largely destroyed by blanching. Vegetables for drying may promote microbial growth if kept at room temperature for too long. Populations ranging from 10^3 to 10^4 cfu g^{-1} have been reported on produce dried by blowing hot air through vegetables on perforated trays or belts (ICMSF, 2005).

Table 2. Calculated and analysed microorganism level in muesli samples

Muesli sample	Total plate count, cfu g^{-1}	Mould count, cfu g^{-1}	Yeast count, cfu g^{-1}
Calculated	1.0×10^5	1.0×10^3	1.0×10^3
UV light treatment	3.8×10^4	4.2×10^2	9.3×10^5
Ozone treatment	4.8×10^2	3.6×10^2	1.1×10^5
Sterilisation	6.5×10^2	9.1×10^1	1.7×10^4

Because the muesli samples were prepared at different times for each treatment experiment, muesli total plate count, mould count, and yeast count analyses were performed prior to each experiment. The calculated results and the results before each analysis are shown in Table 2. According to the written information in the raw material specification, the possible levels of microorganisms in the muesli were calculated. The results are as follows - total plate count 1.0×10^5 cfu g^{-1}, mould count 1.0×10^3 cfu g^{-1}, yeast count 1.0×10^3 cfu g^{-1}.

The results show that the total plate count and mould count were lower than calculated and ranged from 10^1 to 10^4 cfu g^{-1}, while the yeast count was higher than calculated and ranged from 10^4 to 10^5 cfu g^{-1}. It can be seen that the microorganism content does not always meet the specification and may vary from batch to batch. No specific microbiological criteria for ready-to-eat breakfast cereals or muesli are mentioned in European Commission Regulation (EC) No. 2073/2005. Chinese legislation for microbiological limits was used for comparison. The national food safety standard GB 19640-2016 for cereal products is: total plate count 1.0×10^5 cfu g^{-1}, mould count 1.0×10^2 cfu g^{-1} and yeast count 1.0×10^2 cfu g^{-1}. Looking at the calculated and analysed microorganism content in the cereal sample, the total plate count complies with the regulations, but the mould and yeast counts exceed the limits. Nebrink (2007) reported that the allowable value of total plate count of 10^4 to 10^5 cfu g^{-1} is within the acceptable range. As for water activity, Semicenkova et al (2019) reported results of 0.36 for muesli with seeds, raisins, and chocolate. In another study, Senhofa et al. (2014) reported water activity of 0.56 for muesli with seeds.

The content of microorganisms in the cereal after each treatment is shown in Figs 4–6. The content of microorganisms in the muesli after sterilisation is shown in Fig. 4. The results show that the total microbial count, mould count and yeast count were 10 log cfu g^{-1} after sterilisation. The values show a significant difference ($p < 0.05$). Thermal food sterilisation and pasteurization are the most widely used preservation technologies to extend the shelf life of food by inactivating microorganisms and enzymes that can spoil food (Rodrigo et al., 2016). Food sterilisation is divided into two categories: Sterilisation by heating (thermal processing) and sterilisation without heating (non-thermal processing). Thermal processing is widely used nowadays, despite some problems, such as that the heating process could reduce the nutritional value or deteriorate the quality of the food, and that it is ineffective against certain types of bacteria. Non-thermal processing is considered an effective method that does not cause quality deterioration, unlike thermal processing. Nevertheless, there are no reports on the effect of sterilisation without heating (Ramesh, 2003). Some samples had changed their colour, which implies that different sterilisation methods should be tested to ensure low level of microorganisms and consistent quality.

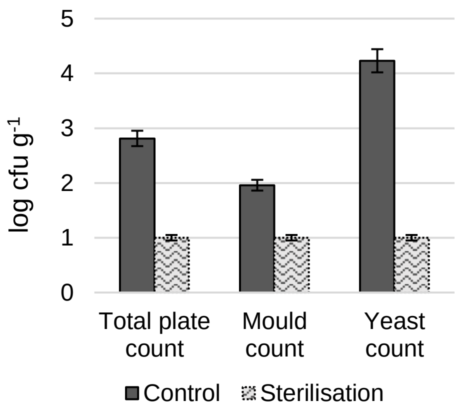

Figure 4. Microorganism level in muesli sample after sterilisation.

The content of microorganisms in the muesli sample after ozone treatment is shown in Fig. 5. The results show that the total microbial count decreased from 2.68 to 2.15 log cfu g^{-1}, mould count decreased from 2.56 to 10 log cfu g^{-1} and yeast count decreased from 5.04 to 4.54 log cfu g^{-1}. The values after ozone treatment showed a non-significant difference ($p > 0.05$). There are not many studies on the effect of ozone treatment on the quality of muesli or breakfast cereals, mostly on the quality of cereal flakes or dried fruits. The study by Wu et al. (2006) showed that gaseous ozone for preservation of stored wheat was a very effective method to inactivate 96.9% of fungal spores associated with wheat. Higher treatment efficacy was achieved when the temperature and water activity of the wheat were increased. In another study, Dodd et al. (2011) reported on the effect of ozonation on malting barley that the treatment did not result in a significant reduction in aerobic plate count but did reduce mould and yeast counts by 1.5 log cfu g^{-1} in the finished malt. Gaseous ozone had no negative effect on any aspect of malt quality. Zorlugenc et al (2008) reported that the efficacy of gaseous ozone on aerobic mesophilic bacteria count (AMB) in figs was reduced by 0.81, 1.0 and 1.42 log cfu g^{-1} at 7.5, 15 and 30 min, respectively. Application at 7.5 min had a significant ($p < 0.05$) effect on the reduction, while application at 15 and 30 min was not significant. Total yeast count was reduced by 0.16, 1.57 and 2.09 log cfu g^{-1} at 7.5, 15 and 30 min, respectively. The reduction in yeast count was statistically significant ($p < 0.05$). Total mould count was reduced by 0.59 log cfu g^{-1} at 7.5 min. Moulds were completely inactivated at 15 min and 30 min. Najafi & Khodaparast (2009) reported the effectiveness of ozone in reducing microbial populations in date fruits. The study showed that the total number of mesophilic microorganisms was reduced from 4.06 to 3.80, 3.60 and 3.50 log cfu g^{-1}. For yeasts and moulds, the initial level was 3.93 log cfu g^{-1} and was reduced to 3.80, 3.63 and 3.50 log cfu g^{-1}. No visual changes for samples were detected.

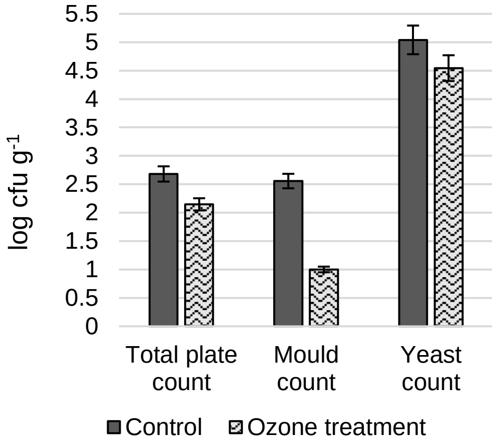

Figure 5. Microorganism level in muesli sample after ozone treatment.

The results after UV light treatment are shown in Fig. 6. It shows that the total bacterial count decreased from 4.58 to 3.89, 3.45 and and 3.38 log cfu g^{-1} after 1, 2 and 5 minutes of treatment, respectively. Mould count changed from 2.62 to 2.66, 2.08 and 10 log cfu g^{-1} after 1, 2 and 5 min of treatment, respectively. Yeast count decreased from 5.97 to 5.26, 5.14, and 5.99 log cfu g^{-1} after 1, 2, and 5 min of treatment, respectively. Pulsed UV light is considered more

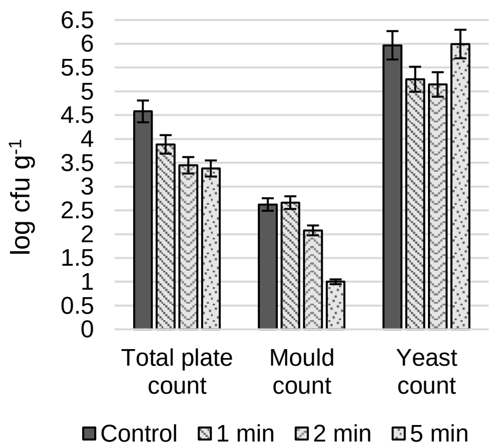

Figure 6. Microorganism level in muesli sample after UV light treatment.

efficient in microbial inactivation than continuous UV light and provides safer and faster decontamination. UV-C light, with the peak of maximum efficacy at wavelengths of approximately 260–265 nm, which corresponds to the peak of maximum DNA absorption, is most effective for inactivating microorganisms. The formation of cyclobutane-pyrimidine dimers during UV light treatment leads to mutagenesis and cell death. Although pulsed UV light is believed not to be a suitable technology for cereals due to the rough and uneven surfaces of cereals, the antimicrobial efficacy of this technology against microorganisms present on stored cereal grains has been demonstrated (Los et al., 2018). Maftei et al. (2013) study on decontamination of naturally occurring moulds on wheat grains achieved a reduction of about 4.0 log cfu g^{-1}, it also showed that the initial mould load of the grains is an important factor for treatment efficacy. No visual changes for samples were detected.

CONCLUSIONS

This study shows that microorganism level is variable, which means that treatment before packing would be preferable to be sure that results always meet the specification. Results show that UV light and ozone treatment had no significant impact on muesli sample microorganism level. Results show that the suggested UV light lamp cannot be used during the production process to decrease microorganism levels. This study also shows that the manufacturer suggested ozone concentration for cereal flakes cannot be applied for muesli treatment. All muesli raw materials have different microbiological parameters and irregular surfaces. The surface properties of food also influence ozone and ultraviolet light inactivation of microorganisms in dried foods. Heat sterilisation had the most significant effect on muesli sample microorganism level. Yet, it is needed to find the most appropriate sterilisation method. Some samples had changed colour what means that different sterilisation regimes or nonthermal methods should be tested to ensure low microorganism levels and equable quality. Nutritional and sensory properties need to be evaluated.

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Evaluation of selected soft winter wheat lines for main ear grain weight

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Abstract. Studies to assess the breeding samples of soft winter wheat by weight of grain from the main ear anddetermine the indicators of adaptability were conducted at the Bila Tserkva Research and Selection Station (BTRSS) in 2011–2013. The study revealed significant differences in breeding lines in the range of variability of grain mass from the main ear and identified small, medium and significantcoefficients of variation, which indicates their excellent response to environmental conditions. It was due to genotype, year conditions and their interaction. Line 42 KS had significantly higher than the standard grain weight of the main ear (0.14 g) and the lowest value of the coefficient of variation (8.7%). According to the indicators of adaptability(GAC, $\sigma^2(GxE)gi$, σ^2SACi, $\sigma SACi$, etc.) the lines 42 KS, 24 KS and 44 KS were distinguished. There was asignificant correlation between the weight of grain from the ear and the weight of 1,000 grains (0.603–0.674) and the direct influence of the weight of grain from the main ear on the weight of grain from the plantand grain yield (0.805–0.942). Selected lines as a result of research (2015–2020) from these populations of soft winter wheat are competitively tested in the conditions of Bila Tserkva Research and Selection Station, forming high grain yields (7.39–8.12 t ha^{-1}) and will be transferred to 2021 for the State variety test for inclusion in the Register of plant varieties suitable for distribution in Ukraine.

Key words: winter wheat, correlation, variability, plasticity, stability, adaptability.

INTRODUCTION

Wheat is grown in most countries, occupying an important place among cereals and is the main food crop (Shpaar, 2012). The sown area of winter wheat (*T. aestivum* L.) in Ukraine is about 5.5–6 million hectares(Litvinenko, 2011). An important factor in the growth and stabilization of crop yields, especially in adverse conditions, is not only the creation and implementation of varieties with high yield potential into production, but also increase their environmental sustainability. Plants with almost the same biological characteristics maydiffer in requirements for environmental conditions, i.e. ecological characteristics (Kochmarsky, 2012; Kulyk et al., 2020). Different environmental conditions were found to affect dry matter remobilization from the leavesand sheath, current photosynthesis, grain yield, and the relative contributions by the stem and the ear to grain yield (Golabadi et al., 20215).

The priority direction is the creation of a source material for selection with improved economically valuable traits, such as increased productivity, early-maturing, disease resistance, balanced chemical composition of grain (Artemchuk, 2013).

Creating wheat varieties with the highest possible level of productivity is the goal of every breeder, as increasing yields is one of the most important tasks due to its considerable difficulty and complexity (Bagan et al., 2012).

Selection played a major role in the increase in winter wheat yield after 1946 in France. The contribution of selection to this increase depended on the agronomic treatment and varied from one third to one half. Reduction of height was the most important factor. The number of grains per unit area had increasedover time without alteration of the weight of the grains. The negative relationship between 1,000 grain weightand grain number per m^2 was therefore shifted and new cultivars were thus able to fill more grains than older entries (Brancourt-Hulmel et al., 2003).

The tasks of adaptive selection can be solved if the methods for studying the plasticity of genotypes ofplants at early stages of selection are developed and varieties and forms of winter wheat that are identified according to these characteristics are included. The problems of adaptive selection of winter wheat are to be solved from the perspective of organization of selection process (finding, storage, identification and usage of appropriate genetic sources) and closely related systems of variety testing with elements of varietal agrotechnics (Bazalii et al., 2019).

During the process of plant breeding and selection of new winter wheat cultivars a lot of attention is paid to the improvement of the main components of grain yield-number of ears/m^2, number of grains per ear and 1,000 seed weight. Production of cultivars that achieve a large number of fertile ears per unit area, a largenumber of grains per ear and a heavy 1,000 seed weight, even with a lower density, would mean that a high grain yield is achieved with lower costs. On the grounds of the results obtained by a statistical analysis it wasestablished that the sowing rate did not have a statistically significant influence on the other two grain yield components - number of grains per ear and 1,000 seed weight. The investigated cultivars had a statistically very significant influence ($P < 0.01$) on the number of grains per ear, and a statistically significant influence on 1,000 seed weight, whereas there were no statistically significant differences between the investigated cultivarsin relation to the number of ears per unit area (Guberac et al., 2000).

Wheat grain yield is determined by three main factors, namely the ear number per square, grain numberper ear, and 1,000 grain weight. Breeding practice has indicated that increase the grain number per ear is the most effective way for improving of yield in China. The increase of grain number per ear will therefore be ofwidespread concern in future yield improvements (Yen et al., 1995).

Most studies have attributed the increased wheat yields in past decades to increases in grain number per ear (Siddique et al.,1989), thousand grain weight, or both (Donmez et al., 2001, Zhang et al., 2007). The number of grains per ear was the yield component that most closely followed the pattern of adaptation observedfor grain yield (Sanchez-Garcia et al., 2012).

In breeding research, Diordiieva et al. (2018) installed positively correlations between grain weights from main ear with yield capacity and can be used in selecting high-yielding genotypes at early stages of breeding work.

The main element of productivity that determine the yield of a particular plant in an ecosystem is the grain weight per ear, which consists of the number and the weight of grains. The elements of productivity have different variability depending on the interaction of the genotype and environment factors (Manukyan et al., 2019).

Studies have shown that grain weight per ear has greatly contributed to genetic improvements in wheatyield (Xiao et al., 2012).

In the conditions of left-bank side of North-east forest steppe of Ukraine a direct relation between: a ripeness group → plant height ($r = 0.96$) → resistance to overwintering ($r = 0.78$) → ripeness group ($r = 0.92$) winter wheat was noted. Having analyzed the received indices as for homeostasis and adaptability made sure that its genetic potential 40–80% under the conditions of north-east forest steppe of Ukraine (Vlasenko et al., 2018).

According to research, Innes et al. (1985) have shown that no differences in number of ears m^{-2} or in number of grains per ear between the early and the late selections. Mean weight per grain of the early selectionswas greater than that of the late selections. There were no differences in number of ears m^{-2} between the shortand the tall selections. The number of grains per ear was greater and mean weight per grain was less for the short selections than for the tall selections.

Feng et al. (2018) determined that the significant increases in grain yield in the past 60 years were mainly due to increases in grain number per ear and grain weight, while ears number per m^2 has not changed significantly. Improvements in thousand grain weight from the 1950s to 2010s have occurred at four grain positions (G1 to G4). The increase in grain number per ear since the 1950s was mainly due to an increase in grain number at G1, G2 and G3, with the relative contribution of grain position to grain number being G1 > G2 > G3 > G4.

While grain numbers and grain weights at the four grain positions increased at different rates from the1950s to 2010s, the total grain weight gradually increased, which greatly improved grain yield. Grain numberand grain weight per spikelet differ between spikelet and grain positions (Li et al., 2016).

Growth rate per grain depended on floret position within the ear, varied between cultivars (those withlarger grains at maturity having a faster rate), and increased with rise in temperature. With cultivars in which grain number per ear was markedly affected by illuminance, light had relatively little effect on growth rate pergrain (Sofield et al., 1977).

The aim of the research was to estimate the breeding samples of soft winter wheat by grain weight from the main ear and to determine the indicators of plasticity and stability.

MATERIALS AND METHODS

The research was conducted at the Bila Tserkva Research and Selection Station (BTRSS) in 2011–2013 (49°72'52.6"N30°09'89.2"E). The soil of the experimental field is typical chernozem, low humus, medium and light loam. The humus content in the soil layer 0–30 cm - 3.4–3.8%, the reaction of the soil solution is close to neutral. This type of soil is common in the Right Bank Forest-Steppe of Ukraine. The sowing period for soft winter wheat is from September 20 to October 1. The experiments were based on complete randomized blocks. Sowing was carried out three replication with a seeder CH-10, the accounting area each plot - 10 m^2. Mineral fertilizer was applied for pre-sowing cultivation at the rate of $N_{16}P_{16}K_{16}$ kg ha^{-1}. In the spring, soft winter wheat (BBCH 30) were fertilized of ammonium nitrate (N_{50} kg ha^{-1}). The predecessor is peas.

The following lines were studied: 7 KS, 8 KS, 42 KS, 29 KS, 26 KS, 24 KS, 12 KS, 44 KS, 54 KS, 22 KS, 17 KS. The standards were varieties Bilotserkivska napivkarlykova, Perlyna Lisostepu and Podolyanka. The experiments were performed according to the methodology (Volkodav, 2003; Rokitsky (1973); Dospekhov (1985); Snedecor (1961)).

Biometric analyzes between the elements of the yield structure weredetermined on the average sample of 25 plants in three replication, selected at the beginning of full maturity of wheat. The strength of the connection between the was determined by Guzhov (1987): $r < 0.3$ - the relationship between the signs is weak, $0.3 < r < 0.5$ -moderate, $0.5 < r < 0.7$ - significant, $0.7 < r < 0.9$ - strong, $r > 0.9$ - very strong, close to functional.

According to Rokitsky (1973) and Dospekhov (1985) the arithmetic mean X, the range of variability (min-max), the variance (S^2) and the coefficient of variation (CV) were determined. The coefficient of ecological plasticity (bi) was determined by Finlay & Wilkinson (1963), the homeostatic index (Hom) and theselection value (Sc) by Khangildin & Litvinenko (1981). General adaptive capacity (GAC), specific adaptive capacity variance (σ^2SACi), nonlinearity coefficient (Lgi), relative genotype stability (Sgi), genotype selection value (GSVi) and compensation-destabilization coefficient (Kgi) according to Kilchevskyi & Khotyleva (1985).

In generalized estimation of the adaptive potential of lines ranking by Snedecor (1961) andcalculations of the rating of adaptability of the variety (RAV) by Vlasenko (2006) were used. The results of the experimental data were processed using Statistica 6.0.

RESULTS AND DISCUSSION

The mass of grain of one ear, i.e. its productivity is the result of the action and interaction of many hereditary factors that determine its components. The variability and ratio of the components of ear productivity determine the level of its manifestation and the nature of inheritance and variability (Orlyuk, 2012).

In 2011, the average value of the weight of grain from the main ear (1.32 g) ranged from 1.01 g (26 KS,17 KS) to 1.91 g - 24 KS (Table 1).

The highest mass of grain in the main ear of the studied genotypes was formed in 2012 with variationin the range of 1.11–2.19 g and the mean 1.71 g. This year, only the line 54 KS significantly exceeded the standards for grain weight from the main ear. In 2013, the mass of grain from the main ear was in the range from 0.97 g (Podolyanka) to 2.04 g (12 KS), the mean 1.41 g. With the exception of the line 7 KS, all others on 0.04–0.83 g significantly exceeded the standards.

A significant excess over the standards for the weight of grain from the main ear, on average for 2011–2013, was set in the samples 42 KS (1.63 g), 24 KS (1.73 g) and 54 KS (1.77 g).

Table 1. Weight of grain from the main ear, g

Lines	Years				± to standard		
	2011	2012	2013	$\bar{x}$	Perlyna Lisostepu	Bilotserkivska napivkarlykova	Podolyanka
7 KS	1.44	1.98	1.05	1.49	-	+0.19	+0.11
8 KS	1.49	1.53	1.25	1.42	-0.07	+0.12	+0.04
42 KS	1.54	1.78	1.56	1.63	+0.14	+0.33	+0.25
29 KS	1.18	1.58	1.77	1.51	+0.02	+0.21	+0.13
26 KS	1.01	1.11	1.32	1.43	-0.06	+0.13	+0.05
24 KS	1.91	1.86	1.42	1.73	+0.24	+043	+0.35
12 KS	1.04	1.13	2.04	1.40	-0.09	+0.10	+0.02
44 KS	1.65	1.76	1.27	1.56	+0.07	+0.26	+0.18
54 KS	1.28	2.19	1.83	1.77	+0.28	+0.47	+0.39
22 KS	1.13	1.74	1.55	1.47	-0.02	+0.17	+0.09
17 KS	1.01	1.85	1.48	1.45	-0.04	+0.15	+0.07
Standards							
Perlyna Lisostepu	1.43	1.83	1.21	1.49	-	-	-
Bilotserkivska napivkarlykova	1.13	1.74	1.04	1.30	-	-	-
Podolyanka	1.26	1.92	0.97	1.38	-	-	-
SD_{05}	0.11	0.06	0.02	-	-	-	-

On average over three years, the lowest variability of grain weight from the main ear (0.24–0.28 g) and insignificant coefficients of variation (CV = 8.7; 10.0%) were observed in the lines 42 KS and 8 KS. At the same time, 42 KS had significantly 0.14 g more grain weight of the main ear than all standard (Table 2).

Table 2. Parameters of variability by weight of grain from the main ear (average for 2011–2013)

Lines	$\bar{x}$, g	Lim (g)		R, g	S^2	CV, %
		min	max			
7 KS	1.49	1.05	1.98	0.93	0.22	31.5
8 KS	1.42	1.25	1.53	0.28	0.02	10.0
42 KS	1.63	1.54	1.78	0.24	0.02	8.7
29 KS	1.51	1.18	1.77	0.59	0.09	19.9
26 KS	1.43	1.01	1.32	0.31	0.03	12.1
24 KS	1.73	1.42	1.91	0.49	0.07	15.3
12 KS	1.40	1.04	2.04	1.00	0.31	39.8
44 KS	1.56	1.27	1.76	0.49	0.07	17.0
54 KS	1.77	1.28	2.19	0.91	0.21	25.9
22 KS	1.47	1.13	1.74	0.61	0.10	21.5
17 CC	1.45	1.01	1.85	0.84	0.18	29.3
Standards						
Perlyna Lisostepu	1.49	1.21	1.83	0.62	0.10	21.2
Bilotserkivska napivkarlykova	1.30	1.04	1.74	0.70	0.15	29.8
Podolyanka	1.38	0.97	1.92	0.95	0.24	35.5

The average coefficient of variation (CV = 12.1–19.9%) was characterized by the lines 26 KS, 24 KS, 44 KS and 29 KS. In lines 7 KS, 12 KS, 54 KS, 22 KS, 17 KS and standards, in yearsof researches, on weight of grain from the main ear, the greatest variability (0.61–1.00 g) with a coefficient of variation is defined (21.2–39.8%).

Significant differences in the studied selection forms, in the amplitude of variability of grain mass from the main ear, as well as insignificant, average and significant coefficients of variation, indicating their differentreaction to environmental conditions were established. It is due to the interaction of genotype with the environment. Using analysis of variance, it was found that the share of variability caused by the interaction of genotype and environment factors had the greatest impact (51.49%) on the formation of grain mass in the main ear. The influence of the conditions of the year was at the level of 25.76%, and the genotype –21.69% (Fig. 1).

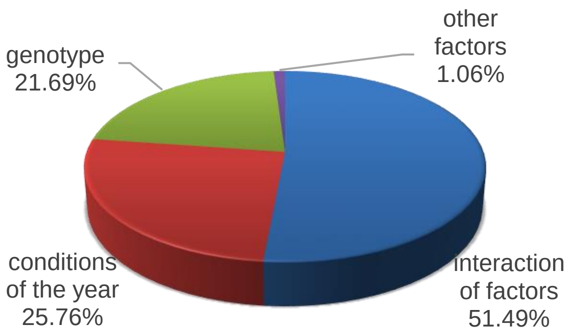

Figure 1. The share of the factors influence in the total variance according to the level of manifestation of the grain mass from the main ear (average for 2011–2013).

The results of our research coincide with the data of the Manukyan et al (2019) which found that the genotypes of the studied samples (the 'cultivar' factor) had the highest impact on the overall variability of productivity - their proportion was 50%. The proportion of variability caused by the influence of environmental conditions (the 'year' factor) was 26.5%.

To identify the mechanisms of plasticity and stability of new genotypes, it is necessary to focus on known cultivars with different types of resistance and plasticity (Kocherina, 2009).

The results of our research show that by the homeostatic indicators of grain weight of the main ear, standards (Hom = 3.93–7.06) exceeded the lines 29 KS (Hom = 7.57), 26 KS (Hom = 8.31), 44 KS (Hom = 9.47), 24 CS (Hom = 11.10), 8 CS (Hom = 13.38) and 42 CS (Hom = 19.87) (Table 3).

According to the selection value (Sc), the excess over the standards (Sc = 0.70–0.99) was observed in 29 KS (Sc = 1.01), 54 KS (Sc = 1.03), 44 KS (Sc = 1.13), 8 KS (Sc = 1.16) and 24 KS (Sc = 1.29).

The analysis showed that the line 54 KS had more than the average weight of the grain of the main ear (1.77 g) and specific adaptability to favorable conditions (bi = 2.03). Podolyanka (bi = 2.05), Bilotserkivska napivkarlykova (bi = 1.75) and samples 17 KS (bi = 1.91) and 7 KS (bi = 1.80) were determined to be the most sensitive to improved growing conditions. Perlyna Lisostepu and 22 KS had coefficients bi at the level of 1.22 and 1.32. Low-plastic were 42 KS (bi = 0.64), 44 KS (bi = 0.62), 29 KS (bi = 0.60) and 8 KS (bi = 0.31). The line 24 KS had a high grain weight of the main ear (1.73 g) and one of the minimum indicators (bi = 0.27).

Indicators of general adaptive capacity of standards (GAC = 1.73–2.18) exceeded lines 7 KS, 44 KS, 42 KS, 54 KS and 24 KS with an indicator (GAC = 2.29–2.76) (Table 4).

Table 3. Homeostaticity and adaptability by weight of grain of the main ear, (average for 2011–2013)

Lines	Grain weight, g	Adaptability parameters			
		Hom	Sc	bi	σ_{di}
7 KS	1.49	4.75	0.79	1.80	0.16
8 KS	1.42	13.38	1.16	0.31	0.04
42 KS	1.63	19.87	1.41	0.64	0.00
29 KS	1.51	7.57	1.01	0.60	0.15
26 KS	1.43	8.31	0.88	0.01	0.05
24 KS	1.73	11.10	1.29	0.27	0.14
12 KS	1.40	3.56	0,72	-0.59	0.58
44 KS	1.56	9.47	1.13	0.62	0.10
54 KS	1.77	6.81	1.03	2.03	0.07
22 KS	1.47	6.95	0.96	1.32	0.05
17 KS	1.45	4.97	0.79	1.91	0.05
Standards					
Perlyna Lisostepu	1.49	7.06	0,99	1.28	0.06
Bilotserkivska napivkarlykova	1.30	4.46	0.78	1.75	0.03
Podolyanka	1.38	3.93	0.70	2.05	0.12
Statistical parameters					
$\bar{x}$	**1.50**	**8.01**	**0.97**	**1.00**	**0.11**
Min	1.30	3.56	0.70	-0.59	0.00
Max	1.77	19.87	1.41	2.05	0.58

Table 4. Parameters of adaptive capacity and stability by weight of grain from the main ear (averagefor 2011–2013)

Lines	Grain weight, g	GAC	σ^2(GxE)gi	σ^2SACi	σSACi	Lgi	Sgi	GSVi	Kgi
7 KS	1.49	2.29	0.11	0.22	0.47	0.23	31.31	0.46	2.66
8 KS	1.42	1.95	0.04	0.02	0.15	0.26	10.55	1.09	0.28
42 KS	1.63	2.36	0.01	0.02	0.13	0.04	8.09	1.34	0.21
29 KS	1.51	1.87	0.08	0.09	0.30	0.27	19.90	0.85	1.10
26 KS	1.15	1.08	0.07	0.02	0.16	0.42	13.69	0.80	0.30
24 KS	1.73	2.76	0.09	0.07	0.27	0.34	15.54	1.14	0.88
12 KS	1.40	1.37	0.40	0.31	0.55	0.72	39.39	0.18	3.74
44 KS	1.56	2.35	0.06	0.07	0.26	0.22	16.43	0.99	0.80
54 KS	1.77	2.60	0.08	0.21	0.46	0.17	25.92	0.75	2.56
22 KS	1.47	1.90	0.03	0.10	0.31	0.09	21.14	0.78	1.19
17 KS	1.45	1.87	0.06	0.18	0.42	0.14	29.07	0.52	2.16
Standards									
Perlyna Lisostepu	1.49	2.18	0.03	0.10	0.31	0.10	21.05	0.80	1.20
Bilotserkivska napivkarlykova	1.30	1.73	0.04	0.14	0.38	0.10	29.18	0.46	1.77
Podolyanka	1.38	2.02	0.11	0.24	0.49	0.22	35.17	0.31	2.89
Statistical parameters									
$\bar{x}$	**1.48**	**2.02**	**0.09**	**0.13**	**0.33**	**0.24**	**22.60**	**0.75**	**1.55**
min	1.15	1.08	0.01	0.02	0.13	0.04	8.09	0.18	0.21
max	1.77	2.76	0.40	0.31	0.55	0.72	39.39	1.34	3.74

The lines 42 KS, 24 KS, 44 KS and 29 KS by grain weight of the main ear exceeded the standards and had lower values of σ²SACi (0.02–0.09).

The indicators of GSVi standards exceeded 42 KS (GSVi = 1.34), 24 KS (GSVi = 1.14), 8 KS (GSVi=1.09), 44 KS (GSVi = 0.99) and 29 KS (GSVi = 0.85).

We investigated that the lower relative stability of the genotype (Sgi) and significantly higher grain weight from the main ear, compared with the standard Pearl of the forest-steppe had 44 KS (Sgi = 8.09) and 24 KS (Sgi = 15.54).

Lines of soft winter wheat, characterized by an above average weight of grain from the main ear, had a predominantly linear response to environmental conditions (Lgi = 0.04–0.34). The compensation-destabilization coefficient (Kgi) varied in the experiment from the compensating (Kgi = 0.21) to a clear destabilizing (Kgi = 3.74) level. The compensating effect (Kgi < 1) among the lines that significantly exceeded the standards for grain weight from the main ear was observed in 24 KS and 42 KS.

The results of ranking the studied genotypes by grain weight of the main ear and indicators of plasticity and stability show that the first place in the rating of adaptability of the variety was taken by the line 42 KS, which by the minimum manifestation of the trait, σ²SACi, Sgi, SGVi, Hom, Sc and σ_{di} was the first, by the average value of the trait, GAC and the coefficient bi was the third, and by the maximum manifestation of the trait was the eighth (Table 5).

Table 5. Ranks by grain weight from the main ear, plasticity, stability and adaptability rating (averagefor 2011–2013)

Lines	Ranks by grain weight from the main ear and adaptability parameters											Average rank	* X / average rank	Rating
	X	min	max	GAC	σ²SACi	Sgi	SGVi	Hom	Sc	bi	σ_{di}			
42 KS	3	1	8	3	1	1	1	1	1	3	1	2	0.75	1
24 KS	2	2	5	1	5	4	2	3	2	7	11	4	0.43	2
44 KS	4	4	10	4	4	5	4	4	4	4	9	5	0.31	3
54 KS	1	3	1	2	11	9	9	9	5	12	8	6	0.28	4
8 KS	11	5	13	8	2	2	3	2	3	6	3	5	0.27	5
Perlyna Lisostepu	7	6	7	6	8	7	7	7	7	1	7	6	0.23	6
29 KS	5	7	9	10	6	6	5	6	6	5	12	7	0.22	7
22 KS	8	8	11	9	7	8	8	8	8	2	5	7	0.20	8
7 KS	6	9	3	5	12	12	12	11	11	9	13	9	0.16	9
17 KS	9	12	6	11	10	10	10	10	10	10	4	9	0.16	10
26 KS	10	13	14	14	3	3	6	5	9	11	6	9	0.13	11
Bilotserkivska napivkarlykova	14	11	12	12	9	11	11	12	12	8	2	10	0.13	12
Podolyanka	13	14	4	7	13	13	13	13	14	13	10	12	0.12	13
12 KS	12	10	2	13	14	14	14	14	13	14	14	12	0.11	14

Second and third place in the RAS, due to the optimal combination of grain weight from the main ear and the parameters of adaptability, took 24 KS and 44 KS, respectively.

Plasticity is to be understood as the ability of a variety to combine a sufficiently high yielding capacity with its stability under varying conditions, while genotypes with hyperreaction to growth conditions are to be considered sensitive to these conditions (Lytvynenko et al., 2013.).

As a result of estimation of lines by the weight of grain from the main ear and indicators of plasticity and stability selection forms, 42 KS, 24 KS and 44 KS were selected, as they are of practical interest for selection work.

The experimental data show that the correlation between grain weight from the main ear and grain yield was at the level of direct strong (0.747–0.871) in 2012–2013 and very strong, close to functional (0.914) in 2011. The direct influence of grain weight from the main ear at the level of strong and very strong, close to functional (0.805–0.942) on the weight of grain from the plant was established. Thus, it can be argued that the main ear plays an extremely important role in shaping the productivity of wheat plants and grain yields (Table 6).

Table 6. Correlation relations (r) of grain mass from the main ear with elements of productivity and grain yield of soft winter wheat

Indicators	2011	2012	2013
Aboveground mass of the plant	0.877*	0,513	0.805*
The mass of the main stem	0.964*	0.780*	0.882*
The weight of the main ear	0.995*	0.870*	0.909*
The mass of straw of the main stem	0.891*	0.387	0.802*
The length of the main stem	0.344	0.202	0.751*
The length of the ear-bearing internode	0.615*	0.451	0.613*
The length of the internode, second from the top	0.315	0.285	0.637*
The length of the main ear	0.678*	0.364	0.778*
The number of spikelets in the main ear	0.531	0.580*	0.532
The number of grains from the main ear	0.804*	0.897*	0.941*
The number of grains in the spikelet of the main ear	0.579*	0.625*	0.887*
The mass of grain from the plant	0.942*	0.887*	0.805*
The mass of 1,000 grains of the main ear	0.672*	0.674*	0.603*
Grain yield	0.914*	0.871*	0.747*

* probably at $P < 0.05$.

Direct correlations at the level of significant and strong (0.513–0.877) are observed between the mass of grain from the main ear and the aboveground mass of the plant. At the level of strong and very strong, close to functional, the correlation between the grain mass of the main ear is determined: with the mass of the main stem (0.780–0.964); mass of the main ear (0.870–0.995); and the number of grains from the main ear (0.804–0.941). The correlation between the grain weight of the main ear and the straw mass was less close (0.387–0.819) and was characterized as moderate and strong.

Studies have shown a significant positive correlation between grain weight per spike and spikelet weight per spike (Green et al., 2012).

There was a significant correlation between the weight of the grain from the ear and the weight of 1,000 grains (0.603–0.674) and the number of ears (0.531–0.580). The correlation between the mass of grain from the ear and the number of grains in the ear was not stable and varied from significant (0.579–0.625) in 2011–2012 to strong (0.887) in 2013.

The correlation between the mass of grain from the main ear was insignificant with the length of the stem (0.202–0.751), the length of the spikelet node (0.451–0.615), the length of the internode that is second from the top (0.285–0.637), the length of the main ear (0.364–0.778).

CONCLUSIONS

Thus, the lines of soft winter wheat 42KS, 24 KS and 54 KS were characterized by high grain weight from the main ear (1.63–1.77 g). As a result of selection by grain mass from the main ear and other economically valuable traits, properties and indicators of adaptability, lines of soft winter wheat 44 KS, 42 KS, 24 KS were involved in 2014 in hybridization in different combinations of crosses. Selected lines as a result of research (2015–2020) from these populations of soft winter wheat are competitively tested in the conditions of Bila Tserkva Research and Selection Station, forming high grain yields (7.39–8.12 t ha^{-1}) and will be transferred to 2021 for the State variety test for inclusion in the Register of plant varieties suitable for distribution in Ukraine.

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Alleviation of technological stresses by a feed supplement

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Abstract. Technological stresses affect the productivity of broiler-type chicken and are related directly to poultry farming intensification. Heat stress occurring in conditions of high density keeping, especially at high summer ambient temperatures, is very important. Stress factors influence the metabolic processes in poultry, while reducing the production parameters of operation and, by extension, the efficiency of broiler farming.
The objective of this research was to identify the efficiency of a feed supplement in alleviating technological stresses in poultry industry.
Use of supplement (the preparation contains succinic acid, L-carnitine, betaine, inorganic salts of zinc, manganese, copper and lithium) preserved the production performance of broiler-type chickens in the pre-slaughter period, by reducing the technological load on the poultry body, as it was indicated by an increase in the efficiency of fattening by 16.2 conditional units, meat yield by 1.4%, gross income by 49.2% and a decline in mortality rate by 1.5%. The data on production efficiency indicators of broiler feeding were obtained on a large sample - 6136 heads.

Key words: pre-slaughter period, technological stress, heat stress, poultry industry, broiler-type chicken, livability, growth, development, meat productivity, economic efficiency.

INTRODUCTION

Quick rearing and broiler fattening is usually associated with technological stress. The body is continuously under stress, since it responds to loading and transportation, deviations from the thermoneutral zone, the quality and quantity of feed, as well as changes in the social structure of the flock. Thus, it is essential to control stress originated from environmental factors in poultry. Feed supplements could serve as an alternative strategy of coping with these stressful treatments (Kavtarashvili & Kolokolnikova, 2010; Shatskikh & Molokanova, 2019; Shatskikh et al., 2020).

According to many authors, technological stresses in poultry industry result in decrease of body weight, livability rates and meat productivity, deterioration of meat quality, and thus- economic efficiency of poultry operation (Oliveira et al., 2018; Cândido et al., 2019). Notably, the greatest losses are observed in the pre-slaughter period (Jacobs et al., 2017b; Di Martino et al., 2017).

Catching stage causes a significant increase in production losses: the bird can be injured, therefore, the level of carcass defects rises and the death rate increases (Queiroz et al., 2015). It has been observed that the longer the bird remains in the hands of the catcher, the greater the chances of various types of injuries (Langkabel et al., 2015; Kittelsen et al., 2015a).

High ambient temperature during transportation is also a great stressor for poultry (Spurio et al., 2016). When the duration of transportation increases, the risk of coat contamination with poultry waste products increases, leading to a decrease in the sanitary condition of carcasses (Jacobs et al., 2017a).

Pre-slaughter period is very crucial and can contribute to great losses of birds. The mortality rate may depend on the immunity of poultry, method of catching, cage density, ambient temperature, conditions and duration of transportation, distance to the slaughter unit and conditions of pre-slaughter treatment (Grilli et al., 2015; Vieira et al., 2015; Jacobs et al., 2017b; Di Martino et al., 2017). The most frequent causes of broiler-type chicken death in the pre-slaughter period are the so-called 'sudden death syndrome' and injuries, including fractures and hepatorrhexis (Kittelsen et al., 2015b).

In view of this, the aim of this study was therefore to examine the effectiveness of a feed supplement in alleviating technological stresses in broilers.

MATERIALS AND METHODS

Research and production experiment was performed following the conditions of an industrial-type poultry plant, with Arbor Acres broiler-type chickens.

The applied feed supplementis protected by copyright (Miftakhutdinov et al., 2019), and included vitamin-like and mineral substances such as: succinic acid - 37.0–38.0%, L-carnitine - 5.0–6.0%, betaine - 14.0–15.0%, inorganic salts of zinc - 11.5–11.7%, manganese - 11.5–11.7%, copper - 2.6–2.8% and lithium - 16.5–16.7%.

Birds were allocated into 2 groups: group I – control (n = 6,136), group II – experimental (n = 6136). Poultry was kept in different units, with similar housing conditions. Broiler-type chickens of the experimental group received feed additive at a dose of 1,269 g L^{-1} ton of feed 5 days before slaughter, the poultry in the control group did not receive any additive in the diet. In the course of the experiment, the clinical status of birds, feeding and keeping conditions were checked. On day 38, broilers were slaughtered. Scheme of the experiment is shown in Table 1.

Table 1. The scheme of the experiment

Group	Heads quantity	Poultry feeding
I group – control	6,136	Main diet
II group – experimental	6,136	Main diet + feed additive 5 days before slaughter

Before slaughter, blood from the bird axillary vein was taken, into vacuum tubes with a coagulation activator. The sample size was 9 heads. The total protein was determined by the refractometric method, urea - by the urease phenol hypochlorite method, creatinine - by the Jaffe color reaction, total lipids - by the color reaction with the sulfophosphovanilic reagent, cholesterol - by the Ilk method, glucose - by the glucose oxidant method, calcium - by the Wilkinson complexometric method, phosphorus - by photometric method with ammonium molybdate.

Poultry growth and development were controlled by individual weighing on Roxell scales. On the basis of weighing, the absolute and average daily gain was calculated.

Broiler-type chicken meat productivity was determined by cutting carcasses and establishing their morphological composition.

Further analyses were conducted in the laboratory of the innovation research center of South Ural State Agrarian University. Production indicators of poultry feeding efficiency and the results of assessing the meat productivity of broiler-type chickens are presented by the poultry farm. Statistical processing of the results of biochemical blood tests and the distribution frequency of causes of mortality in poultry in groups was carried out using the STATISTICA 12 program. Economic efficiency was calculated in accordance with the methodology developed by N.A. Zhuravel and co-authors (Zhuravel et al., 2019).

RESULTS AND DISCUSSION

Biochemical blood tests findings were within the reference values (see Table 2).

In the blood serum of broiler-type chickens of the experimental group, at the level of significant differences, there was a decrease in urea concentration - end product of nitrogen metabolism by 22.54%. The data obtained indicate the maintenance of the protein level in the experimental group of poultry and increased protein utilization in the control.

In the experimental group, the tendency to total lipids content decrease by 6.12% indicated the mobilization of lipid metabolism for the development of adaptive mechanisms, while in the control group, the process of lipid accumulation by the body was observed.

A higher calcium content in the experimental group by 22.5% may indicate an increase in minerals absorption from feed, due to a general improvement in the metabolic functions of the body.

Table 2. Biochemical blood status of broile-type chickens ($X \pm S\bar{x}; n = 9$)

Indicator	Group	
	Control	Experimental
Total protein, g L^{-1}	33.70 ± 0.98	35.65 ± 1.07
Urea, mmol L^{-1}	2.56 ± 0.10	1.99 ± 0.13**
Creatinine, mmol L^{-1}	21.43 ± 1.06	23.88 ± 1.74
Total lipids, g L^{-1}	5.72 ± 0.16	5.37 ± 0.13*
Cholesterol, mmol L^{-1}	4.85 ± 0.14	4.74 ± 0.12
Glucose, mmol L	10.89 ± 0.44	10.12 ± 0.42
Calcium, mmol L^{-1}	2.50 ± 0.13	3.06 ± 0.10**
Phosphorus, mmol L^{-1}	2.17 ± 0.06	2.28 ± 0.12
Calcium-phosphorus ratio	1.15	1.34

Note, hereinafter: * – differences are significant at $P \leq 0.1$; ** – differences are significant at $P < 0.05$.

Average daily and absolute total wight gain, livability and the efficient coefficient are presented in Fig. 1.

Data on production efficiency indicators for broiler-type chickens feeding are presented in average values obtained on a large sample - 6,136 heads. Average daily gain of broiler-type chickens in the experimental group was higher by 0.7%. The absolute increase was 2.3% in comparison with the control group. Data obtained suggested a better feed conversion in the experimental group. Owing to higher indicators of live weight gain and poultry livability in the experimental group, the efficiency coefficient was higher by 16.2 conditional units. The control group was lower than the experimental group in all the studied production parameters: the use of the developed additive had a positive effect on the average daily gain and poultry livability, whereby an additional 288 kg of meat was obtained.

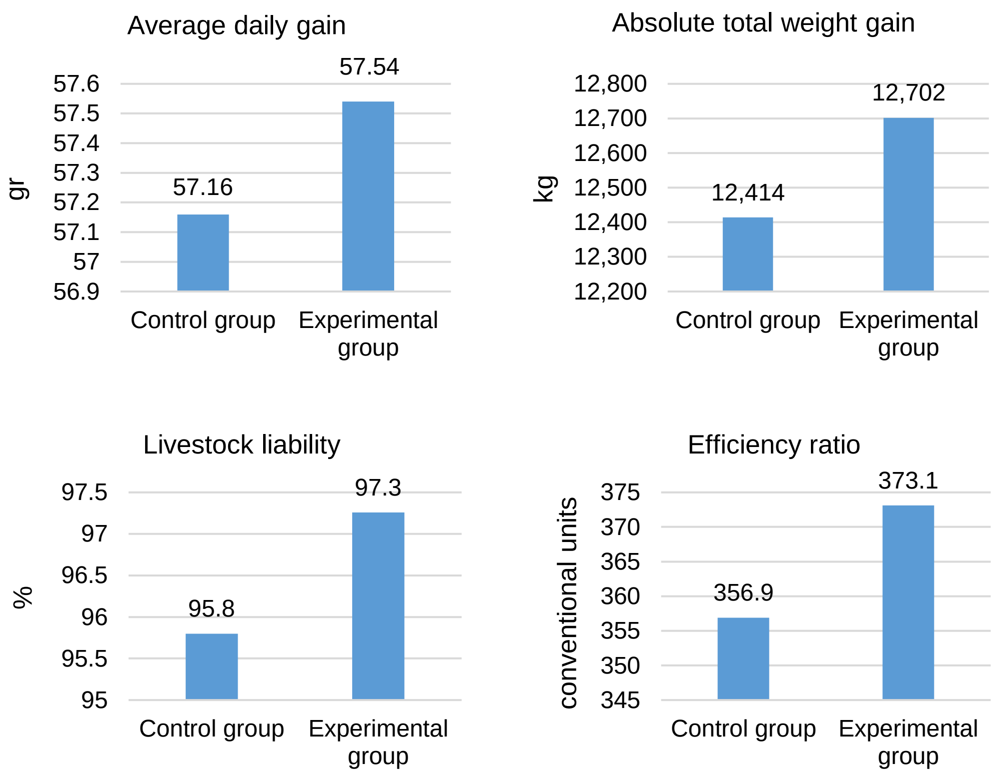

Figure 1. Production indicators of the efficiency of broiler-type chickens ' fattening (n = 6,136).

A living body continuously adapts to changing environmental conditions and maintains the necessary level of homeostasis. The trigger for the development of adaptation is the nervous system in connection with the endocrine system. Started flood of internal reactions is marked by the mobilization of energy and plastic resources of the body, owing to which the formation of an adequate response to the stressor occurs. Nevertheless, the physiological body reserves are progressively depleted, especially when the effect of stress factors does not stop or is reinforced by the appearance of new ones. Disorders of homeostasis are followed by changes in metabolism, dysfunction of organs and their systems. If the changes are nonreversible, it can lead to high mortality rates (Kavtarashvili & Kolokolnikova, 2010).

In the birds of the control group that experienced a high stress load at the peak of their productivity, a reduction in the average daily gain owing to the utilization of the body's resources for the development of adaptive mechanisms was observed, whereas in the experimental group feed additive components were consumed in response to the stress impact. Lithium salts by interfering the conductivity of the nerve fiber (Egbert & Ploeger, 1974) may have contributed to the sparing use of body reserves. The antioxidant properties of the components protected the cells from oxidative stress, which is the basis of free radical damage to tissues and organs (Olukosi et al., 2019).

In the experimental group, at the level of trends, there was a lower mortality rate from diseases such as pulmonary edema, intestinal atony, glandular stomach atony and enteritis (see Fig. 2).

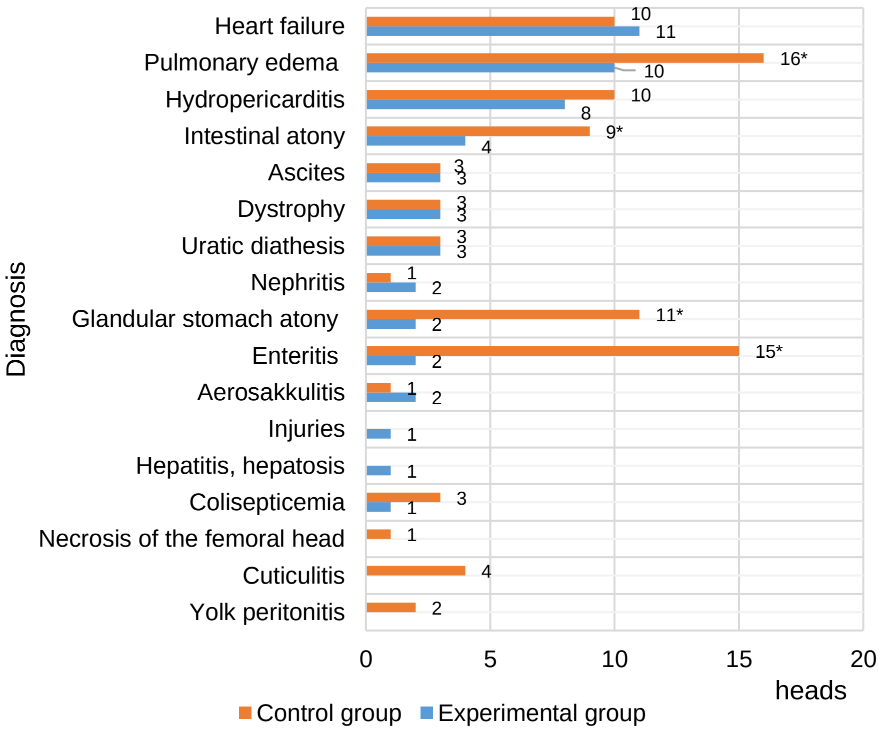

Figure 2. Chicken mortality causes in the experiment period.

Evaluating the reasons for poultry deaths, it was revealed that on day 33–35 in the experimental and control groups, the mortality rate did not exceed 0.1% of the total number of birds, according to the poultry farm requirements for this period. On the 36th day, within the limits of permissible values, there was an increase in the mortality rate of broiler-type chickens in the control group to 0.2%, in contrast to the experimental broiler-type chickens, where the mortality values remained at the same, lower level. By contrast, the mortality rate of broiler-type chickens in the experimental group remained the same. On the final day of fattening process, an increase in mortality rates was observed, with the lowest value of this indicator in the experimental group and the highest in the control group.

No infection episodes were reported during the period of broiler chickens' operation. The same dynamics and etiological factors of death of broiler-type chickens were found in the experimental and control groups. In the experimental group, there was a lower mortality of poultry by 1.5%, owing to higher liability at the final stage of fattening.

Pre-slaughter stage of poultry keeping is characterized by a complex of veterinary, sanitary and technological measures. Prior to slaughter, the poultry is subjected to starvation exposure, clinical examination, catching, loading, transportation, unloading, hanging on the conveyor line, stunning and bleeding. Due to physical impact, the birds could be injured at each stage. Scratches, dislocations, hematomas, hurts, bruises, and fractures appear. Injuries lead to an increase in the defective product level, and meat from stress-sensitive poultry can develop signs of PSE and DFD. By reducing the susceptibility of poultry to the effects of stressors and regulating adaptive mechanisms, a reduction in the number of broiler-type chickens ' injuries and carcass defects is accomplished, as well as an improvement in the meat quality (Miftakhutdinov et al., 2020).

The applied feed supplement appeared to mitigate the negative impact of stress factors on the meat productivity of broiler-type chickens (see Fig. 3).

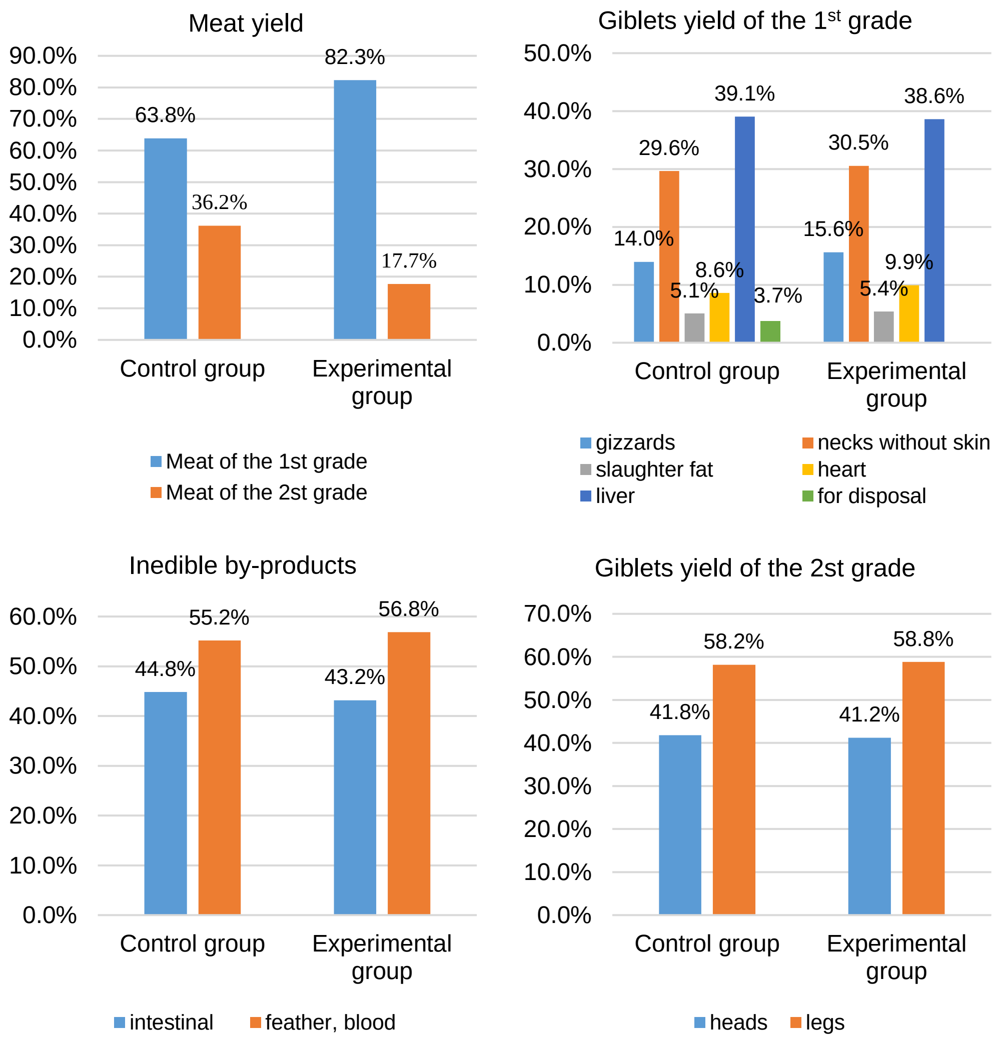

Figure 3. Meat productivity of broiler-type chickens (n = 50).

The data on poultry meat productivity represent the results of anatomical cutting of 50 broiler-type chickens (from each group) and are obtained by summing the total weight of meat, by-products and inedible by-products.

In the experimental group of broiler-type chickens, there was an increase in the total yield of poultry meat by 1.4%, including grade 1 - by 18.5%, a decrease in the yield of by-products of categories 1 and 2 by 0.6 and 0, 9% respectively in relation to the control group.

This positive dynamics in the growth indicators of experimental poultry, livability and meat productivity has led to a reasonable increase in economic efficiency (see Fig. 4).

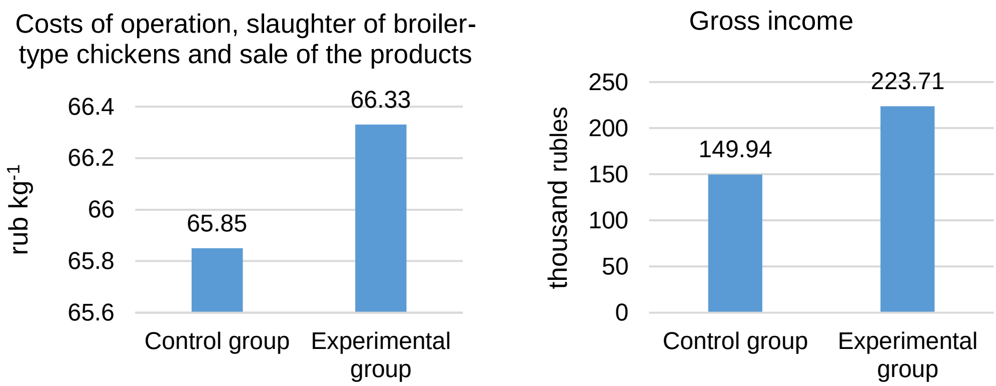

Figure 4. Economic efficiency of the supplement application.

Owing to additional costs for the purchase of components and preparation of feed, the costs of operation, slaughter and sale of products raised and exceeded the control indicator by 0.7% in the experimental group. Nevertheless, operation, livability of livestock, meat yield of the 1st grade enabled to increase the gross income from sales of the experimental group's products by 49.2% and to get additional profit in the amount of 73.77 thousand rubles.

Our research results are compatible with the ones of a number of authors (Fisinin et al., 2016; Kuzminova et al., 2018; Redka et al., 2018; Sobolev et al., 2019; Olukosi et al., 2019; Feng et al., 2020; Tufarelli et al., 2020).

Application of a complex lithium-containing pharmacological supplement had a positive effect on the livability of broiler-type chickens, which in the control group during the study period was lower by 1.24% than in the group where the supplement was used for stress prevention. It is explained by the fact that the components of the pharmacological supplement have adaptogenic properties, forming a mechanism for compensating the resource costs of the body for the development of adaptive processes under stress (Fisinin et al., 2016).

The use of the additive, containing succinic acid, for broiler-type chickens made it possible to achieve high preservation and growth in poultry live weight, by increasing the adaptive potential and optimizing the biochemical processes in the body. By the end of the experiment, the bird of the experimental group surpassed the control in terms of the total protein content in the blood by 9.7–12.2%, live weight - by 7.5%, viability - by 4.0% (Kuzminova et al., 2018).

The addition of lithium salts to the poultry diet increased the intensity of metabolic processes and contributed to higher productivity by increasing the deposition of nutrients in the body. At the same time, the average daily gain in poultry live weight in the experimental groups was higher than the control by 1.6–2.5%. (Sobolev et al., 2019).

Experiments on the influence of copper and zinc salts on metabolic processes showed an increase in the live weight of poultry (Olukosi et al., 2019). A meta-analysis of the evaluation also found a positive correlation between the intake of copper in the diet and the productivity of broilers (Feng et al., 2020). The use of a feed complex with zinc in its composition increased the protein content in the blood of the experimental group by 11.9–13.8% comparing to the control group. The authors determined an increase in the level of calcium and phosphorus in the blood, which they associate with better assimilation of these substances from feed (Redka et al., 2018).

Periods of operation and fattening broiler-type chickens are associated with intensive protein synthesis. The additional introduction of L - carnitine into the poultry diet, which has a positive effect on the growth and development of broiler-type chickens, promotes this process with a high degree of confidence ($p < 0.05$) (Tufarelli et al., 2020).

CONCLUSION

Therefore, application of a stress-inducing supplement in the pre-slaughter period enables to maintain high production rates of poultry breeding and, thereby, increase the efficiency of poultry farming. Owing to this, the use of a stress-inducing supplement in the pre-slaughter period allows to enhance the efficiency of poultry farming. As a result of the removal of technological stresses from poultry body, with the help of feed additive, the intensity of the increase in live weight is maintained, the level of preservation and meat grade is increased.

COMPLIANCE WITH ETHICAL STANDARDS:
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Productivity of winter wheat in the northern Steppe of Ukraine depending on weather conditions in the early spring period

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Abstract. The objective of the research was to develop scientific and methodological bases for adapting the technology of growing winter wheat depending on weather conditions in early spring. Winter wheat was grown at different sowing dates. It is established that the reserves of productive moisture in the soil at the time of renewal of spring vegetation are crucial for the formation of winter wheat harvest. After worse forecrop, the impact of moisture on productivity reaches 49.7–66.4%. The later the renewal of spring vegetation of winter wheat is, the lower the productivity of the crops. The shorter the period from the date of transition of the average daily air temperature above 0 °C to the beginning of active vegetation of plants is, the higher the productivity of winter wheat crops. Therefore, depending on weather conditions of early spring period and the time of renewal of spring vegetation of plants, it is necessary to adjust the technology of growing winter wheat during the spring-summer period.

Key words: winter wheat, weather conditions, sowing dates, timing of vegetation renewal, yield, protein.

INTRODUCTION

The most difficult task in the cultivation of field crops is successful adaptation of the already developed agricultural technologies in accordance with specific agro-climatic conditions. In general, the climate of the northern Steppe of Ukraine is favourable for growing most field plants, including winter wheat (Bazalii et al., 2014; Domaratskiy et al., 2017). It ranks second among cereals in terms of sown area. Most high-productive maize ranks first (Domaratskiy et al., 2017).

Winter wheat plants have the longest growing season among annual field crops. This fact has both its advantages and disadvantages compared to other crops. A long growing season allows managing the production process of crops more effectively. Adverse effects caused by unfavourable weather conditions can, to some extent, be successfully compensated in subsequent periods of plant growth and development.

Active plant growth occurs at all times of the year except winter. During the growing season in the northern Steppe of Ukraine there can be extremely negative factors including moisture deficit, high temperatures, plant diseases and pests (Bazalii et al., 2014; Vasylkovska et al., 2016, Mostipan, 2019).

In most years in the northern Steppe of Ukraine, the consequences of plant damage by below-freezing temperatures in winter are extremely negative factors for the formation of high productivity of winter wheat. Their aftereffect is extremely long, and therefore the process of crop density can occur before the ear stage of plants, and in some years even later. It is known that plants that are damaged in winter but survived have significantly lower individual productivity compared to undamaged plants (Mostipan & Umrykhin, 2018). Therefore, under such conditions, there is always a matter of feasibility and effectiveness of certain agronomic techniques that would increase individual productivity of plants (Mostipan et al., 2017; Pron et al., 2020).

Numerous studies have proven the undeniable role of weather conditions in the early spring period in the formation of winter wheat crop. The time of renewal of spring vegetation of plants is of particular importance (Lykhochvor, 2018; Mostipan & Umrykhin, 2018).

For the first time in Ukraine, V. Medynets established a significant influence of the time of spring vegetation of winter wheat plants on the productivity of its crops (Medynets, 1982). That is, the peculiarities of weather conditions during the renewal of spring vegetation of winter wheat plants have a decisive influence on the productivity of the crops. Moreover, for the first time he proposed effective agricultural techniques to improve grain quality and ensure the level of crop stability depending on the timing of vegetation renewal.

Based on the results of correlation analysis, M. Marenych (Marenych et al., 2010) is convinced that the optimal combination of precipitation and air temperature during grain filling is of high importance for obtaining high-quality winter wheat grain.

Also, studies by M. Marenych (Marenych et al., 2010) and co-authors found the relationship between protein content of winter wheat grain and the amount of precipitation in June (correlation coefficient is 0.74–0.81%). The correlation coefficient between gluten content and air temperature during May is 0.75–0.89%. According to the authors, in the conditions of the left-bank Forest-Steppe of Ukraine, the optimal combination of precipitation and air temperature during grain filling is extremely important for obtaining high-quality grain of winter wheat.

Scientists I. Brazhenko, V. Hanhur and others (Brazhenko et al., 2006) connect the influence of the time of spring vegetation renewal on the productivity of winter wheat crops in the left-bank Forest-Steppe of Ukraine with the circulation of warm or cold atmospheric air masses. It is argued that at different times of renewal of spring vegetation winter wheat plants have different opportunities for soil and air nutrition, which is reflected in their individual productivity. According to Bulgarian researcher E. Roumenina in the early stages of renewal of spring vegetation, plants are better provided with the main factors of life compared to the later dates (Roumenina et al., 2020).

The Polish researcher H. Bujak allows that it is no correlation between the yield of winter wheat and the total precipitation (Bujak et al., 2013). The soil type proved to be the environmental factor of particular importance in determining winter wheat yields. However, our research does not confirm this hypothesis.

Conditions of plant growth and development have a huge impact on the quality of winter wheat grain. According to O. Sozinov and V. Kozlov (Sozinov & Kozlov, 1970), the protein content in the grain of winter wheat by 70% depends on the conditions of the growing environment and only 30% on their genetic characteristics. Later studies by I. Pravdziva and N. Vasylenko (Pravdziva et al., 2017) proved that the influence of weather conditions on the protein content of winter wheat flour reaches 57%, and the effect of genotype does not exceed 5%.

Similar statements follow from the results of research by L. Bozhko and I. Burdeina (Bozhko & Burdeina, 2010). They argue that weather conditions during the growing season have a much greater impact on the protein content of winter wheat compared to agronomic methods of its cultivation.

In different parts of the world, by A. Linina and A. Ruza (Latvia), the total amount of grain varies annually, mainly due to changing climatic conditions owing to drought or excessive moisture (Linina & Ruza, 2018). Wheat yield is influenced by the interaction of a number of factors including cultivar, soil, climate, and cropping practices (Shejbalova et al., 2014).

It is important to know which agronomic techniques provide more rational use of basic plant life factors and, above all, moisture, because Kirovohrad region (Ukraine) belongs to the zone of insufficient moisture (Andriienko et al., 2020).

Therefore, the development of scientific and methodological bases for the application of ecological and adaptive technologies for growing winter wheat and their adjustment during the spring-summer period depending on weather conditions in the early spring is an extremely important issue.

The study of the relationship between the productivity level and moisture consumption in the interphase periods in the northern Steppe of Ukraine was studied for the first time.

In this regard, the main objective of our research was to develop scientific and methodological bases for adapting and adjusting the technology of growing winter wheat during the spring-summer vegetation season depending on weather conditions in early spring.

MATERIALS AND METHODS

Field studies were conducted in experimental crop rotation at the Institute of Steppe Agriculture of the National Academy of Agrarian Sciences of Ukraine. The dependence of winter wheat productivity on the time of spring vegetation renewal was studied during 1986–2005. It was sown after arable land and corn for silage in three terms: September 2nd, 17th and October 2nd. The influence of the level of moisture supply on the productivity of winter wheat crops was studied during 1992–2004. Wheat in this experiment was also sown after arable land and maize for silage, but at different times: August 25th, September 10th and 25th. This was due to the trend towards earlier sowing of winter crops due to climate change (Mostipan & Umrykhin, 2018). Yield calculation and identification of protein content in winter wheat grain were performed by generally accepted methods (USSR State standard specification 10846-91; Beljkaš et al., 2010). Measuring of soil moisture was performed by thermostatic weighting method according to CEN ISO/TS 17892-1:2007.

The soil of the experimental plot is ordinary chernozem with medium humus, heavy loam and deep, which is characterized by a very deep humus profile (80–100 cm) with a significant depth of humus horizon (40–50 cm) and a well-defined granular structure, which gradually turns into granular-fine-lump. The humus content is 4.54%. The content of moving forms of nutrients in the soil is 14.5 mg of hydrolyzed nitrogen, 12.1 mg of phosphorus and 15.7 mg of potassium per 100 g of soil. Identification of hydrolyzed nitrogen by Cornfield (Ukraine State standard specification 7863: 2015: Soil quality). The amount of absorbed bases is 39.4 mg per 100 g of soil, pH is 5.6. The identification was carried out according to ISO 10390:1994 Soil quality - identification of pH. The climate in the study area is temperate continental. The average annual air temperature, according to Kirovohrad meteorological station, is 7.9 °C, and the annual amount of precipitation is 474 mm, the main amount of which falls from May to September. The frost-free period lasts 164 days.

The statistical analysis methods, including MS-Office software, were used for the analytical study.

RESULTS AND DISCUSSION

Productivity of winter wheat crops depending on their level of moisture supply

Conditions for the growth and development of winter wheat plants during the spring-summer vegetation are extremely variable. Calendar terms of transition of average daily air temperature through 0 °C towards positive temperatures vary in a rather wide range. The earliest one can happen in early February, and the latest term takes place in the late third decade of March. Active growth of winter wheat plants begins in the period from the third decade of February to the first decade of April. From the time of renewal of spring vegetation of plants to firm-ripe stage of grain during the period of research there were 182 mm of precipitations on average. In some severely arid years, such as 1987 and 1996, only 79.1 and 60.9 mm fell, respectively. The maximum amount of precipitation during the spring-summer vegetation period fell in 1997 and 2004, respectively 317.8 and 279.9 mm.

The average daily air temperature during the spring-summer period of plant vegetation for the years of research was 15.9 °Con average. However, in cool years it might decrease to 13.3 °C, which was observed in 1990, or rise to the level of 18.1 °C in the hot, arid 1996.

Conditions for moisture supply of winter wheat crops at the beginning of active growth and development of plants differ significantly in different years. On average over the years of research, the reserves of productive moisture in a meter layer of soil at the time of renewal of spring vegetation on arable land was 164.4 mm with fluctuations from 124.2 to 210.0 mm, and after maize for silage - 156.3 mm with variation in different years in the range of 122.5–190.4 mm. Thus, water resources of crops at the beginning of spring vegetation can be characterized from acutely insufficient to the conditions of good moisture.

From the time of the cessation of autumn vegetation to its renewal in spring, the reserves of productive moisture in the soil increase. However, the absolute increases in the amount of available moisture for plants in the soil in different years can be completely different. Of the total amount of precipitation that falls during this period, only 34.2% is recorded in a meter layer of soil. In the years with the fall of rains in the late autumn or winter periods on the background of unfrozen soil, the efficiency of precipitation absorption increases and can exceed 70%.

Winter wheat crops in the northern Steppe of Ukraine are particularly sensitive to the amount of available moisture in the soil at the time of renewal of spring vegetation. When growing on arable land, the share of the influence of productive moisture reserves in a meter layer of soil at the beginning of spring vegetation renewal on crop formation is from 39.7% to 55.2%, and after maize for silage - 49.7–66.4% (Table 1). This pattern is characteristic of all different age crops of winter wheat. At the same time, the shift of sowing dates after maize for silage from early (August 25th) to later (September 25th) dates increases the dependence on the amount of productive moisture at the time of renewal of spring vegetation. If for crops sown on August 25th the share of the impact of moisture reserves on productivity is 49.7%, and for crops sown on September 25th it increases to 66.4%.

Table 1. Dependence of the productivity level of winter wheat crops of different ages on the reserves of productive moisture in the soil (average for 1992–2004), %

Sown after	Crop development phase	Sowing date		
		25.08	10.09	25.09
Arable land	renewal of spring vegetation	39.7	55.2	36.3
	start of booting	42.1	24.8	33.8
	ear stage	9.3	18.7	22.8
	other factors	8.9	1.3	7.1
Maize for silage	renewal of spring vegetation	49.7	51.3	66.4
	start of booting	9.8	9.8	10.6
	ear stage	39.9	34.8	22.3
	other factors	0.6	0.3	0.7

Forecrops have a huge impact on water and nutrient regimes of winter wheat crops. It is known that the better the conditions for plant growth and development are, the greater the density of winter wheat crops at the time of cessation of autumn vegetation. When sowing winter wheat on arable land, its plants have greater bushiness, air-dry mass and a well-developed primary and secondary root system. After the worst forecrops, the biometric performance of plants is lower. The results of the research show that the crops of winter wheat sown on arable land and after maize for silage differ in terms of sensitivity to the reserves of productive moisture in the soil at different stages of plant development. Productivity of winter wheat crops after arable are mainly determined by the amount of available moisture in a meter layer of soil at the time of spring vegetation renewal and at the beginning of plant booting, while productivity of crops after maize for silage is determined by the time of spring vegetation renewal and the ear stage of plants. That is, it possible to state that for more developed crops after arable land, soil moisture reserves at the beginning of the booting phase are also important for crop formation. Their influence on productivity is in the range of 24.8–42.1%.

Different age crops of winter wheat after maize for silage show a specific dependence on the content of productive moisture in the soil at the time of renewal of spring vegetation and in the stage of earing. The shift of sowing dates from August 25th to September 25th increases their dependence on productive moisture reserves for the time of spring vegetation renewal from 49.7% to 66.4% and at the same time reduces their moisture reserves in the ear stage from 39.9% to 22.3%.

The water needs of winter wheat plants change during their growth and development. During the booting period, when the above-ground mass of plants accumulates intensively, and the grain is filled, winter wheat plants are too sensitive to water deficiency. Droughts in these periods lead to significant crop losses. However, the absolute water consumption of winter wheat crops does not always coincide with the physiologically important needs of plants in water for crop formation. The obtained results show that the total water consumption by winter wheat crops for the whole vegetation period from a meter layer of soil is on average 342.9 mm and almost does not depend on the forecrops. At the same time, the transfer of sowing dates from early to late period reduces the total water consumption from 361.3 to 330.3 mm for arable from 361.5 to 330.3 when growing winter wheat after maize for silage (Table 2, Fig. 1, Fig. 2).

The highest water consumption by winter wheat crops is observed in the period of 'renewal of spring vegetation - start of booting' and during the 'ear stage - firm-ripe stage'. Of the total amount of water consumed by crops during the entire growing season, the share of arable costs is 28.4% and 35.2%, respectively, and after maize for silage - 26.4% and 34.1%. For comparison, during the autumn period water consumption was 14.1% after arable land and 17.1% after maize for silage. During the period of plant booting, water consumption increases to the level of 22.0–22.2%, but it is lower compared to the two previously mentioned periods.

Table 2. Water consumption by winter wheat crops from a meter layer of soil depending on sowing dates (average for 1992–2004), mm ha^{-1}

Sowing date	Indicators of water consumption in periods, mm ha^{-1}				
	Sowing - cessation of vegetation	Renewal of vegetation - start of booting	Start of booting - ear stage	Ear stage - firm-ripe stage	For the whole period
Sown after is arable					
25.08	71.1	94.3	74.6	121.2	361.3
10.09	36.6	100.4	77.2	124.0	338.2
25.09	37.4	98.1	77.2	117.7	330.3
average	48.4	97.6	76.3	121.0	343.3
Sown after maize for silage					
25.08	85.3	89.2	70.8	116.2	361.5
10.09	49.5	91.5	75.5	119.1	335.7
25.09	44.6	90.3	79.9	115.5	330.3
average	59.8	90.3	75.4	116.9	342.5

Studies have shown that in the northern Steppe of Ukraine the largest reserves of productive moisture in a meter layer of soil are achieved at the beginning of spring vegetation of winter wheat and then gradually decrease to the firm-ripe stage. Thus, on average over the years of research, the reserves of productive moisture after arable land from the time of spring vegetation to firm-ripe stage of grain decrease from 164.4 to 63.2 mm, and after maize for silage from 156.3 to 59.9 mm. Precipitation that falls during the period of active growth or accumulation of dry matter by plants does not change the existing dependence. Even after heavy rainfall, high soil moisture persists for a short period of time. This is especially evident in the significant development of aboveground vegetative mass of plants. Such plants, in the conditions of high air temperature, intensively transpirate and the amount of available moisture in a meter layer of soil decreases rapidly. This is also facilitated by the intense physical evaporation of water from the soil surface.

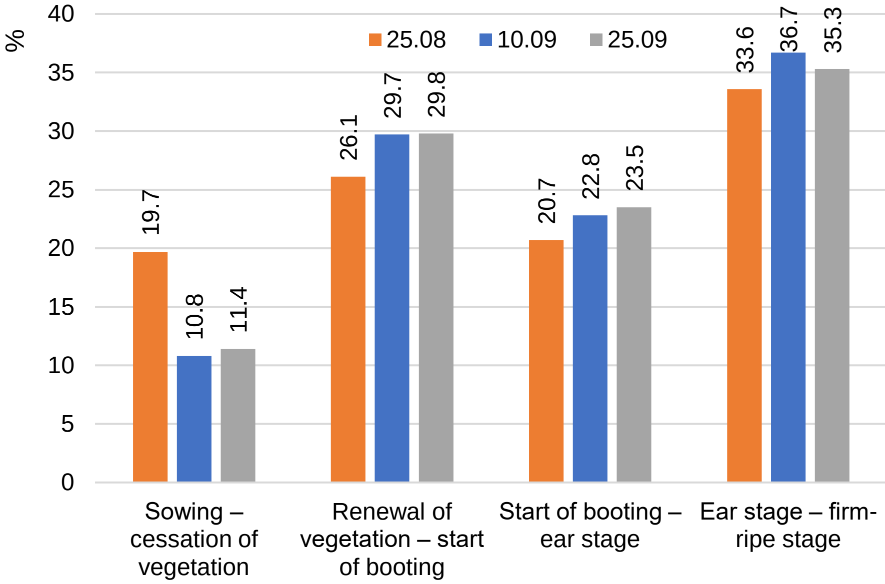

Figure 1. Dynamics of water consumption by winter wheat crops from a meter layer of soil depending on the sowing date (sown after arable land), %.

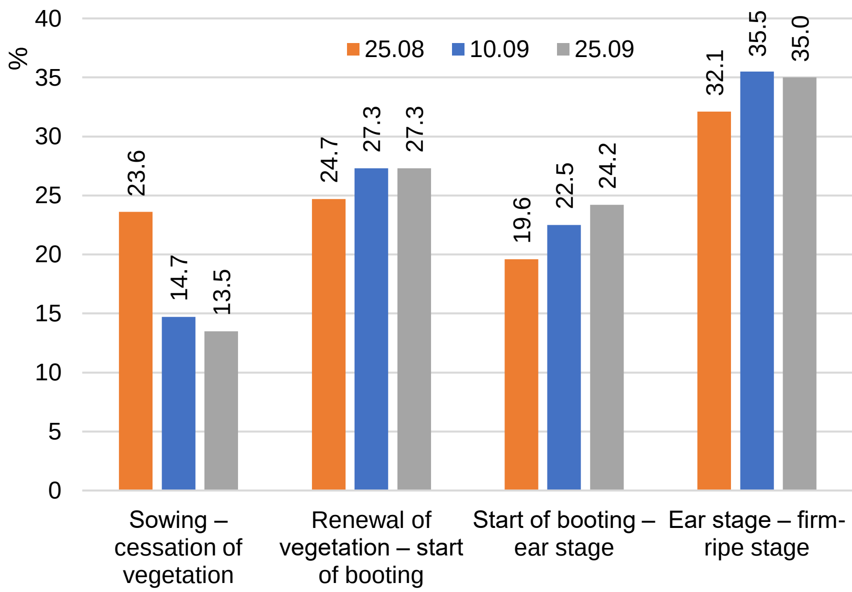

Figure 2. Dynamics of water consumption by winter wheat crops from a meter layer of soil depending on the sowing date (forecrop is maize for silage), %.

Analyzing the two periods of development of wheat crops with the highest water consumption, we note that during the ‘renewal of spring vegetation - start of booting’ water consumption is inefficient and they are mainly due to physical evaporation of water from the soil surface. Therefore, in practice, with the onset of physical maturity of the soil for winter wheat crops should be taken such measures that would reduce physical evaporation of water. This will save it for a later period increasing productivity.

Productivity of winter wheat crops depending on the time of renewal of spring vegetation of plants

In the northern Steppe of Ukraine, the renewal of spring vegetation of winter wheat plants can occur in a very wide range from the beginning of the third decade of February to the first decade of April. Therefore, it is proposed to distinguish very early renewal of vegetation (third decade of February), early (first - second decade of March), middle (third decade of March) and late (first decade of April) (Mostipan, 2019). The average daily air temperature from the time of renewal of spring vegetation to the beginning of booting of plants on average for the years of research was 8.93 °C with variation in different years from 4.78 °C to 12.95 °C. The sum of average air temperatures for the specified period was 260 °C with changes in different years from 57 °C to 437 °C. On average, 26.4 mm of precipitation fell from the time of spring vegetation renewal to the beginning of plant booting. In some years the amount of precipitation reached 83 mm, while in dry years the precipitation was completely absent. The earlier the vegetation of plants is renewed, the higher the productivity of winter wheat is (Table 3). This dependence is characteristic as forwinter wheatsown after arable, so forsown after maize for silage one. When growing winter wheat on arable land, the productivity of wheat in the years with very early renewal of vegetation is 6.74 t ha^{-1} against 3.68 t ha^{-1} in the years with late renewal. After maize for silage, the productivity is 6.11 and 2.9 t ha^{-1}, respectively.

Table 3. Water consumption by winter wheat crops from a meter layer of soil depending on sowing dates (average for 1986–2005), t ha^{-1}, %

Time of renewal of spring vegetation	Average	Arable land				Maize for silage			
		average	2.09	17.09	2.10	average	2.09	17.09	2.10
Productivity, t ha^{-1}									
Very early	6.42	6.74	6.96	6.81	6.44	6.11	6.13	6.18	6.02
Early	4.71	5.39	4.73	6.02	5.41	4.03	3.78	4.35	3.95
Middle	3.46	3.84	3.66	4.25	3.60	3.09	3.07	3.29	2.90
Late	3.29	3.68	2.95	4.38	3.72	2.90	2.55	3.04	3.12
Content of protein in grain, %									
Very early	12.57	13.12	13.29	13.22	13.16	12.02	11.92	12.13	12.47
Early	13.19	13.54	13.75	13.29	13.61	12.84	12.77	12.91	12.87
Middle	13.75	14.12	14.22	13.99	13.84	13.37	13.11	13.56	13.56
Late	13.13	13.90	13.88	13.77	13.74	12.35	12.46	12.16	12.55

The timing of the renewal of spring vegetation of plants can significantly change the impact of sowing dates on the productivity of winter wheat. In case of very early renewal of spring vegetation, the productivity is almost land in sops of different ages it varies from 6.44 to 6.96 t ha^{-1}, and after maize for silage - from 6.02 to 6.13 t ha^{-1}. At later dates of vegetation renewal, the highest productivity of winter wheat sown after both arable land and maize for silage is formed when sowing in the optimal time, that is on calendar dates close to September 17th.

Studies have shown that in the northern Steppe of Ukraine the highest protein content of winter wheat grain is formed in the years with the average renewal time of plant vegetation. This pattern is characteristic of all different age crops of winter wheat sown after both arable and maize for silage. On average, over the years of the research, the protein content of arable land during this period of vegetation renewal was 14.12%, and after maize for silage it was 13.11%. Both earlier and later renewal of vegetation leads to a decrease in the amount of protein in the grain of winter wheat. However, later renewal of vegetation provides higher protein content of winter wheat grain compared to earlier terms.

The weather conditions of the early spring period in the northern Steppe of Ukraine are too changeable. The timing of the onset of active growth of winter wheat plants can vary significantly depending on the conditions of the year. In some years, the duration of the period from the time of transition of the average daily air temperature through 0 °C to +5 °C, when the active growth of plants begins, can be only 2 days, while the maximum duration of this period is 46 days. It is proved that the longer this period is, the lower the productivity of winter wheat. The average productivity of winter wheat in the years with duration of this period up to 10 days is 5.31 t ha^{-1}, while in the years with the length of more than 30 days it decreases to 3.14 t ha^{-1} (Table 4).

Table 4. Influence of the duration of the period from the transition of the average daily above-freezing temperature to the renewal of spring vegetation on the productivity of winter wheat (1986–2005), t ha^{-1}, %

Duration, days	Average	Arable land				Maize for silage			
		average	2.09	17.09	2.10	average	2.09	17.09	2.10
Grain productivity, t ha^{-1}									
Up to 10	5.31	5.96	5.45	6.88	5.56	4.66	4.81	4.78	4.41
10–20	4.51	4.29	4.11	4.73	4.03	4.72	4.51	4.96	4.68
20–30	4.09	4.52	3.88	4.99	4.70	3.65	3.37	3.91	3.68
More than 30	3.14	3.89	3.15	4.52	3.99	2.39	2.07	2.69	2.41
Content of protein in grain, %									
Up to 10	13.25	14.15	14.30	14.01	14.04	12.35	12.47	12.40	12.17
10–20	13.56	14.06	13.89	14.11	13.93	13.06	12.80	13.14	13.37
20–30	13.96	14.57	14.76	14.46	14.14	13.35	13.28	13.43	13.35
More than 30	13.32	13.42	13.51	13.17	13.13	13.21	13.19	13.22	13.30

The decrease in productivity level due to the lengthening of the period from the time of transition of the average daily air temperature through 0 °C to the active growth of plants is characteristic of all different age crops of winter wheat sown after both arable land and maize for silage. In addition, when growing winter wheat on arable land, a sharp decrease in productivity is observed even in the years with duration of this period from 10 to 20 days, while after maize for silage, the productivity is almost unchanged. Therefore, the productivity on arable land in the years with the length of such a period from 10 to 20 days is 4.29 t ha^{-1} against 5.96 t ha^{-1} in the years with duration up to 10 days. After maize for silage, the productivity is 4.72 and 4.66 t ha^{-1}, respectively. The causes of this phenomenon can be significant fluctuations in night and day temperatures, the negative effects of frost, spread of pathogens, insufficient supply of nutrients from the soil to plants, suppression of microbiological processes in the soil, sharp water losses from the soil due to drought, etc.

The analysis of the research results allows stating that the largest amount of protein in wheat grain accumulates in the years with the length of the period from the transition of the average daily air temperature through 0 °C to the active growth of plants from 20 to 30 days. The average protein content in grain when growing winter wheat on arable land in such years is 14.57%, and after maize for silage it is 13.35%. In the years with longer and shorter duration of this period, the protein content decreases. However, in case of arable land, a significant decrease in grain protein is observed only in the years with duration of the specified period of more than 30 days, while after maize for silage a significant decrease is observed only in the years with duration of this period up to 10 days.

CONCLUSIONS

The above-mentioned analysis allows drawing the following conclusions:

– the later the spring vegetation of winter wheat plants is renewed, the lower the productivity of its crops is. In the years with the renewal of spring vegetation in the third decade of February, the productivity of winter wheat averages 6.42 t ha^{-1}, while with its late renewal in the first decade of April, it decreases almost twice and is 3.29 t ha^{-1}. The largest amount of protein in the grain of winter wheat accumulates in the years with the average renewal of spring vegetation in the third decade of March and is on arable on average 14.12% and 13.84% after maize for silage;

– in the years with early renewal of spring vegetation, sowing dates have almost no effect on the level of productivity of winter wheat crops. When growing it on arable land, the productivity of crops of different ages varies in the range of 6.44–6.96 t ha^{-1}, and after maize for silage, the variation of productivity indicators is 6.02–6.18 t ha^{-1};

– crops of winter wheat on arable are more dependent on the reserves of productive moisture in the soil at the time of renewal of spring vegetation and at the beginning of the booting phase and less dependent on the moisture content in the soil in the ear stage. At the same time, when growing winter wheat after maize for silage, its productivity depends more on soil moisture reserves during the renewal of spring vegetation and the ear stage of plants and less on the amount of moisture in the soil at the beginning of plant booting;

– in the northern Steppe of Ukraine, the highest water consumption by winter wheat crops is observed in the period of ‘ear stage - firm-ripe stage’ and is 35.2% of the total water consumption for the entire growing season, and after maize for silage it is 34.1%. Water consumption of winter wheat crops from the time of spring vegetation to the beginning of plant booting is higher compared to the period ‘start of booting - ear stage’ and averages 27.4% and 22.1% of the total for the entire growing season; the shorter the period from the date of transition of the average daily air temperature through 0 °C to the beginning of active vegetation of plants is, the higher the productivity of winter wheat crops. In the years with duration of this period up to 10 days, the productivity of winter wheat crops averages 5.31 t ha^{-1}. And in the years with the length of more than 30 days, it decreases by 2.17 t $ha^{-1}$and is 3.14 t ha^{-1}. At the same time, the largest amount of protein in winter wheat grain accumulates in the years with the length of this period from 20 to 30 days and is 13.96%. Extension of this period over 30 days causes a decrease in grain protein content to the level of 13.32%, and reduction to 10 or less days - to 13.25%.

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Autoencoders for semantic segmentation of rice fungal diseases

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Abstract. In the article, the authors examine the possibility of automatic localization of rice fungal infections using modern methods of computer vision. The authors consider a new approach based on the use of autoencoders - special neural network architectures. This approach makes it possible to detect areas on rice leaves affected by a particular disease. The authors demonstrate that the autoencoder can be trained to remove affected areas from the image. In some cases, this allows one to clearly highlight the affected area by comparing the resulting image with the original one. Therefore, modern architectures of convolutional autoencoders provide quite acceptable visual quality of detection.

Key words: autoencoder, neural network, computer vision, fungal diseases.

INTRODUCTION

According to the data of the Food and Agriculture Organization of the United Nations (FAO), diseases and pests destroy 20–40% of the world's agricultural crops (Food and Agriculture Organization of the United Nations, International Plant Protection Convention, 2017). We observe no less losses when growing rice, especially in the years of epiphytoties. At the same time, fungal diseases cause enormous economic damage. The most common rice diseases are: alternaria leaf spot (causative agent is Alternaria oryzae Har. Ital); helminthosporiosis (causative agent is Helmintosporium oryzae Br. de Haan); blast (causative agent is Pyricularia oryzae Cav.); fusarium, causing root rot (causative agent is Fusarium oxysporum). Rice blast, caused by a fungus, causes lesions to form on leaves, stems, peduncles, panicles, seeds, and even roots. The potential crop losses due to this disease are enormous. That is why it is considered the most dangerous of all (Zelensky, 2016).

Farmers suffer significant financial losses every year due to fungal diseases. It is very important accurately and at an early stage to identify the symptoms of the disease in order to take the necessary measures to combat it in a timely manner. Symptoms of fungal diseases often appear in the form of spots around the infected areas, so the initial detection of the disease is reduced to the analysis of these spots. At present, farmers mainly rely on their own experience, disease-identifying atlases or involve expert agronomists (Vimal et al., 2019). However, the identification is complicated by the fact that different diseases can have similar types of spots and vice versa, the same disease can manifest itself differently in different crops varieties and depending on growing conditions. Even nutritional deficiencies and pests can cause symptoms similar to those of some fungal rice diseases (Barbedo, 2016).

Misidentification usually results in inappropriate, untimely and sometimes uncontrolled use of pesticides. Their use is harmful to the environment and negatively affects biodiversity, including insect, bird and fish populations, as well as soil, air and water quality (Gill & Garg, 2014; Knillmann & Liess, 2019). Pesticide use also poses significant risk to human health with both acute and chronic illnesses (Bassil et al., 2007; Kim et al., 2016). In turn, the laboratory pathogen identification is a laborious process that requires time-consuming cultivation of the pathogen. In any case, both of these methods require the participation of high-level professionals in the identification process, which is often inaccessible to small farms. Automatic identification of plant diseases based on image-processing methods solves the specified problems by automatically searching for diseases or helping both farmers and experts.

The topic of intelligent processing of images of plants affected by various diseases is considered more and more often in scientific studies. The most common approach to the classification and segmentation of plant diseases, until recently, was an approach based on the application of classical machine learning algorithms (Huang, 2007; Tian et al., 2007; Zhao et al., 2007; Dong Pixia et al., 2013; Moshou et al., 2014; Jitesh et al., 2016; Joshi et al., 2016; Ebrahimi et al., 2017; Pantazi et al., 2017; Vimal et al., 2019). The general approaches in these studies are similar. Firstly, images of the disease are obtained using cameras or scanners. Secondly, the spots of the disease are segmented from the background. Thirdly, features of colour, shape or texture are extracted. Finally, the disease is attributed to one of the groups using classification methods such as support vector machines, K-nearest neighbors, etc.

The following authors (Baghel & Jain, 2016; Elangovan & Nalini, 2017; Gayathri Devi et al., 2018; Guiling Sun et al., 2018, Vithiya & Santhi, 2020) use methods such as SVM, Linear Regression to classify plant diseases, and unsupervised K-means to further isolate diseased areas. In (Vinoth Kumar & Jayasankar, 2018), linear iterative clustering and k-meaths unsupervised methods are used to improve the quality of segmentation.

In a recent paper (Jayanthi & Shashikumar, 2020), the segmentation problem was solved from the standpoint of classical computer vision using physical considerations like minimization of some energy function. The alternative active contour model (ACM) built in this paper was validated with various metrics such as Jaccard index, the Dice index, and the Hausdorff distance. As a result, in (Jayanthi & Shashikumar, 2020) the authors managed to get a fairly good model without involving any data-learning algorithms.

The next step towards modeling the detection of plant diseases was the use of more progressive neural network methods of computer vision (Boulent et al., 2019). This approach has a number of advantages over both methods based on classical physical considerations. and over classical machine learning methods. First, one of the main features of convolutional neural networks is automatic feature extraction, whereas classical machine learning methods require manual feature extraction from an image. Secondly, the quality of the model given by deep convolutional neural networks for image processing is usually significantly higher than in classical models. Finally, the recent modern neural network architectures are opening up new approaches to unsupervised segmentation of objects in the image.

The approach we propose here develops a number of researches where deep convolutional neural networks are used to solve problems of classification of plant diseases. So, in (Pardede et al., 2018) convolutional autoencoders are used to replace handcrafted features with atomatic feature inference. The paper notes the importance of both the auto-generation of features itself and the unsupevised manner of this process, which is provided by the specifics of autoencoders. The authors validate their results using the features given by the autoencoder as input to the SVM model for classifying plant diseases. The authors (Pardede et al., 2018) also note an increase in the quality given by autoencoders with convolution layers compared to conventional deep autoencoders, which is quite natural for image processing tasks.

In (Zilvan et al., 2019), autoencoders are also used to highlight signs of plant diseases and denoise. The authors note that with the help of a variational autoencoder, one can obtain even more informative features from the image. Further, as in the paper (Pardede, 2018), the signs automatically given by the encoder part of the autoencoder are used to classify plant diseases. The results (Zilvan et al., 2019) differ from those by (Pardede et al., 2018) due to the use of a more advanced autoencoder architecture, which allows, in particular, to achieve better denoise compared to conventional convolutional autoencoders.

Other studies propose modern solutions and platforms for automated crop production that cover the most important parts of the production process on the farm and include disease detection and classification models (Hakojärvi et al., 2010), as well as solutions for remote visual examination of agriculture in real time (Komasilovs et al., 2018).

Proposal of this paper and its significance

The aim of this paper is to propose a new approach to the problem of disease segmentation based on deep convolutional autoencoders, while using the autoencoder not as a regular feature extractor, as suggested in the above-mentioned papers, but as a closed unsupervised image processing system, which is able to highlight areas of plant disease damage automatically and without the participation of experts.

The rest of the paper is constructed as follows. Section 2 thoroughly previews modern methods for plant disease identification based on neural network technologies. These methods refine and automate the conventional visual detection method used by plant pathologists to identify rice diseases. Then we overview main neural network architectures suitable for solving this problem. Section 3 provides the basic methodology and gives a brief description of the basic principles of convolutional neural networks and autoencoders used in this study. We list main advantages and prospects of the chosen approach. Section 4 describes the details of the autoencoder model used as well as image post-processing. Также приведено a description of the used image dataset, as well as details of the technical implementation of the used autoencoder model. We offer a description and discussion of the main results of the performed numerical experiments.

CURRENT METHODS FOR PLANT DISEASE DETECTION

Preliminary visual analysis remains one of the most basic methods for detecting diseases in a variety of crops. Before sending samples to the laboratory, an experienced plant pathologist tries to establish the external symptoms of the disease, its degree of development and prevalence (Bidaux, 1978). This part of research is extremely important, and it is this stage that has enormous potential for automation up to the complete exclusion of human experts and their replacement with automated algorithms for determining the required defects on plants.

The main tool for such automation at present is computer vision - a set of automatic and semi-automatic approaches based on intelligent image processing (Yao et al., 2009; Xiao et al., 2018). Until recently, here the so-called classical computer vision was widely used. At present it has given way to the modern one - based on neural network architectures (Zeiler & Fergus, 2014; Wang, 2017; Zhang, 2018; Too et al., 2018).

The neural network approach to the detection of plant diseases is based on a rather simple idea to bring the work of a computer with an image closer to how the human eye does it. The vision of humans and animals generally works as follows: when a person tries to classify an object in front of him, he sequentially focuses on separate parts of the object and compares them with the forms in his memory, and does this from smaller parts to larger ones. This process is mimicked by convolutional neural networks, which began with the revolutionary work of Y. LeCun et al. (1989). In subsequent works, these ideas were significantly developed and in the last decade, convolutional neural networks confidently hold the leadership both in the competition for image detection and in solving specific applied problems. The quality of classification that neural networks currently provide is quite comparable to that of a human, and in some cases even surpasses it.

It is crucial to mention several neural network architectures, which were a kind of milestones in the history of the development of this approach and which are used in the present paper to detect the areas of rice fungal diseases. After LeCun et al. (1989) the next big breakthrough was AlexNet. It is notable for the fact that in 2012 it reached a test accuracy of 84.6% in the problem of classifying 1.2 M images into 1,000 different classes, which is a very impressive result (Alom, 2018). In 2013, He et al. (2016) significantly increased the computing power and changed the network architecture to a heavier one - VGG, while achieving the 92.7% test accuracy, which is already comparable with the visual acuity of a human eye. Further, the experts wondered whether it was possible to reduce the model without losing its quality. It turned out that using a more thought-out model, the amount of memory required for its storage can be reduced by more than 20 times, and the quality will even increase. In 2014, the GoogLeNet Inception model with 93.3% test accuracy was presented (He et al., 2016) and had only 6M parameters, instead of 138M for VGG. Subsequent improvements, ResNet (He et al., 2016), SqueezeNet (Wu et al., 2016), and DenseNet (Gao Huang et al., 2018), improved test accuracy to 96.43%.

Versatility, determined by the architecture, is the main advantage of the above-mentioned models. They cannot be directly used to identify plant diseases, since they were trained for other tasks, but they can be re-trained for the required task by showing a sufficient number of examples - images of healthy and diseased plants. The larger the number of images presented to the model, the more accurate will be its predictions in the future. Liu et al. (2009), Phadikar et al. (2013) point out that the stage of collecting a sufficient number of high-quality images is extremely important and in fact determines the success or failure of solving the problem of detecting diseases of various agricultural crops. So, for example, if the images are very noisy, made with insufficient / excessive lighting, then this will significantly degrade the overall quality of the final model.

MATERIALS AND METHODS

Autoencoders have a special place among modern neural network image processing models. They are specific types of neural networks, a pair of neural networks actually - encoder and decoder, interconnected by a thin bottleneck (Bank, 2020).

The idea behind autoencoders is as follows (see Fig. 1): some image is fed to the input of the network, which is compressed by the first network - encoder into a vector of dimensions less than the dimension of the original image, giving it a dense representation. Further, this vector is fed to the input of the second network - decoder, which tries to decode it back into the original image.

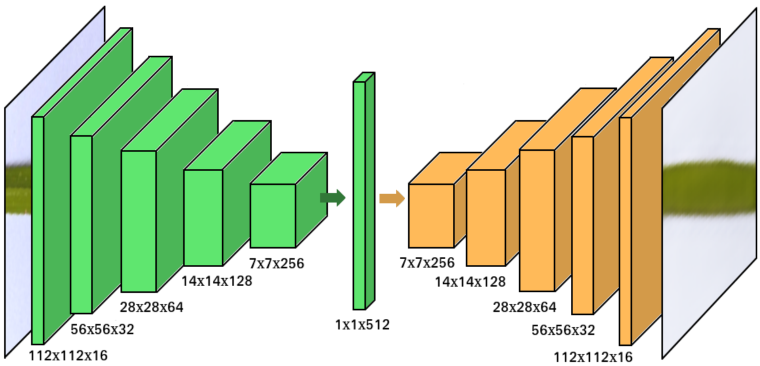

Figure 1. Autoencoder architecture: the input is a 224×224 image, which is encoded in the center with a 512-vector length. Then the resulting vector is decoded back into the image of the original size by a sequence of inverse operations.

Thus, at the output, the original image is compared with itself. If the original image is similar enough to the output, this has a number of advantages at once. Firstly, it has a compression effect of some sort. If we are ready to neglect some loss of image quality, then we can easily replace the image with its dense representation - the vector that gives the encoder a part of the network at the output. Indeed, in this case, we can easily restore (with some accuracy) the original image by feeding it dense to the decoder input of the neural network autoencoder part. Secondly, we extract, in an encoded form, useful features that fully or partially characterize our image. In this case, we talk about data projections - the presentation of original images in a space of a lower dimension without significant loss of information reflected on them. If we are not faced with the task of directly interpreting these vectors of lower dimension, then they may well be used further in other models, for example, predicting a specific type of disease affecting a plant or lesion size.

Thirdly, autoencoders are often used to smooth out an image noise. Due to its specificity, the autoencoder memorizes in a dense representation the most essential features of each image fed to it, and noise is usually ignored.

Ian Goodfellow and his co-authors (Goodfellow et al., 2016) used for the first time the above-mentioned concepts for conventional fully-connected neural networks in noise suppression tasks. Further, it was noticed that these ideas can be transferred without changes to convolutional neural networks, which use the convolution operation to switch between layers instead of all neurons of the previous layer. Convolution acts on the original image and subsequent layers of the neural network as a learning filter. Mathematically, it can be represented as

$$\mathrm{conv}(x) = \sum_{i,j} w_{\mathrm{ij}} x_{i-a,j-b}, \qquad (1)$$

where x is the original image, or the output of any inner layer of the network, w are the weights of the kernel that defines the convolution, and the summation does not apply to all indices, but only to some 'window' from 0 to 2a on the first index and from 0 to 2b on the second (Murphy, 2013; Goodfellow et al., 2016). The physical meaning of such an operation is that it acts as a filter that simplifies the original image and allows to select all the necessary details in it. This is exactly what convolutional neural networks do in the course of their training - they adjust the weights of all such filters so that the final result matches the initial data as much as possible. The combination of these ideas yielded convolutional deep autoencoders, which are now widely used for intelligent image processing. One can find more information regarding convolutional neural networks in the classic books by (Murphy, 2013 and Goodfellow et al., 2016).

All the above-mentioned features of autoencoders are widely used in our study and help to get an acceptable quality of segmentation. We demonstrate that the autoencoder can be trained in such a way that it will remove the disease lesions from the original image. Thus, comparing the image at the output of the model with that supplied to the input, in a number of cases it is possible to quite clearly automatically localize the focus of the plant lesion by a fungal disease.

Finally, it is worth noting the importance of post-processing of the results of neural networks in a number of tasks. For example, in the well-known real-time object detection problem (Redmon et al., 2016), this happens for a number of reasons. Firstly, the chosen neural network may have too few parameters, and we may not want to complicate it, striving for its simplicity and lightness. Secondly, data post-processing after the operation of an algorithm can be dictated by the very nature of the problem, for example, the physics of the process. In this study, we've noticed that preprocessing in the form of overlaying additional color filters has a positive effect on the final quality of segmentation of plant diseases. In accordance with the general methodology for adjusting the parameters of models in machine learning, we configure the parameters of additional filters on lazy data sampling, thus avoiding their adjustment to the current data and increasing the generalizing ability of the model.

In this study, we use a dataset by Huy Do (2019) to train our models. It contains about 3,500 photographs of both healthy rice leaves and those affected by three types of diseases - hispa, brown spot and leaf blast. The images in this dataset are of sufficient quality for the use of computer vision; they are practically free of extraneous noise and other shooting defects.

Augmentations were applied to the input data in order to increase the generalizing ability of the model. Augmentations are various distortions and transformations such as rotations by small angles, reflections near the coordinate axes and addition of a little noise. This increased both the overall quality of the model and the confidence of its predictions.

MODEL DESCRIPTION AND TRAINING

The operation of the convolutional autoencoder used in the present study is schematically shown in Fig. 1. The original image is fed to the input of the first convolutional layer, which, using the convolution operation, transforms it into a new tensor that is half the size along the axes, but with greater depth. Then this operation is repeated, each time giving tensors of smaller and smaller resolution, until a one-dimensional vector is obtained in the very middle. This vector serves as a coded representation of the original image. The resulting vector is then converted into an image by means of inverse operations. Layer labels in Fig. 1. mean their dimensions - height, width and depth. These numbers, like the number of layers, may be different for other architectures, but the general concept remains the same.

In this paper, we considered the standard autoencoder model - Convolutional Autoencoder. It is less heavyweight than its advanced version - Deep Variational Autoencoder (Vahdat, 2020). Convolutional Autoencoder is a multilayer convolutional encoder and a convolutional decoder symmetric to it. From a number of possible architectures, we experimentally chose the following:

Encoder:

conv2d(3, 16, 8×8) → conv2d(16, 32, 8×8) → conv2d(32, 64, 8×8) →
conv2d(64, 128, 8×8) → conv2d(128, 256, 8×8) → conv2d(256, 512, 7×7)

Decoder:

conv_t2d(256, 512, 7×7) → conv_t2d(128, 256, 8×8) → conv_t2d(64, 128, 8×8) →
conv_t2d(32, 64, 8×8) → conv_t2d(16, 32, 8×8) → conv_t2donv2d(3, 16, 8×8)

Here conv2d (i, o, wxw) is a convolution layer with kernel of size wxw, which inputs a block of data of depth i and outputs a block of depth o; conv_t2d is a symmetric convolutional layer giving the inverse operations to conv2d. Between all convolutional units are ReLU activated units commonly used in image analysis tasks. The number of parameters of this model is 18.4M.

We used the PyTorch v1.6.0 framework to train the model. It is widely used due to its simplicity and functionality. Nevertheless, the architecture of the used autoencoder described above is quite simple, and it can be easily repeated on any other

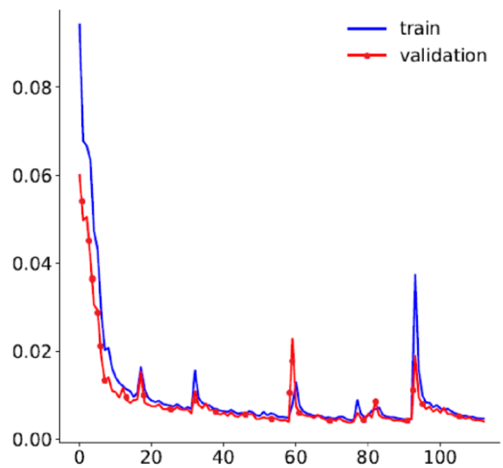

Figure 2. The process of training the autoencoder.

framework, for example TensorFlow, Caffe etc. The neural network was trained on a stationary computer with the following configuration: Core i7 / GTX 1660, GPU - GTX 1080 4 Gb.

It took about 120 epochs (approximately 6 hours of counting on the above-mentioned hardware configuration) for the model quality to reach its maximum level (Fig. 2). In the process of training, the error function experiences characteristic fluctuations due to the fact that the neural network uses minibatches for its training, but in general the error level drops. Generally, in regression problems, the values of the error function have no direct interpretation, in contrast to the usual percentage of accuracy in classification problems. In our case, one can also only observe the drop in the values of loss-function and watch when it reaches a certain plateau. After that, it is necessary to evaluate the quality of the model in general.

After training the model, the results given to it were subtracted from the original ones, and then they were subjected to additional post-processing, that is the application imposition of filters by colors and brightness for a clearer detection of the area affected by the disease. We identified the most optimal thresholds for color channels to identify the affected areas:

$$|red - r_h| < r_t, \ |green - g_h| < g_t, \ |blue - b_h| < b_t. \quad (2)$$

Here $r_h = 0.14$, $g_h = 0.29$, $b_h = 0$, $r_t = 0.1$, $g_t = 0.1$, $b_t = 0.08$. The problem of selecting such external parameters of the model, also called hyperparameters, often arises in machine learning, since almost all algorithms have such. It is crucial to select them not on the training or test set. Otherwise, the effect of overfitting may occur, when the model adjusts well to the training set, but then works poorly on new data. It is better to adjust hyperparameters on a sample that is specially postponed in advance and is not used in training and testing. In our case, these values were selected on a deferred sample in the amount of 10% of the training sample.

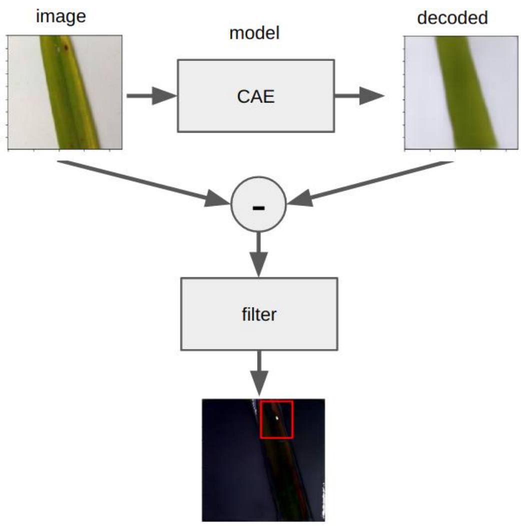

Figure 3. The final model for highlighting the affected plant areas.

Thus, the final pipeline of image processing can be represented as follows (see Fig. 3). Primarily, a 224×224 image is fed to the input of the previously trained autoencoder. This autoencoder, as described above, first converts this image into an encoded vector-512 and then expands it back to its original size. The resulting image is visually close to the original (this is the main idea of the autoencoder training), but some details are missing. After subtracting the resulting image from the original, we apply the above-described special color filters and get the final result - an image in which fine noise has been removed and areas of plant damage are clearly visible.

RESULTS AND DISCUSSION

Here we are not interested in the classification of plants by types of diseases, but in the presence or absence of such a disease in general. This task is easier, since it is not required to additionally train the neural network to distinguish plant diseases among themselves. However, it is solved here together with the task of determining the most affected area, which is already much more difficult. What may be obvious to a phytopathologist can be challenging for a computer.

As mentioned above, autoencoders have a number of interesting features that distinguish them from other neural networks. Thus, they allow, by compressing the data obtained from the image, to eliminate minor things that are insignificant for a given dataset. Moreover, if the lesion on the leaf is not too large, the autoencoder can, after proper training, 'erase it' from the original image. After comparing the original image with the obtained one, it is quite easy to clearly distinguish the affected area. It is noteworthy that the described image processing scheme is possible (and even preferable) precisely at the early stages of the development of the disease. This increases the value and potential benefits of the proposed approach.

The above image processing scheme (Fig. 3) contributes in a number of cases to clearly segment the image with a diseased plant, highlighting the areas of disease lesion on it Fig. 4 shows a typical example of brown spot rice as well as the result of processing it with our algorithm. Obviously, the inclusion of a neural network autoencoder along with conventional filters in the image processing pipeline, makes it possible to clearly detect the area of plant damaged by a harmful fungus.

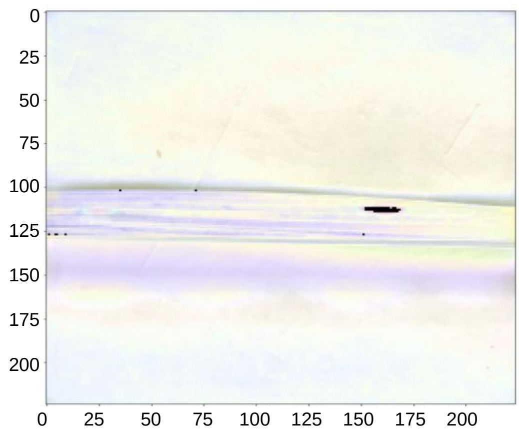

Figure 4. Highlighting of the affected plant areas (black).

According to our estimates, the proposed model makes it possible to automatically visualize diseased areas on rice leaves in 40–50% of cases. We consider it to be a fairly good result. Moreover, the described method is perfect in the early stages of infection, when the affected areas are still small. In this case, the autoencoder removes them from the image most accurately, taking them for insignificant noise, thereby increasing the possibility of detection of these areas after subtracting the resulting image from the original one. Thus, the method is particularly applicable for plant diseases detection at an early stage of vegetation.

Pardede and co-authors (Pardede et al., 2018) use a deep convolutional autoencoder similar to our architecture (see Fig. 1 above and Fig. 2 in (Pardede et al., 2018)). However, we are not trying to use an autoencoder to get only hidden features from images. In this study, we investigate a slightly more advanced problem of disease segmentation, where we are no longer concerned with the central layer of the autoencoder, which, in fact, reveals hidden signs. We show that an autoencoder can be useful in general, and not just as a feature extractor. This approach allows us to interpret the problem of the segmentation of plant diseases in a completely unsupervised manner, without using hidden features directly.

In contrast to the study (Zilvan et al., 2019), where a variational autoencoder is also used to obtain features and subsequent classification, we use a simpler deep convolutional autoencoder architecture. Here we specifically focus on lighter architectures that can be used effectively on mobile devices if needed.

Heavier architectures require more complex solutions for implementation in production, for example, a dedicated computing server and constant access to the Internet. Our solution is designed to significantly simplify the work of phytopathologists in identifying rice diseases and detecting specific areas of plant damage.

CONCLUSIONS

In this paper, we propose a new approach to the problem of segmentation of plant diseases based on the deep convolutional autoencoder model. In this case, the autoencoder does not work like a regular feature extractor, as suggested in the above-mentioned papers, but as a closed unsupervised image processing system, which is able to highlight areas of plant disease damage automatically not involving qualified phytopathologists. The complete pipeline of image processing also includes the use of specially selected color filters that improve the final quality of the model without the use of heavier architectures such as variational autoencoders. The authors show that training of such models is quite possible without the involvement of serious computing power. The final proposed solution, due to its lightness, may well be used on mobile devices when computing resources are limited.

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Fatty oil accumulation in vegetable soybean seeds and its thin-layer chromatography

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Abstract. This paper studies the accumulation of crude oil (triacylglycerides, monoacylglycerides, diacylglycerides, free fatty acids, phospholipids, tocopherols, pigments, sterols, waxes) in soybean vegetable samples. Samples were taken from two groups: grown in an experimental field and in protected ground of the Federal Scientific Center for Vegetable Growing in the Moscow Region. Both groups were observed in the phase of technical ripeness and in the phase of complete biological ripeness (finally ripe seeds). Soxhlet method as arbitration in analysis was used as suitable for the extraction of lipophilic substances. It was determined that the fat content in the technical ripeness phase in most soybean samples averaged 10.5%. In the phase of biological ripeness, the highest accumulation of fatty oil was observed in Hidaka and Nordic (17.6%). The oil content in vegetable forms of soybeans was consistently lower than that of grain varieties: in the phases of technical and biological ripeness by 55.6% and 22.0% (in relative values) respectively. Thus, he accumulation of oil in seeds is determined mainly genetically. The refractive index of vegetable and oil soybean was established equal on average 1.4755. According to this finding the soybean oil can be classified as semi-drying.
Thin layer chromatography (TLC) was used to study the lipophilic components of soybean fatty oil. It was found experimentally that the best separation of the components is achieved using an eluent system: carbon tetrachloride: chloroform in a 2: 3 ratio. It was found that the main fat-soluble compounds are the following (in order of increasing Rf in the chromatogram): phospholipids, monoacylglycerides, triacylglycerides, tocopherols, fatty acid esters. As a finding of the research vegetable soybean cultivated at 55 °N in both technical and biological ripeness phases significantly accumulate crude oil in the seeds. This crude oil contained ω-6, ω-3, phospholipids, and vitamin E.

Key words: vegetable soybean, *Glycine max* (L.) Merr., oil content, crude oil, fat, thin layer chromatography.

INTRODUCTION

The aim of the research is to study the oil content in vegetable-type soybean seeds in the R6 and R8 phases under conditions of 55 °N, and the selection of eluents in thin layer chromatography.

Soybean is a crop of dual industrial use: a source of protein and fat content varies in the range of 16–27% (Hymowitz et al., 1972; Degola et al., 2019; Novytska et al., 2020). Soybean oil is one of the most biologically valuable among all vegetable oils: a high content of polyunsaturated fatty acids (PUFA) - about 55–63%, a low proportion of saturated fatty acids - no more than 15% (Dornbos et al., 1992; Carson et al., 2011). The composition of fatty acids significantly affects the organoleptic qualities of vegetable soybeans and the nutritional value of oil (Haun et al., 2014; Li Qing-Tian et al., 2017).

Soybean oil contents 50–60 % linoleic oil. Its content has a close correlation with the amount of α-linolenic acid (up to 8%), which gives the oil a peculiar taste, aroma and contributes to its rapid oxidation (Kostik et al., 2013; Miao Long et al., 2020). The fat content in vegetable soybeans in the technical ripeness phase varies within 8.0–14.6% (on dry matter) (Rao et al., 2002; Shafigullin et al., 2020a) the active synthesis occurs almost until the onset of full biological ripeness (Rubel et al., 1972; Nadtochii et al., 2015).

Moisture evaporation under high air temperature causes water deficiency and decrease in fat synthesis. At the same time, the accumulation of protein increases (Tretyakov et al., 2005; Wijewardana et al., 2019) and respiratory processes intensify. A large amount of oxygen spends on the synthesis of PUFA. That's why the iodine number decreases.

Fat content in soybean seeds from south (45–50° N) to north (55–59° N) regions increases in more than 2% The fraction of oleic acid decreases by 15.4% in absolute values. The proportion of linoleic acid increases by 5.5% in absolute values. The proportion of linolenic acid increases almost 3 times. The result of these biochemical processes is a general decrease in the ratio of ω-6 and ω-3 (3–4: 1) (Kucherenko et al., 2008). For this reason, soybean seed oil grown in northern conditions is less stable during storage, but more suitable for medicinal purposes and functional nutrition.

Oil content of vegetable forms increased, in comparison with oilseeds by an average of 2% in soybean cultivars (Glycine max (L.) Merr.), that grown in the Krasnodar region of Russia. The content of ω-6 and ω-3 decreased by 4% and 50% respectively because of the rejection of samples with an increased accumulation of linolenic acid, which imparts a bitter taste to vegetable oil and soybeans in the phase of technical ripeness (Petibskaya et al., 2006).

Gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR) methods are most often used to analyze the chemical composition of vegetable oils (Tariq et al., 2011; Goryainov et al., 2012). Nevertheless, thin layer chromatography (TLC) is the simplest and most inexpensive for identification of fatty oil (Lobaeva et al., 2013). TLC is an analytical and operational method characterized by high efficiency and versatility, widely used in scientific research (Nazi et al., 1998). For the analysis of lipophilic components, it is important to select the optimal eluents for most qualitative separation the lipophilic components on a chromatogram, where a thin layer of adsorbent is used as a stationary phase (Fuchs et al., 2011).

METHODOLOGY

The soils of the experimental field are sod-podzolic medium loamy. The humus content is low, 1.3–4.6%. The reaction of the soil solution varies from acidic to close to neutral: pH_{KCl} = 4.5–6.9. The content of mobile phosphorus (P_2O_5) is sufficient, and averages 320–840 mg kg^{-1} of soil, and potassium (K_2O) is much less - 100–250 mg kg^{-1} of soil (Knyazkov et al., 2014). Soil cultivation included plowing in the fall, disking, early spring harrowing and pre-sowing cultivation. The predecessor was complete fallow.

The study of *Glycine max* (L.) Merr., samples was carried out in the laboratory of physiology and biochemistry of the Federal Scientific Vegetable Center during 2016–2018. Ten lines of soybeans were studies: two oilseeds, two universal and six vegetable ones. Most of the collection material was provided by the Federal Research Center 'Vavilov All-Russian Institute of Plant Genetic Resources'; breeding material from the Federal Scientific Vegetable Center collection was also used (Table 1).

There are morphological and economic characteristics, biological characteristics, biochemical parameters use to determine vegetable form (Shafigullin et al., 2020b). Universal cultivars have features of vegetable and oil varieties.

Soybean cultivar 'Okskaya' was a control as registered in the Russian State Register. The soybean cultivars were sown manually in open ground in the third decade of May in 2016 and 2018, in 2017 - in the first decade of July in protected ground (greenhouse) in three rows 1.5 m long, 45 cm interrow, standing density 40 pcs m^{-2}, harvesting area 15 m^3.

Table 1. Origin of soybean samples and directions of use

No.	Varieties	Origin	Type
1	Okskaya (standard)	Russia	grain (oilseed)
2	Soer-5	Russia	grain (oilseed)
3	Gokuwase Hayabusa Edamame	Japan	vegetable
4	Japaneese sampleA	Japan	vegetable
5	Nordik	Russia	universal
6	Hidaka	Japan	vegetable
7	740-1	Sweden	vegetable
8	Fiskeby III	Sweden	vegetable
9	Tundra	Canada	universal
10	Cha Kura Kake	Japan	vegetable

The crude oil content was determined according to Redfern et al. (2014). N-hexane (C6H14) was used as solvent due to its low toxicity, good dissolving properties and low boiling point (68.7 °C).

Analysis of variance was used for statistics using Microsoft Office Excel (2010) and then validated in Origin 9.1.

Thin layer chromatography (TLC) was performed on Merck plates (TLC Silica gel 60 F254) on silica gel (7×15) cm with a total area of 105 cm^2. The system was chosen as the most optimal eluent: carbon tetrachloride-chloroform in the ratio (8:12) mL. Chromatograms were developed in a desiccator with iodine vapor; the saturation time of the chromatogram in the chamber was about 30 min. The temperature of separation of lipid fractions by TLC is about 22 °C. The witnesses (taps) were: 10% solution of vitamin E (LLC 'Tula pharmaceutical factory') and 97% solution of lecithin (LLC 'Vitaprom'). The Rf index was calculated as the ratio of the distance (l) traveled by the substance to the distance (L) traveled by the solvent: l:L.

The refractive index (refractive index) was determined at a temperature of 25 °C using a laboratory refractometer RL 3 (Poland) in four replicates.

The purpose of the work was to study the accumulation of crude oil in vegetable soybean seeds at the technical and biological stages of development in the central region of Russia, as well as to select the optimal eluents for separating obtained oils by TLC and to study the lipophilic fractions of these compositions.

RESULTS AND DISCUSSION

Studying the oil content of soybean seeds

Increase in the accumulation of fat in seeds was observed in all. Thus, there was a twofold increase in the oil content, while it was slightly lower and amounted to 36.2% relative values. Oil content of seeds in grain varieties was higher in technical and biological ripeness. Technical ripeness (R6), the difference in fat content between the breeding forms decreased by almost 3 times in the phase of biological ripeness compare to technical ripeness (R6). This may indicate a more intensive process of oil synthesis in the initial stage of generative development in oilseeds, as well as a later onset of accumulation of triacylglycerides in vegetable forms. An increase in seed oil content, similar to protein content, goes to the terminal stage in ontogeny, i.e. shedding of leaves and a decrease in moisture content in seeds below 10%.

Accumulation of oil in the R6 phase in most of the vegetable forms of soybeans was almost at the same level and averaged 10.5% (in absolute values). Thus, in the phase of technical ripeness, vegetable soybean contains, in addition to protein, also a large proportion of polyunsaturated fatty acids in seeds (Fig. 1).

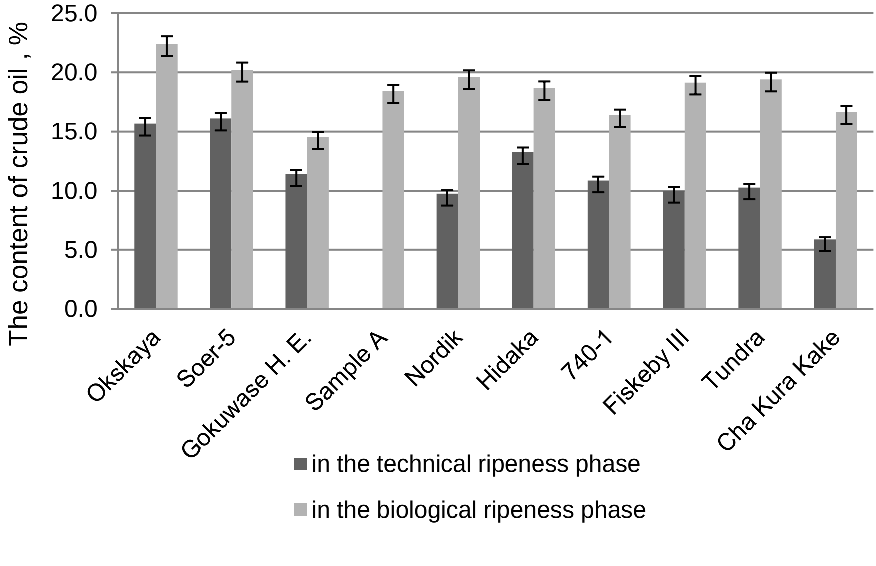

Figure 1. The content of crude oil in soybean seeds in the phase of technical and biological ripeness of samples, average 2016–2018 (dry weight).

Importantly, in 2017 the influence of non-standard growing conditions in greenhouses on biochemical processes was observed, which led to a decrease in the oil content of seeds in all samples by 30.4% (in relative values) compared to 2016 and 2018.

Over the course of 3 years, oilseed samples consistently averaged 22% higher crude fat than vegetable samples. The maximum oil content among vegetable samples was observed in the Hidaka and Nordic lines (17.6%). The Gokuwase Hayabusa Edamame sample had a lesser oil content than other cultivars (33.3% lower than average mean in group of vegetable). It belongs to the ancient species cultivated more than 40 years ago. Fat content is mainly determined by hereditary factors; therefore this indicator can also be considered as one of the biochemical characteristics for selection. These findings correspond to earlier research (Chen Liang et al., 2018). The total crude oil yield was slightly lower than total crude oil yield of the vegetable soybean samples. The highest yield of crude oil was observed in Nordic breeding line and 740-1 vegetable form. In 2016, there was a higher fat collection in almost all samples, which is explained by increased seed productivity (Table 2).

Table 2. Content and yield of crude oil in seeds of soybeans in the phase of biological ripeness

No.	Varieties	Crude oil content, %				Vσ, %	Crude oil yield, g plant^{-1}		
		2016	2017	2018	Average in 3 years		2016	2018	Average in 2 years
1	Okskaya	19.7 ± 0.6	16.0 ± 0.5	22.4 ± 0.7	19.4 ± 1.8	16.4	3.6 ± 0.1	2.5 ± 0.1	3.0 ± 0.5
2	Soer-5	20.5 ± 0.6	17.5 ± 0.5	20.2 ± 0.6	19.4 ± 1.0	8.6	3.4 ± 0.1	4.0 ± 0.1	3.7 ± 0.3
3	Gokuwase Hayabusa Edamame	13.2 ± 0.4	9.2 ± 0.3	14.5 ± 0.4	12.3 ± 1.6	22.6	4.2 ± 0.1	1.4 ± 0.0	2.8 ± 1.4
4	Sample A	17.6 ± 0.5	12.8 ± 0.4	18.4 ± 0.6	16.3 ± 1.8	18.7	3.4 ± 0.1	2.6 ± 0.1	3.0 ± 0.4
5	Nordik	18.7 ± 0.6	13.2 ± 0.4	19.6 ± 0.6	17.2 ± 2.0	20.3	6.7 ± 0.2	3.3 ± 0.1	5.0 ± 1.7
6	Hidaka	17.9 ± 0.5	17.4 ± 0.5	18.7 ± 0.6	18.0 ± 0.4	3.5	2.5 ± 0.1	3.6 ± 0.1	3.1 ± 0.6
7	740-1	17.1 ± 0.5	13.8 ± 0.4	16.4 ± 0.5	15.8 ± 1.0	11.0	5.1 ± 0.2	2.8 ± 0.1	4.0 ± 1.1
8	Fiskeby III	15.5 ± 0.5	12.9 ± 0.4	19.1 ± 0.6	15.9 ± 1.8	19.7	4.7 ± 0.1	2.4 ± 0.1	3.5 ± 1.1
9	Tundra	17.6 ± 0.5	12.2 ± 0.4	19.4 ± 0.6	16.4 ± 2.2	22.8	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.1
10	Cha Kura Kake	17.0 ± 0.5	13.0 ± 0.4	16.6 ± 0.5	15.5 ± 2.2	14.4	6.1 ± 0.2	1.8 ± 0.1	3.9 ± 2.2
	LSD_{05}	2.1	2.5	2.2	2.1				

In the course of studying the refractive index (refractive index) of the crude oil of the samples, it was found that in soybean seeds of both breeding directions, the fat has almost the same values in the ability to refract a ray of light. It can be concluded that soybean oil is semi-drying in terms of the content of unsaturated fatty acids, since the refractive index is proportional to the content of the latter (Table 3).

Table 3. Refractive index of crude oil of soybean samples, average for 2016–2018

Type of soybean varieties	Refractive index
oilseed	1.4765 ± 0.00001
vegetable	1.4744 ± 0.00001

Thin layer chromatography of oils.

The isolated crude oil from the seeds was studied by thin layer chromatography (TLC), in which vitamin E (tocopherol) and soybean lecithin standards were used as witnesses, as well as flaxseed and olive unrefined oils for comparison. Eluents of non-polar and polar nature were tested in various ratios for the selection of the mobile phase in order to optimally separate all the main lipophilic fractions.

It was experimentally found that the mobile phase CCl_4:$CHCl_3$ in the ratio (10:10) cm^3, (15: 5) cm^3, (15:5) cm^3 n-hexane: ethanol (95%) in the ratio (20:0.5) cm^3, as well as pure carbon tetrachloride and chloroform (20 cm^3 each), did not separate fractions, or were separated, but not completely. For example, using pure carbon tetrachloride (20 cm^3), or the system: n-hexane: ethanol (95%) in the ratio (20:0.5) cm^3, the chromatogram almost did not 'stretch' the fat, but only chloroform 20 cm^3, or the system: carbon tetrachloride: chloroform in the ratio (05:15) cm^3- on the contrary, the length of the plate was not enough even to separate compounds at the level of triacylglycerides (TAG) (Fig. 2).

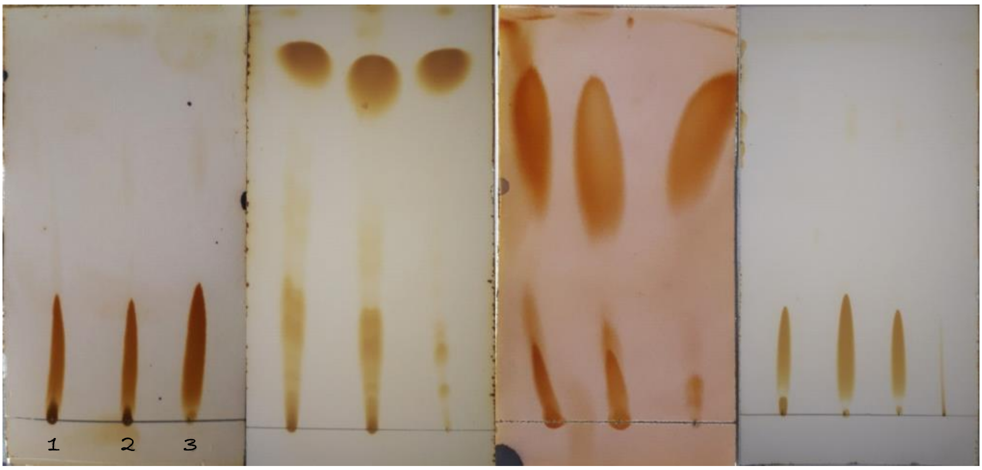

Figure 2. Thin layer chromatography of the obtained crude fat from soybean seeds using eluents in various ratios: 1 – n-hexane: ethanol (95%) in the ratio (20:0.5) cm^3; 2 – chloroform 20 cm^3; 3 – carbon tetrachloride: chloroform in the ratio (5:15) cm^3; 4 – carbon tetrachloride 20 cm^3 (left to right).

The transit time of the eluent from the start line to the finish line was 50 minutes using pure carbon tetrachloride, or the system: n-hexane: ethanol (95%) in the ratio (20:0.5) cm^3, about 30 min., using the system CCl_4:$CHCl_3$ in the ratio (8:12) cm^3, and about 20 min. using chloroform 20 cm^3, or the mobile phase CCl_4:$CHCl_3$ in the ratio (5:15) cm^3.

It has been proved that the eluents: carbon tetrachloride-chloroform in the ratio (8:12) cm^3 have the optimal separation of all the main fat-soluble fractions. The diagrams of these chromatograms are shown in Figs 3–4. The most significant spots in terms of area and size belong to TAG - the main components of vegetable oils, incl. soybean. Linseed and olive oils, taken for comparison, visually showed similar chromatographic results.

Figure 3. Thin layer chromatography of the obtained crude fat from soybean seeds: 1 – crude oil from oil seeds of the Soer-5 variety, 2016; 2 – unrefined linseed oil; 3 – vitamin E solution; 4 – lecithin solution.

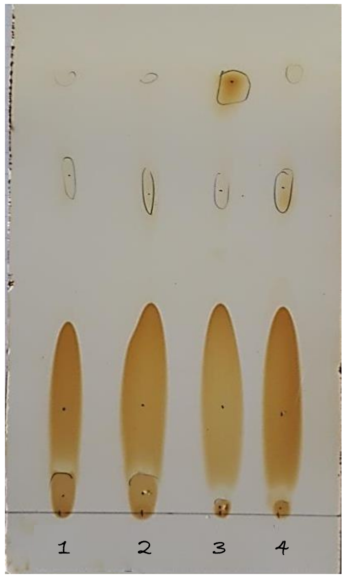

Figure 4. Thin-layer chromatography of the obtained crude fat from soybean seeds: 1 – crude oil from the seeds of the oil variety Soer-5, 2016; 2 – crude oil from the seeds of the vegetable sample Cha Kura Kake, 2016; 3 – unrefined olive oil; 4 – vitamin E solution.

In accordance with the standards and literature data, the separated lipid fractions were analyzed and the Rf index was calculated (Sherma & Rabel, 2018). It was found that the standards (tocopherol and lecithin) appear as spots with Rf 0.66 and 0.01, respectively. Based on the obtained chromatograms, a conclusion was made about the presence of tocopherol and phospholipids in crude soybean oil, based on similar Rf values, which is also confirmed by literature (Touchstone et al., 1980). Also, other adsorption spots were found on the chromatogram, which are supposed to correspond to monoacylglycerides and fatty acid esters (Table 4).

Table 4. Rf index of separated lipid fractions from crude oil of soybean samples, average for 2016–2018

No.	Separated lipid fractions	Value Rf
1	Phospholipids	0.01 ± 0.00
2	Monoacylglycerides	0.04 ± 0.01
3	Triacylglycerides	0.20 ± 0.03
4	Tocopherols	0.66 ± 0.10
5	Fatty acid esters	0.95 ± 0.14

CONCLUSSION

The oil content of seeds in vegetable forms of soybeans, in comparison with cereals, in the phase of technical and biological ripeness is less by 55.6 and 22.0%, respectively (in relative values). It does not drop below 16% under standard growing conditions. The accumulation of fatty oil is an important biochemical characteristic of vegetable-type soybeans. The most valuable lines (Sample A, Hidaka) in the phase of complete biological maturity showed a fat content of 17.1%. Oil accumulation is mainly dependent on genetic factors and is one of the main biochemical characteristics suitable for selection.

It can be concluded that thin layer chromatography is used in a qualitative analysis for the content of the main fat-soluble components (phospholipids, monoacylglycerides, triacylglycerides, tocopherols, fatty acid esters); their different content is shown in comparison with olive and linseed oils.

Optimal separation of fat-soluble fractions by thin layer chromatography was achieved by using eluents carbon tetrachloride: chloroform in a ratio of 2: 3. The use of this mobile phase made it possible to separate the main components of a lipophilic nature that are part of the fatty soybean oil: phospholipids, monoacylglycerides, triacylglycerides, tocopherols, fatty acid esters.

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Differentiated application of nitrogen fertilizers based on optical sensor readings

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Abstract. The article considers the method of variable rate application of top dressing with nitrogen fertilizers in spring barley crops in the system of precise agriculture. The principle of is based on the in-process diagnosis of plants state in key phases of development and the introduction of necessary dose of top dressing in specific field areas. To assess the plants state, a GreenSeeker optical sensor, which measures the NDVI (Normalized Difference Vegetation Index). The tailored application of top dressing increases the yield of spring barley grain by 14.2% compared to the application of fertilizers with one calculated rate for the entire plot or field (Skudra, 2017, Hamann, 2020).

Key words: fertilizers, agriculture, nitrogen, top dressing, precision agriculture, ammonium nitrate, spring barley.

INTRODUCTION

Modern technologies in agriculture are aimed at obtaining high and stable yields with the rational use of energy and other resources. In agricultural production, attempts are being made to reduce the consumption of fuels and lubricants, heat energy, gas and electricity. Nevertheless, fertilizers, pesticides, and seeds are also a type of consumables. Rational approaches to their consumption can significantly reduce the cost of product unit manufacturing (Conway, 2019; Demestichas, 2020). The most successful experience was the use of fertilizers, pesticides and seeds in precision farming system, in which agricultural machinery manufacturers offer solutions for their differentiated application (Brisco, 1998; Afanacyev, 2012; Belousova, 2015; Monastyrskiy, 2019; Hernández-Clemente, 2019; Jelínek, 2020; Sychev, 2020). These include: Application of the main comples fertilizer with a rate differentiated by area. This method is used based on the results of pre-defined task maps based on the data of the agrochemical survey, taking into account the content of phosphorus (P_2O_5), kalium (K_2O), and nitrogen

(NO_3, NH_4) in the soil. Application of liquid and dry fertilizing with nitrogen fertilizers with a rate differentiated by area. Nitrogen fertilizers are one of the most expensive items of expenditure. But at the same time, top dressing of vegetative plants is a necessary stage for obtaining high yields and high-quality products. Therefore, their rational use is most relevant in agricultural production. Differentiated application of fertilizers can be carried out both in the off-line mode, which is most acceptable for the main application, according to pre-built maps, and in the on-line mode according to data obtained using sensor photo detectors installed on the sprayer or fertilizer spreader. In this case, the data is read and processed at the time of top-dressing.

The purpose of the research: to study the effectiveness of the method for obtaining data on the state of crops using the GreenSeeker optical sensor. Hypothesis: the possibility to use the data of the GreenSeeker optical sensor for the differentiated application of nitrogen fertilizers.

MATERIALS AND METHODS

Studies on various crops were carried out at the Field Experimental Station of the Russian State Agrarian University-Moscow Agricultural Academy named after K.A. Timiryazev (city of Moscow) and in the Department of remote hybridization of the Tsitsin Main Botanical Garden RAS (Moscow region) in 2009–2016. The soil of the experimental site is sod-podzolic medium loamy. To monitor the dynamics of fertilizer elements, an annual agrochemical survey of the soil was conducted, the results of which indicate that it is well provided with mobile forms of phosphorus (P_2O_5) - 183–304 mL kg of soil^{-1}; and exchangeable potassium (K_2O) - 102–148 mL kg of soil^{-1}; average humus content - 1.9–2.0%; and pH_{KCl} - 4.8' The microrelief of the field is present. This field terrain makes it possible to differentiate the application of nitrogen fertilizers by experimental plots every year. The variability in the main elements of nutrition among plots was the following: for potassium - 13%, for phosphorus - 26.8%, for nitrogen - 46.7%.

The scheme of the experiment each year consisted of three treatments: without top-dressing with nitrogen fertilizers (Control); top-dressing with the recommended fixed dose; differentiated application of nitrogen fertilizers. The differentiated dose was calculated on the basis of data previously obtained from the Trimble GreenSeeker (Trimble; Sunnyvale; CA; USA) optical sensor (Shshuklina, 2017), with a dose varying from the average recommended dose. Top-dressing was carried out manually with ammonium nitrate (NH_4NO_3). The area of the accounting plot is 10 m^2. The repetition in the experiment is 4-fold with 8 repetitions in each variant, which was 32 plots for each of the three variants. A large number of repetitions was necessary to calculate the correlation dependencies of the experiment indicators.

The principle of the GreenSeeker system operation is based on the readings read by optical sensors that have independent light sources. This allows to read information at any time of the day, and regardless of weather conditions (fog, cloudy weather). GreenSeeker emits rays in the red and near-infrared (IR) spectrum, which are reflected from the surface of plants and fall on the photodiode located in the head of the sensor. The data obtained characterize the so-called vegetative index of biomass NDVI (Normalized Difference Vegetative Index). This indicator allows to effectively assess the condition of plants and their potential yield level (Barbosa, 2019). This is achieved by initializing the reflection in the red and infrared spectra. Simultaneously with taking readings by GreenSeeker optical sensor, photometric diagnostics were performed with the portable N-tester 'Yara' (Konica Minolta, Japan), as well as plant (stem, tissue) diagnostics using the modified method of V.V. Tserling (Tserling, 1990) using 0.5 g of diphenylamine in 100 mL of concentrated H_2SO_4 on a 3-point scale. This method is used to detect the amount of NO_2, NO_3 ions in the juice of living plants directly in the field. All measurements were carried out in three phases of spring barley plant development: BBCH 32, BBCH 55, and BBCH 77. The application of fertilizers according to the results of the GreenSeeker measurement was carried out after the studies in the BBCH 32 phase. The differentiation consisted in the fluctuation of the dose of nitrogen fertilizers calculated by the coefficient of deviation from the average NDVI reading for the entire experimental site (Afanasyev, 2006). The fixed top-dressing was applied at a dose of 90 kg ha of nitrogen.The object of the study is spring barley variety TSHA4. The seeds were sown with an Amazon seeder within the time limits accepted for this zone. The seeding rate of spring barley is 5.0 million germinating seeds per 1 ha. Harvesting was carried out by the continuous method of the Sampo Rostov 2000 combine harvester with a working width of 1 m. Statistical processing was carried out according to the method of variance analysis, adopted in Russia when calculating the data of exploratory agricultural work. (Dospekhov, 1976). It is similar to the statistical methods developed by R.A. Fisher (1954). and statistical methods ANOVA.

RESULTS AND DISCUSSION

The indications for applying top-dressing were taken in the phase of stem elongation (BBCH 32) on all experimental plots Greenseeker optical system' In different parts of the field, the NDVI index was low (0.25–0.31) or high (0.68–0.71). It was noted that a low NDVI index was observed in erosion-hazardous areas of the field with a low content of nitrogen and phosphorus. On average, the NDVI index of spring barley crops was 0.49, which is a fairly low value, during the release phase. In most of the plots, the spring barley plants needed to be fed with nitrogen fertilizers. The differentiated dose of nitrogen fertilizers was calculated by deviation coefficient of the NDVI index from the average for all areas of the field wihh differentiated application . At the same time, the nitrogen dose ranged from 64 to 117 kg ha.

During the growth stage Inflorescence emergence (BBCH 55) of spring barley, readings were again taken from the entire experimental area with the GreenSeeker optical sensor. After top-dressing, the average field vegetation index of spring barley biomass was 0.62, which is 0.13 conventional units higher than before the introduction of nitrogen fertilizers. At the same time, it continued to vary widely from 0.27 to 0.68 on plots without top-dressing. Higher rates were recorded in terrain depressions with good water availability. In the variant with a fixed dose of top-dressing, the values ranged between 0.52 and 0' On plots with a fixed dose of nitrogen fertilizers, the biomass index was even higher and ranged from 0.55 to a maximum value of 0.79. This suggests that the application of fertilizers with rate differentiated by area favorably affected the general condition of spring barley plants.

Why no time-series data of GreenSeeker measurements is presented? It would be interesting to see how the NDVI values evolved during the duration of the experiment, and especially before and after fertilization. I suggest including such figure. It is very important that the figure will present standard error (or standard deviation) in order to see the variability within replicates). Values of Yara measurements could also be presented in a time series plot.

Accounting for the yield in the experiment was carried out on a plot basis. At the same time, the average yield in the experiment was the following: on plots without top-dressing - 4.01 kg ha^{-1}, on plots with a fixed dose of top-dressing - 4.58 kg ha^{-1}, on plots with a differentiated dose of fertilizers - 5.21 kg ha^{-1} (Fig. 1). The increase in relation to the control for all variants, as well as between two variants with a different method of application, was statistically significant ($HSD_{.05}$ = 0.17).

The results of the correlation analysis between the yield and various indicators before and after fertilization are presented in Table 1. For this purpose, simultaneously with taking readings with the GreenSeeker optical sensor, stem (tissue) diagnostics according to Tserling (Tserling, 1990), measuring of plant height in the plot, and photometric diagnostics with the Yara N-tester were carried out. The scores of stem (tissue) diagnostics carried out in the phase of leaf-tube formation were most strongly correlated with the yield on plots without fertilization and with the application of their fixed dose.

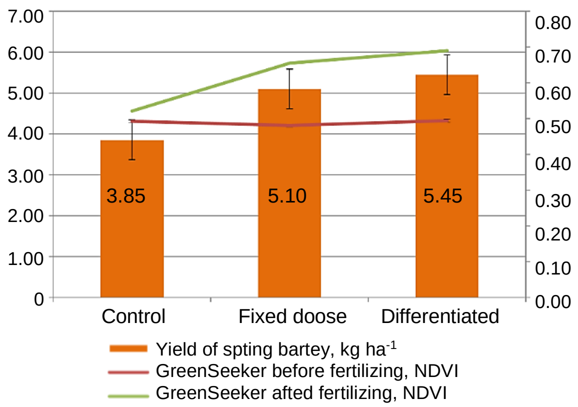

Figure 1. Barley yield for the different treatments, kg ha^{-1}.

The NDVI index recorded by the GreenSeeker optical sensor correlated most strongly with the grain yield of spring barley during filling phase on plots without fertilization (r = 0.70). This confirms the fact that the higher grain yield in this variant depended on the agrochemical composition of the soil of the site. The obtained readings in this phase reflect plant state and with a high degree of probability predict the value of the future yield on a particular plot.

Table 1. Correlation of photometric and stem (tissue) diagnostics data with spring barley grain yield (*: $p < 0.05$)

Indicator	Control (without top-dressing)	Fixed dose	Differentiated dose
Measurement of readings, before fertilization (BBCH 32)			
GreenSeeker, NDVI	-0.05	0.16	-0.15
Yara, c.u.	-0.09	0.06	-0.39
Tissue diagnostics, score	0.30	0.24	0.02
Plant height, cm	0.06	0.25	0.67
Measurement of readings, after fertilization (BBCH 53, 77)			
GreenSeeker, NDVI (BBCH 53)	0.70	0.12	0.37
GreenSeeker, NDVI (BBCH 77)	-0.14	-0.24	0.04
Yara, c.u. (BBCH 77)	0.70	0.51	0.36
Tissue diagnostics, score(BBCH77)	-0.01	0.01	-0.17
Plant height, cm (BBCH 77)	0.52	0.33	0.56

The scores of stem (tissue) diagnostics, as well as the readings of the GreenSeeker optical sensor recorded during milk-ripe phase, do not correlate with the grain yield of spring barley, since in this phase most plants have a high content of dry matter and a low content of chlorophyll, which complicates the romoval of measurement data. At the same time, the correlation coefficient between plant height in milk-ripe phase and yield had a weak bond ($r = 0.33$) in the variant with a fixed dose of fertilizer, and an average ($r = 0.56$) in the variant with a differentiated application of top-dressing. This suggests that taller plants in leveled crops, including in control crops, form a higher grain yield and vice versa.

Since with the help of the Yara N-tester, it is possible to approach the readings in different phases in more detail and find green leaves for manual measurements in the late vegetative phase, its data had the strongest correlation with the yield during the milk-ripe phase. This suggests that plants with a longer growing season, and therefore provided with nitrogen and moisture, gave a higher grain yield.

As I understand, some of the measurements were carried out before fertilization, and that fertilization was carried out for 'differenciated' treatments according to the values of the Greenseker measurements. In this case, the negative correlation between GreenSeeker NDVI and yield at early stages is logical. But it is important to clarify what measurements were carried out before fertilization (only leaf-tube formation phase?). This should be made clear in Table 1 too, maybe by adding an '*' to the measurements carried out before fertilization or a similar way.

CONCLUSIONS

It was found that the yield of spring barley obtained by applying differentiated top-dressing with nitrogen fertilizers was higher than on plots without fertilizers by 29.9%. And higher than the average yield on plots with a fixed dose of nitrogen fertilizers by 14.2%. It should be noted that the total amount of fertilizers applied with this method of calculation does not differ from the continuous application of the calculated dose, but allows to more efficiently apply top-dressing with nitrogen fertilizers, distributing it according to the plant needs, which has a favorable effect on grain yield. When calculating the physical mass of fertilizers used in the experiment, 9.2 kg of ammonium nitrate was used on plots with a fixed dose, and 9.7 kg of ammonium nitrate was used on plots with a differentiated dose.

The results of the readings of the GreenSeeker optical sensor and the Yara N-tester do not correlate with each other, both before and after fertilization. The GreenSeeker is a more user-friendly device. Since the results of its readings do not depend on the weather (cloudy or clear), and the device itself can be attached to the tractor. Readings are taken instantly. The N-tester Yara is a hand-held device that is used when you need to check the condition of plants at a certain point in the field. In order to get the result, it is necessary to take readings from 30 leaves of plants located nearby. This takes about 10–15 minutes

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Fixed nitrogen in agriculture and its role in agrocenoses

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Abstract. On typical low-humus black soils in short crop rotations with legumes (25–33%) and without them, it was found that depending on the set of crops in crop rotation and application of fertilizer rates, nitrogen yield per crop is from 355 kg ha^{-1} to 682 kg ha^{-1}. The recommended fertilization system provided nitrogen compensation for crop yields by only 31–76%. Hence, in the plant-fertilizer system nitrogen deficiency varies from 161 to 370 kg ha^{-1}. The greatest nitrogen deficiency in the soil is observed in crop rotation without the use of fertilizers with the following crop rotation: peas-winter wheat-grain maize-spring barley. The main source of nitrogen for plants is soil nitrogen. In crop rotations with legumes, biological nitrogen is supplied from the air, which in quantitative terms per rotation in crop rotations with peas is 109–288 kg ha^{-1}, with soybeans 264–312, and with alfalfa 486 kg ha^{-1}. Biological nitrogen in crop rotations with peas and soybeans is reimbursed from 25 to 62%, in crop rotation without legumes - 9% (non-symbiotic nitrogen fixation), and in crop rotation with alfalfa - 89% of the total nitrogen removal with the crop.

Key words: biological nitrogen, legumes, nitrogen balance, nitrogen fixation, short crop rotations.

INTRODUCTION

The level of soilfertility and crop yieldlargely depend on the reserves and mobility ofnitrogen in the soil. Insufficient application of fertilizers and the absence of legumes reduce the content of organic matter and nitrogen in the soil. The current state of nitrogen balance in the soils of Ukraine does not correspond to its optimal parameters. Alfalfa, sainfoin and cloverare the most powerful assimilation of biological nitrogen. The nitrogen fixation coefficient of these crops is 0.7–0.8. In general, perennial legumes attract from 100 to 300 kg ha^{-1} of nitrogen to the biological cycle.

Nitrogen is a biogenic element of the Earth planet, and the main component of living matter, which plays a crucial role in the lives of plants and animals. A powerful nitrogen reservoir is the Earth's atmosphere, where its reserves are about 4 trillion tons. There is on average about 80 thousand tons of molecular nitrogen above each hectare of land in the air, which is a source of replenishment of bound nitrogen in the soil (Patyka et al., 2015; Toom et al., 2019; Karpenko et al., 2020; Tonkha et al., 2021).

The productivity of terrestrial and aquatic ecosystems, as well as the biosphere in general, depends on the sources of nitrogen. Providing green plants with nitrogen is a rather difficult task, as higher plants are not able to use free nitrogen in the air as a source of nitrogen nutrition. The studies have shown that all living organisms, including plants, constantly need available forms of nitrogen and have no ways to reserve it. They are in the ‘ocean’ of molecular nitrogen. Of all biodiversity of living nature, only a small number of organisms are able to supply themselves with nitrogen, while microorganisms-nitrogen fixers provide not only themselves but also the entire biosphere with biological nitrogen, as well as its reservation in the form of various nitrogen compounds (Eckert et al., 2001; Aras Mohammed Khudhur & Kasim Abass Askar, 2013; Manabu Itakura et al., 2013; Frans de Bruij, 2015; Steenhoudt & Vanderleyden, 2000; Hryhoriv et al., 2020; Novák et al., 2020).

The only way to create nitrogen reserves is to convert it into a specific soil organic matter - humus. Only soils have the ability to accumulate bound nitrogen during biological fixation and play the role of the only long–term bank of this element. In terms of significance for wildlife, the phenomenon of nitrogen fixation carried out by microorganisms in symbiosis with plants is compared with another global process of the Earth planet, namely photosynthesis(Zakharchenko et al., 1974; Kudeyarov, 1989; Patyka et al., 2015; Karpenko et al., 2020a).

The scientific researches have shown that under the conditions of field crop with insufficient application of fertilizers and the absence of legumes there is a gradual decrease in the content of organic matter and nitrogen in the soil. This is due not only to mechanical tillage and intensification of oxidative processes, but also the alienation of nitrogen with crop yields. Thus, the cost of humus (mineralization) for the cultivation of cereals reaches 0.5–0.7 and even 1.0 t ha^{-1}, including - 25–50 kg ha^{-1} of nitrogen. Under row crops and especially in conditions of pure vapors, the mineralization of organic matter and the lack of nitrogen in the soil is further enhanced (1.5–2.0 t ha^{-1}, including 75–100 kg ha^{-1} of nitrogen), which subsequently leads to general deterioration of physical, physicochemical properties and nutrient status of the soil (Zakharchenko & Shilina, 1968; Zakharchenko et al., 1974; Karpenko et al., 2019; Telekalo & Melnyk, 2020).

Due to comprehensive and in–depth studies of the cycle and balance of plant nutrients in agriculture of Ukraine, the parameters of the cycle are set, the complex multifaceted transformation of nitrogen in the system 'soil-fertilizer-plant-water' is studied, and the optimal levels of return of nutrient elements to the soil with fertilizers in crop rotation of different climatic zones of Ukraine are determined. This, in turn, largely defined the high level of crop productivity and farming (Pirozhenko, 1963; Zakharchenko & Medvid, 1972; Shilina et al., 1983; Tsvei et al., 2008; Litvinov et al., 2020; Karbivska et al., 2020). If it is permissible on black soils for some time to increase yields due to the use of soil nitrogen reserves, then on poor soils of Polissia it is necessary to create a deficit–free and even excess nitrogen balance.

Hence, there is an urgent need to address the problem of restoring and preserving soil fertility, obtaining environmentally friendly agricultural products of high quality. The growing interest of scientists in the biological conversion of molecular nitrogen into biological one under the action of diazotrophic microorganisms in symbiosis with plants in natural ecosystems and agrocenoses is quite clear (Aras Mohammed Khudhur and Kasim Abass Askar, 2013; Manabu Itakura et al., 2013; Steenhoudt & Vanderleyden, 2000).

The most powerful assimilate of biological nitrogen in crop production is perennial legumes - clover, sainfoin, alfalfa - whose nitrogen fixation coefficient is 0.7–0.8. During the growing season, these crops accumulate from 30 to 38 kg of biological nitrogen for each ton of products in the dry matter. In general, perennial legumes attract from 100 to 300 kg ha^{-1} of nitrogen to the biological cycle, of which 75–200 kg is a pure product for the plant; the rest of the nitrogen is consumed by the plants from the soil. The assimilation capacity of legumes is slightly lower than perennial legume grasses. The nitrogen fixation coefficient is 0.4–0.5, and the rest of the nitrogen they use from the soil. For each ton of dry matter of the main and by-products lupine and fodder beans assimilate 20–27 kg from the air; peas, soybeans and others - 10–15 kg of nitrogen. Much of the biological nitrogen (sometimes up to 90%) during the growing season is concentrated in the aboveground mass, usually alienated from the field, and the rest remains in the soil in the roots and post–harvest residues, causing nitrogen gain in the soil after mineralization (Hamkalo, 2004; Kots & Patyka, 2009; Patyka et al., 2001; Patyka et al., 2003; Kots, 2011; Patyka, 2014; Yermolaev et al., 2014; Tanchyk et al., 2017; Karbivska et al., 2019).

Due to the ability of legumes to enter into symbiosis with specific for a particular species or group of species nodule bacteria, they can absorb up to 125–350 kg ha^{-1} of nitrogen during the growing season in the soil and climatic conditions of Ukraine (Zakharchenko & Shilina, 1968; Shilina et al., 1983; Pirozhenko,1993; Patyka et al., 2015; Litvinov et al., 2019). Due to symbiotic nitrogen fixation, legumes form high yields of cheap vegetable protein without the use of expensive, energy–intensive and environmentally hazardous mineral nitrogen fertilizers. After harvesting, more than 30% of biologically fixed nitrogen remains in post-harvest and root residues and is used by subsequent crops.

The goal of the work is to reveal the parameters of biological nitrogen fixation depending on the saturation of short-term crop rotations with the legume component.

MATERIALS AND METHODS

Field studies were conducted in a stationary field experiment during 2004–2011 in the subzone of unstable humidification of the Forest–Steppe of Ukraine at the Panfil Research Station of the National Scientific Centre ‘Institute of Agriculture of National Academy of Agrarian Sciences’ (Panfily village, Yahotyn district, Kyiv region) (Table 1). The experiment started in 2001, included 16 variants, with a four-time replication. The cultivation area was 90 m^2, the tested area under crops was 60 m^2. Cattle’s dung with litter was used for grain maize and sunflavers 10 t ha^{-1} if calculate for 1 hectare of crop rotation fields, the other crops used an afteraction.

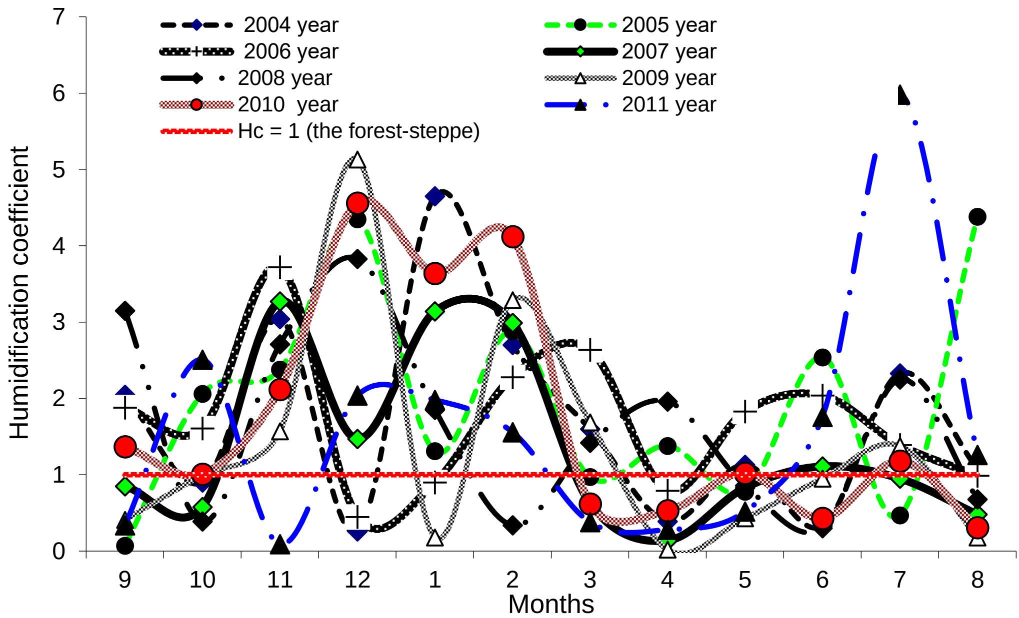
Table 1. Scheme of stationary experiment on the study of short crop rotations

Variant of rotation	Alternation and fertilization of crops in crop rotation					Average crop rotation rate of fertilizers			
	I	II	III	IV	V	Organic, t	Mineral, kg d.r.		
						manure	N	P	K
1	peas	winter wheat	grain maize	spring barley		–	–	–	–
2	peas $P_{30}K_{40}$	winter wheat $N_{60}P_{60}K_{60}$	grain maize $N_{60}P_{40}K_{60}$	spring barley $N_{60}P_{40}K_{60}$		–	45	42	55
3	peas $P_{30}K_{40}$	winter wheat $N_{60}P_{60}K_{60}$	grain maize $N_{60}P_{40}K_{60}$ + 40 t $ha^{-1}$of manure	spring barley $N_{60}P_{40}K_{60}$		10	45	42	55
4	perennial legumes $P_{30}K_{40}$	winter wheat $N_{60}P_{60}K_{60}$	grain maize $N_{60}P_{40}K_{60}$	barley + grasses $N_{60}P_{40}K_{60}$		–	45	42	55
5	peas $P_{30}K_{40}$	winter wheat $N_{60}P_{60}K_{60}$	sunflower $N_{90}P_{60}K_{90}$ + 20 t $ha^{-1}$of manure	spring barley $N_{60}P_{40}K_{60}$	grain maize $N_{60}P_{40}K_{60}$ + 30 t $ha^{-1}$of manure	10	54	46	62
6	soybean $P_{30}K_{40}$	winter wheat $N_{60}P_{60}K_{60}$	grain maize $N_{60}P_{40}K_{60}$ + 30 t $ha^{-1}$of manure			10	40	43	53
7	buckwheat $N_{30}P_{40}K_{40}$	winter wheat $N_{60}P_{60}K_{60}$				–	45	50	50

The soil cover of the experimental area is typical low-humus coarse-grained light loamy black soil. The humus content in the arable layer (0–20 cm) varies from 3.08 to 3.15 % (by the Tyurin method). The soil is characterized by a high content of phosphorus - 233–270 mg kg^{-1}, high and medium content of exchangeable potassium (80–100 mg kg^{-1} of soil) by the Chirikov method. The reaction of the soil solution is weakly acidic - pH_{KCL} - 5.7 by potentiometry, the degree of saturation of the absorption complex with bases is high (85–99%). The agronomic value of organic fertilizers (cattle’s dung without litter) according to the main food compounds in 1 ton of dung is: N - 4.0 kg, P_2O_5 - 2.0 kg, K_2O - 4.0 kg.

According to the observations of Yahotyn Meteorological Station (Yahotyn), the average annual air temperature is 7.3 °C; the average long-term precipitation is 442 mm and it varies from 250 to 670 mm; the relative humidity is 78%; the average duration of the growing season is 202 days.

Meteorological conditions during the years of research were quite contrasting, as evidenced by significant deviations from the long-term average in terms of precipitation and average monthly air temperature. Analysis of heat and moisture supply by the humidification coefficient (Hc) by Ivanov (Ivanov, 1946) shows that in all years of research the driest conditions were in April and May. This fact affected the vegetation of the studied crops to some extent (Fig. 1).



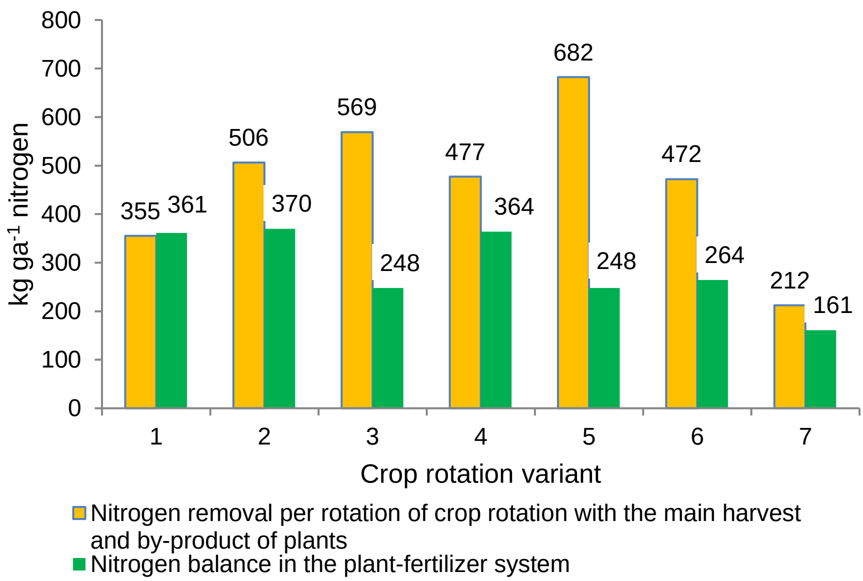
Notes: Humidification coefficient - the ratio of annual precipitation to evaporation for the same period Hc = P/f, where P is the amount of precipitation (mm), and f is the evaporation for the same period (%). Evaporation calculation according to Ivanov: f = 0.018 × (t + 25) 2 × (100 - R), where t – is the average temperature for the period (°C/year), R – is the average relative humidity (%).

Figure 1. Characteristics of heat and moisture supply of the stationary experiment during the growing season in the years of research on the humidification coefficient by N.M. Ivanov.

In general, the most favorable for the formation of crop productivity were 2004–2006, 2008 and 2009 and 2011. Arid conditions of crop vegetation developed in 2010, which was characterized by the most significant and extreme deviation of air temperature during the growing season. Based on the quantitative indicators of individual components of crop rotation in the soil-fertilizer-plant system obtained in the experiment, its full balance in the system of short crop rotations for 2004–2011 was compiled. The calculations are based on the method of comparing (ratio) of its expenditure and compensation items (Zakharchenko et al., 1974, Shilina et al., 1983). When compiling the balance, we did not consider the input of nitrogen with atmospheric precipitation and carried out by infiltration waters from the root layer of the soil, since these sources of 'input' and 'consumption' of nitrogen in terms of parameters, as studies have shown, do not play a significant role in the balance. Therefore, in addition to accounting for its amount removed from the field by crops, the item 'costs' includes losses of nitrogen from fertilizers in gaseous form, and the item of income includes the amount of nitrogen in mineral and organic fertilizers and crop seeds. The input of biological nitrogen into the crop rotation system due to symbiotic and non-symbiotic nitrogen fixation was determined by the difference between the final indicators of nitrogen balance in the plant-fertilizer system and indicators of quantitative changes in the total nitrogen content in the soil during rotation.

RESULTS AND DISCUSSION

Biological nitrogen fixation is a powerful factor in the system of preservation and reproduction of soil fertility, increasing the productivity of agrocenoses. According to the results of the studies in short crop rotations with legumes (25–33%) and without them, it was found that depending on the set of crops in crop rotation and NPK application in the norm of 136–142 kg ha^{-1} of crop rotation area with legumes, crop nitrogen removal by rotation was from 355 kg ha^{-1} in the control variant (without fertilizers) up to 443–682 kg ha^{-1} when applying fertilizers (Fig. 2). This fertilizer system provided reimbursement of nitrogen costs for crop production by only 31–76%. Thus, a significant nitrogen deficit is created in the plant-fertilizer system, the value of which varied from 161 to 370 kg ha^{-1}.



Note. Crop rotation variant: 1, 2, 3 – peas-winter wheat-grain maize-spring barley; 4 – alfalfa-wheat winter-grain maize–spring barley; 5 – peas-winter wheat–sunflower–spring barley–grain maize; 6 – soybean-winter wheat-grain maize; 7 – buckwheat-winter wheat.
Fertilizer rate per 1 ha of crop rotation area: 1. without fertilizers; 2. $N_{45}P_{42}K_{55}$; 3. manure, 10 t + $N_{45}P_{42}K_{55}$; 4. $N_{45}P_{42}K_{55}$; 5. manure, 10 t + $N_{54}P_{46}K_{62}$; 6. manure, 10 t + $N_{40}P_{43}K_{53}$; 7. $N_{45}P_{50}K_{50}$.

Figure 2. Nitrogen balance in the plant-fertilizer system, average for 2004–2011.

With such a significant nitrogen deficiency in the plant-fertilizer system, a decrease in the total nitrogen content in the soil was observed in the control crop rotation: pea-winter wheat-grain maize-barley (without fertilizers), where the main source of nitrogen for plants was soil nitrogen. In a similar crop rotation, under the mineral fertilization system ($N_{45}P_{42}K_{55}$ per 1 ha of crop rotation area) only 32% of nitrogen removal was compensated by fertilizers. This pattern was observed in two-field crop rotation: buckwheat–winter wheat, without legumes. The absolute values of nitrogen consumption in the soil in these crop rotations were much lower than its deficit in the plant-fertilizer system, which indicates an additional supply of nitrogen to the soil and plants from natural sources, namely due to biological nitrogen (symbiotic and non-symbiotic nitrogen fixation).

The studies have shown that in the soil–plant-fertilizer system (Table 2), in all variants of crop rotations due to the receipt of biological nitrogen, fixed from the air by legumes and partially existing in the soil microorganisms - nitrogen fixers, the result was positive, which in quantitative relation to crop rotations with peas was 109–288 kg ha^{-1}, with soybeans - 312, and in crop rotations with alfalfa - 468 kg ha^{-1}.

Table 2. Biological nitrogen, average for 2004–2011

Fertilizer system (rate of fertilizers per 1 ha of crop rotation area)	The amount of biological nitrogen				
	Biological nitrogen (symbiotic and non-symbiotic nitrogen fixation) total		Including by fixing legumes		
	kg ha^{-1}	% from removal of crops	kg ha^{-1}	% from removal of crops	kg ha^{-1} per year
Peas-winter wheat-grain maize-spring barley					
control (without fertilizers)	+109	28	+69	18	+17.3
$N_{45}P_{42}K_{55}$	+261	48	+221	39	+55.4
manure, 10 t + $N_{45}P_{42}K_{55}$	+294	48	+254	41	+63.5
alfalfa-winter wheat-grain maize-spring barley					
$N_{45}P_{42}K_{55}$	+468	89	+428	81	+107.0
peas–winter wheat–sunflower–spring barley-grain maize					
manure, 10 t + $N_{54}P_{46}K_{62}$	+288	40	+238	33	+47.6
soybean–winter wheat-grain maize					
manure, 10 t + $N_{40}P_{43}K_{53}$	+312	62	+282	56	+94.0
buckwheat–winter wheat					
$N_{45}P_{50}K_{50}$	+21*	9	-	-	-

Note. * – due to non-symbiotic nitrogen fixation.

Due to biological nitrogen in crop rotations with peas and soybeans it was reimbursed from 28% to 62%, in crop rotation without legumes - 9% (non-symbiotic nitrogen fixation), and in crop rotation with alfalfa - 89% of the total nitrogen removal with the crop. Nitrogen increase due to fixation from the atmosphere by legumes was the highest in crop rotation with alfalfa - 107 kg ha^{-1} per year and soybeans - 94 kg year^{-1}; lower increase was in crop rotation with peas - from 17.3 to 63.5 kg ha^{-1} per year. Nitrogen fixed by legumes prevailed in the total increase of biological nitrogen in crop rotations. Thus, biological nitrogen in crop rotations (20–50%, and in crop rotations with perennial legumes up to 90%) is able to compensate for nitrogen costs and thus significantly reduce the need for mineral nitrogen. Thus, nitrogen fixation in crop rotation largely depends on the role and saturation of the crop rotation with legumes.

The current state of nitrogen balance in agriculture in Ukraine does not correspond to its optimal parameters. According to the State Statistics Committee of Ukraine, the current level of fertilizer application in the fields of the country is: rganic fertilizers - 0.5 t ha^{-1}, mineral fertilizers - 80 kg ha^{-1}, of which mineral nitrogen is 67%. The share of the area on which fertilizers are applied is for organic ones - 1.2%, for mineral fertilizers - 39.8% of the total area of agricultural land (State Statistics Committee of Ukraine. http://www.ukrstat.gov.ua.).

Scientifically substantiated level of compensation for nitrogen removal from all sources of income, on average in Ukraine, according to the results of long experimental studies and calculations based on them should be at least 110% (in Polissia 130, Forest-Steppe - 100–110, Steppe - 100%) (Patyka, 2014; Shilina et al., 1983). This means that with high crop productivity, adequate nitrogen removal of 100 kg ha^{-1} to create its positive balance, which will ensure the reproduction of soil fertility, for each hectare of arable land you need to return at least 110 kg of nitrogen Potential opportunities for the supply of biological nitrogen due to symbiotic nitrogen fixation are: perennial legumes - 30–35 kg per 1 ton of dry matter of the main product; legumes (peas, soybeans, beans) - 10–15 kg per 1 ton of dry matter of the main and by-products; lupine, fodder beans - 20–27 kg per 1 ton of dry matter of the main and by-products (Zakharchenko et al., 1952; Zakharchenko & Shilina, 1968; Zakharchenko et al., 1974).

Calculations of biological nitrogen supply showed that about 10% of legumes in the structure of sown areas (including 2.83% of perennial legumes) and the average yield of legumes - 1.80–2.64 t ha^{-1}, perennial legumes - 4.01 t ha^{-1} due to symbiotic nitrogen fixation for each hectare of arable land in 2018 we received additionally only 6.08 kg of nitrogen (Table 3).

That is, the total supply of biological nitrogen due to symbiotic nitrogen fixation in the country's agriculture is determined solely by the area under legumes and in particular perennial legumes in the structure of sown areas.

CONCLUSIONS

Effective in the system of short-rotation crop rotations on chernozem is nitrogen replenishment due to symbiotic and associative nitrogen fixation. Against the background of the use of fertilizers at high crop yields, the intake of biological nitrogen due to nitrogen fixation in crop rotations with peas is 261–294 kg ha^{-1}, soybeans - 312 kg ha^{-1}, perennial grasses (alfalfa) - 468 kg ha^{-1}.

By introducing a legume component into the crop rotation, it makes it possible to compensate, due to nitrogen fixation, from 40 to 89% of the nitrogen consumption from fertilizers and soil for the growing crops.

The priority measure for the effective use of biological nitrogen of legumes in agriculture should be a scientifically grounded structure of sown areas with the optimal share of these crops at the national level and at the level of each farm, regardless of its land ownership form.

Table 3. Biological nitrogen in agriculture of Ukraine (legumes), 2018 (State Statistics Committee of Ukraine. http://www.ukrstat.gov.ua.)

Legume crop	Sowing area		Crop yield of main products, t ha^{-1}	Yield of dry matter of the main product, cha^{-1}	The ratio of the main and by-products (in dry matter), c	Yield of dry matter of by-products, t ha^{-1}	Total dry matter yield of main and by-products, t ha^{-1}	Gross collection of dry matter of main and by-products, t	Fixation of nitrogen from the air	
	Thousands of ha	% to the arable land							kg per 1 ton of dry matter of the main and by-products	Total kg
Grain legumes (peas, beans, vetch etc.)	568.4	1.75	1.80	1.55	1:1.3	2.02	3.57	2,029.188	10	20,291.880
Soybeans	1,709.0	5.25	2.64	2.28	1:1.6	2.65	4.93	8,425.370	10	84,253.700
Perennial legumes (clover, alfalfa, sainfoin)	920.6	2.83	4.01 (hay)	3.37	–	–	3.37	3,102.422	30	93,072.660
Total of legumes	3,198.0	9.84								197,618.240
Arable land of 2018	32,500.0	100								
On 1 ha of arable land										6.08

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Productivity, heritability and stability analysis of a Moroccan sugar beet germplasm

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Abstract. Progeny testing is the second part of maternal recurrent selection scheme adopted by INRA-Morocco for the national sugar beet breeding programme. The objective of this study is sugar beet germplasm productivity, heritability and stability analysis. The studied material concern 18 half-sib families (HSF) preselected initially for their seed production potential. Trials were conducted using randomised complete blocks designs during, 2013/14, 2014/15, 2015/16, 2017/18 campaigns in two experimental fields of INRA-Morocco; Sidi Allal Tazi (34° 30' N, 6° 19' W) and Larache (35° 11' N, 6° 09' W). Evaluated parameters concern the vigour, root weight (RW), leaf biomass yield (LBY), and sugar content (Sc). Data analysis by comparative procedures explores different accordance degrees of HSF versus controls. Good vegetative growth was observed, 85.6% closer to the maximal indicated scale level. The RW was significantly influenced by the genotype and reached a maximum of 1.06 kg versus 1.08 kg average recorded by controls. Sugar content recorded mean was 20.97% in HSF versus 21.39% in the controls. Most of HSF revealed mean values close to Z-type variety. Estimated heritability was 0.5 for RW, 0.2 for the LBY, and 0.02 for Sc. Sugar content was influenced by the environment and explained by the AMMI model (73.6%) versus 53.9% and 44.4% for root weight and leaf biomass yield respectively. The AMMI stability values showed F11, F12, F16, and F17 families as the most performing and stable HSF. Results demonstrate the relevance of the maternal recurrent selection scheme of the on-going national breeding programme.

Key words: half-sib family, heritability, germplasm, productivity, stability, sugar beet.

INTRODUCTION

Sugar beet is a major economic crop that provides wide range of products, especially sucrose, pulp for animal feed, and bio products such ethanol (FAO, 2019). Sugar beet crop is mainly cultivated in temperate zones. At nationally level, sugar beet covers more than 80% of the total sugar crops area. Morocco imports sugar beet seeds and sugar in large quantities every year to meet the national need. The local sugar beet germplasm enhancement will have significant contribution for sugar beet cropping extension and the development of locally adapted cultivars.

The average sugar beet root weight usually ranges between 0.5 to 1 kg and sugar content between 13–22 percent. Sugar yield as a combined trait between root yield and sugar content represents the most important parameter for farmer and breeder (Hoffmann et al., 2009). Sugar yield is strongly influenced by the environment and is highly correlated to root yield and sugar content (Powers et al., 1963; Schneider et al., 2002; Hoffmann et al., 2009). Several types of sugar beet varieties exist; they differ mainly in term of their vegetation season, root yield and sugar content. Sugar beet genotype has a significant effect on sugar yield. Z-type hybrids have better stability and sugar yield and a relatively shorter vegetation period (Curcic et al., 2018).

In plant breeding, crop productivity and heritable variation are key criteria in germplasm enhancement. Heritability for plant breeders and geneticists measures the precision of the experiments (Piepho et al., 2008), and evaluates the selection relevance (Schmidt et al., 2019). It measures the variance due to the genetic causes and predicts crop improvement progress (Songsri et al., 2008).

Multiple-sites trials are required to characterise and structure germplasm according to their behaviour in different environments. The environment and varietal effects produce substantial variation in the genotype expression between sites, which decreases the correlation between phenotypic and genotypic forces (Delacy et al., 1990). The genotype by environment interaction (GEI) is always acting in crop production by causing variation in the varietal performance and ranking under the environmental testing conditions (Ndhlela et al., 2014). The genotype x environment interaction is of foremost significance; it evaluates the environments' effect on the breeding genotypes performance and assesses their stability (Moldovan et al., 2000). Previous studies evaluate sugar beet germplasm stability through multi-variate analyses and AMMI (Additive Main effect and Multiplicative Interaction) model (Paul et al., 1993; Ranji et al., 2005). The AMMI model is the most used method; it interprets a major part of the total deviation of GEI and assesses the genotypes performance and stability (Ebdon & Gauch, 2002).

The present study aims to evaluate the HSF progeny for productivity, heritability, and stability of a Moroccan sugar beet germplasm. The evaluation corresponds to the second phase of three selection cycles performed on 18 half-sib families preselected initially for their seed production potential under local climate conditions. This study was performed to analyse the INRA-morocco breeding programme progress for sugar beet germplasm enhancement through the maternal pedigree selection method.

MATERIALS AND METHODS

Studied material

The plant material is composed of 18 sugar beet half-sib families of the national sugar beet germplasm collection received from USDA-ARS breeders. It is multigerm germplasm developed from a sugar beet population carrying a normal cytoplasm that confers several resistance genes to rhizomania. Adopting a maternal pedigree selection method, the 18 sugar beet half-sib families (HSF) were selected to constitute the initial material for the progenies evaluation. This evaluation represents the second phase of three selection cycles, C1, C2, and C3. Seed production potential improvement (first part of a selection cycle) was based on several selection criteria, disease resistance, the vernalisation capacity, the plant vigour at the vegetative growth phase, and yield components performance. The half-sib progenies were duplicated in the field by open pollination in a polycross design between selected individuals belonging to 18 half-sib families. Advanced individuals are considered as potential parents and elected to improve sugar beet next generation. The preselected progenies were characterised through their seed production potential under local conditions during the first phase of the selection cycles (Table 1). The half-sib families were compared to three monogerm sugar beet varieties listed in the official catalogue by the National Office for Food Safety: VERDI type E, CANDIMAX type N and ELVIS type Z. E-type control variety has 230 days cycle duration, low sugar content, and high root yield. N-type control variety is intermediate for the cycle duration of 210 days, the root yield, and the sugar content. Z-type control cultivar is a short cycle (180 days), low root yield, and high sugar rate. These three controls are coded as TE, TN and TZ respectively. The control varieties above have good potential and plasticity under the Moroccan climate conditions, while the 18 half-sib families have wide genotypic variability for seed production.

Table 1. Main characteristics of the half-sib families for seed production potential

Half-sib family	NdB (day)	NdM (day)	GY (g)	Gr (%)
F1	257.89	332.75	112.30	86.29
F2	256.00	327.45	102.56	89.18
F3	251.55	322.09	120.54	87.73
F4	250.86	324.00	136.08	88.29
F5	262.40	334.70	170.19	87.90
F6	252.86	324.71	114.11	89.71
F7	254.24	325.12	92.52	88.32
F8	266.33	333.94	115.28	87.17
F9	259.78	328.00	123.85	87.56
F10	255.50	320.38	88.51	88.50
F11	259.40	324.00	106.16	89.20
F12	254.86	328.64	79.20	83.86
F13	247.00	316.95	107.88	88.89
F14	247.74	319.87	124.74	88.91
F15	255.78	326.78	135.48	84.78
F16	248.82	324.18	122.41	84.64
F17	252.65	327.25	85.95	87.90
F18	263.56	330.11	255.28	89.00
Average	255.11	326.40	116.67	87.57

NdB = Number of days to bolting; NdM = Number of days to maturity; GY = Grain yield per plant in gram; Gr = Germination percentage.

Experimental sites

Two experimental fields of the National Institute of Agricultural Research (INRA-Morocco) were targeted for this study. Sidi Allal Tazi's first site is situated at Gharb-Chrarda-Beni Hssen region (34° 30' N latitude, 6° 19' W longitude, and 10.5 m elevation). It has clay soil and sub-humid bioclimate types. It's annual rainfall averages 520 mm, and means minimum and maximum temperatures 4 and 34 °C respectively. Larache's second site is located in the Tangier-Tetouan-Al Hoceima region at 35° 11' N latitude, 6° 09' W longitude, and 38 m elevation and has sandy soil type. Its climate is sub-humid with an average annual rainfall of 630 mm and minimum and maximum temperatures of 9 and 28.3 °C.

The study was conducted during three selection seasons and in two sites considered as having independent environments. Therefore, these environments were symbolised as E1, E2, E3, E4, E5, and E6. The meteorological data during the vegetative growth phase are presented in Table 2 into three periods: P1 'Winter' December to February, P2 'Spring' March to May, and P3 'Summer' June to July.

Methods and techniques

Trials were conducted according to randomised complete block designs during 2013/14, 2014/15, 2015/16, 2017/18 cropping seasons. The studied HSF were distributed randomly in the elementary parcels. The sowing was carried out manually in December according to four simple flat rows spaced 0.80 m apart and 25 cm between individual seeds and placed at 3 cm depth. Since the HSF were multi-germ, excess plants were discarded at 2 to 3 true leaf stage. The roots harvest was carried out in July, 200 days after sowing, at approximately the average controls growth cycle duration. Once harvested, the plants in central rows were used to carry the measurements and the data collection and analysis.

Table 2. Rainfall means (mm), maximum temperatures mean (°C), minimum temperatures mean (°C) and relative humidity means (%) during the vegetative growth phase in 6 environments, E1, E2, E3, E4, E5 and E6

Environ-ment	Period	Tmax (°C)	Tmin (°C)	Rainfall (mm)	Relative humidity (%)
E1	P1	18.3	8.3	33.3	78.3
	P2	21.7	12.3	42.3	77.7
	P3	26.5	17.5	2.0	72.0
E2	P1	17.7	8.3	45.3	78.7
	P2	20.7	12.0	66.3	80.0
	P3	26.5	17.5	4.5	73.5
E3	P1	21.3	11.3	27.3	75.0
	P2	22.7	13.3	23.0	71.0
	P3	30.0	20.5	4.0	65.0
E4	P1	20.7	11.0	35.3	79.0
	P2	21.7	12.7	38.0	74.0
	P3	30.0	21.0	6.5	64.0
E5	P1	19.0	9.0	36.3	78.7
	P2	24.7	14.3	26.0	69.7
	P3	30.5	21.0	8.0	63.0
E6	P1	19.7	9.0	35.0	78.3
	P2	24.7	14.7	19.0	68.3
	P3	28.5	19.5	6.0	66.0

P1 = From December to February; P2 = From March to May; P3 = From June to July; Tmax = Maximum temperature; Tmin = Minimum temperature.

Data recording

Plant vigour (Vg) was evaluated 3 months after the sowing using a 1 to 5 scale. A score of 5 corresponds to the best vegetative growth and 1 to plants presenting the lowest. The yield components were measured at harvest: leaf biomass yield (LYB) and root weight (RW) were recorded in kilogrammes on 5 individual plants per plot. The sugar content was assessed using 6 randomly selected plants per plot and expressed in percentage. A field refract-metre (ATAGO) was used to assess the total extract, expressed in Brix degrees (°B) where 1 °B corresponds to a refraction index of 1% sucrose solution in water. This procedure was used as a comparative approach to the controls.

Statistical analysis

Collected data were analysed using statistical software R, version 3.6.2, and the STATISTICA 6FR. For the root and sugar productivity analysis, the factorial ANOVA model was used to test the HSF and locality and their interactions' main effects on the total variance. Tukey's HSD test was performed to assess the significant differences between group means (Tukey, 1953). Dunnett's test was carried out also in the HSF groups' comparison to the controls (Dunnett, 1955). The independent factors considered in the heritability analysis are the HSF without the controls. The environments are represented by the combination of the experimental sites and the years.

The heritability (h2) in its narrow sense was calculated by extracting the variance components using the summer package, using the solving Mixed Model Equations in R (MMER) (Covarrubias-Pazaran, 2019). The core of this package represents the function, whereas the multivariate and the univariate mixed model were performed according to Maier et al. (2015). The heritability value is expressed as the fits rate (0 to 1) or as the percentage fits (0 to 100%).

The additive main effects and multiplicative interaction (AMMI) model (Gauch 1992) is used to select the best genotype and environment combinations with respect to the variables' response by analysis of variance. The non-additive residual that is attributed to the GEI (genotype-environment interactions) is analysed by the principal component analysis (Zober et al., 1988). The sum of squares of the GEI is divided into the Interaction of the Principal Component Axis (IPCA). The Biplot of the GEI (Gabriel, 1971; Bradu & Gabriel, 1978; Zobel et al., 1988) is used to explore and interpret the underlying structure and causes of interaction. The IPCA scores estimate the genotypes' stability; the best performing and stable genotypes have a high yield and an IPCA value close to zero. Yield stability was calculated using the AMMI stability value (ASV) as described by Purchase et al. (2000). The ASV represents the deviation from zero in the two-dimensional scattergram (IPCA 1, IPCA 2). The genotypes more stable in different environments record an ASV closer to zero.

RESULTS

Productivity and heritability analysis of the national sugar beet germplasm

As initial data analysis to identify sources of variation, factorial analysis of variance was performed to reveal the individual main effect of locality, HSF, and their interaction on yield components (Table 3). The locality has shown a significant effect on plant vigour and leaf biomass. The HSF revealed a significant effect on root weight and leaf biomass. There was no significant effect of the locality*half-sib family interaction on the yield components. A post-hoc test was used to elucidate the productivity variation.

Table 3. Factorial analysis of variance of yield components versus two factors, locality and half-sib family

		Vigour		Root weight (kg)		Leaf biomass yield (kg)		Sugar content (%)	
Variable	*DF*	*MS*	*F-test*	*MS*	*F-test*	*MS*	*F-test*	*MS*	*F-test*
Locality	1	2.39	4.80*	0.31	3.45	2.33	55.74***	17.39	2.23
HSF	20	0.61	1.23	0.18	2.00***	0.08	1.81*	7.95	1.02
Locality*HSF	20	0.42	0.85	0.09	0.98	0.03	0.73	5.55	0.71
Error	290	0.50		0.09		0.04		7.79	

DF = Degrees of Freedom; *MS* = Mean Square; * = significant at $p < 0.05$; *** = significant at $p < 0.001$.

The Dunnett's test for pair comparisons of HSF versus controls exhibits significantly consistent values (Table 4). The first measurements reveal the good vegetative growth; the best vigour values (4.53) were recorded by two HSF, F2 and F7, which were closer to the control TE (4.58), and exceeded TN and TZ ones. All the HSF were vigorous at 100%, 88.8%, and 94.4% in comparison with TZ, TE, and TN respectively.

For the leaf biomass, the HSF and the controls showed only one group of means. The pair comparisons of HSF means versus the controls indicated significant correlations (Table 4); the similar HSF's values had leaf biomass mean of 0.48 kg. Three HSF had significantly higher values exceeding the controls; F1, F11, and F18 produced respectively 0.62, 0.54, and 0.57 kg per plant compared to the control average value of 0.22 kg.

Table 4. Multiple comparisons of means for the vigour and leaf biomass yield. Tukey's HSD test and Dunnett's test p-values for the pairs of means comparisons of the HSF versus a fixed control, TE, TN and TZ

	Vigour				Leaf biomass yield (kg)			
		p-values for pair comparisons				*p-values* for pair comparisons		
HSF	Mean	HSF vs. TE	HSF vs. TN	HSF vs. TZ	Mean	HSF vs. TE	HSF vs. TN	HSF vs. TZ
F1	4.31^a	0.97	1.00	1.00	0.62^a	0.01*	0.02*	0.11
F2	4.53^a	1.00	1.00	1.00	0.45^a	0.65	1.00	0.98
F3	4.31^a	0.97	1.00	1.00	0.56^a	0.11	0.02*	0.44
F4	4.44^a	1.00	1.00	1.00	0.53^a	0.33	0.96	0.80
F5	4.26^a	0.86	1.00	1.00	0.40^a	1.00	0.21	1.00
F6	4.42^a	1.00	1.00	1.00	0.41^a	0.99	1.00	1.00
F7	4.53^a	1.00	1.00	1.00	0.53^a	0.08	0.42	0.39
F8	4.42^a	1.00	1.00	1.00	0.52^a	0.10	0.62	0.45
F9	4.32^a	0.96	1.00	1.00	0.49^a	0.25	0.44	0.72
F10	4.44^a	1.00	1.00	1.00	0.42^a	1.00	0.05	1.00
F11	4.47^a	1.00	1.00	1.00	0.54^a	0.01*	0.80	0.10
F12	4.31^a	0.97	1.00	1.00	0.47^a	0.88	0.17	1.00
F13	3.62^a	0.00***	0.01*	0.15	0.52^a	0.15	0.45	0.49
F14	4.31^a	0.98	1.00	1.00	0.39^a	1.00	1.00	1.00
F15	4.15^a	0.65	0.92	1.00	0.50^a	0.31	1.00	0.75
F16	4.30^a	0.99	1.00	1.00	0.55^a	0.49	0.13	0.88
F17	4.30^a	0.99	1.00	1.00	0.57^a	0.33	0.16	0.74
F18	3.77^a	0.02*	0.08	0.44	0.57^a	0.03*	0.36	0.19
TE	4.58^a	-	1.00	0.98	0.34^a	-	1.00	1.00
TN	4.47^a	1.00	-	1.00	0.35^a	1.00	-	1.00
TZ	4.31^a	0.98	1.00	-	0.37^a	1.00	1.00	-

HSF = Half-sib family; * = significant at $p < 0.05$; *** = significant at $p < 0.001$; Same lower letter (^{a}) indicates a non-significant difference between half-sib families.

The root weight revealed two groups (Table 5) and the HSF average values fluctuated between 0.72 and 1.06 kg for F15 and F11 respectively. The HSF comparison with controls showed that all HSFs were closer to TZ type control, except F15 which had the lowest root weight.

For sugar content, there was no significant difference between HSF, and significantly comparable values with the controls TE, TN, and TZ (Table 5). The controls represented sugar content mean values of 21.23%, 20.60%, and 22.33% respectively, while the half-sib families' average values were between 19.47% and 22.07% recorded respectively by F8 and F15. The HSFs reveal a higher sugar content potential compared to new cultivated cultivars. The enhanced HSFs were likely closer to the TZ varieties with a high sugar content and lower root weight.

Table 5. Multiple comparisons of means for the root weight and sugar content; Tukey's HSD test and Dunnett's test p-values for the pairs of means comparisons of the HSF versus a fixed control, TE, TN and TZ

	Root weight (kg)				Sugar content (%)			
		p-values for pair comparisons				*p-values* for pair comparisons		
HSF	Mean	HSF vs. TE	HSF vs. TN	HSF vs. TZ	Mean	HSF vs. TE	HSF vs. TN	HSF vs. TZ
F1	0.88^{ab}	0.02^{*}	0.01^{*}	0.81	21.28^{a}	1.00	0.80	1.00
F2	0.84^{ab}	0.02^{*}	0.01^{*}	0.84	21.56^{a}	1.00	0.97	1.00
F3	0.86^{ab}	0.01^{*}	0.01^{*}	0.73	20.96^{a}	1.00	0.95	1.00
F4	0.89^{ab}	0.02^{*}	0.02^{*}	0.85	20.77^{a}	1.00	1.00	1.00
F5	0.74^{b}	0.00^{***}	0.00^{***}	0.23	20.66^{a}	1.00	1.00	0.99
F6	0.88^{ab}	0.07	0.04^{*}	0.99	21.14^{a}	1.00	1.00	1.00
F7	0.86^{ab}	0.04^{*}	0.02^{*}	0.95	21.94^{a}	1.00	0.86	1.00
F8	0.90^{ab}	0.16	0.11	1.00	19.47^{a}	0.43	0.88	0.23
F9	0.90^{ab}	0.11	0.08	1.00	20.49^{a}	1.00	1.00	0.95
F10	0.83^{ab}	0.01^{*}	0.00^{***}	0.51	20.33^{a}	1.00	1.00	1.00
F11	1.06^{ab}	1.00	1.00	1.00	20.07^{a}	0.69	0.98	0.41
F12	0.94^{ab}	0.09	0.06	0.99	21.10^{a}	1.00	0.87	1.00
F13	0.80^{ab}	0.06	0.04^{*}	0.94	20.65^{a}	0.99	1.00	0.86
F14	0.87^{ab}	0.22	0.16	1.00	21.10^{a}	1.00	1.00	0.99
F15	0.72^{b}	0.00^{***}	0.00^{***}	0.42	22.07^{a}	1.00	0.99	1.00
F16	1.00^{ab}	0.50	0.40	1.00	21.80^{a}	0.98	0.73	1.00
F17	0.96^{ab}	0.20	0.15	1.00	20.93^{a}	1.00	1.00	1.00
F18	0.86^{ab}	0.16	0.11	1.00	21.21^{a}	1.00	1.00	1.00
TE	1.14^{a}	-	1.00	0.76	21.23^{a}	-	1.00	1.00
TN	1.16^{a}	1.00	-	0.64	20.60^{a}	1.00	-	0.98
TZ	0.95^{ab}	0.76	0.64	-	22.33^{a}	1.00	0.98	-

HSF = Half-sib family; * = significant at $p < 0.05$; *** = significant at $p < 0.001$; Lower case letters (^{ab}) indicate a statistical difference ($P < 0.05$) between half-sib families; Same lower letter indicates a non-significant difference between half-sib families.

Performing germplasm in a breeding programme should have significant heritability variation of the selection key traits. So, besides the variability analysis, the heritability was evaluated on the three main yield components, the root weight, leaf biomass, and sugar content. The controls barring in this analysis purpose was to disclose effectively the studied parameters heritability. Since the controls are monogerm registered improved cultivars, while the studied germplasm are basically multigerm still under genetic enhancement.

Variance components were assessed through the estimation of the heritability (Table 6). The Z-ratio represents the comparison score of the studied variables by standardising the distribution. The *z*-score is positive when the HSF score is higher than the average. The half-sib family had the largest effect on root weight in comparison with the two other variance components. The RW heritability reaches almost 0.5; which is the greatest value that confirms also the selection efficiency of this variable. The LBY has an h2 value of about 0.2. On the other hand, sugar content has the lowest heritability score of 0.02 since it's a trait significantly influenced by the environment. Such results are relevant for the selection purposes; root weight, then leaf biomass are valuable criteria for sugar yield selection. More analyses are needed to explain the sources of variability, to detect the genotype-environment interaction effect on the behaviour of the studied germplasm and to discriminate the more stable and productive HSFs.

Table 6. Variance components and narrow sense heritability (h2) estimation

Variable	Variance component	Variance	Variance *SE*	*Z-ratio*	Heritability	
					Estimate	*SE*
Root weight (kg)	HSF	0.013	0.006	2.274	0.466	0.227
	Env	0.001	0.002	0.324		
	Env* HSF	0.006	0.003	1.810		
	Env*Block	0.000	0.000	0.000		
	Error	0.028	0.003	8.339		
Leaf biomass yield (kg)	HSF	0.006	0.003	2.143	0.196	0.152
	Env	0.004	0.005	0.777		
	Env* HSF	0.000	0.002	0.164		
	Env*Block	0.001	0.001	1.034		
	Error	0.024	0.003	8.268		
Sugar content (%)	HSF	0.235	0.172	1.363	0.017	0.025
	Env	8.191	5.398	1.518		
	Env* HSF	0.096	0.238	0.406		
	Env*Block	0.128	0.131	0.979		
	Error	3.515	0.362	9.700		

HSF = Half-sib family; Env = Environment; *SE* = Standard error.

Stability study by AMMI analysis of genotype × environment interaction

The variance analysis displayed significant effect of genotype-environment interaction on the yield component parameters which validates the genotypes differential performance between the environments (Table 7). The first interaction principal component IPCA1 gathered 53.9%, 44.4%, and 44.6% of the total variance for respectively the root weight, the leaf biomass, and the sugar content. The IPCA scores were used to explain the half-sib families' behaviour with regard to the environments accordingly throughout the three selection cycles, Recalling that, the HSF were analysed E1 and E2 for the third selection, E3 and E4 for a second selection cycle, and E5 and E6 for the first selection cycle. The climatic data were different mainly between seasons, winter and spring (Table 2). For E1, the average rainfall was 33.3 and 42.3 mm respectively during the winter and spring periods. For E2, the values were 45.3 and 66.3 mm respectively during winter and spring.

Table 7. Analysis of variance for the AMMI model for 18 half-sib families, three controls and six studied environments

	Variable	DF	*SS*	*MS*	% explained	*F-test*
Env	Root yield (kg)	5	5.47	1.09		1.86
Rep (Env)		13	7.64	0.59		12.93***
Génotype		20	4.25	0.21		4.67***
Env x Gen		78	5.44	0.07		1.53**
IPCA1		24	3.76	0.16	53.9	3.33***
IPCA2		22	1.44	0.06	20.7	1.44
Residuals		215	9.77	0.05		

Table 7 continued

Env	Leaf biomass yield (kg)	5	5.65	1.13		11.92^{***}
Rep (Env)		13	1.23	0.09		3.76^{***}
Génotype		20	1.88	0.09		3.72^{***}
Env x Gen		78	2.76	0.03		1.40^{*}
IPCA1		24	1.57	0.06	44.4	2.59^{***}
IPCA2		22	0.85	0.04	24.1	1.53
Residuals		215	5.42	0.02		
Env	Sugar content (%)	5	1,244.76	248.95		21.49^{***}
Rep (Env)		13	150.53	11.58		3.57^{***}
Génotype		20	146.92	7.35		2.26^{**}
Env x Gen		78	362.19	4.64		1.43^{*}
IPCA1		24	205.69	8.57	44.6	2.64^{***}
IPCA2		22	133.75	6.08	29.0	1.87^{*}
Residuals		215	697.88	3.25		

DF = Degrees of Freedom; *MS* = Mean Square; *SS* = Sum of Squares; Env = Environment; Rep = Repetition; Gen = Genotype; * = significant at $p < 0.05$; ** = significant at $p < 0.01$; *** = significant at $p < 0.001$.

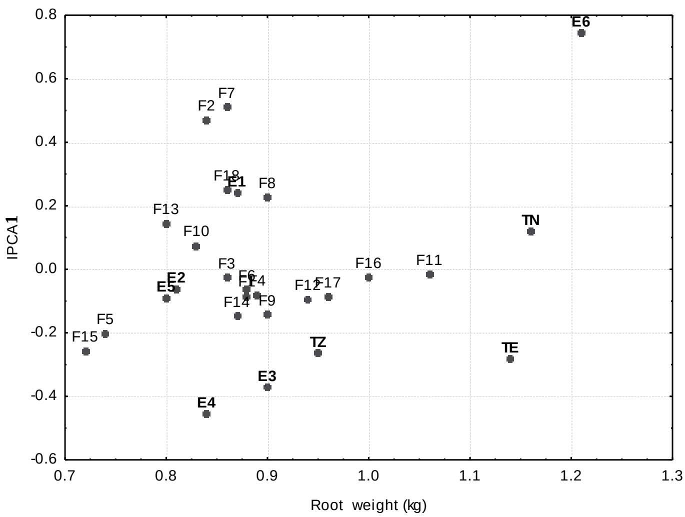

Figure 1. AMMI biplot for IPCA1 scores versus root weight (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

This analysis aims to discriminate the most performing stable HSFs and reveals the genetic progress lengthwise the selection cycles. For a better assessment of the data, every dependant variable is presented in scatter plots (IPCA1, dependant variable). Also, the scatter plots IPCA1 vs. IPCA2 are accomplished for the three yield components. High IPCA scores, negative or positive, indicate the good adaptation of the genotype to a special environment. The more stable is a genotype over the tested environments; the closer is the IPCA scores to zero. The biplot of the first interaction principal component (IPCA1) versus the root weight (Fig. 1) showed significant variation among the studied environments. Half-sib families F11, F12, F16, and F17 displayed better stability; their root weight ranked between 0.94 and 1.06 kg and IPCA1 scores between -0.017 and -

0.096. The four HSF have good adaptation and genetic flexibility to the experimental environments. The environments matching the third selection cycle E1 and E2 are less interactive as they recorded close IPCA1 scores of 0.24 and -0.062 respectively (Figs 1, 2).

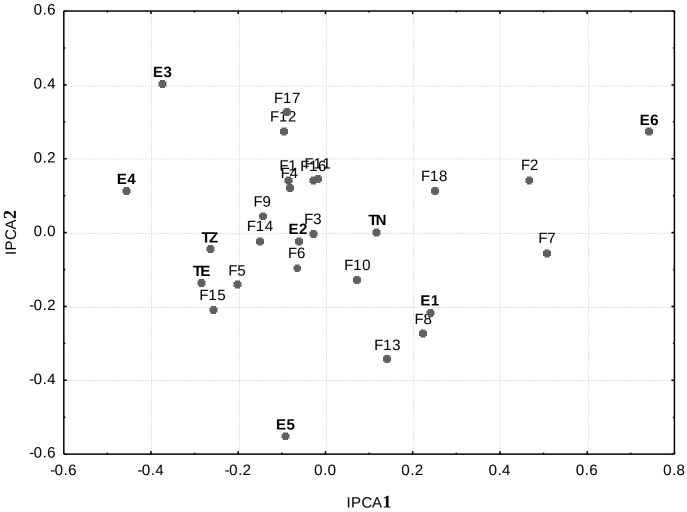

Figure 2. AMMI plotted IPCA1 and IPCA2 scores for root weight (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

Such data reveal that the improved germplasm heterogeneity has decreased via the panmictic crosses during the two first selection cycles. The root weight's HSF ranking according to the AMMI stability values (ASV) is illustrated in Fig. 3. As shown, the more the ASV value is close to zero, the more stable is HSF across environments. Root weight and its corresponding ASV value reveal differences between half-sib families in terms of stability. The families F11, F12 F16 and, F17 were the most stable presenting ASV values stand between 0.15 and 0.39 and RW values of 1.06, 0.94, 1.0, and 0.95 kg respectively, exceeding the 0.91 kg overall average as are also the three controls.

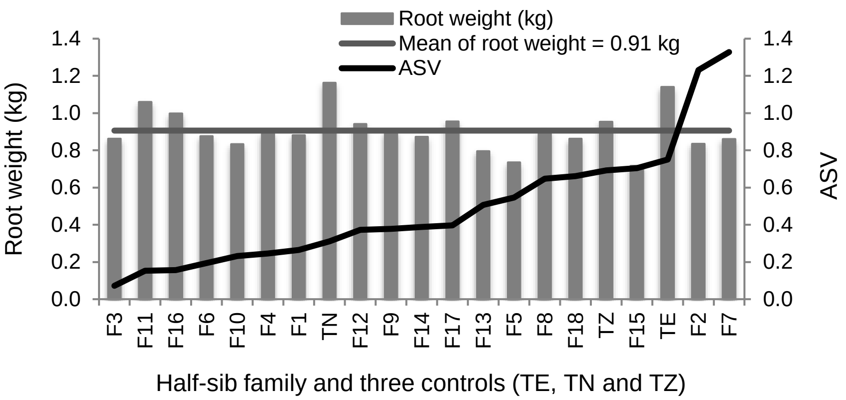

Figure 3. Ranking of 18 half-sib families and three controls according to the AMMI stability values (ASV) for root weight (kg).

The IPCA1 versus leaf biomass biplot (Fig. 4) shows that third selection cycle E1 and E2 environment data has IPCA1 scores close to zero, equal to 0.013 and 0.036 respectively and leaf biomass values of 0.33 and 0.59 kg correspondingly. The IPCA1 vs. IPCA2 elucidate further these results (Fig. 5). The most stabsle and adapted half sib-families are F17, F16, and F12; they present leaf biomass yields of 0.57, 0.55, and 0.47 kg respectively, and IPCA1 values between -0.008 and -0.0023. The HSF F14 and F5 are closer to the controls with means close to 0.40 kg.

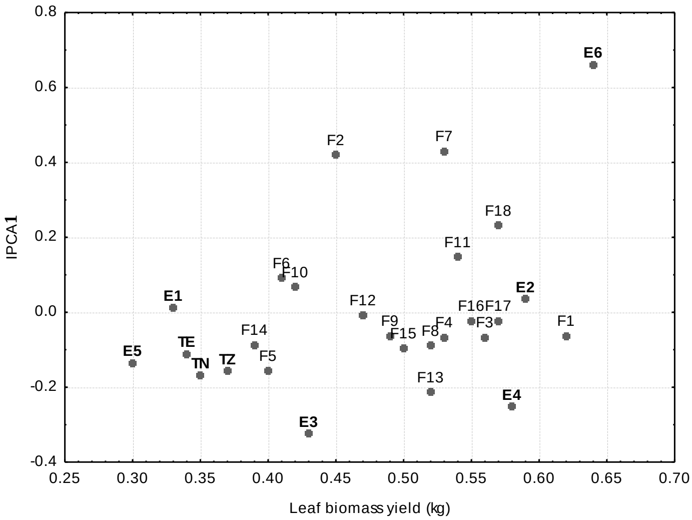

Figure 4. AMMI biplot for IPCA1 scores versus leaf biomass yield (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

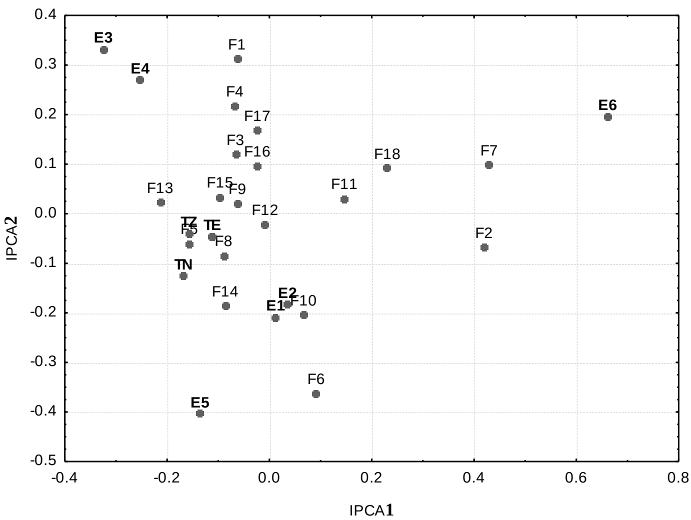

Figure 5. AMMI plotted IPCA1 and IPCA2 scores for leaf biomass yield (kg) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

The foliar biomass stability values (Fig. 6) showed that the majority of the half-sib families have stability values as good as the controls and less than 0.3. The most stable families are F12, F16, F9, F3, F17, F15, and F8, all their ASV values less than 0.18 and biomass yield sizes between 0.47 and 0.57 kg.

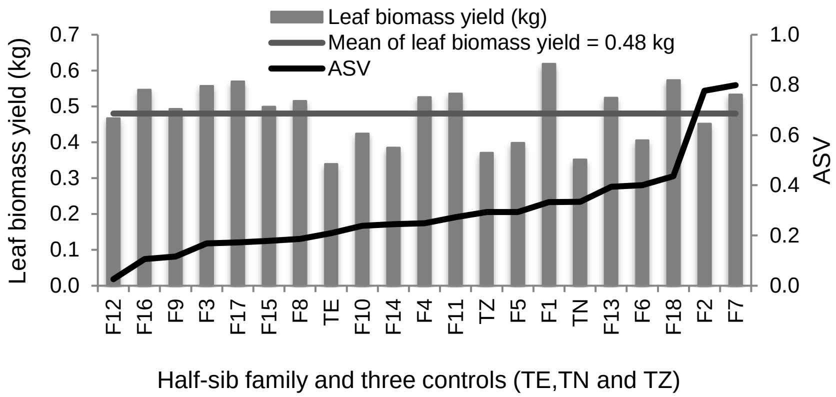

Figure 6. Ranking of 18 half-sib families and three controls according to the AMMI stability values (ASV) for leaf biomass yield (kg).

The sugar content vs. the IPCA1 biplot shows that the most stable and adapted HSF are F2, F4, F9,F13, F10, F11, F12, F16,and F17; their average sugar contents fit between 20.07 and 21.79%, and their IPCA1 scores between -0.047 and 0.271 (Fig. 7). The E1 and E2 IPCA1 values for sugar content are close to each other in comparison to the other environments. The IPCA1 vs. IPCA2 biplot adds more explanation (Fig. 8); IPCA1 scores for E1and E2 are equal to 0.25 and 0.51 respectively.

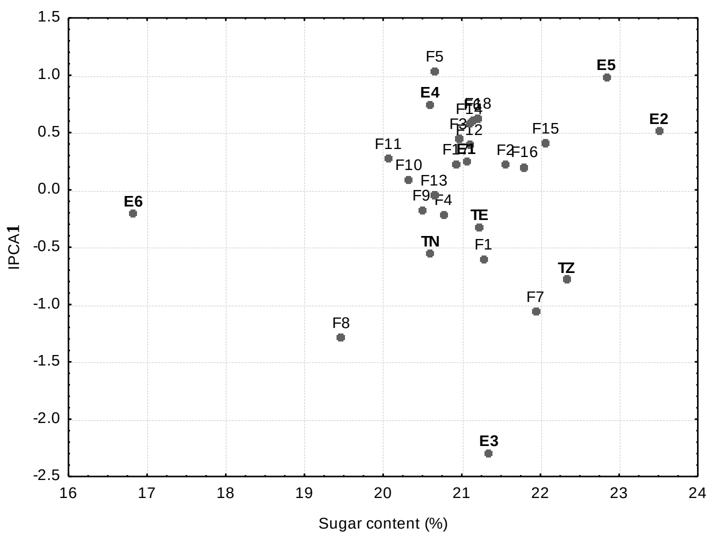

Figure 7. AMMI biplot for IPCA1 scores versus sugar content (%) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

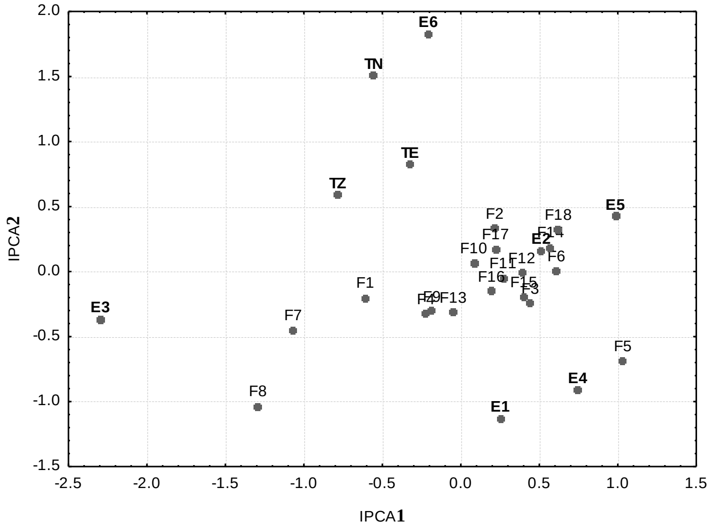

Figure 8. AMMI plotted IPCA1 and IPCA2 scores for sugar content (%) of 18 half-sib families, three controls TE, TN and TZ for 6 environments, E1, E2, E3, E4, E5 and E6.

The sugar content's ASV ranking (Fig. 9) shows the HSF F16, F17, F2, F11, and F12 being the most stable; their ASV values are below 0.61. These HSFs recorded sugar concentration averages between 20.07 and 21.79% recorded respectively by F11 and F16. The F11 HSF, with 20.07% sugar content and 1.06 kg root weight, is the closest to the TN; the intermediate control variety which sugar level is 20.60% and root weight 1.16 kg.

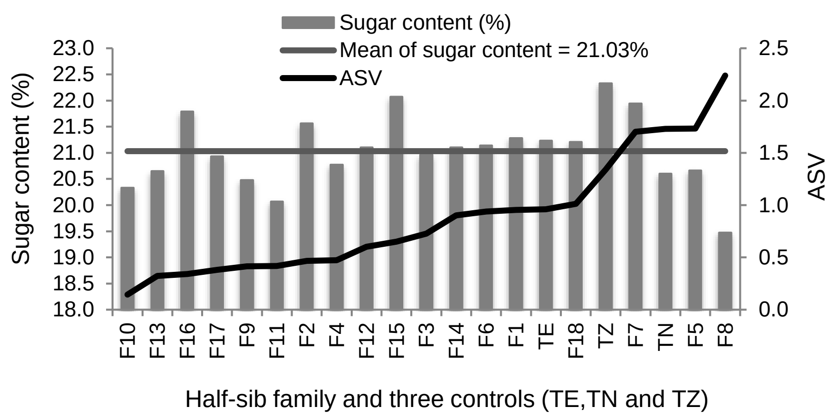

Figure 9. Ranking of 18 half-sib families and three controls according to the AMMI stability values (ASV) for sugar content (%).

DISCUSSION

The present study aims to analyse the selection efficiency of Moroccan sugar beet multigerm germplasm comparatively with three monogerm varieties used as controls, under local limited vernalising climate. The 18 preselected HSF standing for the analysed plant material, were evaluated in two environments during three recurrent maternal selection cycles to gatherers multi-trials database. The HSFs did not show any specific adaptation to the experimental localities, Larache and Sidi Allal Tazi. The Locality-HSF interaction ANOVA values were not significant. The plants' vigour showed 95% significance in it correspondence to the controls. The seeds' quality and especially the germination rate, which are linked to the plants' vigour, seem to have an appealing effect on vegetative development. The maternal plants selected at the two firsts selection cycles exceeded a germination rate of 83% (Table 1). The plant vigour is assessed by using different methods at different stages of the vegetation. Among these methods, plantlet's rise is used as an indicator for seed vigour evaluation (Podlaski & Chomontowski, 2020). Low seed vigour is described to decrease root yield as seedling emergence is slower and less uniform compared to high vigour seeds. Generally, vegetative vigour is a robust indicator for seed quality. Improved germplasm with high and homogeneous vigour is a prerequisite in successful breeding programmes.

Root weight and leaf biomass are mainly influenced by the plant genotype; they both presented significant variability. The combined root weight and sugar content data enlightens TZ control type of the majority of the half-sib families and approves the negative correlation between sugar content and root weight as reported in many studies conducted on sugar beet (Curcic et al., 2018; Ahmadi et al., 2011; Biancardi et al., 2010; Hoffmann, 2010; Schneider et al., 2002). In sugar beet germplasm enhancement, when root weight is increased by the selection, sugar content tends to decrease, and vice versa (Biancardi et al., 2010). The 18 HSFs recorded significantly similar values with the two other controls, TE and TN. The HSFs' variability reported is principally due to the genotypes, and can be explained by the differences in the maturation stage and accordingly to the storage sugar content in the roots (Meier et al., 1993). Also, the trend towards an intermediate type variety will be optimal to provide flexibility for the harvesting schedule. Among the hybrid cultivars, there is the TZ- type that has balanced root yield and sugar content and is set for intermediate and late harvest (Ludecke, 1953; Bosemark, 2006). Harvest dates delayed up to 210 days is reported to increase significantly root weight and sugar content (Hussein et al., 2012).

The sugar-beet roots harvest was carried at 200 days, which is 10 and 30 days earlier to TN and TE controls growth cycle but was close to TZ control (180 days). Z-type sugar beet hybrids are considered more stable, having higher sugar yield and shorter vegetation periods (Curcic et al., 2018). Early harvesting is prised to avoid summer early drought and to seek high sugar yield and economic expenses. Such data proclaim opportunities to select and establish locally adapted cultivars. The variance decomposition reveals a large contribution of the half-sib families on the root weight and leaf biomass yield variability. Previous studies reported significant variability of root yield among several sugar-beet genotypes (Ulaković et al., 2015; Curcic et al., 2018). Leaves' size is influenced by the genotype, growth stage, or climatic conditions (Klotz, 2005). The heritability reaches 0.5 values for root weight and 0.2 for leaf biomass; both variables are slightly affected by the environment in comparison with sugar content which heritability value is as low as 0.002. Several research works validate sugar yield dependence on the environment and its high correlation to the root yield and sugar content (Powers et al., 1963; Schneider et al., 2002; Hoffmann et al., 2009). Genotype × environment interaction studies on sucrose, as total dissolved solids in table beet, showed strong environmental effects and limited heritability (Goldman et al., 1996). The genotype-environment interaction

(GEI) analyses in the present study were relevant to interpret the variability and in structuring the sugar beet germplasm through their stability and sugar yield efficiency. The AMMI model shows high significant values for studied parameters exceeding 44%. Many studies record a significant effect of GEI in sugar beet field trials (Moradi et al., 2012; Hoberg et al., 2015; Al Jbawi et al., 2017). Significant GEI is valuable since it helps to discriminate the genotypes through their genetic and production potentials in different environments (Aghaee-Sarbarzeh et al., 2007). The stability explains the sugar content heritability recorded the highest significant AMMI value of 73.6% against 53.9% for root weight and 44.4% for leaf biomass. Significant genotype main effect can also be weighty for the sucrose (as total dissolved solids) in table beet; this happened when the genotype × environment interactions are significant (Hanson & Goldman, 2019).

In our case, the environment E3, E4, E5, and E6 registered higher IPCA1 and IPCA2 scores than E1 and E2. E1 and E2 environments were less interactive and displayed the closest IPCA scores. These results express the heterogeneity declined at the third selection cycle through the open crosses between selected genotypes for seed production potential evaluation during early selection cycles. The most performing and stable HSFs were identified; F11, F12, F16, and F17 recorded high yield component values and were stable in comparison to the rest of HSFs. These analyses reveal the efficiency of the selection and the evaluation of the sugar beet genotypes. The present study reports promising HSFs and substantial germplasm advancement through three recurrent selection cycles.

CONCLUSIONS

This comparative study confirms that the germplasm denotes a large yield components variability and constitutes a rich database for the national sugar beet breeding programme with probable wide and narrow adapted material. The studied Half-Sib Families recorded significant performing traits comparatively to the controls, especially for root yield and sugar content. Most HSFs are close to TZ control, highlighting the trend of the performing families that have a short cycle (200 days). The narrow-sense heritability values showed different levels for studied parameters; the root weight shows the highest values of 0.5, while sugar content reveals a value of 0.02 being as significantly influenced by the environment. The most performing and stable half-sib families are F11 (closer to TN control), F12, F16, and F17 (closer to TZ control) with high yield component values and high stability. This study is of great support to the national sugar beet breeding programme.

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The food security concept as the state support basis for agriculture

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Abstract. The article discusses the problem of the country (territory) food security formation and its relationship with the state support size for agriculture. The work purpose is to determine the features of the food security formation in the Samara region and the relationship with the state support size for agricultural production in the region. Within this study framework, it is necessary to solve the following tasks: - study the features of the food security concept and its application in the Samara region conditions; - the optimal parameters of the region's self-sufficiency determination in food products; - establishing a link between food security and the optimal amount of state support for the agro-industrial complex. Taking into account only the data on the region self-sufficiency, the region produces a sufficient amount of potatoes and vegetables. Comparing these indicators with rational consumption rates, it can be seen that the residents' demand for agricultural products is provided mainly by potatoes, the consumption of which is 1.5 times higher than the rational. The subsidies existing system in the Samara region is not optimal from the point of view for ensuring food security, which indicates either the underfunding of the production for both these and other product groups. In 2017–2018 the amount of state support, based on the above calculations, had to be increased by at least 100–500 million rubles.

Key words: criterion, food security, PSE, self-sufficiency, subsidies.

INTRODUCTION

Food security is an essential element of the economic security of a country, region or other strictly limited area. It is formed due to the country (region) ability to provide the required amount of the state (limited territory) population needs in agricultural products in amounts that allow for rational life. In order to ensure an optimal level of life for the region population, the state must provide such a set of food products, which, on the one hand, is sufficient in composition and quantity to fulfill this task, and on the other hand, corresponds to the peculiarities of the inhabitants national composition, takes into account the natural and climatic properties of the territory, i.e. has the ability to adapt to these parameters (Zhichkin et al., 2019; Bryukhovetskaya et al., 2020; Glushchenko et al., 2020). The territory food security functional combines the actual (physical and economic) provision of agricultural products, as well as its security (Naidanova & Polyanskaya, 2017; Lakomiak & Zhichkin, 2019; Khayrzoda et al., 2020). These parameters should be guaranteed at all levels of the region's government in full through the establishment of quantitative values (standards). The list of these indicators should include: the optimal rate of food consumption, the subsistence minimum, indicators of the quality of life, guaranteed prices, etc. At the same time, it is necessary to consider the fact that the degree of food security and its quantitative assessment is influenced by many interdependent factors, the ratio of which varies across territories:

– the actual income of the population and the quality of life in certain territories;

– the nutrients imbalance presence in the territory population due to national, climatic and other factors (Ripoll-Bosch et al., 2013; Falcone et al., 2019);

– insufficient implementation of control mechanisms for the agricultural products quality or their insufficient level (Michalk et al., 2019; Morkovkin et al., 2020; Rasva & Jürgenson, 2020);

– the risky nature of the territory natural conditions as a result of which there are significant fluctuations in domestic agricultural production (Herrero et al., 2013);

– a significant share of imported agricultural products (Trotsuk et al., 2018; Pismennaya et al., 2019);

– the foreign policy situation and its impact on the possibility of purchasing food outside the territory (sanctions and counter-sanctions) (Yen et al., 2008; Nieto & Reyes, 2019; Gibadullin et al., 2020).

MATERIALS AND METHODS

The work purpose is to determine the features of the food security formation in the Samara region and the relationship with the state support size for agricultural production in the region. Within this study framework, it is necessary to solve the following tasks: - study the features of the food security concept and its application in the Samara region conditions; - determination of the region's self-sufficiency optimal parameters in food products; - establishing a link between food security and the state support optimal amount for the agro-industrial complex. Elements of the food security modern concept are shown in Fig. 1. Taking into account the territorial boundaries of application, the food security following levels are distinguished: - international; - country; - regional; - territorial; - individual (Fedotova et al., 2018; Abramov et al., 2020).

When implementing this technique, food security at various levels (state, subject, territory) is closely interrelated. These interconnections are very close, since they are controlled by means of life quality uniform standards for the entire state as a whole and each specific subject separately. Ensuring these standards is a fundamental function of public administration at government all levels. It is implemented through a system of equalizing the individual regions income through the organizational measures system and support for subsidized regions.

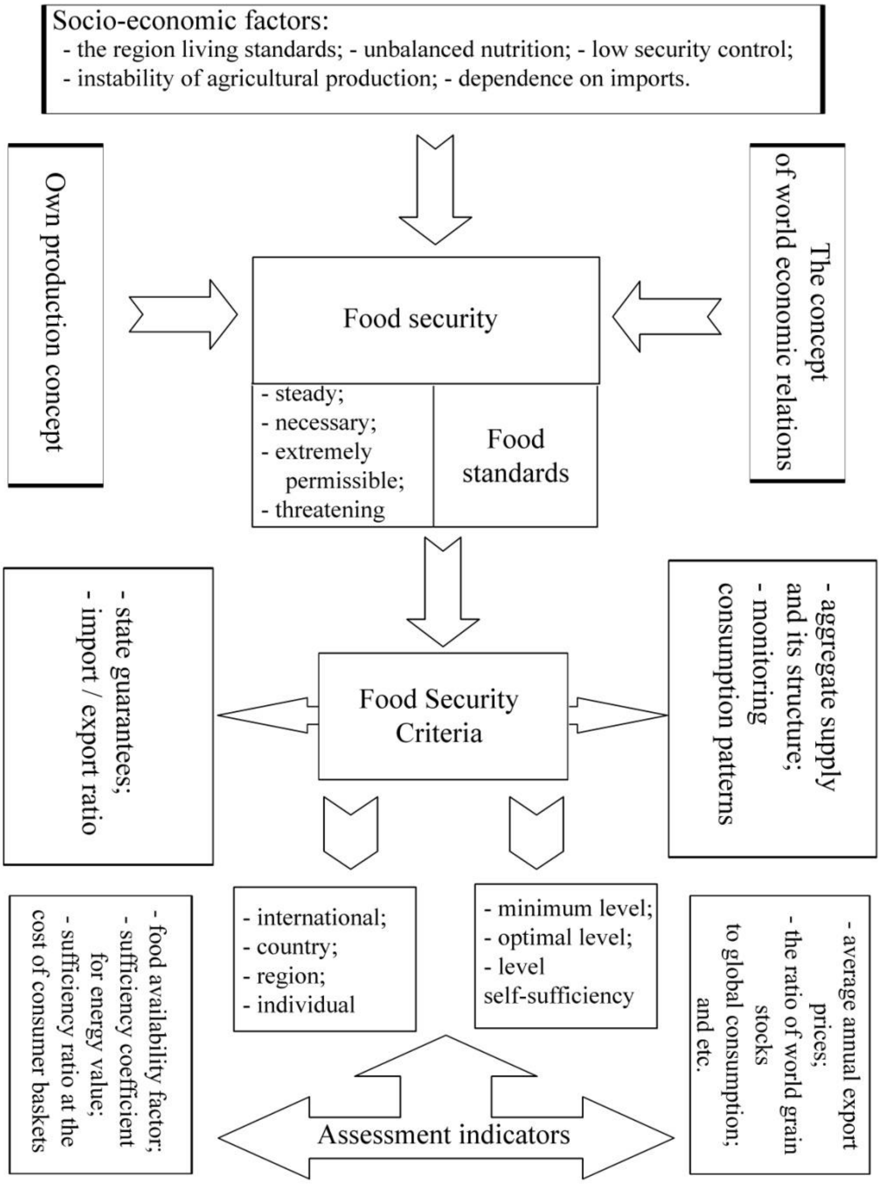

Figure 1. Food Security Concept System.

At the same time, it is also influenced by the ability of the region itself to form a income certain level, described through indicators: income per 1 inhabitant, the food prices level, etc.

Abstract-logical and statistical methods were used in the study. In particular, we used absolute, relative and average values. The research results are presented in tabular and graphical forms.

RESULTS AND DISCUSSION

When planning the food security of the territory, it is proposed to take into account a number of indicators that form the region's self-sufficiency in agricultural products: the dynamics of the region's population size and demographic characteristics; the food resources structure and volume in the region; the local agricultural production size; environmental factors of agricultural production; food export and import policies of federal and regional authorities (Shagaida & Uzun, 2015). Additionally, the level of the region self-sufficiency with food products can be estimated on the basis of the regional coefficient of agricultural products' production - the consumption. It shows how much agricultural products of a given type are produced per inhabitant.

$$I = \frac{(P + Z_1 - Z_2 - V)}{Ch \cdot Norm} \qquad (1)$$

where I – regional coefficient of agricultural products' production - consumption; P – the amount of food product *i* produced in the region during the period *t*; Z_1 and Z_2 I carryover quantity of food product *i* on the territory of the region at the beginning and end of period *t*; V – export of food product *i* outside the region during period *t*; Ch – the actual population of the estimated area during the period t; $Norm$ – the legislatively enshrined norm of food rational consumption taking into account climatic, national and other characteristics of the region population consumption.

The values of this indicator can be explained as follows: I > 1.1 - this type of food is available for the population of the region, but its consumption is higher than rational (due to the relatively low price, consumer habits of residents, etc.), therefore, there is a violation of the consumption optimal structure; 1.1 > I > 1.0 - the product is available to the population, the consumption structure is optimal; 1.0 > I > 0.8 - the product is practically available to the residents of the region, but for some reason, consumption is somewhat lower than rational; 0.8 > I > 0.5 - the food product is limitedly available to the residents of the region, there are problems with its consumption; I < 0.5 - significant problems with the availability of this product, violation of the optimal structure of consumption in the region, search for replacement products (Bukhtoyarov et al., 2020).

Analyzing the data of the Samara region (Figs 2, 3), it can be seen that the region produces a sufficient amount of potatoes and vegetables. Potatoes belong to the first group, the provision for which is complete, but leads to a violation of the consumption optimal structure, since it displaces other food groups from the diet. Vegetables belong to the third group - there is an almost complete supply of needs. But for meat and milk (belonging to the fifth group), one can clearly see the failure of self-sufficiency and a high degree of dependence on the food products delivery. At the same time, there is a constant growth in the consumption of meat, milk, vegetables.

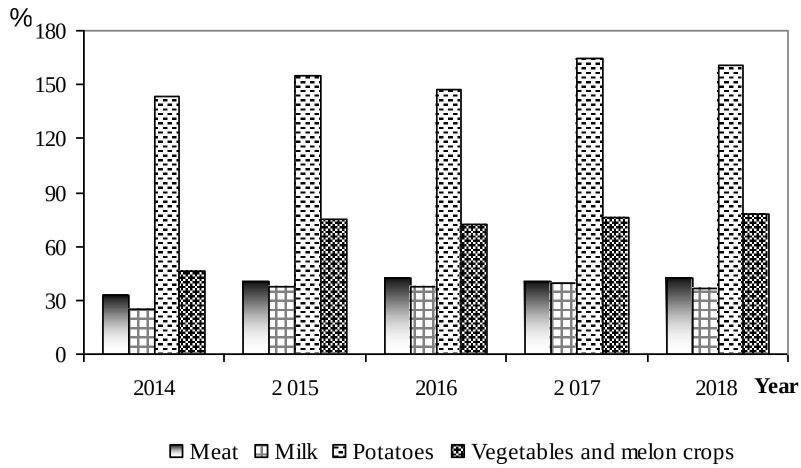

Figure 2. The coefficient of providing the territory with its own production products in the Samara region (calculated by the authors according to the data of the Territorial Body of the Federal State Statistics Service for the Samara region).

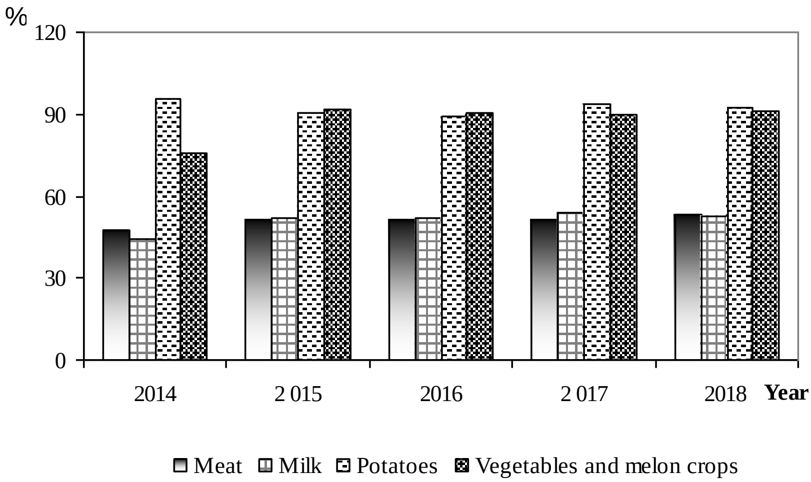

Figure 3. Food security in the Samara region (calculated by the authors according to the data of the Territorial Body of the Federal State Statistics Service for the Samara region).

In accordance with Order of the Ministry of Health of the Russian Federation No. 614 dated August 19, 2016 'On the approval of recommendations on rational norms for the consumption of food products that meet modern requirements for a healthy diet' the values of food consumption rational norms were established. They were: for meat - 73 kg year^{-1} per person, for milk - 325 kg, for potatoes - 90 kg, for vegetables and melons - 140 kg.

To ensure the food security required level, it is necessary to maintain an appropriate level of state support for each type of product. To calculate the amount of government subsidies, it is proposed to use the following methodology.

Initially, to determine the required amount of products produced, it is proposed to calculate the territory's need for a given type of product, taking into account the declared level of self-sufficiency in kind and in value.

$$Y = \sum_{i=1}^{L} (Norm_i \cdot Ch \cdot Pr_i) \cdot I_i \qquad (2)$$

where Y – the cost of agricultural products necessary to ensure the territory food security in accordance with the current doctrine, rubles; $Norm_i$ – rational consumption rate of the i food product necessary for an active and healthy lifestyle, kg per person; L – the number of food products types included in the population diet, pcs; Pr_i – the price of the i food product necessary for an active and healthy lifestyle, rubles kg^{-1}; Ch – the population of the territory, persons; I_i – the level of self-sufficiency for the *i* food product in accordance with the current food safety doctrine, %.

At the second stage, we determine the need for subsidies.

$$Sub = Y \cdot PSE \qquad (3)$$

where Sub – the required amount of subsidies to ensure the food security level in accordance with the current doctrine, rubles; PSE – the support level for agricultural producers, %.

Based on the agricultural products average prices (Table 1), the population size (Table 2) and the average PSE value (on average over 5 years equal to 7.8%), the need for subsidizing the production of selected food products can be calculated. Estimated data show that to fully provide the region population, it is necessary to produce about 230 thousand tons of potatoes, 1,040 thousand tons of vegetables, 287 thousand tons of cattle and poultry meat, and 447 thousand tons of milk (Table 3).

Table 1. Average producer prices for agricultural products*

Product	2014	2015	2016	2017	2018
Potatoes	10,846	10,849	7,983	10,439	10,662
Vegetables	19,020	23,784	19,637	17,151	16,097
Livestock and poultry (live weight)	58,758	73,348	72,665	94,566	97,706
Milk	19,717	21,314	22,525	25,193	22,745

*– data of Territorial body of the Federal State Statistics Service for the Samara region.

Table 2. Initial indicators for calculation*

	2014	2015	2016	2017	2018
Population, total	3,212.7	3,206.0	3,203.7	3,193.5	3,183.0
State support level indicator (PSE), %	9.8	7.0	6.3	7.6	8.3

*– calculated by the authors according to the data of the Territorial Body of the Federal State Statistics Service for the Samara region.

Table 3. The required amount of agricultural products for the region self-sufficiency, thousand tons

Product	2014	2015	2016	2017	2018
Potatoes	234.5	234.0	233.9	233.1	232.4
Vegetables	1,044.1	1,042.0	1,041.2	1,037.9	1,034.5
Livestock and poultry (live weight)	289.1	288.5	288.3	287.4	286.5
Milk	449.8	448.8	448.5	447.1	445.6

From the data in Table 4 it can be seen that the cost of products required for self-sufficiency in the region ranges from 46,058.2 million rubles in 2014 to 58,861.6 million rubles in 2017, depending on the population size and market conditions.

Table 4. The cost of agricultural products necessary for the region self-sufficiency, million rubles

Product	2014	2015	2016	2017	2018
Potatoes	13,780.3	17,166.2	16,994.2	22,045.7	22,702.9
Vegetables	20,587.1	22,208.1	23,453.1	26,147.5	23,529.1
Livestock and poultry (live weight)	3,136.0	3,130.4	2,301.8	3,000.3	3,054.3
Milk	8,554.8	10,675.2	8,807.5	7,668.0	7,173.1
TOTAL	4,6058.2	53,179.9	51,556.6	58,861.6	56,459.5

To assess the results obtained, it is necessary to introduce the concept of a state support sufficiency criterion, taking into account the provisions of the food security doctrine.

The criterion for the adequacy of the government funding amount, taking into account the provisions of the current food security doctrine:

$$\sum PSE_i \cdot I \cdot (Norm_i \cdot Ch \cdot Pr_i) - \sum PSE_i \cdot K_i \cdot (Norm_i \cdot Ch \cdot Pr_i) > 0 \quad (4)$$

When a negative value is obtained, we are talking about the underfunding of the industry, and the absolute value of the indicator indicates the subsidies amount that have not been received by agricultural producers. Based on the data in Table 5, it can be seen that, since 2017 there has been an underfunding of agricultural production, based on this criterion.

Table 5. Amount of subsidies required to ensure food security in the region, million rubles

Product	2014	2015	2016	2017	2018
Potatoes	1,074.9	1,339.0	1,325.5	1,719.6	1,770.8
Vegetables	1,605.8	1,732.2	1,829.3	2,039.5	1,835.3
Livestock and poultry (live weight)	244.6	244.2	179.5	234.0	238.2
Milk	667.3	832.7	687.0	598.1	559.5
Total for selected types of products	3,592.5	4,148.0	4,021.4	4,591.2	4,403.8
Actual subsidies for all agriculture in the region	6,029.4	4457.2	4,129.4	3,900.0	4,354.3

A positive aspect of this indicator is its comprehensive nature, taking into account not only the subsidies size allocated to agriculture, but also the level of existing production. Positive values of this criterion can be obtained only if both conditions are met simultaneously.

CONCLUSIONS

Based on the above calculations, it can be seen that the existing subsidy system in the Samara region is not optimal from the ensuring food security view point. As can be seen from the data in Table 5, the amount of optimal state support for the four selected product groups practically coincides with the full amount of state support for the region agro-industrial complex, which indicates either underfunding of production for both these and other product groups. In 2017–2018 the amount of state support, based on the above calculations, had to be increased by at least 100–500 million rubles. The above calculations imply that the amounts determined in the article are characteristic of the existing system of agricultural support in the region and extrapolate the current laws. The calculated standards will change with the improvement of the agricultural production system in the region, a set of state support measures and production potential.

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Airflow resistance of two hop varieties

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Abstract. The quality of hops used in brewing is substantially reliant upon the processing step of drying. To ensure effective drying in kiln as well conveyor-belt dryers, homogeneous distribution of air is of particular importance. Uneven air distribution often results in inefficient drying and nonuniform moisture content of the hop cones. The air distribution naturally is governed by the airflow resistances in the individual floors or belts of a dryer. Hence, in order to quantify the airflow resistance of hop cones at different air velocities and bed heights, systematic measurements were carried out. In addition to determining the bulk densities of hops, the investigations included trials with fresh and dried hop samples. Clear differences were observed between hop varieties both in measured pressure drops and in bulk densities. Moreover, in the case of fresh hops, a non-linear increase in pressure drop with bed height was ascertained. Semi-empirical equations were developed to describe pressure drop as a function of air velocity. This work will contribute to the design of dryers with optimum airflow distribution and thus enhance the efficiency of drying as well as the product quality.

Key words: airflow resistance, bulk density, drying, hops, pressure drop.

INTRODUCTION

Hop provides the typical flavour to beer and thus is an essential raw material in brewing. In order to increase the shelf life of the hops, freshly harvested hop cones must be dried immediately. For this purpose, kiln dryers and conveyor-belt dryers are used which are usually operated at drying temperatures of up to 70 °C (Münsterer, 2020). Higher temperatures cannot be used to expedite the drying, as drying temperatures well below 65 °C have been proposed to reduce the loss of essential oils and other heat sensitive substances (Heřmánek et al., 2017; Rybka et al., 2018). Even though partially overdrying is commonly used as a measure against the occurrence of nests of moist hops, it adversely affects the product quality and energy consumption (Rybka et al., 2017; Heřmánek et al., 2018). Alternative approaches including technological improvements were suggested by several researchers (Rybka et al., 2019a; Rybka et al., 2019b), but the operation of conveyer-belt dryers is essentially based on experience.

Nowadays, model-based process analysis and smart control systems offer the most promising options in terms of increasing the energy efficiency of hops drying whilst improving product quality. Nevertheless, there is a lack of studies relating to the change of physicochemical properties of hops during drying. The mass and length of hop cones vary according to variety, during the growing season and also from year to year (Čeh et al., 2012). Little is known about the density or bulk density of hop cones at different moisture contents, although it is an important parameter for harvesting, processing and storage (Kumhála & Blahovec, 2014). One study investigated the relationships between the dielectric properties of bulk hops and bulk density. However the investigations were limited to freshly harvested and subsequently compressed cones, as opposed to investigating those during drying (Lev & Kumhála, 2017). With regards to hops quality, for example, investigations of a pilot scale drying system revealed that colour changes depended strongly on the bulk weight and resulting bulk thickness. The research demonstrated that the specific mass flow rate of drying air plays a critical role in determining the quality of the final product, as well as the processing time required (Sturm et al., 2016; Sturm et al., 2020). These findings have established that, therefore, it is important to consider optimum bulk and process parameters, to optimize the hop drying process and to improve process efficiency as well product quality (Raut et al., 2020).

It is well known that for any convection drying, having a better understanding and more importantly a better control over the airflow patterns is paramount. On that front, the prediction of airflow resistance is fundamental to the design of efficient drying and aeration systems. Hence, several theoretical, semi-theoretical, and empirical models have been developed which relate pressure drop to airflow (Górnicki & Kaleta, 2015). In a comprehensive fundamental work, Matthies (1956) investigated the airflow resistance of different agricultural crops and the independent variables effecting this resistance. The research included numerous grain and root crops as well as some grass and foliage crops. However, to the best of the authors' knowledge, no such studies have yet been carried out on hops. Therefore, the objective of this work was to obtain a better understanding of the bulk densities and airflow resistances of hops, particularly in relation to drying.

MATERIALS AND METHODS

The investigations described in this paper were carried out during the harvest period of the year 2020, at a drying facility located in Saxony (Saaz variety: August 26–28; Perle variety: September 07–09). The hops were dried with a three-belt dryer of Czech design (type PCHB-750). The fresh hop samples (green hops) were taken from the pile in front of the feed belt, whereas the dried hop samples were collected after continuous conditioning. In addition to the measurements to determine airflow resistance, moisture content and bulk density of the two examined hop varieties were determined.

A simple test system was set up to determine flow resistances at different air velocities, bed heights and moisture contents (Fig. 1). Essentially, the test system consists of a radial blower equipped with a frequency converter, a measuring section for the air volume flow rate and a sample container with a sieve grate. The measuring section and the sample container were realized with ventilation pipes made of galvanized sheet steel (standard diameter: 300 mm).

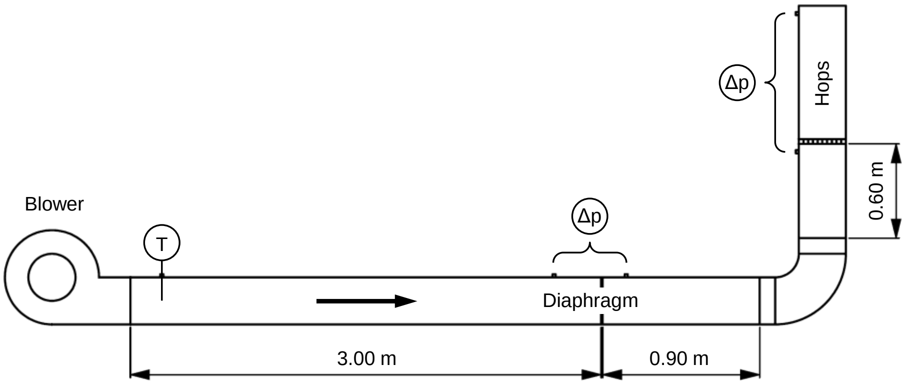

Figure 1. Simplified schematic of the test facility.

The air volume flow rate was determined by measuring the differential pressure and the air temperature on a standard diaphragm (inner diameter: 50 mm) in accordance with EN ISO 5167-2 (2003). At the same time, the pressure loss of the ventilated bed was measured (differential pressure before and after the sample container). Two differential pressure sensors (measuring range 0-1,250 Pa; Ahlborn FD A602-S1K), a temperature sensor (thermocouple type K; Ahlborn ZA 9020-FSK) and a data logger (Ahlborn Almemo 2890-9 by Ahlborn Mess- und Regelungstechnik GmbH) were used for the measurements.

At the beginning of each experiment, the sample container was filled with hop cones up to a bed height of 25 cm. During the subsequent ventilation, seven different air volume flow rates were set via the frequency converter of the fan (10–70 Hz). Each volume flow rate was held constant for about three minutes. The two differential pressures and the air temperature were measured every second and recorded as mean values every five seconds. The mean values were then used for subsequent analysis for each of the seven volume flow rates. The sample container was then filled with hop cones first to a bed height of 50 cm and finally to 75 cm. The procedure described above for varying the air volume flow was repeated for the other two bed heights as well. Each trial was carried out three times with different hop samples. With two hop varieties, three bed heights, two moisture contents and three repetitions, a total of 36 trials were conducted.

The following equation of resistance for bulk grain and bulbous crops was developed by Matthies (1956):

$$\Delta p = \frac{C_0}{2} \cdot k \cdot \frac{h}{\varepsilon^4} \cdot \eta^n \cdot \rho_a^{1-n} \cdot \frac{w^{2-n}}{d^{1+n}} \quad (1)$$

where Δp denotes pressure loss in the bed; C_0 is the drag coefficient; k is a material specific empirical constant for the respective crop; h represents the bed height; ε is the void fraction of the bed (porosity), η stands for the dynamic viscosity of the air; ρ_a denotes the density of the air; w is the air velocity through the empty sample container, and d is the diameter of an equivalent sphere with the same volume as the bulk body (equivalent diameter). Concrete values for the exponent n resulted from extensive theoretical and experimental investigations of the drag coefficient of flows through the beds as a function of the Reynolds number. By rearranging the above Eq. (1)

to express the bed-height specific pressure drop and simplifying it further by consolidating the unknown quantities C_0, k, ε and d in C_1, we get:

$$\frac{\Delta p}{h} = C_1 \cdot \eta^n \cdot \rho_a^{1-n} \cdot w^{2-n} \qquad (2)$$

Alternatively, to consider the void fraction (porosity) as a variable, by excluding it from the consolidation, Equation (1) reduces to:

$$\frac{\Delta p}{h} = \frac{C_2}{\varepsilon^4} \cdot \eta^n \cdot \rho_a^{1-n} \cdot w^{2-n} \qquad (3)$$

The mathematical description of the bed-height specific pressure drop $\Delta p/h$ according to Eqs (2) and (3) was essentially contingent upon the experimental determination of the factors C_1 or C_2 and the exponent n. The systematically varied air velocities were calculated from the differential pressures measured on the standard diaphragm in accordance with EN ISO 5167-2 (2003). A description of the iterative calculation method is omitted here for brevity.

RESULTS AND DISCUSSION

Table 1 shows the moisture content and bulk density of the two hop varieties. Each value in Table 1 represents the mean value from three individual measurements. The moisture contents were determined by weighing and drying in a drying cabinet (manufactured by Memmert GmbH) at 105 °C for 24 h.

Table 1. Moisture contents w.b. (wet basis) and bulk densities of the hop varieties Saaz and Perle before and after drying

	1	2	3	Mean	B/A*	P/S**
Saaz fresh						
Moisture content w.b. (%)	78.8	79.1	78.9	78.9		
Bulk density A (kg m^{-3})	81.7	78.9	82.2	80.9		
Bulk density B (kg m^{-3})	91.4	89.3	94.9	91.8	114%	
Saaz dry						
Moisture content w.b. (%)	10.8	12.0	12.4	11.8		
Bulk density A (kg m^{-3})	22.5	22.1	24.5	23.0		
Bulk density B (kg m^{-3})	25.4	24.6	27.8	25.9	113%	
Perle fresh						
Moisture content w.b. (%)	78.9	78.4	78.4	78.6		
Bulk density A (kg m^{-3})	90.8	84.1	88.9	87.9		109%
Bulk density B (kg m^{-3})	105.9	98.8	102.9	102.6	117%	112%
Perle dry						
Moisture content w.b. (%)	10.6	11.4	10.7	10.9		
Bulk density A (kg m^{-3})	22.7	23.4	23.3	23.1		101%
Bulk density B (kg m^{-3})	26.8	27.8	27.1	27.2	118%	105%

* B/A = bulk density of hops compacted by manual shaking (B) as compared to hops loosely filled (A); ** P/S = bulk density of Perle (P) as compared to Saaz (S).

The bulk densities were determined by weighing in a container with a volume of exactly 20 L. The cones were initially loosely filled (bulk density A) and then compacted by manual shaking (bulk density B). The shaking resulted in approx. 13–18% greater values. In addition, the difference in bulk density among the two investigated hop varieties was also observed. The bulk density of the freshly picked cones of the Perle variety was around 9–12% greater than that of the Saaz variety.

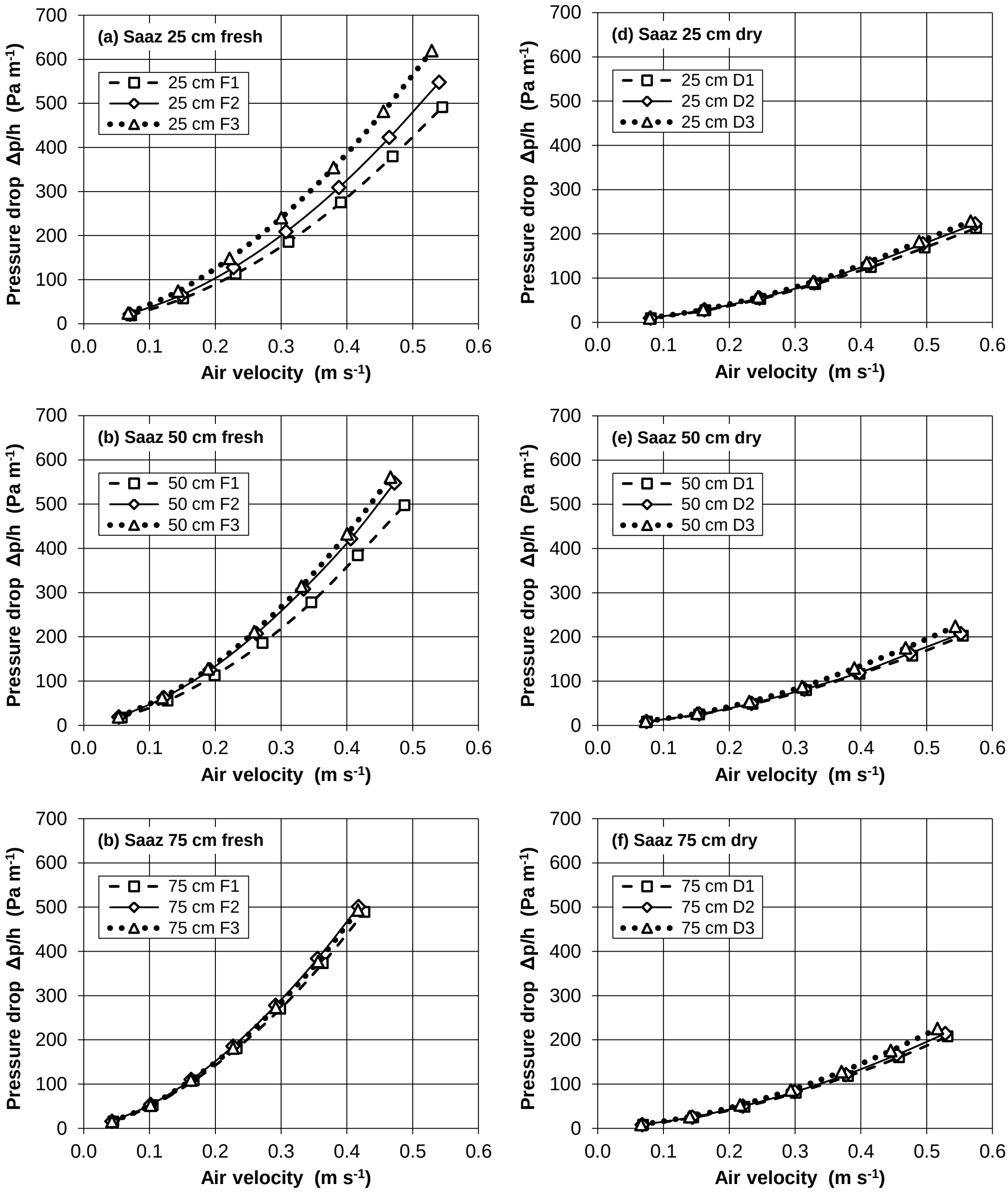

Figure 2. Pressure drop of the hop variety Saaz, measured at different air velocities, bed heights, and moisture contents.

The pressure losses of Saaz and Perle hops as a function of air velocity are shown in Fig. 2 and Fig. 3 respectively, both before and after drying ('fresh' und 'dry'). The values measured in the individual tests were related to the respective bed height and grouped accordingly. As already mentioned, each measurement was repeated three times. In Fig. 2 and Fig. 3, F1, F2, F3 denote measurements with fresh hop cones and D1, D2, D3 measurements with previously dried samples.

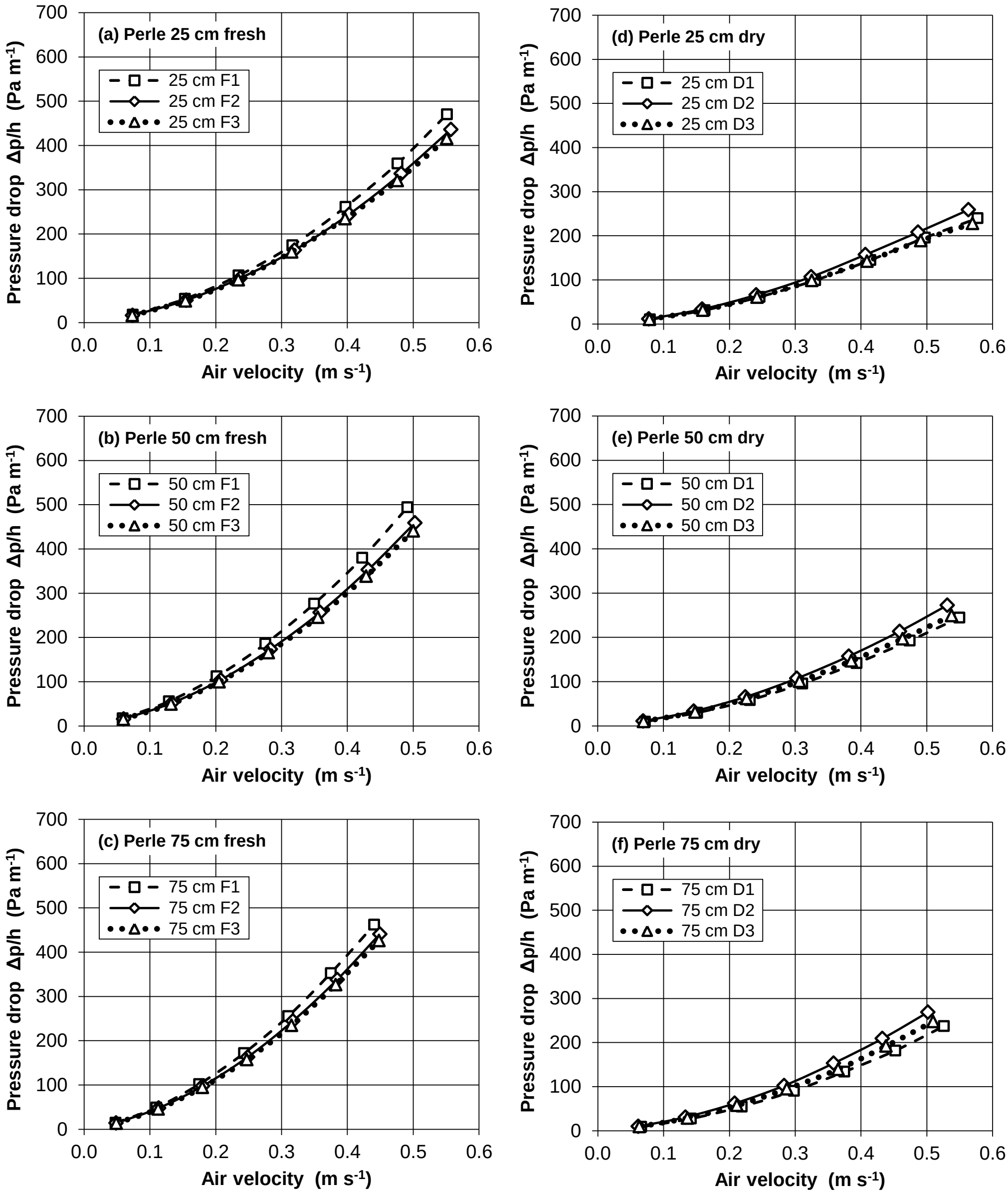

Figure 3. Pressure drop of the hop variety Perle, measured at different air velocities, bed heights, and moisture contents.

With a few exceptions (e.g. Fig. 2, a), the pressure loss curves lie generally very close to one another. The measurements with previously dried cones also give quantitatively very similar values (see Fig. 2, d–f and Fig. 3, d–f). In contrast, measurements with the fresh cones show quantitative differences in bed-height specific pressure drops (see Fig. 2, a–c and Fig. 3, a–c). In a double-logarithmic plot, pressure losses as a function of air velocity appear as virtual straight lines. Fig. 4 shows mean values formed from the individual measurements for the two hop varieties. The bed-height specific pressure drops recorded for fresh cones were significantly greater than that for previously dried cones. The values for fresh cones of both hop varieties increased with the height of the bed.

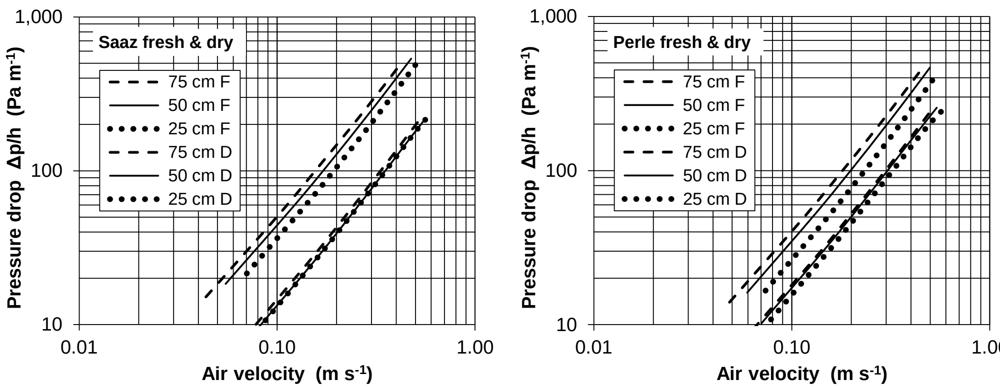

Figure 4. Pressure drop of the hop varieties Saaz and Perle, mean values at different air velocities, bed heights, and moisture contents.

In practice, the air velocity used for drying freshly harvested hops lies in the range of 0.3–0.4 m s^{-1}, although the actual values are subject to strong fluctuations (Münsterer, 2020). The cause of these fluctuations are ultimately differences in the structure of the packing and thus in the airflow resistance of the beds. Even within one hop variety, individual cones differ considerably in terms of size, shape and surface properties. Added to this are the size distribution of cones, the proportion of voids in the bed, as well as leaves and stems. In addition, changes in moisture content and bulk density during the drying process affect the effective airflow resistance. Hence, airflow resistance remains a dynamic variable throughout the course of drying.

In order to determine the parameters of Eqs (2) and (3), the slopes of the straight line in Fig. 4 were first calculated. The slopes in the double-logarithmic representation correspond to the exponent of air velocity, as shown below:

$$2 - n = \frac{\ln\,(\Delta p/h)_6 - \ln\,(\Delta p/h)_2}{\ln\,(w)_6 - \ln\,(w)_2} \tag{4}$$

The indices 2 and 6 denote the values of the corresponding height specific pressure drop and air velocity measured at 20 Hz and 60 Hz, respectively. The exponents calculated according to Eq. (4) are listed in Table 2. The mean value of the exponents was used for all further calculations ($2 - n = 1.63$).

The air temperature fluctuated only slightly around 22 °C in all measurements. Therefore, constant values for the dynamic viscosity ($\eta = 18.5 \cdot 10^{-6}$) N s m^{-2} and the density of the air ($\rho_a = 1.18$) kg m^{-3} were used, as opposed to temperature dependent ones. However, it must be noted that at higher temperatures, the temperature dependence of η and ρ_a must be considered. With the factors C_1 given in Table 2, all measured pressure losses could be predicted satisfactorily based on Eq. (2). The calculated pressure loss depends on air density and thus on temperature. Temperature deviations of less than 5 K lead to deviations in pressure loss of less than 1.1 % under the test conditions described. The freshly picked cones of the Perle variety caused significantly lower pressure loss values than the Saaz variety. On the other hand, the measured bulk density exhibited the opposite trend (see Table 1). Hence, the correlation between the pressure loss and the bulk density suggested by Matthies (1956) for stalk and leaf-shaped crops could not be satisfied.

Table 2. Parameters for calculating the pressure drop according to Eq. (2) and Eq. (3)

Hop variety		Saaz fresh	Perle fresh	Saaz dry	Perle dry
Exponent of air velocity					
$2-n$ at 25 cm		1.64	1.67	1.66	1.62
$2-n$ at 50 cm		1.61	1.63	1.65	1.62
$2-n$ at 75 cm		1.58	1.60	1.64	1.63
Mean of exponents	Ø 1.63	1.61	1.63	1.65	1.62
Factor C_1 in Eq. (2) *					
C_1 at 25 cm		$76.1 \cdot 10^3$	$56.4 \cdot 10^3$	$27.9 \cdot 10^3$	$32.2 \cdot 10^3$
C_1 at 50 cm		$93.5 \cdot 10^3$	$73.2 \cdot 10^3$	$28.2 \cdot 10^3$	$35.9 \cdot 10^3$
C_1 at 75 cm		$106.6 \cdot 10^3$	$85.1 \cdot 10^3$	$30.9 \cdot 10^3$	$37.9 \cdot 10^3$
Porosity ε (assumed)					
ε at 25 cm		0.420	0.420	0.420	0.420
ε at 50 cm		0.405	0.405	0.415	0.415
ε at 75 cm		0.390	0.390	0.410	0.410
Factor C_2 in Eq. (3) *					
C_2 at 25 cm		2,369	1,755	870	1,003
C_2 at 50 cm		2,490	1,970	836	1,066
C_2 at 75 cm		2,466	1,970	874	1,071

* Factors C_1 and C_2 calculated with mean of exponents $2-n$ = 1.63.

Matthies (1956) pointed out in particular that airflow resistance is inversely proportional to the fourth power of porosity. Since the porosity of hop beds is unknown, an attempt was made to estimate the influence. For this purpose, it was assumed that the volume of the void decreased proportionally to the height of the bed when the bed settled, more so for the fresh samples than for the dried ones. The assumed values for porosity ε and the resulting factors C_2 for Eq. (3) are given in Table 2. The factors C_2 were found to lie close to each other for the different bed heights investigated, albeit with differences between the two hop varieties. The absolute values depend strongly on the assumed porosity.

CONCLUSIONS

Knowledge of physical product properties is an important basis for both the design and the operation of dryers. The physicochemical properties of most agricultural products change considerably in the course of drying and hops are no exception to this. The airflow resistance of the material to be dried is not only a decisive factor for the choice of blowers, but also determines the distribution and utilization of the airflow inside the dryer, and thus the uniformity of the drying process. The throughput of conveyor-belt dryers, for example, is often controlled by monitoring the bed height. Consideration of the bulk density of different hop varieties facilitates an improved adjustment of belt speed. However, the different physical properties of hops depend not only on the variety. In addition, the growing conditions, ripening time, weather conditions and moisture contents also markedly affect the physical properties.

In this work, the airflow resistance of hop cones at different air velocities and bed heights was investigated experimentally. The key findings obtained from the measurements carried out with the varieties Saaz and Perle can be summarized as follows:

- The fresh samples of the two varieties showed markedly different bulk density. In contrast, only minor differences were observed among the dry samples.
- The airflow resistances of beds consisting of two hop varieties differed considerably. However, differences in pressure drop did not positively correlate with differences in bulk density.
- No linear relationship was ascertained between bed height and airflow resistance.
- The void fraction of a packed bed generally has a major influence on its airflow resistance. However, it would hardly be possible to experimentally establish the porosity of hops beds. The calculation of pressure loss curves with estimated porosity values confirmed that moist hop cones are presumably compressed, so that the void fraction decreases and the airflow resistance increases.

For modelling and simulation of hops drying, especially with computational fluid dynamics (CFD), sound knowledge of airflow resistance and bulk density is essential. This work has shown how targeted experiments can lead to valuable insights into predicting the dynamic airflow resistance of a hops bed. However, further systematic investigations are required to gain better understanding of the changes in bulk density during the course of drying, and to achieve an accurate model to reflect the reality within reasonable tolerance.

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Analysis of the *Foeniculum vulgare* Mill. collection by the complex of features in the conditions of the Crimea foothills

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Abstract. *Foeniculum vulgare* Mill. is a valuable essential oil plant, which raw materials and derived products, and, above all, essential oil, are widely used in the perfume and cosmetics, liquor, paint and varnish industries, in the food industry and medicine. The source material for cultivated plants selection, including *F. vulgare*, is the collections of the gene pool. The objective of this study was a comparative study of *F. vulgare* samples collection by the complex of features to clarify the possibility of identifying sources of economically valuable characteristics for creating promising breeding material. The study of the *F. vulgare* collection supported by the Research Institute of Agriculture of Crimea, which includes 75 samples rom 28 countries, was conducted in 2017–2019 at an experimental base located in the Crimea Foothills (Krymskaya Roza village, Belogorsky district). The collection samples were analyzed according to morphobiological parameters and productivity indicators. The work was guided by generally accepted methods, including those developed for essential oil plants. Statistical processing of the obtained data was carried out. The wide variability of the collection is shown, according to individual indicators (variation coefficients - from 8.3 to 54.4%). In this way, the mass fraction of essential oil (one of the most important indicators) varies within a wide range in the collection - from 1.09 to 3.86% (of absolutely dry mass) in whole plants and from 4.16 to 8.53% in fruits. The composition of the essential oil depends on the raw material. The anethole content reaches 80% in fruit oil, and the content of fenchone and terpene compounds is increased during the plant processing. The results of the collection analysis are basic, allowing preliminary sampling with high productivity indicators for inclusion in breeding studies.

Key words: collection, essential oil, *Foeniculum vulgare*, productivity parameters, variability.

INTRODUCRION

For the essential oils production in the world, about 300 species of cultivated and wild-growing essential-oil-bearing plants are used (Pashtetsky et al., 2018). Essential oils and products derived from essential-oil raw materials are used in the perfumery and cosmetics, pharmaceutical, liquor, paint, and varnish industries, in medicine and the food industry (Voitkevich, 1999; Tkachenko, 2011; Pashtetsky & Nevkritaya, 2018). One of the well-known essential oil crops is *Foeniculum vulgare* Mill. (Pashtetsky et al., 2018).

The herbaceous raw materials and fruits of *F. vulgare*, as well as the products of their processing, are widely used as a medicine and as a spice (Mehra et al., 2021). Valuable qualities are given to them by the essential oil and other valuable biologically active compounds that accumulate in them (Voitkevich, 1999; Timasheva & Gorbunova, 2012). The essential oil content in the *F. vulgare* fruits varies from 3.5% to 10.0% (Voitkevich, 1999). It contains more than 20 components, the main of which are anethol (60–80%), which gives a sweet taste and fenchon (up to 20%), which causes the bitter taste of the fruits. Methylchavicol is in significant amounts in the composition of the essential oil (3–15%) (Voitkevich, 1999; Hussein et al., 2016). The ratio of components that determines the quality of the essential oil, including its antimicrobial and antioxidant activity, varies depending on the type of raw material – whole plants or fruits. The phase of plant development and, accordingly, the state of fruit maturity is of great importance (Anwar et al., 2009).

In medicine, the fruits and essential oil of *F. vulgare* are used as a carminative, diuretic, lactic, aromatic stimulating and strengthening gastric remedy (Choi & Hwang, 2004; Rather et al., 2016; Akhbari et al., 2019). *F. vulgare* essential oil is a strong antiseptic. In terms of its bactericidal action, it is 13 times higher than phenol (Voitkevich, 1999). The antiamnestic, antidepressant, and anxiolytic effects of fennel have been proven (Perveen et al., 2017; Abbas et al., 2020). There is information that *F. vulgar*e essential oil not only exhibits antibacterial activity, in particular, against gram-negative strains of *Pseudomonas aeruginosa, Escherichia coli*, and *Shigella dysenteriae*, but also has high antitumor activity against human breast cancer (MDA-Mb) and cervical epithelial carcinoma (Hela) cell lines (Akhbari et al., 2019).

The possibility and prospects of using *F. vulgare* essential oil in the development of environmentally safe insecticides designed to protect plants from aphids have been experimentally demonstrated (Pavela, 2018).

The *F. vulgare* fruits are used in cooking as a seasoning for cheese, meat.

In the 80–90s of the twentieth century, *F. vulgare*, as an essential oil crop, was grown in many European countries and in the United States. The production of its essential oil in France alone reached 500 tons per year (Nazarenko & Afonin, 2008). Now it has significantly decreased due to competition with synthetic anethol. Currently, *F. vulgare* is cultivated in many countries: in the northern and southern regions of Europe, including Ukraine, Moldova, and Russia; in Asia (India, China, Japan), North and South America, in the oases of Africa (Pashtetsky et al., 2018).

F. vulgare is a perennial herbaceous plant of the Apiaceae family, reaching a height of 80–250 cm, with a hollow, branched, annually dying stem. The shoots are repeatedly branched and end, like the main stem, in inflorescences. The leaves are strongly divided. The flowers are small yellow in color. The inflorescence is a compound umbel, consisting of 10–25 simple umbels. The fruit is an ovoid-oblong, greenish-brown cremocarp, splitting into two half-fruits (Pashtetsky et al., 2018).

In Russia, *F. vulgare* is most often grown as an annual plant. In regions characterized by a warm long period and mild winters, including in the Crimea, *F. vulgare* can be cultivated as a perennial crop - up to 5–7 years (Nazarenko & Afonin, 2008). In comparison with the first year, in the following years of the growing season, the development of plants occurs 10–15 days earlier, and a higher yield is formed.

*F. vulgare*is a highly profitable crop, but, as for other agricultural crops, it is periodically necessary to carry out variety changes, including new promising varieties in production. The main direction of breeding is the development of effective varieties with high adaptive capacity, increased fruit yield and high content of high-quality essential oil.

The source of promising genotypes and donors of valuable traits of cultivated plants are the gene pool collections (Tkachenko, 2019).Evaluation and selection of collection material, represented by forms and samples of various origins, are important at the initial stages of the selection process (Saxena et al., 2016; Krivda et al., 2020; Nevkrytaya et al., 2020). At the same time, it is important to consider the variability of indicators depending on the regional soil and climatic conditions, meteorological conditions during plant growing season and agricultural techniques used in fennel cultivation (Shafeiee et al., 2019; Akbari-Kharaji et al., 2020).

The assessment of differences between genetically similar genotypes in breeding studies is very effective using molecular markers (Choudhary et al., 2018). Thus, Iranian scientists assessed 10 fennel genotypes from different regions for economically valuable traits. Twelve PCR primers were used to analyze genotypic diversity. Special attention was paid to the dependence of the main indicators on water stress. Obtained results made it possible to identify the most promising genotype for cultivation (Poudineh et al., 2018a, Poudineh et al., 2018b).

The study of the collection material of *F. vulgare* and the creation of varieties based on it has been carried out in the Crimea for decades (Pashtetsky et al., 2018). The State Register of Selection Achievements Authorized for Use for Production Purposes of the Russian Federation includes two varieties of the Research Institute of Agriculture of Crimea (RIAC) - Mertsishor and OxamitKryma (State Register of Selection Achievements Authorized for Use for Production Purposes, 2020). Currently, breeding studies of *F. vulgare* are continuing, using both traditional and modern biotechnological techniques for creating valuable source material (Zolotilova et al., 2017). Much attention is paid to the replenishment and study of the *F. vulgare* collection, as a source not only of donors of high productivity, but also of samples that differ in the component composition of the essential oil (Saxena et al., 2016; Zolotilova et al., 2019).

The objective of this study was a comparative study of *F. vulgare* samples collection by the complex of features in order to clarify the possibility of identifying sources of economically valuable characteristics for creating promising breeding material.

MATERIALS AND METHODS

In 2017–2019, in order to clarify the nature of the intraspecific diversity of the *F. vulgare* collection in the Crimea Foothills conditions and to determine its breeding value, a comprehensive assessment of samples by morphobiological and economically valuable characteristics was carried out. The collection includes 75 samples of various ecological and geographical origin (from 28 countries of the world), including varieties of domestic and foreign selection (Table 1).

The collection includes 16 RIAC samples, including the varieties Mertsishor and Oxamit Kryma, hybrids and regenerants obtained in culture *in vitro*.

Table 1. Samples of the *F. vulgare* collection

Region	Number ofsamples	Region	Number ofsamples	Region	Number ofsamples
Russia	19	USA	3	Israel	1
Italy	4	Germany	2	Morocco	1
India	4	Poland	2	Belgium	1
Canada	4	CzechRepublic	2	Hungary	1
Afghanistan	4	Switzerland	2	ArabRepublicofYemen	1
Argentina	3	Syria	2	Iran	1
Azerbaijan	3	Sweden	1	Pakistan	1
Bulgaria	3	Uzbekistan	1	Tunisia	1
France	3	Algeria	1		
Ukraine	3	China	1		
Total	75				

The study was conducted at the experimental base of the department of essential-oil-bearing and medicinal crops of RIAC in the village of KrymskayaRoza (Belogorsky district), located in the eastern part of the Crimea Foothill zone. This region belongs to one of the five agro-climatic regions - the upper foothill (Table 2) (Savchuk, 2006).

Table 2. Soil and climatic conditions of the study region

Indicator	Crimea Eastern Foothill
Type of climate	moderate continental
Annual sum of effective temperatures, °C	3,200–3,400
Annual precipitation, mm	450–500
Average annual evaporation from the surface, mm	460–484
Hydrothermal coefficient of humidification (G.T. Selyaninova)	0.92
Total annual solar radiation, kcal/cm^2	112–128
Average daily temperature of the warmest month, °C	+22.3 (July)
Average daily temperature of the coldest month, °C	-0.8 (January)
Duration of the period with an average daily temperature of > 0 °C, days	292
Soil type	southern carbonate chernozems
Granulometric composition of soils	Heavy loam
Acidity, pH	8.0

The collection nursery was founded in 2016. The samples were placed on plots with a length of 1 m with row spacing of 0.6 m. The area of the accounting plot is 0.6 m^2. The samples were analyzed in two replicates, which is acceptable when studying the collection.Sample placement randomized.The assessment was carried out in 2017–2019 on plants of 2–4 years of age.

The analysis of the collection samples was performed according to the developed methods (Beydeman, 1974; Arinshteyn, 1977). The following indicators were analyzed:

– phenological - period from the beginning of growing season to flowering, from flowering to maturation, total duration of vegetation period;

– morphobiological - winter hardiness, plant height, height of the lower umbrella attachment, number of shoots of the first order, weight of 1,000 fruits;

– productivity indicators - harvest from the plot, collection of essential oil;

– biochemical - content of essential oil in whole plants and in fruits.

Statistical processing of the obtained data was performed using the Microsoft Office Excel 2010 software package (Dospekhov, 2012).

RESULTS

The years of research (2017–2019) differed significantly by the hydrothermal conditions of the period of active vegetation of plants (Figs 1, 2).

The wettest period was the spring-summer period of 2017, and the hottest and driest period was 2018 (the exception was July, but precipitation during flowering did not favor pollination and fruit formation). Compared to the previous year, 2019 was generally more favorable. At the same time, the temperature regime in June was higher, and in July - significantly lower than in previous years. In addition, heavy precipitation fell in July. The peculiarities of weather conditions in the years of the research led to differences in the nature of the morphobiological parameters and productivity indicators of the studied samples of the *F. vulgare* collection.

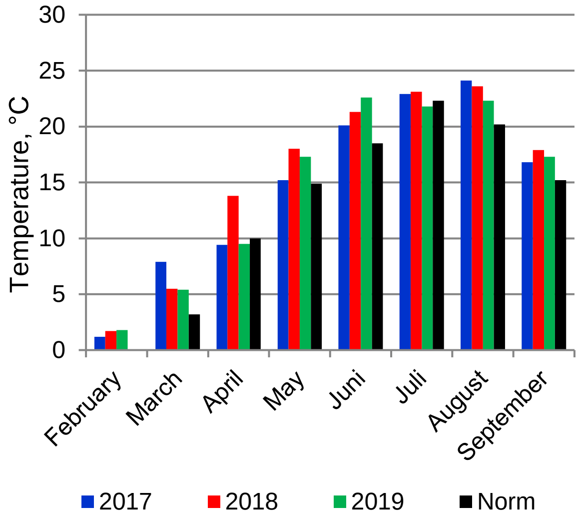

Figure 1. Average monthly air temperature during the period of active vegetation, 2017–2019.

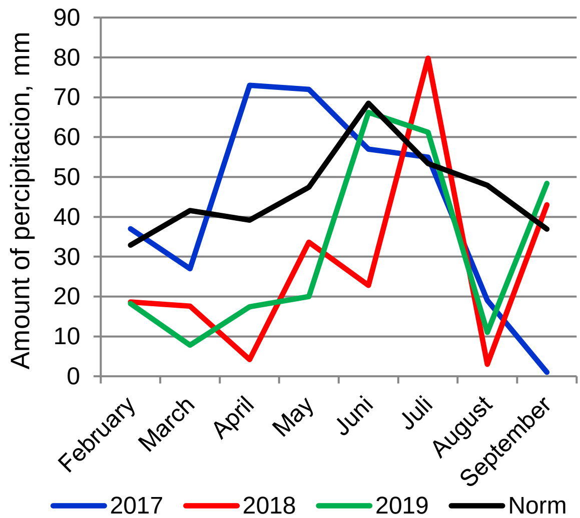

Figure 2. Average monthly precipitation during the period of active vegetation, 2017–2019.

The terms of passing the plants vegetation phases also differed in years. Spring regrowth of collection samples was observed in the period from March 3 to March 17, 2017, in 2018 - from February 26 to March 5, in 2019 - from February 24 to March 10. The difference between the samples at the beginning of the vegetation restoration was 14–16 days in 2017 and 2019, and 8 days in 2018.

The duration of the vegetation period from spring *regrowth to fruit maturation in F. vulgare* is significantly longer compared to the related annual species of the Apiaceae family - *Coriandrum sativum* L., *Anisum vulgare*Gaertn. and *Anethum graviolens* L. When sown in the third decade of March, the fruit ripens in these crops in the third decade of July, and the mass ripening of fruits in *F. vulgare* is at least a month later. Accordingly, *F. vulgare* plants experience fluctuations in weather conditions for a longer time. Plants are particularly sensitive during flowering, fruit formation and ripening. Heavy precipitation during this period prevents normal fruit formation and accumulation of essential oil, and extremely high temperatures do not favor the development of fruits, reducing the time of their maturation, but stimulate the oil-forming process (Coban et al., 2018). The duration of the vegetation period in the studied samples of the collection for all years varied, on average, from 161 to 186 days. In 2017–2018, the difference between the samples was 17 and 16 days (161–178 and 164–180 days), and in 2019, due to the low temperature regime in June and heavy rains, this period was extended to 23 days (163–186 days) due to different genotype responses. According to the results of the analysis of three-year data, the samples were divided into three groups: early - up to 170 days - 5 samples (6.7%), mid - 171 to 175 days - 33 samples (44.0%), and late - more than 176 days -37 samples (49.3%). The varieties Mertsishor (174 days) and OxamitKryma (175 days) are included in the group of medium-ripened samples.

The general characteristics of the main economically valuable indicators of collection samples are given in Table 3.

Table 3. Characteristics of the *F. vulgare* collection by complex of features, 2017–2019

Indicator value	Indicator									
	Winter hardiness, point	Plant Height, cm	Number of shoots 1 order, pcs.	Weight o f 1,000 fruits, G	Yield from the plot0 ($0.6\ m^2$)		Mass fraction of essential oil, % of absolutely dry mass		Collection of essential oil, g from the plot ($0.6\ m^2$)	
					Green raw materials, kg	Fruits, g	In green raw materials	In fruits	From green raw materials	From fruits
Average indicator in the collection	4.2 ± 0.1	149.7 ± 1.5	6.9 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	40.9 ± 2.7	2.32 ± 0.06	5.92 ± 0.11	18.4 ± 0.7	2.6 ± 0.2
Indicator range	1–5	115.4–179.1	5.4–8.4	2.0–5.4	1.6–6.7	5.2–108.1	1.09–3.86	4.16–8.53	6.2–34.3	0.4–6.3
Variation coefficient, (v),%	21.4	8.9	8.3	16.6	28.2	54.4	21.1	14.7	31.9	54.1

Since *F. vulgare* can be cultivated in a perennial culture, the winter hardiness indicator is of particular importance, as a guarantee of plants overwintering in certain climatic conditions. The winter hardiness of the samples was determined in the spring at the beginning of the growing season by the degree of damage to the plants on the accounting plots after overwintering. The highest winter hardiness (5 points) for three years of study showed 24 samples - 32.0%, the lowest winter hardiness (1–3 points) was noted in 14 samples - 18.7%, the average winter hardiness (4 points) have 37 samples or

49.3% of the number of studied samples. The varieties Mertsishor and OxamitKryma were characterized by the highest winter hardiness - 5 points. As the results of the evaluation show, it is possible to select forms in the collection that are promising for creating winter-hardy varieties.

The range of variability of the average height of the samples over three years is quite large - 63.7 cm (115.4–179.1 cm) with an average height of 149.7 ± 1.5 cm in the collection. The main number of samples (51 out of 75 or 68.0%) - medium-sized (131–160 cm). This group also included the variety Mertsishor (on average, 145.2 ± 5.9 cm). Significantly fewer samples (17 or 22.7%) were characterized as tall (more than 161 cm). This group includes the variety OxamitKryma (on average, 160.9 ± 3.8 cm). Undersized specimens with a height of up to 130 cm are least present in the collection (7 or 9.3%).

The number of shoots of the 1st order is an indirect indicator of the potential productivity of *F. vulgare*, since they form the main productive inflorescences. The average number of shoots per plant varied from 5.0 to 8.6 pieces in the samples of the collection. Varieties of Mertsishor and OxamitKryma - 7.2 ± 0.3 and 7.8 ± 0.3 pcs. A sample from Iran with an average number of shoots - 8.4 ± 0.1 pcs, is notable.

For mechanized harvesting, it is important to know the height of the cut of plants. The study showed that the height of the lower inflorescence attachment in the collection samples is, on average, 77.5 ± 0.1 cm. At the same time, the range of variability of this indicator is quite high - from 57 to 106 cm, on average, over three years. When sampling for analysis, the plants of each sample were cut considering this indicator.

F. vulgare essential oil is obtained both from whole plants (in the phase of the end of flowering - the beginning of fruit formation) and from mature fruits.

The yield of green mass, on average, in the collection was 3.5 ± 0.1 kg from the accounting plot (0.6 m^2). 74 samples were evaluated by this trait. One, with low winter hardiness, did not form a generative sphere in 2017-2018 and was not evaluated in 2019 due to the lack of seed reserve. Fluctuations in the yield of green mass in the collection ranged from 1.6 to 6.7 kg per plot (V = 28.2%). The largest number of samples refers to medium-yielding - 38 (51.4%). Their harvest was 3.1–4.5 kg from the plot. This group includes both RIAC varieties - Mertsishor and OxamitKryma, with an average yield of 4.2 kg per plot. In the group of low-yielding (less than 3 kg per plot) - 28 samples (37.8%). The most interesting is the high-yielding group, which included 8 (10.8%) samples with an average yield of more than 4.6 kg from the plot (4.6–6.7 kg). Samples of this group can potentially serve as donors of high yield.

During the period of full maturation, the harvest was recorded in 67 samples that formed the fruits. Eight samples did not form fruit due to poor overwintering and poor plant development. The range of variability, on average for 2017–2019, was in the range from 5.2 to 108.1 g per plot. According to this indicator, all samples are divided into three groups: high-yielding (over 60 g per plot) - 14 samples out of 67 (20.9%), medium-yielding (30–60 g per plot) - 31 samples (46.3%) and low-yielding (less than 30 g per plot) - 22 samples (32.8%). Mertsishor variety with an average yield of 66.5 ± 20.5 g per plot it is included in the group of high-yielding, and the OxamitKryma variety - 50.1 ± 2.4 g per plot - in the group of medium-yielding samples. The high variability of the collection by this feature (V = 54.4%) and the presence of samples with a high yield of fruits exceeding the varieties Mertsishor and OxamitKryma indicate the prospects of working with the collection in order to create valuable breeding material.

The weight of 1,000 fruits (m_{1000}) varied quite widely in the collection, on average, over three years, from 2.0 to 5.4 g. The bulk of the samples (47 out of 67–70.1%) had medium-sized fruits (m_{1000} from 3.1 to 4.0 g). 9 out of 67 samples (13.4%) were assigned to the small-fruited group (m_{1000} up to 3.0 g). Large fruits (m_{1000} more than 4.0 g) were formed by 11 samples (16.5%). OxamitKryma and Mertsishor varieties form a fruit of medium size - 3.8 ± 0.1 and 4.1 ± 1.0 g, respectively. The weight of the fruits depends on both genetic determination and hydrothermal conditions during their formation. The smallest fruits were formed in extremely dry and hot conditions in 2018. It should be noted that there are quite stable samples by this feature, which remained belonging to the same size group for all three years: 12 from the group of medium-sized, 4 from the group of large-sized, and 2 from the group of small-sized.

The most important indicator of essential oil plants is the content of essential oil in the raw material. The mass fraction of essential oil in the whole plants of the collection samples of *F. vulgare* ranged quite widely - from 1.09 to 3.86% (of the absolutely dry mass). The average figure in the collection for three years was 2.32 ± 0.06%. For breeding purposes, samples with an essential oil content of less than 2.0% are not of interest. In this regard, all samples (74) of the collection are divided into three groups: low-oil, with a mass fraction of essential oil up to 2.0% - 20 samples (27.0%); medium-oil (2.1–3.0%), the main group - 48 (64.9%) samples and high-oil (more than 3%) - 6 (8.1%) samples. The most valuable are samples from the high-oil group, as donors of this trait. Both varieties - Mertsishor (2.32 ± 0.34%) and OxamitKryma (2.72 ± 0.03%) were included in the middle group.

The content of essential oil in mature fruits is much higher than in whole plants. This is primarily due to the fact that the massive stem, which occupies a significant share in the total mass of raw materials, practically does not contain glandular conceptacles in which essential oil accumulates, and, in fact, is a ballast. The essential oil content in the fruits of 65 samples was analyzed. (The remaining samples either did not form fruit or were not sufficient for analysis.) On average, over three years, the mass fraction of essential oil in the fruits of the studied samples was 5.92 ± 0.11% (of the absolutely dry mass) and was characterized by a fairly wide variability range - from 4.16 to 8.53%. Taking into account the value for breeding purposes, the samples are conditionally divided into three groups: low-oil (up to 5.5%) - 22 samples (33.8%), medium-oil (5.5–6.5%) - 29 samples (44.6%), and the most valuable for breeding - a group of high-oil (more than 6.5%) - 14 samples (21.5%). The Mertsishor variety (7.25 ± 1.46%) is included in the group of high oily specimens, and the OxamitKryma (6.30 ± 0.55%) variety is in the group of medium oily samples.

The main indicator of the productivity of essential oil crops varieties is the collection of essential oil. This is the resulting indicator, depending on the yield of raw materials and the content of essential oil in it.

The collection of essential oil during the processing of whole plants in the *F. vulgare* collection ranged, on average, from 6.2 to 34.3 g per plot. The average value in the collection is 18.3 ± 0.7 g per plot. Samples with the collection of essential oil of up to 15 g per plot, inclusive, formed a low-oil group (23 samples out of 74–31.1%). In 50.0% of the samples, the collection of essential oil was in the range of 15.1 to 25.0 g per plot. The high-oil group (from 25.1 g per plot) included 14 (18.9%) samples, including the varieties Mertsishor (25.1 ± 8.0 g per plot) and OxamitKryma (27.7 ± 10.1 g per plot).

The variability range of the collection indicator of essential oil from fruits in the collection was, on average, 0.4–6.3 g per plot, and the average indicator was 4.3 ± 1.0 g. The group with a low indicator (less than 1.5 g $plot^{-1}$) includes 16 samples - 24.6% of the number analyzed (65), the group with an average collection of essential oil (1.5–3.5 g $plot^{-1}$) - 33 samples (50.8%). The group with a high indicator (over 3.5 g $plot^{-1}$) consists of 16 samples (24.6%), including the Mertsishor and OxamitKryma varieties.

The collection of essential oil from whole plants is far superior to that of whole fruits.

The high variability of the samples in terms of essential oil collection (V = 31.9% when processing whole plants and 54.1% when processing fruits) indicates the prospects of working with the collection when creating varieties, providing a high collection of essential oil from a unit area.

The component composition of the essential oil depends on the processed raw materials. In this way, the content of anethole can reach 80% in essential oil from fruits, and when processing whole plants, the content of fenchone and terpene compounds is increased in essential oil. In this regard, the choice of raw materials for processing is determined by the subsequent use of essential oil.

In this way, the analysis of the *F. vulgare* collection showed its high variability in terms of the main productivity indexes, which is consistent with the conclusions of similar studies by a number of scientists, including the use of genetic markers (Maghsoudi Kelardashti et al., 2015; Hadli et al., 2021). Obtained results indicate the possibility of identifying sources of valuable traits in the collection in accordance with the breeding tasks.

The study was carried out on the basis of the RIAC collection of the gene pool of spicy-aromatic, essential oil and medicinal plants, registered in the Russian Federation as a unique scientific installation USI No. 507515 (http//www.ckp-rf.ru).

CONCLUSIONS

The analysis of the *F. vulgare* collection, which includes 75 samples of various origins (from 28 regions), has been carried out for a complex of morphobiological and economically valuable traits.

Wide variability of the collection samples for the studied parameters is shown (the variation coefficients are from 8.3 to 54.4%). The groups of samples that are promising for the creation of valuable source material in accordance with the selection tasks have been identified.

The results of the collection analysis are basic, for preliminary selection of samples with high indicators for inclusion in breeding studies.

The obtained characteristics of collection samples will be used to create a reference manual for researchers conducting breeding studies of *F. vulgare* and related species of the Apiaceae family - *A. vulgare* Gaertn. and *C. sativum* L., *A. graviolens* L., *Carum carvi* L., etc.

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