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Effect of hemp fibre length on the properties of polypropylene composites

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Abstract. Hemp fibre (HF) is a natural fibre that has gained increased application in interior material for automobile industries (Sanjay et al., 2016). However, good interfacial bonding between fibre/matrix is necessary to enhance the mechanical properties of the composite (Pickering et al., 2007). This study focuses on the effect of fibre length, alkali and silane treatments on the mechanical and physical properties of hemp fibre reinforced polypropylene composites. Compression moulding technique was used to produce the composite, fibre lengths of 50, 100 and 150 mm were selected and combined with polypropylene powder at a fibre/PP ratio of 60/40%, a pressure of 1.67 MPa and temperature between 160–200 °C. The results obtained show that longer fibres enhanced mechanical strength. The tensile test result, for instance, shows a 21% increase in flexural strength at 150 mm compared to the fibre length of 50 mm. The modification resulted in a 46% decrease in strength, especially for 150 mm long fibres. This may have been as a result of fibre damage, inadequate modification, less quality fibre or higher initial moisture content in the modified fibres as observed from FTIR spectroscopy. Further investigation of these factors is required to be able to conclusively determine if they may have affected the mechanical performance (Alao, 2018).

Key words: hemp fibre, interfacial bonding, polymer composite, modification, polypropylene, fibre length, moisture content, mechanical properties.

INTRODUCTION

Increasing research are ongoing to develop environmentally friendly, sustainable and reusable composite materials such as hemp, flax and sisal as replacement to glass fibres and other carbon-based materials used as reinforcements for plastic polymers. (Placet, 2009). The drawback comes from the fact that these materials are mainly obtained from hydrocarbon fuel which are considerably highly air polluting, not sustainable and hardly recyclable, especially when combusted (Masuelli, 2013).

According to (Alao, 2018), researchers such as (Wambua et al., 2000) identified that the suitability of these natural fibres as reinforcement for thermoplastic composites requires analysis and comparison of the mechanical properties with that of their glass fibre counterpart. Comparable results of the strength properties have been obtained between these fibres and that of glass. Other factors that must be determined are the temperature and humidity that ensures the sustainability of fibre integrity (Davies &

Bruce, 1998). The polymer viscosity is the main deciding factor for the selection of the temperature (Gassan & Bledzki, 2001).

Alao (2018) in this project focused on researching hemp fibre, a variety of cannabis sativa plant species. Islam et al. (2010) identified the constituent of the fibre as crystalline cellulose (55–72 wt.%), hemicellulose (8–19 wt.%) and lignin (2–5 wt.%) and comparison between other natural fibres shows that industrial hemp is a strong and stiff material with the ability to reinforce polymer.

Suardana et al. (2011) defined this hemp fibre (*Cannabis Sativa L*) as a cheap, high-quality natural fibre that has been increasingly applied as a suitable interior material in the automobile industry because it possesses outstanding mechanical properties. While Sisti et al. (2017) corroborated the fibre content as shown in Table 1 below to be cellulose, hemicelluloses and lignin. They are generally extracted by retting before use as a composite reinforcement (Alao, 2018)

Table 1. The chemical composition of hemp fibres. Source: (Suardana et al., 2011)

Hemp Fibre	Cellulose	Pectin	Hemicelluloses	Lignin	Waxes & oils
wt. %	70.2–76.12	0.9–1.55	12.28–22.4	3.7–5.7	0.8–1.59

It was asserted by (Alao, 2018) that the polymer matrix is an essential material in fibre reinforced polymer (FRP) composite. These polymers are classified into thermosetting and thermoplastics, but the latter according to (Malkapuram et al., 2011) are the most widely used. These includes, Polypropylene (PP), Polyethylene (PE) and Poly (vinyl) chloride (PVC). The origin of these polymers can further be used to categorize them into synthetic or Bio based. Those obtained from petroleum-based products are called synthetic polymers while carbohydrate-rich substances like corn and sugar cane are termed Bio based, or biodegradable polymers (Mohanty et al., 2005).

Factors such as commercial availability, low density (0.92 g cm⁻³), good heat stability, impact resistance, ease of processing and low investment input have made polypropylene (PP) the most commonly used polymer matrix. This polymer also has the ability to improve the chemical and stain resistance of the final composite material (Denis et al., 2016). But, (Harutun, 2003) noted that the mechanical properties of the resulting composite are most likely dependent on the reinforcing material and production parameters.

This was further corroborated by (Ho et al., 2012) in which factors such as effective fibre/polymer matrix interface adhesion, fibre content, processing parameters and conditions were emphasized as the important factors influencing the mechanical performance of FRP composites produced from natural fibres like hemp fibre.

Alao (2018) stressed the set-back of using natural fibres with polymer matrix as pinpointed by Wambua et al. (2000). Natural fibres in FRP composites combined with these matrices causes poor fibre/matrix adhesion which is because of the heterogenous nature and high-water sorption rate of the fibre. This issue leads to poor stress transfer between the fibre and matrix, causing weak mechanical performance of the final composite material. Following recent research, surface treatment was identified as a means to improve the adhesive fibre-matrix interface bonding.

The presence of cellulose in natural fibres causes hydrophilic properties while polymer matrices are hydrophobic leading to incompatibility when combined. There is low wetting of the fibres by the molten polymer resulting in low dispersion, inadequate

reinforcement and bad mechanical properties (Harutun, 2003). Better adhesion between the fibre and polymer can be enhanced by chemically modifying the fibre surface to increase the hydrophobicity (Malkapuram et al., 2011; Denis et al., 2016). Currently, modification with a solution of alkaline and silane are being researched (Alao, 2018). To extract cellulose fibres, mild treatments with alkaline have been used. This has led to improved fibre packing and orientation of the chain molecules (Gassan & Bledzki, 1999).

Although combined treatment of hemp fibres with alkaline and coupling agent such as maleic anhydride (MA) grafted PP (MAPP) produced composite with better tensile properties, modification with 25% of alkaline alone was found to increase the young modulus of the composite by almost 50% (Pickering et al., 2007). Ho et al. (2012) thus, defined a coupling agent as any substance that adheres two materials together and serves to improve the reaction between the fibre and the matrix. These chemicals can modify the mechanical properties of the thermoplastic matrix making them more polar. They react with both the polymer and fibre surface to improve adhesion (Harutun, 2003).

In this research, the aim was to determine how fibre length affects hemp fibre reinforced polypropylene composites, the effective treatment method and hemp fibre amount required to produce a functional FRP composite. The properties of the produced hemp fibre reinforced polypropylene (HFRP) composites were determined.

MATERIALS AND METHODS

Materials

The hemp bale was supplied by Hempson OÜ. ICORENE supplied Polypropylene powder with trade name ICORENE® PP CO14RM having a density of 0.9 g cm⁻³ and melt flow rate (MFR) of 13 g per 10 min. NaOH granules (98% concentration), ethanol (96.7% concentration), acetic acid (Lachner: 99.8% concentration and molar mass of 60.05 g mol⁻¹), silane (3 – Aminopropyl-triethoxy silane: 98% concentration), tap water and distilled water were used to modify the fibre while, litmus paper was used to confirm the removal of NaOH after modification.

Modification of the hemp fibres

Hemp fibres were separated from the hurd by hand and cut into lengths of 50, 100, and 150 mm. For modification, 150 g of the fibres were first soaked at room temperature 23 °C in a solution of 1,000 mL tap water and NaOH granules 5 wt.% HF for 30 min, thoroughly washed with tap water, checked with litmus paper to ensure there was no residual alkaline, before drying in the oven at 80 °C for 24 h. The modification was completed by washing these fibres with a solution of Silane 3 wt.% HF in 9/1 ethanol and distilled water before oven drying at 80 °C for 24 h. The solution was first neutralized with 20 mL acetic acid and steered for 30 mins to activate the silane.

Production of test specimen

A mixture of HF and PP powder 60/40 was used. 135 g of modified/unmodified hemp fibres and 54.6 g of polypropylene powder were weighed using a Mettler Toledo PL202-s and then combined in a hot press. To ease processing of the unmodified hemp fibres, they were first immersed in water for 10 min, drained, cold-pressed at 1.65 MPa for an additional 10 min before drying at 80 °C for 24 h.

The fibres were then combined with PP before placing the mixture in the press at a temperature of 190 °C for 15 min without pressure and then a pressure of 1.65 MPa was introduced for 10 min at temperatures between 190 °C and 210 °C.

Tensile test

The tensile test was performed according to EN-ISO 527-4 (1997) using an Instron 5688 tensile testing machine and test specimens with a dimension of 150 mm x 25 mm. Test condition was at a temperature of 23 °C, relative humidity of 20% and a test rate of 5 mm min⁻¹. In carrying out the test, the load was applied to a test specimen placed between two grips until failure.

Compressive test

The test was done according to EVS-EN-ISO 14126 (2000) test standard for composites. Instron 5688 and test specimen with a length of 110 ± 1 mm and width of 10 ± 0.5 mm was used to conduct the test. Prior to testing, all specimens were covered with veneer sheets of 50 x 10 x 2 mm at 50 mm from each end using a polyvinyl acetate (PVA) glue. Test speed was 1 mm min⁻¹ ± 0.5 mm min⁻¹.

Flexural test

The flexural test was based on EN-ISO 14125 (1998) test standard. The dimension of specimens was (80 x 10) mm. The test was carried out using Instron 5688 machine at standard laboratory atmosphere of 23 °C and relative humidity of 20%.

Water absorption and swelling test

The water absorption and thickness swelling test was performed according to international test standard EVS-EN 317 (2000). Test specimens had a dimension of (50 x 50 mm) while the thickness was measured using the veneer calliper before the test. Specimen edges were first dipped in wax to prevent water from directly absorbing through them. To calculate the percentage change in mass (C) and thickness (T) of the specimen the initial mass and thickness were determined using the following equations:

$$C = \frac{m_2 - m_1}{m_1} \cdot 100\%, \quad (1)$$

where m_1 is the mass (g), after initial drying and before immersion; m_2 is mass (g), after immersion.

$$T = \frac{t_2 - t_1}{t_1} \cdot 100\%, \quad (2)$$

where t_1 is the average thickness (mm), after initial drying and before immersion; t_2 is the average thickness (mm) after immersion.

Air permeability

International standard EN 12114 (2000) was used to perform the test. The influence of fibre modification on the insulation properties of the composite was determined using airflow resistivity. Specimens dimension was (100 x 100) mm with special air permeability tape called seal flex from tesa used to seal the edges to prevent air leakage. Fig. 1. shows the schematics of the test apparatus.

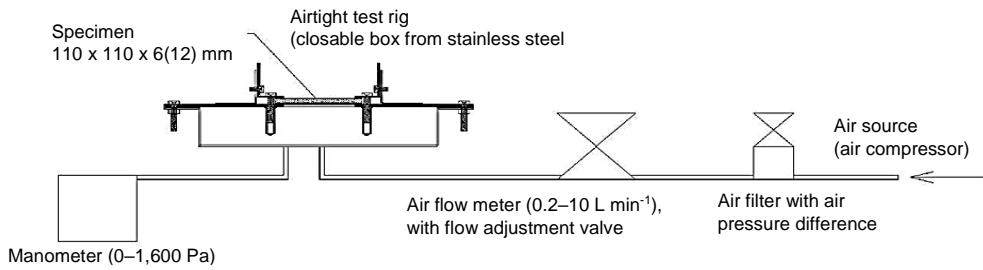


Figure 1. Equipment's complex scheme for carrying out the air permeability test. Source: (Kukk, 2016).

The pressure was introduced through a small pipe from the bottom of the apparatus. The maximum and minimum pressure difference (Δp_{max} & Δp_{min}) were 1,000 Pa and 50 Pa. Three pulses of pressure were administered to the specimens and maintained for at least 2 mins. Each pulse produced a pressure difference of 1,100 Pa. While some specimens were airtight at this pressure. Further testing was done at pressures of 1,000, 652, 425, 277, 181, 118, 77 and 50 Pa for specimens with air flow until there was no airflow recorded. Specimens that are airtight at 1,100 Pa (Stage 1) required no further testing at these pressures (Stage 2). Second phase test pressures were calculated based on the following equation.

$$\Delta p_i = 10^i \frac{\log \Delta p_{max} - \log \Delta p_{min}}{N} + \log \Delta p_{min} \quad (3)$$

where Δp – pressure difference (Pa); N – total number of pressure steps; i – number of pressure steps.

For this test, $\Delta p_{max} = 1,000$ Pa, $\Delta p_{min} = 50$ Pa

Fourier transform infrared spectroscopy (FTIR)

The spectroscopy was performed using fibre strands and thin sheet specimen of modified and unmodified HFRP composites cut with a scalpel. Each was separately placed under the clamp and measured with peak points marked. The spectra range and resolution were 4,000–500 cm^{-1} and 4 cm^{-1} respectively.

RESULTS AND DISCUSSION

The data presented in results shows modified samples as S(length)M and where numbers represent fibre lengths. i.e. S50M (composites of modified hemp fibres of 50 mm) S50 (composites of unmodified hemp fibres).

Tensile properties

The maximum tensile strength of all specimen is illustrated in Fig. 2. The result shows an increased tensile strength as fibre length increased but not with modification. S150 produced the best result (25 MPa) compared to a 47% decrease for S150M. The most significant decrease was 59% for 50 mm HF after modification. This overall poor result may suggest that it was not sufficiently modified or that these fibres were weaker compared to those of unmodified HFRP as they were produced about 60 days later. According to (Pickering et al., 2007), the time of harvest of HF can affect the strength

properties of the composites. The tensile results obtained for the unmodified composites especially S150 conform slightly with (Puech et al., 2018) were tensile strength of 24.5 ± 0.1 MPa and modulus of 2.6 GPa were reported for untreated HF, but these fibres were only 2 mm long and the composites were produced using co-rotating twin screw extruder. Hence, with appropriate modification long HF may perform better than shorter ones since 40% more strength is obtained at 150 mm.

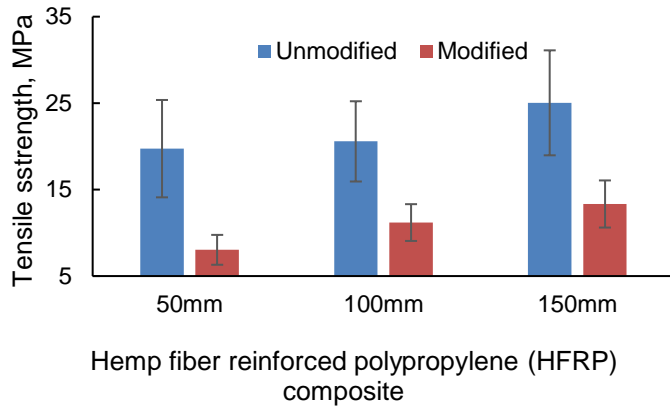


Figure 2. Tensile strength of modified and unmodified HFRP composites with varying fibre lengths (Alao, 2018).

The Young’s modulus of the HFRP composite is shown in Fig. 3. The result is similar to that of tensile properties where 150 mm unmodified fibre composites performed better. S150 had the highest elastic modulus at approximately 4.5 GPa and S50M was the lowest at 1.8 GPa. Overall modulus increased by 56.8% and 20% for modified and unmodified HFRP composites as fibre length increased from 50 to 150 mm.

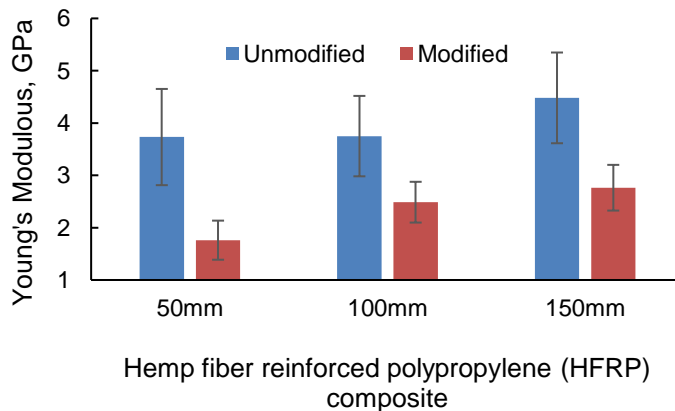


Figure 3. Young’s modulus of unmodified & modified HFRP composites with varying fibre lengths (Alao, 2018).

These results are not exactly unexpected as recent research by (Sepe et al., 2018) on hemp fibre reinforced epoxy composites produced by vacuum infusion process showed a 25% decrease in tensile strength and 7% lower tensile modulus after treatment with 5% alkaline solution. The research also presented a 10 and 15% increase in the tensile modulus when silane was used to treat the fibres as compared to results from untreated and alkali-treated fibre composites. The decrease in tensile strength after alkali modification is purely attributed to excessive removal of lignin and hemicellulose content of the fibre, while treatment with silane is shown to improve bonding between the matrix and fibre. It may thus be inferred that for this research, the bond between the modified HF and PP powder was poor.

Compressive properties

The compressive strength result is shown in Fig. 4. Here, modification did not enhance compressive properties. The best result was obtained for S150, 21 MPa and S150M 18 MPa. A decrease of 35% was recorded for 50 mm fibres after modification.

Strength increased with fibre length from 50 to 100 mm to 16% and 27% from 100 to 150 mm. The modified fibres showed a slightly different trend with compressive strength decreasing by 12% from 50 to 100 mm but an increase of 16% from 50 to 150 mm conclusively shows that an increase in the fibre length, enhances the compressive strength of the composite.

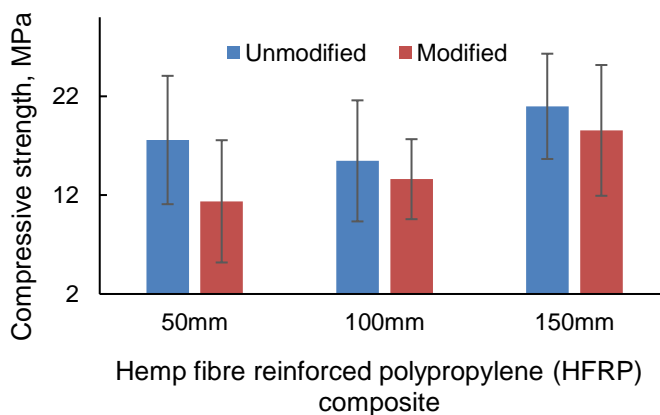


Figure 4. The maximum compressive strength of unmodified & Modified HFRP composites with varying fiber lengths (Alao, 2018).

In Fig. 5 where the compressive modulus is depicted, a similar result is also seen where higher modulus was obtained for the unmodified HFRP composites compared to those of the same length. The compressive modulus for S50, S100 and S150 were 39%, 28% and 29% higher than S50M, S100M and S150M accordingly. Showing that 50 mm fibres had the most significant decrease in strength after modification and also produced 27 and 7% lower performance than S150M (1.26 GPa) and S100M (0.98 GPa).

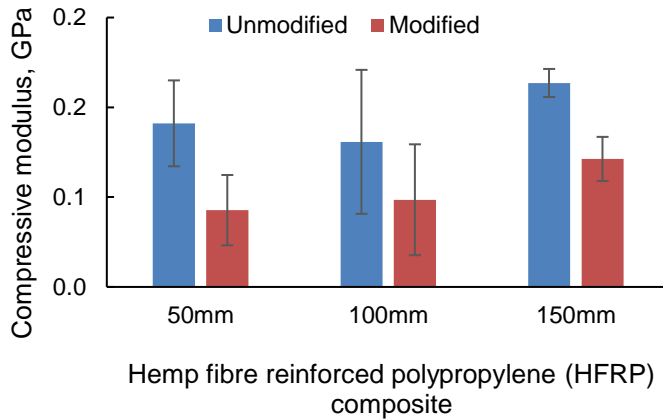


Figure 5. Compressive modulus of HFRP composite from modified and unmodified hemp fibres (Alao, 2018).

Flexural properties

Flexural test results shown in Fig. 6, presents poor outcome for the modified compared to unmodified HFRP composites. It decreased on average by 56%. 150 mm HF showed the most significant decrease of 63%. An increase in fibre length favoured higher flexural performance in particular for the unmodified fibre composites where S150 produced 32.67 MPa. It was 11 and 38% more than S100 and S50. There was no significant difference in the flexural strength for 100- and 150-mm fibres after modification but both were 12% more than 50 mm long hemp fibres.

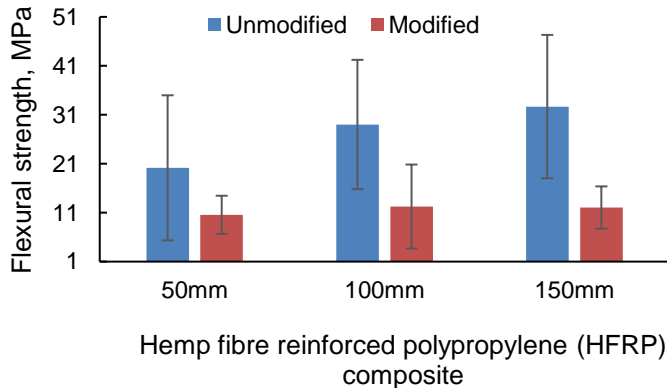


Figure 6. Flexural strength of HFRP composites with varying fibre lengths (Alao, 2018).

The flexural modulus from the modified and unmodified hemp fibre composite is shown in Fig. 7. The result is also identical to that of flexural strength. The best modulus, 3 GPa by S150 decreased 84% after fibre modification (S150M). Commonly, flexural modulus increased with fibre length except for modified fibre where it is unclear why 150 mm fibre yielded 13% less than 50 mm.

These results affirm some past research that flexural strength increases with fibre length. Thomason et al. (1996), Joseph et al. (2002) and Sathishkumar et al. (2012) have shown that using fibres with higher initial lengths enables the composite material to carry higher bending loads. The poor results for the modified HFRP composite, on the other hand, may be attributed to reduced load sharing ability caused by low interaction between polymer and matrix as a result of the ineffective modification. Combined treatment of hemp fibre with NaOH and silane from previous research have shown improvement of bonding between fibre surface and matrix leading to improved flexural properties even when compared to treatments with only NaOH (Sood & Dwivedi, 2017).

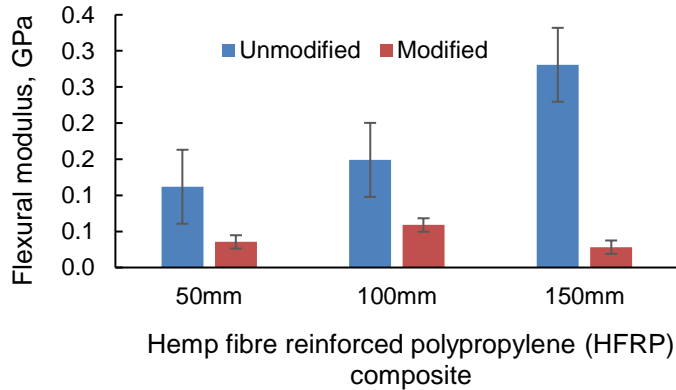


Figure 7. Flexural modulus of HFRP composites with varying fibre lengths (Alao, 2018).

Water absorption and swelling properties

The water absorption results and image of S150M are as shown in Fig. 8. Test standard allows 672 hr. (28 days) of immersion, however, an additional 672 hr. was used for this research. During the first period of soaking (24 hr.), modified fibre composites had higher water uptake than unmodified ones. S150M absorbed 65% while S150 increased by only 13%. At the end of the additional 672 hr of immersion, S150M had gained 85%, showing massive water sorption. Although, this may have been partly due to the wax coming off the edges during the first hours of immersion as shown in the image.

From the result, there appears to be no logical correlation between the lengths of the fibres and water uptake, especially for modified fibre composites. However, if wax removal in S150M is regarded and the fact that S100M absorbed 8% less moisture than S50M is highlighted, it could be concluded, that the water absorption of all the composite examined decreases with increase in fibre length. Higher water uptake recorded in this research could be inferred from (Pickering et al., 2015) which is shown to be influenced by large fibre volume fraction. Hargitai et al. (2008) in their research with a nonwoven fleece of PP fibres using fibre combinations of 30, 40, 50, and 70%, discovered that water sorption characteristics of a composite were affected by the fibre content with a composite of 70% hemp fibre showing 42% water absorption after about 19 days of immersion.

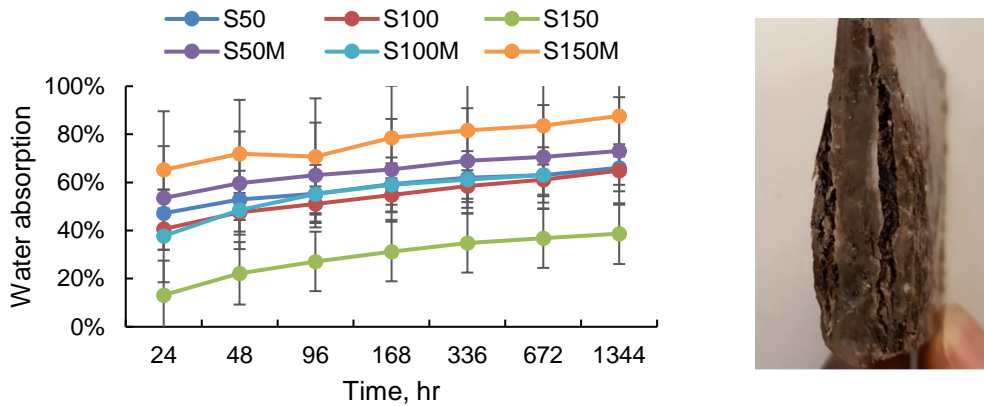


Figure 8. Water absorption of unmodified/modified HFRP composites with varying length of hemp fibres (Alao, 2018).

In Fig. 9, the swelling was constant at 14% for S150 during the first 48hr. 29%-dimensional change shown by S50 was the most significant of all the samples, but this was only 4% higher than that of the composite of the same length. There was no significant difference in thickness swelling results for S100 and S100M, though S150M increased by 2% compared to S150.

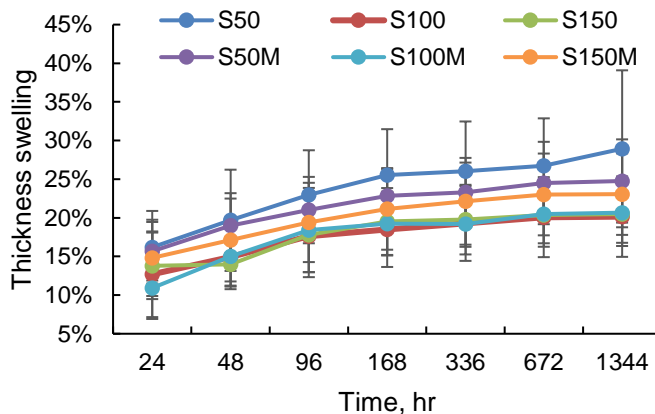


Figure 9. Thickness swelling of HFRP composite with varying length of hemp fibres (Alao, 2018).

Air permeability properties

The mean values of air flow in the first and second stage pressure test are shown in Table 2 below. Specimens from unmodified HFRP composite performed better. This confirms (Nazire et al., 2012) research where it was stated that alkaline treatment causes a drop-in basis weight and a decrease in airflow resistivity.

Table 2. Mean values of air flow in first and second pressure test stages (Alao, 2018)

Pressure stage	Test pressure (Pa)	Mean values of air flow of specimen S50 (L min ⁻¹)	Mean values of air flow of specimen S50M (L min ⁻¹)	Standard deviation S50M	Mean values of air flow of specimen S100 (L min ⁻¹)	Standard deviation S100	Mean values of air flow of specimen S100M (L min ⁻¹)	Standard deviation S100M	Mean values of air flow of specimen S150 (L min ⁻¹)	Standard deviation S150	Mean values of air flow of specimen S150M (L min ⁻¹)	Standard deviation S150M
1	1,100	0	0.3	0.09	0.12	0.20	0.25	0.27	0.09	0.15	0.36	0.12
	1,100	0	0.3	0.09	0.12	0.20	0.25	0.27	0.09	0.15	0.36	0.12
	1,100	0	0.3	0.09	0.12	0.20	0.25	0.27	0.09	0.15	0.36	0.12
2	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	77	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	118	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	181	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	277	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	425	0	0.00	0.00	0.00	0.00	0.07	0.13	0.00	0.00	0.07	0.12
	652	0	0.08	0.14	0.07	0.12	0.11	0.19	0.00	0.00	0.10	0.17
	1,000	0	0.27	0.09	0.11	0.19	0.17	0.29	0.08	0.14	0.32	0.11

Only unmodified HFRP composites of 50 mm long HF were totally airtight at the 1st stage of the pressure test. There seems to be no direct relationship between the fibre lengths and the air permeability and at 425 Pa, S100M and S150M were still not airtight. The growth rate at 425 Pa for S150M/S100M was constant with 652 Pa, 0.9, but then increased by more than 100% to 1.94 at 1,000 Pa. Compared to S100M, the airflow rate of S150M was twice much. This may be attributed to the longer fibre length. Further analysis may be required to assume this because the flow at 1,000 Pa is 41% less for S100M compared to S50M.

The correlation between fibre volume fraction and porosity was emphasized by (Pickering et al., 2015) to be maxed at fibre contents of 50–60 m %, with higher causing increased porosity. Hence, the general lack of airtightness in this research may have been because of the hemp fibre content used (Alao, 2018).

FTIR properties

The FTIR spectrum of the treated and untreated hemp fibres, as well as their composites, are shown in Fig. 10. The spectrums are all similar. The region between 3,500 to 3,000 cm^{-1} , shows wide stretched peak of hydrogen bond for water which is particularly obvious for the modified fibre because of the high absorbance. This shows it contains higher moisture than the unmodified fibre.

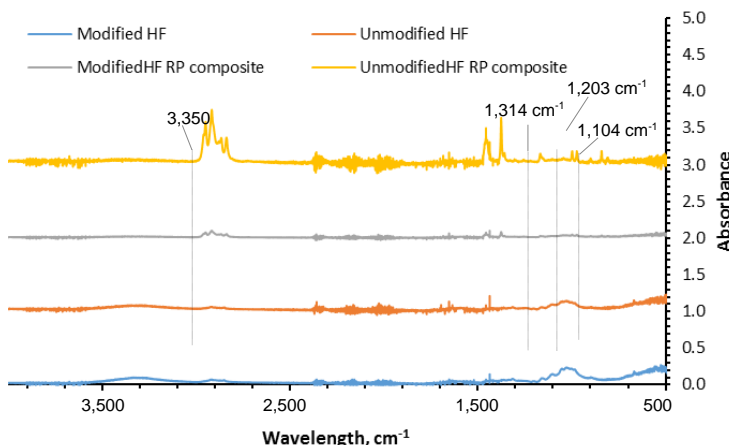


Figure10. FTIR spectra of modified and unmodified hemp fibres (Alao, 2018).

The spectra show that all specimen have similar features within the wavelengths of 1,500–2,500 cm^{-1} . Although, the peak seems to intensify in the wavelengths below 1,500 cm^{-1} for the unmodified fibres compared to modified ones which indicate chemical treatment induced the C-O-C stretching causing a reduction in peak intensity. Theresa et al., 2017, published that NaOH modification is especially responsible for this. Another study by Sepe et al., 2018 on the influence of chemical treatments on mechanical properties of hemp fibre reinforced composites showed a decrease in weak and strong peaks of 1,734 cm^{-1} and 1,373 cm^{-1} respectively for hemp fibre composites modified with different concentrations of alkali (1% wt., 5% wt. and 20% wt.) which increases with the concentration of NaOH. This treatment caused the removal of a part of the

hemicellulose from the surface of the fibre. However, as seen, no new peaks were observed after silane modification which may indicate ineffective treatment.

The dislocation of natural fibres was analysed using Fourier-transform infrared spectroscopy by Dasong & Mizi, 2011. It was deduced that significant differences in the spectra are obtained between bands below $1,500\text{ cm}^{-1}$, although this spectra show CH₂ rocking vibration ($1,314\text{ cm}^{-1}$), C-O-C symmetrical stretching ($1,203\text{ cm}^{-1}$), and C-C, C-OH, C-H ring and side group vibrations ($1,104\text{ cm}^{-1}$), no evidence of silane attachment was observed (Alao, 2018).

CONCLUSIONS

It could be concluded that 60% fibre content may have led to the overall poor mechanical performance and increased porosity of the final composite material due to poor impregnation and inadequate compaction in the composite material. This is because previous research have determined that fibre mechanical properties and porosity is maxed at a fibre content of 50% and starts to decrease. Based on air permeability result, alkalization treatment may have caused further low outcome for modified composite fibres because of the low rigidity arising from lignin and hemicellulose removal.

The FTIR analysis showed some evidence of ineffective modification with silane and possible higher moisture content in the treated fibres.

Although properties improved with fibre length, further investigation is required to ascertain the main reason for the general unsatisfactory performance.

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Effects of nitrogen, phosphorus and vermicompost fertilizers on productivity of groundnut (*Arachis hypogaea* L.) in Babile, Eastern Ethiopia

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Abstract: Though groundnut (*Arachis hypogaea* L.) is a main cash crop for smallholder farmers in several districts of eastern Ethiopia, its yield is very low. Farmers apply little or no fertilizers to grow the crop possibly because no generic or recommended fertilizers rates available for farmers. Therefore field experiments were conducted for two consecutive cropping seasons in Babile district, to prove the hypothesis that one of the major factors that severely reduce the yield of the crop was lack of nutrients in the soil with the objective of investigating the effects of applying mineral NP fertilizers and vermicompost on the productivity of improved groundnut variety 'BaHa-Gudo'. The experiment was 3×3×3 factorial with three replications laid down in a randomized complete block design comprising nitrogen (0, 23 and 46 kg N ha⁻¹), phosphorus (0, 46 and 92 kg P₂O₅ ha⁻¹) and vermicompost (0, 2.5 and 5 t vermicompost ha⁻¹). The data on crop growth, nodulation and yield were collected and subjected to analysis of SAS 9.2 software. Means separation were made using Duncans Multiple Range Test (DMRT) at 5% level of significance. The results revealed that growth, yield attributes, and yields were significantly ($P < 0.01$) affected by the main and interactions effect of the treatments. The pod yield obtained from combined application of 46:46 kg N: P₂O₅ ha⁻¹ and 2.5 t vermicompost ha⁻¹ exceeded the pod yield produced from nil application of fertilizers by about 100%. The economic analysis also indicated that the highest marginal rate of return (671%) and net benefit (\$ 1,830) ha⁻¹ were obtained from combined application of 46:46 kg N: P₂O₅ ha⁻¹ and 2.5 t vermicompost ha⁻¹. From the results it could be concluded that applying the aforementioned doses of fertilizers combination would enable farmers increase productivity of groundnut so as to enhance farmers' income and livelihoods.

Key words: groundnut; mineral NP fertilizers; vermicompost; combined application.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a monoecious annual legume widely grown in tropical and sub-tropical regions for direct use as food, oil, high protein meal and animal feed (Pande et al., 2003; Taru et al., 2010). It is among important oil crops in Ethiopia, with Oromia region as the major producer in the country contributing 55% to the national groundnut production (CSA, 2017). Moreover, as a cash crop, groundnut is high value

crop with relatively high return on a limited land area, and it is well adapted to hot semi-arid conditions (Wangai et al., 2001).

The lowland areas of Ethiopia have immense potential for groundnut production. The estimated production area and yield of groundnut in the country during 2016/2017 cropping season were 74,861.4 ha and 129,636.4 tonnes, respectively, and the largest production areas are in Oromia (41,055.3 ha), Benshangul-Gumuz (19,729.0 ha) and Amhara (7,104.4 ha) (CSA, 2017). However, the productivity of the crop in the country remained low. The mean dry pod yield of groundnut on farmers' field (1.73 t ha^{-1}) is lower than the mean potential dry pod yield of $1.80\text{--}2.50 \text{ t ha}^{-1}$ recorded at research field under rain-fed (Teklemariam et al., 2012; Chala et al., 2014). In fact, there have been some efforts to improve the productivity of groundnut in Ethiopia. However, the effort was not boosted the crop productivity as expected (Sahelamedhin & Taye, 2000). The low groundnut yield is, therefore, related to poor agronomic practices (Page et al., 2002; Chala et al., 2014) particularly soil fertility management in addition to other biotic and abiotic production constraints.

Declined soil fertility due to continuous land cultivation with little fertilizers inputs is a major threat to crop production in Africa, including Ethiopia (Sanchez & Jama, 2002; Negassa et al., 2005; Tesfaye et al., 2011). Inadequate fertilizers supply accompanied with low rate of return of biomass to the soil in a cultivated land aggravated soil degradation leading to low productivity (Zelleke et al., 2010; Samuel, 2013). This is a major problem particularly in East Hararghe Zone, where crop residues are rigorously used for competing ends such as animal feed and fuel wood while fertilizers input is insufficient. Most Ethiopian soils are deficit in major nutrients, especially nitrogen (N) and phosphorus (P) (Maheswar & Sathiyavani, 2012) and N (Mohamed & Abdalla 2013; Samuel, 2013). Although groundnut can satisfy part of its N need through beneficial N_2 fixation; the crop showed response to external N fertilizer supply (Singh & Singh 2001; Kandil et al., 2007). Besides, in semi-arid regions, N_2 fixation by legumes is limited by moisture stress (Devi et al., 2013; Collino et al., 2015). Furthermore, N_2 fixation potential of groundnut is restricted by P as it is essentially required for growth of efficient root system and nodulation (Ndakidemi et al., 2006; Zafar et al., 2013; Abdulkadir et al., 2014). Thus, external source of N and P is essentially required for growth and yield of the crop.

Groundnut is best grown in semi-arid eco-systems where rainfall is low and erratic (Hamidou, 2012); soils are sandy and sandy loam which with poor fertility and low water holding capacity (Mohsen et al., 2015); low soil organic matter (Samuel, 2013) and thus poor fertilizer use efficiency. The nutrient and water holding capacity of such soils can be improved through adding organic materials (Mohsen et al., 2015). Thus combined use of mineral and organic fertilizers like manures, compost and vermicompost (VC) is becoming increasingly important (Saadatnia & Riahi, 2009; Chouichom & Yamao, 2011). Gursum and Babile districts of eastern Ethiopia are major areas of groundnut production (Chala et al., 2014). These districts are mainly semi-arid and commonly influenced by water stress arising from low, highly variable and erratic rainfall. Due to moisture scarcity, the soil organic matter turnover and fertilizer use efficiency is very low. Furthermore, the soil is sandy or sandy loam with low water holding capacity and deficient in organic carbon, total N and available P (Argaw et al., 2015). To cope up with the problems some farmers of the study area have been commonly using di-ammonium phosphate (DAP) and urea [$\text{CO}(\text{NH}_2)_2$] in groundnut production (Chala et al., 2014).

However, despite, the generally low content of soil organic matter, no organic fertilizer is applied by farmers or no recommended rate of application for such fertilizers is available. Moreover, to exploit the yielding potential of recently released improved groundnut varieties a specific fertilizer recommendation need to be developed. Therefore, this study was conducted to elucidate the nodulation, growth and yield response of groundnut to the combined application of mineral NP fertilizers and vermicompost in Babile District of eastern Ethiopia.

MATERIALS AND METHODS

Description of the Experimental Site

Field experiments were conducted in East Hararghe Zone, Babile district. The district is known for a significant share of groundnut production in the East Hararghe Zone and the country. It is located at 9° 13' 13.5'' N latitude and 42° 19' 20.9'' E longitude and at an altitude of 1,647 meters above sea level. The experiment was undertaken during the main rainy season 2015 and 2016. The area has mean annual minimum and maximum temperatures of 16 °C and 31 °C, respectively and annual rainfall of the area varied within the range 500 to 700 mm with erratic nature (Samuel, 2013). The area has soil texture of sandy loam with low water holding capacity. The soil physical and chemical properties of the experimental site were described in Table 1. The experimental fields were planted sorghum in the previous years of cropping seasons.

Table 1. Soil physical and chemical properties of the experimental site

Physico-chemical properties	Value	Rating	References
pH (1:2.5 H ₂ O)	7.11	Neutral	Tekalign Tadese (1991)
Organic carbon (%)	0.68	Very low	Tekalign Tadese (1991)
Total N (%)	0.11	Low	Tekalign Tadese (1991)
Available P (mg/kg soil)	2.61	Very low	Olsen et al. (1954)
CEC (cmol (+)/kg soil)	24.55	Medium	Landon (1991)
Exchangeable Ca (cmol (+) kg ⁻¹ soil)	7.18	High	FAO (2006)
Exchangeable Mg (cmol (+) kg ⁻¹ soil)	3.63	High	FAO (2006)
Exchangeable Na (cmol (+) kg ⁻¹ soil)	0.17	Low	FAO (2006)
Exchangeable K (cmol (+) kg ⁻¹ soil)	1.04	High	FAO (2006)
Physical properties (texture)			
Clay (%)	20		
Silt (%)	15		
Sand (%)	65		
Textural class	Sandy loam		

H₂O: water; N: nitrogen; P: phosphorus; CEC: cation exchange capacity; Ca: calcium; Mg: magnesium; Na: sodium; K: potassium.

Experimental Procedures

Groundnut (*Arachis hypogaea* L.) variety, BaHa-Gudo (NC-AC-2748×Chico) was used as a test crop. Nitrogen in the form of urea (46% N), P in the form of tri superphosphate (TSP) (46% P₂O₅) and vermicompost (VC) were used as fertilizer. The VC was prepared at Haramaya University from animal manure and plant waste materials by using earthworm (*Eisenia fetida*) for decomposition. The analysis of the VC before application revealed slightly alkaline pH of 7.48, total organic carbon content of 29.25%,

total N content of 1.59%, total P content of 986.74 part per million (ppm), and electric conductivity of 8.96 millisiemens per centimeter (mS cm⁻¹). The treatments consisted of three doses of nitrogen (0, 23, and 46 kg N ha⁻¹), three doses of phosphorus (0, 46, and 92 kg P₂O₅ ha⁻¹) and three doses of VC (0, 2.5, and 5 t VC ha⁻¹). Thus, there were 3×3×3 = 27 treatment combinations. The treatments were laid out in randomized complete block design (RCBD) in a factorial arrangement with three replications.

Two groundnut seeds per planting hole were planted at row spacing of 60 cm and plant spacing of 10 cm in both seasons on gross plot size of 7.2 m² (3.6 m×2 m). Each plot was consisted of 6 plant rows. Plots and blocks were separated by 0.50 m and 1.00 m spaced pathways for intercultural operations. Full doses of P fertilizer were applied in the form of TSP at planting time. Vermicompost was applied to the plots in band at time of planting. Nitrogen was applied in two splits, one at planting, and the second one at flower initiation stage in the form of urea. All other production and agronomic practices were kept uniform for all treatments in both seasons. From the four central rows of each plot one randomly selected row was used for destructive sampling while the rest three central rows per plot (i.e., net plot of 3.6 m² = 1.8 m×2 m size) were harvested for the crop yield data analysis.

Data collection and analysis

The crop canopy (a layer of branches and leaves at the top of the crop) spread, height was taken from randomly selected and pre tagged five plants per plot, measuring from the last leaf on one side to the last leaf on the other side across and along plant row using a measuring tape and the average taken as canopy spread. Height and number of branches of the pre tagged plants were taken at 75% maturity of the crop. Destructive sampling raw for nodule data was randomly selected from central rows of each plot. Five plants from sampling rows of each plot were gently uprooted at complete flowering stage and washed on a fine sieve with tap water to remove soil particles. The nodules were carefully removed from roots, the number of nodules on each plant was counted and the average nodules number plant⁻¹ was determined. Ten nodules were randomly taken from the total number nodules of each plant and effective nodules and non-effective nodules were identified by their pinkish or red color on cutting (Unkovich et al., 2008). The weight of dry pods from the respective net plot was recorded after plucking the pods, drying them, and adjusting their moisture contents to 13% (Konlan et al., 2013). The weight of pods harvested from each net plot area was then was converted to total pod yield (t ha⁻¹). The dried pods were hand-shelled, the seed weighed, and the differences between the pod and seed weights of the treatments were used to compute shelling percentage (%). Shelling percentage was determined as the weight of groundnut seed divided by the weight of pods as shown below:

$$\text{Shelling (\%)} = \frac{\text{Weight of seeds}}{\text{Weight of pods}} \times 100 \quad (1)$$

Five plants were randomly selected and harvested with their pods from the sampling row of each plot to determine total dry biomasses yield at physiological maturity.

Then the aboveground part plus the pods were oven dried at 65 °C to a constant weight to determine the total dry biomass yield (t ha⁻¹). The harvest index was calculated in percentage as the ratio of the economic yield to the total dry biomass yield of the plant multiplied by 100.

All the collected data were subjected to analysis by SAS (statistical analysis system) version 9.2 (SAS Institute, 2004). Homogeneity test of error variances for the two years was performed using the F-test as described by Gomez & Gomez (1984). In all cases, the variations in the two years were not significantly different for all the parameters and the error variances were homogeneous. Thus, a combined analysis of variance (ANOVA) for the two years data was performed. Differences among treatment means were delineated using the Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Economic Analysis

Economic evaluation of the effect of N (urea), TSP (Tri-superphosphate) and VC were performed on the pod yield. Thus, the economic gains of the different treatments were calculated to estimate the net returns, considering the costs of fertilizers N, P, VC and labor related to them, and the income from the harvest following procedures of CIMMYT (1988).

RESULTS

Crop Growth and Nodulation

Canopy spread. The main effects of N, P, and VC significantly ($P < 0.001$) affected canopy spread. However, all of these factors did not interact to influence canopy spread. The canopy spread of groundnut improved by application of N or VC application over the control. The result also showed that medium level (46 kg P_2O_5 ha⁻¹) of P fertilizer resulted in maximum (43.3 cm per plant) crop canopy spread (Table 2).

Plant height. The analysis of variance revealed that the main effect of N, P, and VC as well as the two-factors interactions effect except P×VC, significantly ($P < 0.001$) influenced plant height at harvest. Moreover, the interaction of N×P×VC significantly ($P < 0.001$) affected the height of groundnut. Plant height was significantly enhanced in response to increased doses of N and P across the increasing doses of vermicompost. The tallest (27.2 cm) height was obtained at combined application of 23:46 kg N: P_2O_5 ha⁻¹ with 5 t VC ha⁻¹ as well as at 46:46 kg N: P_2O_5 ha⁻¹ with 2.5 t VC ha⁻¹ (Table 3).

Table 2. Main effects of nitrogen (N), phosphorus (P) and Vermicompost (VC) doses on canopy spread (CS) of groundnut at Babile (2- year's pooled data)

Treatment	Canopy spread (cm)
Nitrogen (kg N ha ⁻¹)	
0	40.4 ^b
23	41.8 ^a
46	42.0 ^a
F-test	***
Phosphorus (kg P_2O_5 ha ⁻¹)	
0	40.7 ^b
46	43.3 ^a
92	40.3 ^b
F-test	***
Vermicompost (t ha ⁻¹)	
0	39.2 ^b
2.5	42.2 ^a
5.0	42.9 ^a
F-test	***
CV (%)	5.15

Means followed by the same letter within a column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test (DMRT) at 5% level of significance.

***: significant at $P < 0.001$; CV: coefficient of variation.

Number of branches per plant. The combined analysis of variance showed significant ($P < 0.001$) differences in the number of branches plant⁻¹ due to the main effect of N, P and VC as well as all their interactions effect. Increasing the dose of P led to significantly ($P < 0.001$) increased plant height as well as number of branches plant⁻¹ across the increasing doses of N and VC (Table 3). The highest (6.4) numbers of branches plant⁻¹ were recorded at the combined application of 23:46 kg N: P₂O₅ ha⁻¹ with 5 t VC ha⁻¹ as well as at 46:46 kg N: P₂O₅ with 2.5 t VC ha⁻¹. Thus, the number of branches plant⁻¹ produced at the aforementioned combined doses of fertilizers significantly exceeded the number of branches plant⁻¹ obtained from non fertilized plots by about 25% (Table 3).

Table 3. Interaction effects of nitrogen (N), phosphorus (P) and vermicompost (VC) doses on number of branches and plant height of groundnut at Babile (2-year's pooled data)

Fertilizers (kg ha ⁻¹)		Vermicompost (t ha ⁻¹)					
		number of branches plant ⁻¹			plant height (cm)		
N	P ₂ O ₅	0	2.5	5.0	0	2.5	5.0
	0	5.1 ^f	5.6 ^{cde}	5.9 ^{bc}	19.9 ^l	20.5 ^{kl}	20.8 ^{i-l}
0	46	5.7 ^{b-e}	5.5 ^{cde}	5.6 ^{cde}	20.6 ^{kl}	21.1 ^{i-l}	21.2 ^{h-l}
	92	5.8 ^{bcd}	5.7 ^{bcd}	5.7 ^{b-e}	19.8 ^{kl}	21.5 ^{f-j}	21.9 ^{d-j}
	0	5.5 ^{df}	5.7 ^{b-e}	6.0 ^b	20.9 ^{i-l}	21.7 ^{e-j}	23.3 ^{cde}
23	46	5.7 ^{b-e}	6.0 ^b	6.4 ^a	22.3 ^{c-i}	23.0 ^{c-f}	27.2 ^a
	92	5.7 ^{b-e}	5.7 ^{b-e}	5.6 ^{b-e}	20.7 ^{kl}	23.3 ^{cde}	22.9 ^{c-g}
	0	5.7 ^{b-e}	5.9 ^{bc}	5.4 ^{ef}	21.3 ^{g-k}	23.2 ^{cde}	22.9 ^{c-g}
46	46	5.8 ^{bcd}	6.4 ^a	5.6 ^{cde}	22.4 ^{c-i}	26.8 ^a	25.4 ^b
	92	5.6 ^{cde}	5.8 ^{bcd}	5.8 ^{bcd}	22.7 ^{c-h}	23.5 ^{cd}	23.5 ^c
F-test		***			***		
CV (%)		5.39			6.27		

Means sharing the same letter are not significantly different according to DMRT at 5% level of significance. CV: coefficient of variation; ***: significantly at $P < 0.001$.

Nodulation. The combined analysis of variance of nodulation assessment at 50% flowering stage revealed that the main factors N, P and VC and their interactions significantly ($P < 0.001$) influenced both total and effective number of nodules plant⁻¹. The number of total and effective nodules obtained in response to the combined application of 23:46 kg N: P₂O₅ ha⁻¹ without VC ha⁻¹ exceeded the total and effective number of nodules plant⁻¹ obtained control plots by about 47% and 50%, respectively (Table 4).

Yield

Dry pod yield. The three-factor interaction significantly ($P < 0.001$) influenced pod yield of groundnut. Increasing the doses of N across the increasing doses of P and VC increased pod yield of groundnut. The highest (3.05 t ha⁻¹) pod yield was obtained at combined application of 46:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹ (Table 6). Thus, the results have demonstrated that the maximum pod yield was obtained at the highest dose of mineral N fertilizer, medium doses of mineral P and vermicompost fertilizers.

Dry biomass yield. The combined analysis of variance revealed that the main factors N, P and VC as well as their interactions significantly ($P < 0.001$) influenced dry biomass (DBM) yield of groundnut (Table 5). Similar to pod yield, all the fertilizer

treatments gave significantly higher DBM yield over the control. The highest DBM (6.91 t ha⁻¹) was obtained in the plot received combination of 23:46 kg N: P₂O₅ ha⁻¹ with 5.0 t VC ha⁻¹ which was statistically at par with the biomass (6.82 t ha⁻¹) obtained at combined application of 46:0 kg N: P₂O₅ ha⁻¹ with 5 t VC ha⁻¹. The lowest DBM yield (3.53 t ha⁻¹) was recorded in the control treatment (Table 5).

Table 4. Interaction effects of nitrogen (N), phosphorus (P) and vermicompost (VC) doses on number of total and effective nodules plant⁻¹ of groundnut at Babile (2-year's pooled data)

Fertilizers		Vermicompost (t ha ⁻¹)					
N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	number of total nōdules plant ⁻¹			number of effective nodules plant ⁻¹		
		0	2.5	5.0	0	2.5	5.0
0	0	113.7 ^{e-h}	124.1 ^{c-g}	118.2 ^{d-g}	100.4 ^{e-h}	106.8 ^{d-g}	103.1 ^{e-h}
	46	126.3 ^{c-f}	169.1 ^a	136.7 ^{bc}	112.9 ^{c-f}	150.6 ^a	118.8 ^{cd}
	92	120.3 ^{d-g}	127.5 ^{c-f}	123.4 ^{c-g}	108.2 ^{c-g}	113.4 ^{cde}	108.8 ^{c-g}
23	0	111.0 ^{gh}	136.3 ^c	122.3 ^{c-g}	99.5 ^{f-i}	121.4 ^{bc}	108.5 ^{c-g}
	46	166.9 ^a	151.2 ^b	117.6 ^{d-g}	150.3 ^a	134.1 ^b	104.1 ^{e-h}
	92	126.9 ^{c-f}	128.6 ^{cd}	127.0 ^{c-f}	113.9 ^{cde}	111.4 ^{c-g}	111.8 ^{c-f}
46	0	95.5 ⁱ	110.9 ^{gh}	128.2 ^{cde}	84.1 ^j	98.3 ^{ghi}	112.9 ^{c-f}
	46	101.4 ^{hi}	99.6 ^{hi}	100.4 ^{hi}	91.2 ^{hij}	86.2 ^{ij}	86.2 ^{ij}
	92	120.9 ^{d-g}	113.6 ^{fgh}	120.4 ^{d-g}	106.2 ^{d-g}	101.0 ^{e-h}	106.3 ^{d-g}
F-test		***			***		
CV (%)		10.34			10.76		

Means sharing the same letter are not significantly different according to DMRT at 5% level of significance. CV: coefficient of variation; ***: significant at $P < 0.001$.

Table 5. Interaction effects of nitrogen (N), phosphorus (P) and vermicompost (VC) doses on dry pod yield and dry biomass yield of groundnut at Babile (2-year's data pooled)

Fertilizers		Vermicompost (t ha ⁻¹)					
N (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	dry pod yield (t ha ⁻¹)			dry biomass yield (t ha ⁻¹)		
		0	2.5	5.0	0	2.5	5.0
0	0	1.50 ⁿ	2.09 ^{kl}	2.29 ^{hij}	3.53 ^g	5.76 ^{b-e}	5.48 ^{b-e}
	46	2.06 ^l	2.36 ^{e-i}	2.34 ^{f-i}	4.66 ^f	5.62 ^{b-e}	5.65 ^{b-e}
	92	2.12 ^{ijkl}	2.40 ^{e-h}	2.51 ^{ef}	5.58 ^{b-e}	5.41 ^{b-e}	5.80 ^{b-e}
23	0	1.81 ^m	2.30 ^{g-i}	2.38 ^{e-h}	5.59 ^{b-e}	5.23 ^{def}	5.93 ^{bc}
	46	2.34 ^{f-i}	2.81 ^{bc}	2.98 ^{ab}	5.66 ^{b-e}	5.94 ^{bc}	6.91 ^a
	92	2.41 ^{e-h}	2.44 ^{e-h}	2.45 ^{e-h}	5.74 ^{b-e}	5.69 ^{b-e}	5.56 ^{b-e}
46	0	2.05 ^l	2.18 ^{i-k}	2.26 ^{h-k}	5.19 ^{ef}	5.33 ^{c-f}	6.82 ^a
	46	2.55 ^{de}	3.05 ^a	2.72 ^{cd}	5.87 ^{b-e}	5.93 ^{bd}	6.05 ^b
	92	2.41 ^{e-h}	2.49 ^{efg}	2.52 ^{ef}	5.60 ^{b-e}	5.90 ^{bcd}	5.73 ^{b-e}
F-test		***			***		
CV (%)		7.13			10.45		

Means sharing the same letter are not significantly different according to DMRT at 5% level of significance. ***: significant at $P < 0.001$.

Pod harvest index. The main effects of P and N as well as the interaction effects of VC with either N or P were significant ($P < 0.01$) on pod harvest index. The highest pod harvest index (51.9%) was obtained from combined application of 46:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹, which was statistically identical with plots that received combination of 23:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹ (Table 6). On the other hand the lowest pod

harvest index (32.4%) was obtained from sole application of 23kg N ha⁻¹, which was statistically at par with plots that received 46kg N ha⁻¹ and 2.5 t VC ha⁻¹ without P₂O₅ (Table 6).

Economic Analysis

The economic analysis revealed that the highest net benefit of \$ 1,830 ha⁻¹ with MRR of 671% was obtained from combined application of 46:46 kg mineral N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹, followed by net benefit of \$ 1,683 with MRR of 55% and \$ 1,620 with MRR of 565%, \$ 1,496 with 322% and \$ 1,213 with MRR of 391% which were recorded for 23:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹, 46:46 kg N: P₂O₅ ha⁻¹ with no VC, and 23:46 kg N: P₂O₅ ha⁻¹ with no VC and 23:00 kg N: P₂O₅ ha⁻¹ with no VC respectively (Table 7). Since the minimum MRR assumed in this study was 100%, the treatment with application of 23:46 kg mineral N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹ was not considered rewarding treatments as it gave MRR of 55% (Table 7).

Table 6. Interaction effects of nitrogen (N), phosphorus (P) and vermicompost (VC) doses on pod harvest indices of groundnut at Babile (2-year's data pooled)

Fertilizers (kg ha ⁻¹)	P ₂ O ₅ (kg ha ⁻¹)	Vermicompost (t ha ⁻¹)		
		0	2.5	5
0	0	42.7 ^{cde}	36.3 ^{fgh}	41.8 ^{c-f}
	46	44.5 ^{bcd}	42.2 ^{cde}	41.6 ^{c-f}
	92	38.3 ^{efg}	45.2 ^{bc}	43.4 ^{b-e}
23	0	32.4 ^h	44.9 ^{bcd}	40.2 ^{c-f}
	46	41.9 ^{cde}	48.9 ^{ab}	43.3 ^{cde}
	92	42.2 ^{cde}	42.9 ^{cde}	44.3 ^{bcd}
46	0	39.6 ^{def}	41.5 ^{c-f}	33.2 ^{gh}
	46	45.0 ^{bcd}	51.9 ^a	45.2 ^{bc}
	92	43.1 ^{cde}	42.2 ^{cde}	44.5 ^{bcd}
F-test		**		
CV (%)		11.47		

Means sharing the same letter are not significantly different according to DMRT at 5% level of significance.

** : significant at $P < 0.01$; CV: Coefficient of variation.

Table 7. Results of the economic analysis for combined application of nitrogen (N), phosphorus (P) and vermicompost (VC) in groundnut at Babile (2-year's data pooled)

N (kg ha ⁻¹)	P (P ₂ O ₅) (kg ha ⁻¹)	VC (t ha ⁻¹)	Ad PY (t ha ⁻¹)	TVC (\$ ha ⁻¹)	GFB (\$ ha ⁻¹)	NB (\$ ha ⁻¹)	MRR (%)
0	0	0	1.50	—	1042	1042	-
23	0	0	1.81	44	1257	1213	391
23	46	0	2.34	132	1628	1496	322
46	46	0	2.55	154	1774	1620	565
23	46	2.5	2.81	267	1950	1683	55
46	46	2.5	3.05	289	2119	1830	671

AdPY: Adjusted pod yield; TVC: Total variable cost; GFB: Gross field benefit; NB: Net benefit; MRR: Marginal rate of return.

DISCUSSION

Crop growth and nodulation

Crop growth. In this study mineral N, P and VC application significantly ($P < 0.001$) increased canopy spread (Table 2) while their combined application significantly increased plant height and number of branches plant⁻¹ (Table 3). Similar results have been reported by Bala et al. (2011), Mathivanan et al. (2013) and

Mukhtar et al. (2014) that application of N in soybean, manures and VC in groundnut, respectively, significantly increased canopy spreads. Similarly, Abbasi et al. (2008) and Ahiabor et al. (2014) reported improved canopy spread due to P application in soybean. The synergistic effects of combined application of mineral NP fertilizers and VC on plant height and number of branches plant⁻¹ in the current experiments have been also reported by Malligawad (2010) and Singh et al. (2017). Similarly, Rajkhowa et al. (2002); Channaveerswami (2005); and Yagoub et al. (2012) reported better growth response of green gram, groundnut and soyabean, respectively, to combinations of organic and inorganic fertilizers. Bhosale et al. (2017) reported higher number of branches plant⁻¹ of groundnut over the control due to combined application of different proportion mineral NP fertilizers and VC. Furthermore, Singh et al. (2011) & Armin et al. (2016) also reported that combined application of inorganic fertilizers which led to the highest number of branches plant⁻¹ of French bean and mungbean, respectively.

Nodulation. The highest numbers of both total and effective nodules were obtained at the combined application of 23 kg N ha⁻¹ and 46 kg P₂O₅ ha⁻¹ without VC ha⁻¹ (Table 4). However, higher doses of inorganic N fertilizer and VC application suppressed the number of nodules. In support with the present result, Basu et al. (2007) reported that application of 20:40:30 kg NPK ha⁻¹ along with 2.5 t FYM ha⁻¹ significantly increased the nodule formation as compared to mineral NPK alone while, at high dose, NPK (40:80:60 kg ha⁻¹) decreased the nodule formation and nitrogen accumulation in nodules over low level of NPK (20:40:30 kg ha⁻¹) in groundnut. Similarly, Ouslim et al. (2015) & Islam et al. (2016) reported reduced nodulation at adequate or high N application in different legumes. The result conclusively indicated that nodule formation could be reduced by high doses of mineral N, while small starter doses of applied N may stimulate nodule formation (Biswas et al., 2003; Abayomi et al., 2008). Amba et al. (2013), Weisany et al. (2013) & Taruvinga (2014) reported increased nodulation in groundnut with application of P. Contrarily, Latif et al. (2014) reported that P did not directly involved in the process of nodulation and N₂ fixation of groundnut suggesting that the role of P varies with crop, growing conditions and time of measurement.

Yield. The dry pod yield (3.05 t ha⁻¹) obtained in this study was higher by about 43% compared to pod yield of groundnut obtained in farmers' fields (1.74 t ha⁻¹, regional average yield, reported by CSA, 2017). The maximum yields obtained in these experiments either at 46:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹ or at 23:46 kg N: P₂O₅ ha⁻¹, with 5.0 t VC ha⁻¹ (Table 5). As depicted in Table 3 the aforementioned doses of fertilizers resulted in high plant height and number of branches plant⁻¹ of groundnut. Therefore, the maximum yield recorded might be due to additive effects of combined use of inorganic NP fertilizers and VC on nutrient supply that promote growth in plant height and number of branches leading to high pod yield. The number of branches was more important in determining the node number which in turn determines pod number and yield. In addition to the additive effect on nutrient supply, VC adds soil organic matter; hence improve soil physical conditions to conserve moisture and resulting in better growth, pod formation and greater yields (Oke, 2015; Sengupta et al., 2016). Murthy et al., 2009 and Sengupta et al. (2016) reported improved growth and yield components

leading higher pod yield of 3.32 ha⁻¹ from application of recommended dose of NPK ha⁻¹ with vermicompost at 2.5 t ha⁻¹ along with gypsum and 3.21 ha⁻¹ application of recommended dose of NPK ha⁻¹ with manure, respectively.

Dry biomass. As depicted in Table 5 statistically similar DBM yields obtained at 23:46 kg N: P₂O₅ ha⁻¹ with 5.0 t VC ha⁻¹ and 46:0 kg N: P₂O₅ ha⁻¹ with 5 t VC ha⁻¹. The favorable effect of optimum level of nutrients through both inorganic and organic nutrient sources in improving vegetative growth could be one of the reasons for higher DBM production of groundnut as stated by Ramana et al. (2002). Particularly optimum N level promotes the development of source system and increases its photosynthetic efficiency leading to higher DBM production (Satapathy et al., 2005). The current finding aligned with that of Karunakaran et al. (2010) and Khaim et al. (2013) who reported that combined application of inorganic and organic fertilizers in groundnut and in soybean, respectively, improved biomass production. Olasan et al. (2018) revealed that plant biomass in the field has significant effect on yield as well as sizes of pods and seeds. However, it is imperative to note that high biomass production may not necessarily indicate high pod or seed yield. Agele et al. (2017) stated that it was dry matter partition, but not only biomass production that greatly determines the pod yield of cowpea. It is the dry matter partitioned in to harvestable organs contribute yield of groundnut. In the current experiment, the high biomass recorded in treatment received 23:46 kg N: P₂O₅ ha⁻¹ with 5.0 t VC ha⁻¹ led to high pod yield, but the equivalently high biomass recorded at application of 46kg N ha⁻¹ and 5 t VC ha⁻¹ without P did not follow the same trend. The difference might be strongly related with the role P in assimilate partition to pods and seed filling. Though the biomass production was high the low pod yield might have also been attributed high fruit abortion and excessive vegetative growth on the expense of fruiting (Agele et al. (2017).

Harvest index. Harvest index (HI) is the partitioning of the assimilated products to the economically important parts that determines the productivity of any crop (Ramana et al., 2002). It is evident from the results that combined application of 46:46 kg N: P₂O₅ and 2.5 t VC ha⁻¹ resulted in 21.5% increases in pod harvest index over the control (Table 6). The highest yield was also recorded at the above mentioned dose of fertilizers. This indicated that balanced nutrient supply through combination of 46:46 kg N: P₂O₅ and 2.5 t VC ha⁻¹ improved efficiency of the crop to convert photosynthesized products into pods.

Economic Analysis. The maximum net benefit obtained at 46:46 kg N: P₂O₅ with 2.5 t ha⁻¹, gave high MRRs of 671% suggesting for each \$ invested, the producer would collect \$ 0.25 after recovering the cost (Table 7). On the other hand, either 46:46 kg mineral N: P₂O₅ or 23:46 kg mineral N: P₂O₅ ha⁻¹ (Table 7) could be profitably used by the groundnut farmers as alternatives when VC may not be accessible. This result agreed with the study by Girma & Gebreyes (2017) who reported high net benefit from the combined application of mineral NP fertilizers with VC for teff (*Eragrostis teff*). Similarly, Meena et al. (2015) reported that combined application of 75% RDF and 2.5 t ha⁻¹ of VC with bio-fertilizers resulted in 42% increase net return over the control treatment in mungbean.

CONCLUSION

Groundnut (*Arachis hypogaea* L.) is produced with little or no fertilizer application in the study area. However, the soil in the study area has been shown to be low in the contents of N, P, and organic carbon, which necessitates application of fertilizers from external sources of fertilizers. Groundnut canopy spread was improved by application of N or VC application over the control. Combined application of 23:46 kg N: P₂O₅ ha⁻¹ with 5 t VC ha⁻¹ or 46:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹ was resulted in high plant height and number of branches plant⁻¹ of groundnut. The highest pod yield (3.05 t ha⁻¹), pod harvest index (51.9%), marginal rate of return (565%) of ground nut were obtained from combined application of 46 kg N, 46 kg 2.5 t VC ha⁻¹. It could thus, be concluded that combined application of 46:46 kg N: P₂O₅ ha⁻¹ with 2.5 t VC ha⁻¹ is optimum and economical to attain better productivity of groundnut in the study area and similar agro-ecologies.

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Theoretical research of force interaction of a flexible cleaning blade with a beet root head

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Abstract. The most common technology of removing the sugar beet haulm in the world is a continuous cut of the entire mass of the green haulm with further additional removal of the upper parts of the sugar beet heads, which is carried out without extracting the roots from the ground. This is the scheme according to which most top harvesting machines, manufactured in the world, now operate. However, we have found in our studies that, due to additional cutting off the upper parts of the root crop heads, up to 10% of the sugar-bearing mass is lost. Besides, there is an urgent need for immediate processing of the sugar beet root crops, as losses of the sugar juice occur, and bacteria enter inside of the root crop through the cut-off part, causing rotting. Therefore, a more favourable operation for harvesting root crop tops is not cutting off their heads but cleaning them from the residues of the foliage. In addition, the operation of cleaning the sugar beet roots from the residues of the foliage is subject to rather high requirements due to the absence of the green and dry residues on the heads of the roots, as well as the losses and damage of the root crops themselves. The purpose of this investigation is to develop a theory of the force interaction of the flexible cleaning blade with the sugar beet head in the process of its cleaning when the blade is mounted on the vertical driving shaft. The methods used of the investigation are those of modelling, higher mathematics and theoretical mechanics, as well as programming and numerical calculations on the computer. As a result of the research, an equivalent scheme was developed and a mathematical model was constructed describing the force interaction of the flexible cleaning blade with the surface of the sugar beet root.

Keywords: sugar beet, haulm, cleaning blade, impact.

INTRODUCTION

Sugar beet is the most important source of sugar. In Europe its plantations occupy more than 6 million hectares, which accounts for 70% of the world's plantations of this crop. The largest areas of this crop are located in Russia, France, Germany, Ukraine and Poland. In addition to the sugar-containing roots, the yield of sugar beet includes haulm which is a good animal fodder or a raw material for generation alternative energy from

the bio-raw materials (Eichhorn, H. 1999; Pogorely & Tatjanko, 2004; Bentini et al., 2005).

Sugar beet harvesting is a complex and expensive process which largely determines the profitability of the sugar beet production (Helemendik, 1996; Gruber, 2005). Separation of the haulm from the root is an important process with regard to obtaining a high-quality material for its processing into sugar, reducing losses and production of high-quality fodder.

Most machines for harvesting sugar beet work according to a scheme when at first complete cutting of the entire mass of the green haulm is carried out without extraction of the roots from the ground, with additional separation of the upper parts of the root crop heads from the residues. However, due to additional separation of the upper parts of the root crop heads, up to 10% of the sugar-bearing mass is lost (Bulgakov et al., 2016). In addition, there arises an urgent need for immediate processing of sugar beet roots because losses of sugar juice occur, and bacteria enter the inside of the root crop through the cut-off part, which worsens the preservation of the material. In view of this, it is more efficient not to cut the root crop heads but to clean them from the residues of the haulm. In this case the operation of cleaning the sugar beet roots from the haulm residues is subject to sufficiently strict requirements which provide that there should not be any green and dry residues on the root crop heads, as well as losses and damage to the root crops themselves.

To implement the process of stripping the haulm residues from the sugar beet heads from which the main massive of the haulm has been previously removed and collected without extraction of the roots from the ground, we developed a series of new root head cleaners (Vasilenko, 1996; Pagorely & Tatjanko, 2004; Bulgakov et al., 2017). A distinctive feature of these cleaners of the root crop heads from the haulm residues is a vertical drive shaft, on the console end of which there are cleaning elements placed in the form of several flexible blades, directed downwards towards the heads of the root crops. This cleaner moves along the rows of the sugar beet plantations, as a result of which the console ends of the flexible cleaning blades, performing a rotational and simultaneously a forward movement, strike the heads of the root crops, stripping (knocking off) the haulm residues from them and without inflicting damage to the spherical surfaces of the root crops heads. Consequently, this cleaner is intended for cleaning only one row of root crops. In the case of a multi-row embodiment an identical cleaner should be installed on each beet root row.

Although there are many scientific studies devoted to the separation of the sugar beet haulm (Helemendik, 1996; Zhang et al., 2013; Bulgakov et al., 2016; Bulgakov et al., 2018; Bulgakov et al., 2019), yet the interaction force of the flexible cleaning blade with the surface of the sugar beet head has been little disclosed.

The purpose of the work is theoretical research of the interaction of the flexible cleaning blade with the sugar beet head and determination of optimal geometrical parameters of the blades.

MATERIALS AND METHODS

Fig. 1 presents a design and technological scheme of one of the layout variants of a cleaner of this type with a vertical drive shaft carrying flexible cleaning blades, and in Fig. 2 an experimental installation in operation. The cleaner consists of a frame 1 on

which a vertical drive shaft 2 is mounted on the lower end of which a disk 3 of a pre-set diameter is fixed. On the outer generatrix of the disk 3, with a pre-set step along the circumference, there are fixed spherical joints 4 of the disk, in which double-arm levers 5 are installed, at the lower short ends of which, on axes 6, there are installed flexible cleaning blades 7 with cantilever ends pointing down. The upper ends of the double-arm levers 5 also have spherical joints 8 of the levers, which, through the pairs of screws 9, by means of kinematic mechanisms 10 are connected with the drive shaft 2. Using the pairs of screws 9, it is possible to prechange and fix the length of the kinematic mechanisms 10, which provides a possibility to pre-install flexible cleaning blades 7 with different angles of their arrangement in a vertical plane. The flexible cleaning blades 7, in their turn, can rotate around the axles 6 and deviate during operation in a radial direction with respect to the drive shaft 2.

The technological process of cleaning the root crop heads from the haulm residues without extraction from the ground by a cleaner of this type proceeds in this way. The vertical drive shaft 2, extending in cantilever on the frame 1, moves in a forward direction along a row of the sugar beet roots from which the main mass of the haulm has already been removed. Due to the rotation of the shaft 2 at a pre-set angular speed ω , the flexible cleaning blades 7, under the action of the centrifugal forces, deviate from the vertical position at a certain angle, as a result of which they create the so-called ‘cleaning cone’ (the top of which is on the axis of the drive shaft 2, the base is directed downwards, and the generatrix surface is composed of the end surfaces of the cleaning blades 7), which ensures the necessary width of the cleaning zone. The cleaning working tool, i.e. the vertical drive shaft 2, together with the cleaning Blades 7, installed at a predetermined height above the soil surface (with a

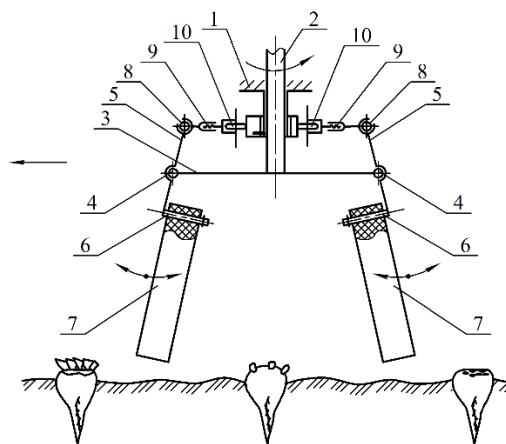


Figure 1. A design and technological scheme of the new cleaner of the root crop heads without extraction from the ground, with a vertical drive shaft (Bulgakov et al., 2018): 1 – the frame; 2 – the vertical drive shaft; 3 – the disk; 4 – the spherical joints of the disk; 5 – the double-arm levers; 6 – the axles; 7 – the flexible cleaning blades; 8 – the spherical joints of the levers; 9 – the pairs of screws; 10 – the kinematic mechanisms.



Figure 2. An experimental installation – a cleaner of the root crop heads from the haulm residues.

significant amount of residues on the heads of the root crops and with other plant residues in the row; this height should be as small as possible), moves in a forward direction along the row of the root crops and, due to the rotation of the drive shaft 2, its flexible cleaning blades 7 perform strikes at the front part of the root crop heads. Bending further, they move with their plane already along the upper surfaces of the root crop heads and cover also the rear part of the head; thus stripping the existing residues from the entire surface of the root crop heads.

The research was conducted applying the methods of theoretical mechanics, higher mathematics, the elasticity theory, as well as the methods of compiling and solving differential equations of the movement on the PC. The main design parameters of the new working tool for cleaning the sugar beet heads from the haulm residues are the width and the length of the blade, under the action of which the stalks of the haulm, remaining on the spherical surface of the sugar beet heads, are separated after the main massive of the haulm has been cut off without extraction of the roots from the ground.

Let us construct an equivalent scheme of the contact interaction of the flexible cleaning blade with the sugar beet head (Fig. 3). First of all, let us present the body of the root crop head in the form of a truncated cone, the shape that the sugar beet heads assume in most cases after complete cutting of the main massive of the haulm without extraction of the roots from the ground, their collection and transportation from the field. Let us suppose that the sugar beet root lies motionless in the soil (actually fixed in it) after cutting off the upper part of the haulm; therefore its head also remains motionless.

Let us connect the surface of the head of the root crop with a fixed (absolute) Cartesian coordinate system $x_1O_1y_1$. We place the origin of this coordinate system (point O_1) so that the horizontal axis O_1x_1 of this coordinate system passed through point O of attachment of the flexible blade to the cleaning tool and is directed towards the forward movement of the cleaner. It should be noted at once that, despite the fact that the cleaning blades are set to pivot on the axes, when driving upon the head of the root crop, moving along the surface of the head, and from it, the flexible cleaning blade moves forward with its front part, which gives a reason to consider it conditionally fixed on the vertical cleaning drive shaft. In this case axis O_1y_1 of the mentioned coordinate system is directed vertically downwards and passes through the leftmost point A of the root crop head (the truncated cone). In the plane, which is formed by the coordinate system $x_1O_1y_1$, this sugar beet head (the truncated cone) is shown as an equilateral trapezium $ABCD$, the lower base of which has a length greater than the length of the upper base, and the angles between the base and the lateral sides are indicated by α_o (Fig. 3).

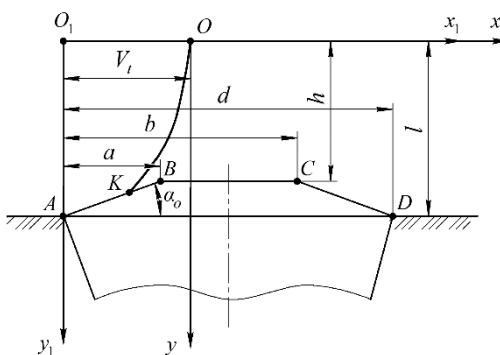


Figure 3. An equivalent scheme of a contact interaction of the flexible cleaning blade with the head of the beet root.

The equations of the broken line $ABCD$ in the coordinate system $x_1O_1y_1$ will be the following equations of straight lines:

$$y_1 = \begin{cases} l - x_1 \tan \alpha_o, & 0 \leq x_1 < a, \\ h, & a \leq x_1 < b, \\ h + (x_1 - b) \tan \alpha_o, & b \leq x_1 \leq d, \end{cases} \quad (1)$$

where α_o is the angle between the lower base of the cone (the lower base AD of the trapezium $ABCD$) and the generatrix AB of the cone; a, b, d, h, l – the parameters characterising the position, size and shape of the surface of the truncated head of the root crop in the coordinate system $x_1O_1y_1$, shown in Fig. 3.

Next, in Fig. 3 we will show a flexible cleaning blade in the form of curve OK , where O is the point of attachment (fixing) of the blade) to the shaft of the cleaning tool. Point K is the end of the cleaning blade, which is also a contact point of the end of the blade with the head of the sugar beet root. In Fig. 3 the contact point K is shown in an arbitrary position on the surface of the root crop head, i.e. it is depicted at an arbitrary moment of time t .

When the shaft of the cleaning tool rotates, the point K of the end of the flexible blade moves in a circle with a certain radius r ; however, the contact point K , because of the forward movement of the cleaner, describes a more complex path on the surface of the root crop head; in the first approximation it describes a certain cycloid. Thus, owing to the simultaneous rotational and forward movements, the cleaning blade, due to the elasticity of the material from which the cleaning blade is made, strips the haulm residues off the head of the root crop.

Let us connect the moving coordinate system xOy with the point of attachment O of the blade. In addition to this, the origin of the coordinates of this system is at the attachment point O of the flexible blade to the vertical shaft of the cleaner, axis Ox is directed along axis O_1x_1 , axis Oy is directed down, parallel to axis O_1y_1 .

The coordinates of point K at the end of the flexible cleaning blade will be denoted by x and y . In the coordinate system $x_1O_1y_1$ coordinates x_1, y_1 of point K are connected with coordinates x, y of this point in the coordinate system xOy as follows:

$$x_1 = V \cdot t + x, \quad (2)$$

$$y_1 = y \quad (3)$$

where $V = \frac{\pi n}{30} r$ is the linear (circumferential) speed of point K of the end of the blade, $m \text{ s}^{-1}$; n is the number of revolutions of the blade, min^{-1} ; r is the radius of the circle along which point K of the end of the blade moves during the rotation of the cleaning tool, m ; t is the time of the forward movement of the cleaner, s .

By substituting the values of x_1 and y_1 , defined by expressions (2) and (3), into expression (1), we obtain:

$$y = \begin{cases} l - (Vt + x) \tan \alpha_o, & 0 \leq (Vt + x) < a, \\ h, & a \leq (Vt + x) < b, \\ h + (Vt + x - b) \tan \alpha_o, & b \leq (Vt + x) \leq d. \end{cases} \quad (4)$$

RESULTS AND DISCUSSION

In order to determine the parameters of the flexible cleaning blade, let us construct an equivalent scheme of forces that act upon a certain selected element in the cleaning blade during its interaction with the head of the beet root (Fig. 4). We will select an element with a length of ds on the uniform cleaning blade at a distance s from the beginning of the blade. During the interaction of the cleaning blade with the sugar beet head from the side of the root crop, a reaction arises from this interaction, which can be decomposed into two components $Q = Q(S)$ and $N = N(S)$, where force $Q(S)$ is directed parallel to axis Ox , and force $N(S)$ is directed parallel to axis Oy . In addition to it, a bending moment $M = M(S)$ arises from the said interaction of the blade with the beet head. These forces, which are shown in Fig. 4, and the bending moment are transmitted to the cleaning blade; and these forces act upon the element with a length of ds ,

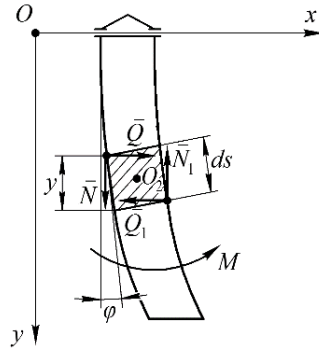


Figure 4. A diagram of forces acting upon an element of the blade during its interaction with the beet root head.

selected in the body of the cleaning blade. From one side force Q acts upon the selected element, from the other side force $Q_1 = Q + \frac{dQ}{ds} \cdot ds$. In a similar way, from one side force N acts upon the selected element, from the other side force $N_1 = N + \frac{dN}{ds} \cdot ds$ (Fig. 4). Since the selected element is in a state of equilibrium, it is possible to write the equilibrium equations of this element into the projections on axes Ox and Oy :

$$\left. \begin{aligned} Q - \left(Q + \frac{dQ}{ds} \cdot ds \right) &= 0, \\ N - \left(N + \frac{dN}{ds} \cdot ds \right) &= 0, \end{aligned} \right\} \quad (5)$$

or

$$\left. \begin{aligned} -\frac{dQ}{ds} \cdot ds &= 0, \\ -\frac{dN}{ds} \cdot ds &= 0. \end{aligned} \right\} \quad (6)$$

Since $ds \neq 0$, then from the system of Eq. (6) we obtain:

$$\left. \begin{aligned} \frac{dQ}{ds} &= 0, \\ \frac{dN}{ds} &= 0. \end{aligned} \right\} \quad (7)$$

From this one can draw a conclusion that forces Q and N are constant values along this blade, starting from $s = 0$ to $s = L$, where L is the length of the blade.

The bending moment in the section of an elastic-viscous rod in accordance with the Focht law (Timoshenko, 1975; Dreizler & Lüdde 2010) is determined by the formula:

$$M = c \left(k + \eta \frac{\partial k}{\partial t} \right), \quad (8)$$

where $c = EI$ – the bending stiffness of the blade section; E – the elasticity modulus of the material of the blade; I – the inertia moment of the cross section; η – the viscosity coefficient of the blade; k – the curvature of the curved axis of the blade.

The curvature value of the curved axis of the blade is determined by the formula:

$$k = \frac{d\varphi}{ds}. \quad (9)$$

In the first approximation we take $\eta = 0$. Then from expressions (8) and (9) we obtain:

$$M = c \frac{d\varphi}{ds}. \quad (10)$$

Further, from the equilibrium condition of the selected element with respect to its rotation around the transverse axis, which passes through the centre of mass (point O_2) of this element, we can write the following equality of moments of the forces indicated in Fig. 4:

$$dM = Q \cdot dy - N \cdot dx, \quad (11)$$

or

$$dM = Q \cos \varphi \cdot ds - N \sin \varphi \cdot ds, \quad (12)$$

where φ – the angle of rotation of the blade.

Then from expression (12) we obtain:

$$\frac{dM}{ds} = Q \cos \varphi - N \sin \varphi \quad (13)$$

Thus, an equation of the rotation of the element of the cleaning blade relative to the transverse axis, which passes through its centre of mass (point O_2), is obtained.

The surface of the root crop, stripped by the end of the blade, is determined by three linear sections AB , BC and CD (Fig. 5). Stripping of the haulm residues by a flexible cleaning blade starts at point A and ends at point D .

The horizontal and the vertical reactions at the end of the blade from the side of the surface of the root crop head in section AB will be equal to:

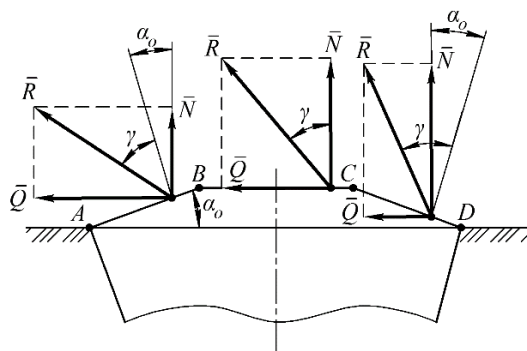


Figure 5. A diagram of the calculated contact areas of the flexible cleaning blade with the surface of the root crop.

$$\begin{aligned} N &= R \cdot \cos(\gamma + \alpha_o), \\ Q &= R \cdot \sin(\gamma + \alpha_o), \end{aligned} \quad (14)$$

in section *BC*:

$$\begin{aligned} N &= R \cdot \cos \gamma, \\ Q &= R \cdot \sin \gamma, \end{aligned} \quad (15)$$

in section *CD*:

$$\begin{aligned} N &= R \cdot \cos(\gamma - \alpha_o), \\ Q &= R \cdot \sin(\gamma - \alpha_o), \end{aligned} \quad (16)$$

where γ – the angle of friction.

In general, it is convenient to write thus:

$$\begin{aligned} N &= R \cdot \cos(\gamma + \alpha), \\ Q &= R \cdot \sin(\gamma + \alpha), \end{aligned} \quad (17)$$

where angle α is written in the following way:

$$\alpha = \begin{cases} \alpha_o, & 0 \leq (Vt + x) < a, \\ 0, & a \leq (Vt + x) < b, \\ -\alpha_o, & b \leq (Vt + x) \leq d. \end{cases} \quad (18)$$

Let us substitute values N and Q from (17) into expression (13); we obtain:

$$\frac{dM}{ds} = R \sin(\gamma + \alpha) \cos \varphi - R \cos(\gamma + \alpha) \sin \varphi = -R \sin(\varphi - \gamma - \alpha). \quad (19)$$

After substituting the value of the moment M from (10) into expression (19), we obtain:

$$c \frac{d^2 \varphi}{ds^2} = -R \sin(\varphi - \gamma - \alpha), \quad (20)$$

or

$$\frac{d^2 \varphi}{ds^2} = -p^2 \sin(\varphi - \gamma - \alpha) \quad (21)$$

where $p^2 = \frac{R}{c}$.

Let us multiply each term of the left and the right side of Eq. (21) by $2 \frac{d\varphi}{ds} ds = 2d\varphi$. We will have:

$$2 \frac{d\varphi}{ds} \frac{d^2 \varphi}{ds^2} ds = -2p^2 \cdot \sin(\varphi - \gamma - \alpha) d\varphi \quad (22)$$

Since:

$$2 \frac{d\varphi}{ds} \frac{d^2 \varphi}{ds^2} = \frac{d}{ds} \left(\frac{d\varphi}{ds} \right)^2, \quad (23)$$

then Eq. (22) will obtain the form:

$$d \left(\frac{d\varphi}{ds} \right)^2 = -2p^2 \cdot \sin(\varphi - \gamma - \alpha) d\varphi \quad (24)$$

We integrate the resulting Eq. (24). We have:

$$\int d\left(\frac{d\varphi}{ds}\right)^2 = 2p^2 \int d[\cos(\varphi - \gamma - \alpha)] \quad (25)$$

After which we will have:

$$\left(\frac{d\varphi}{ds}\right)^2 = 2p^2 \cdot \cos(\varphi - \gamma - \alpha) + C \quad (26)$$

where C – an arbitrary constant.

We find the arbitrary constant C that enters into expression (26). If we take into account the limiting conditions $M(L) = 0$, then, according to expression (10), we will have:

$$\frac{d\varphi}{ds}(L) = 0 \quad (27)$$

where L – the length of the blade.

Then, using the resulting expression (27), we can write:

$$0 = 2p^2 \cdot \cos(\varphi_1 - \gamma - \alpha) + C \quad (28)$$

hence the arbitrary constant C will be equal to:

$$C = -2p^2 \cdot \cos(\varphi_1 - \gamma - \alpha) \quad (29)$$

where φ_1 – the angle of rotation of the end of the blade.

Substituting expression (29) into Eq. (26), we will have:

$$\left(\frac{d\varphi}{ds}\right)^2 = 2p^2 [\cos(\varphi - \gamma - \alpha) - \cos(\varphi_1 - \gamma - \alpha)] \quad (30)$$

After certain transformations of expression (30) we obtain:

$$ds = \frac{d\varphi}{\sqrt{2} p \sqrt{\cos(\varphi - \gamma - \alpha) - \cos(\varphi_1 - \gamma - \alpha)}} \quad (31)$$

By integrating Eq. (31) within the range from 0 to L , and from $\varphi = 0$ to $\varphi = \varphi_1$, we will obtain:

$$\int_0^L ds = \frac{1}{\sqrt{2} \cdot p} \cdot \int_0^{\varphi_1} \frac{d\varphi}{\sqrt{\cos(\varphi - \gamma - \alpha) - \cos(\varphi_1 - \gamma - \alpha)}} \quad (32)$$

where L – the length of the blade.

Thus, integrating Eq. (32) along the elastic line, we find the desired length L of the flexible cleaning blade:

$$L = \frac{1}{\sqrt{2} \cdot p} \cdot \int_0^{\varphi_1} \frac{d\varphi}{\sqrt{\cos(\varphi - \gamma - \alpha) - \cos(\varphi_1 - \gamma - \alpha)}} \quad (33)$$

The resulting expression (33) makes it possible to determine the length of the blade L through an integral that can be calculated using a PC.

However, the length of the blade L , determined by expression (33), is exactly that long flexible cleaning blade which is rational and necessary in order, based on its deformations and the forces applied at the head of the root crop, to ensure a maximum complete contact with the surface of the root crop head without extracting it from the ground, that will ultimately ensure its high quality of cleaning.

Using expression (33), as well as the blade section parameters: width – 0.02 m, length – 0.04 m, the reaction value – $R = 15$ N, we constructed a calculation model for the removal process of the sugar beet haulm in the MatLab system. The value of reaction R is chosen from the destruction condition of the haulm by the cleaning blade on the root crops. The dependencies, shown in Fig. 6, indicate that, with the increase in the length L of the cleaning blade, it is necessary to increase the elasticity modulus E of its material. This is explained by the fact that, as the length of the blade increases, the moment in its elementary sections increases from the components of reaction R ; and, in order to overcome it, greater resistance of the material to deformation is needed. To ensure that

the range of the protruding heights of root crops above the soil surface up to 0.1 m is copied, the required blade length L must be at least 0.2 m. In addition to the length of the blade, it is necessary to substantiate the value of its maximum deflection angle φ_1 . From Fig. 6 it is evident that, with increasing angle φ_1 , the maximum length L of the blade decreases, and for $\varphi_1 = 80^\circ$ – 90° it does not exceed 0.2 m, but for $\varphi_1 = 60^\circ$ – 70° , it ranges from 0.12 m to 0.28 m. Since it is difficult to achieve large values of the elasticity modulus E by selecting properties of the material and by reinforcement, one should strive for smaller values of the required elasticity modulus. Therefore, as a rational value for the deflection angle of the cleaning blade, angle $\varphi_1 = 60^\circ$ was selected, at which the permitted values of the elasticity modulus are $E = 3$ – 11 MPa, and the working length L of the cleaning blade may be selected from a range of 0.2–0.24 m.

The design of the sugar beet root crown cleaner with a vertical drive shaft developed by the authors incorporates the possibility of installing cleaning blades of different lengths L and with different physical and mechanical properties of the materials that can be used for their production.

Fig. 7 presents one cleaning element, which can hold cleaning blades of different lengths L and with different properties, for example, with different elastic moduli E .

The further experimental investigations carried out by the authors have proved that the best quality of the cleaning of sugar beet root crowns from haulm residues according to the criterion of their amount left after the pass of the cleaner is achieved when using a cleaning blade made of bossed rubber (with bosses of hemispherical shape positioned chequer-wise on the blade's cantilever end) and sized as

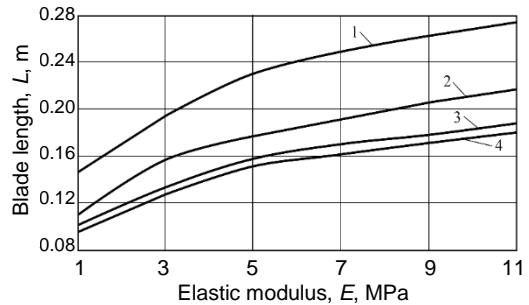


Figure 6. Dependence of length L of the cleaning blade upon its modulus of elasticity E : 1) $\varphi_1 = 60^\circ$; 2) $\varphi_1 = 70^\circ$; 3) $\varphi_1 = 80^\circ$; 4) $\varphi_1 = 90^\circ$.

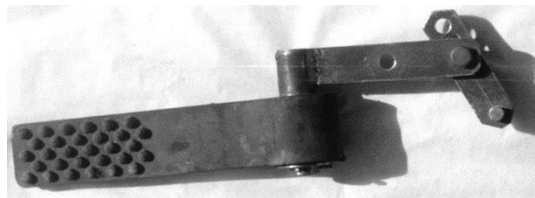


Figure 7. Cleaning element that can hold flexible blades of different lengths and with different elastic moduli (manufactured from different materials: rubber, plastic, cord-reinforced rubber, belts).

follows: the length is $L = 0.20$ m, the breadth is 0.06 m and the thickness is 0.02 m.

The data obtained during the experimental investigations and processed in the MatLab environment are presented in Fig. 8.

As is seen in the presented graphs, the minimum amount of haulm residues is left on the root crowns, when blades with the length L equal to 0.20–0.21 m are used. And the preferable angular velocity of rotation in this case is 734 rad s^{-1} . Further increase of the blade length L is not advisable, since the continuing increase of the quality of cleaning will at greater lengths be outweighed by the excessive wear and tear of the cantilever ends of the cleaning blades as a result of their possible contacts with the soil surface. That results also in the excessive material consumption.

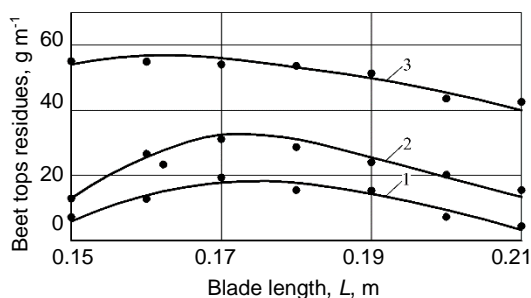


Figure 8. Relation between amount of haulm residues on sugar beet root crowns after their cleaning and cleaning blade length L at different angular velocities of rotation of cleaner's drive shaft: 1– 734 rad s^{-1} ; 2– 540 rad s^{-1} ; 3– 448 rad s^{-1} .

The data obtained as a result of the experimental investigations lend full support to the completed analytical calculations.

Next, we will define analytically what the width of the flexible cleaning blade should be. The main kinematic parameter of the movement of the cleaning blade is the kinematic condition λ , which is determined by means of the following expression (Grote & Antonsson, 2008):

$$\lambda = \frac{V_b}{V_c} = \frac{\omega R}{V_c} \quad (34)$$

where V_b – the linear speed of the end of the cleaning blade, m s^{-1} ; V_c – the forward speed of the cleaner of the root crop heads, m s^{-1} ; ω – the angular speed of rotation of the cleaning blade, rad s^{-1} ; R – the radius of the working tool on which the cleaning blades are installed, m. Let us consider conditions when: $\lambda > 1$, $\lambda = 1$ and $\lambda < 1$.

If $\lambda < 1$, that is, $\omega \cdot R < V_b$, then, as the results of experimental investigations show, the haulm residues will remain after the passage of the cleaner, which does not satisfy the agro technical requirements; therefore this case is excluded. Besides, the speed of the forward movement of the cleaner is so great that there remain areas on the heads of the root crops which the cleaning blades do not touch. Therefore, to achieve high-quality cleaning of the heads of root crops from the haulm residues, it is necessary to observe a condition when $\lambda \geq 1$. Let us consider an extreme case when there is no overlap of the zone of action of the cleaning blades, i.e. $\lambda = 1$, yet there are no above-mentioned areas on the root crop heads that are not captured by the blades. Multiplying the numerator and the denominator of expression (34) by the time t of the interaction of the flexible cleaning blade with the head of the beet root crop, we will obtain:

$$\lambda = \frac{t \cdot \omega \cdot R}{t \cdot V_c} = \frac{\Theta \cdot R}{t \cdot V_c} \quad (35)$$

In expression (35), the product ($t\omega$) shows at what angle the cleaning blade will rotate relative to the vertical axis of rotation during the interaction of the blade with the head of the beet root crop. Let us denote the said angle as Θ . Consequently, $\Theta = \omega \cdot t$.

On the other hand, angle Θ will be part of a full revolution (the working period) of the cleaning blade. This angle, as such, will mean through what angle of rotation of the working tool the next cleaning blade comes into action. In this case, when the design of the cleaner has 8 blades, we find that: $\Theta = \frac{360^\circ}{8} = 45^\circ$.

Then the time from the start of the interaction of this cleaning blade with the surface of the head of the beets till the beginning of the interaction of the next blade (on condition that there is no overlapping of the area of action of the cleaning blades, i.e. $\lambda = 1$) will be:

$$\Delta t = \frac{\Theta}{\omega} \quad (36)$$

On the other hand, it is necessary to observe a condition when the forward movement of the root head cleaner during the time Δt should not exceed the width of the cleaning blade b , i.e. it is necessary that:

$$b = V_c \cdot \Delta t \quad (37)$$

or, taking into account expression (36), we will have:

$$b = V_c \frac{\Theta}{\omega} \quad (38)$$

From the expression (38) it follows that:

$$\frac{\omega}{V_c} = \frac{\Theta}{b} \quad (39)$$

Having substituted correlation (39) into expression (34), we will have:

$$\lambda = \frac{R\Theta}{b} \quad (40)$$

Because of the requirement to satisfy the fulfilment of inequality $\lambda \geq 1$, from expression (40) we will have:

$$\frac{R\Theta}{b} \geq 1 \quad (41)$$

As it is evident from expression (41), the maximum width b_{\max} of the cleaning blade should not exceed the value:

$$b_{\max} \leq R \cdot \Theta \quad (42)$$

On the other hand, the minimum width b_{\min} of the cleaning blade should exceed the maximum size of the existing haulm residues on the heads of the beet roots. Considering (Bulgakov et al., 2018), we have the following inequality:

$$R \cdot \Theta > b > 0.01 \text{ m.} \quad (43)$$

The width of the working part of the cleaning blade should be within the range from 10.7 cm to 1.0 cm. For practical purposes, we use a 5 cm width of the cleaning blade.

CONCLUSIONS

1. There is obtained a mathematical model of the rotation of any element of the flexible cleaning blade around the transverse axis, which passes through its centre of mass.

2. There is obtained an expression for determination of the rational length of the blade depending on the rigidity of the blade, the angle φ_1 of rotation of the end of the blade, the angle of friction γ , and angle α that characterises the shape of the head of the beet root. A rational length of the cleaning blade will ensure the greatest coverage of the surface of the beet root head which guarantees the quality of its cleaning from the haulm residues during the force interaction. Based on the analysis of the kinematic condition, a rational width of the blade has been determined.

3. Based on the analysis of the kinematic mode λ , when $\lambda \geq 1$, a rational width of the blade was obtained, which should be within a range from 0.107 m to 0.01 m.

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Use of thermography for the evaluation of the surface temperature of Japanese Quail submitted at different temperatures

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Abstract. Thermography has been gaining more space in analyzes of the superficial thermal profile of birds since it is a non-invasive way of evaluating thermal comfort. This study aims to evaluate the influence of different air temperatures (t_{air}) from 20 °C to 32 °C on the maximum, average and minimum surface temperature (ST_{max} , ST_{average} and ST_{min}) of Japanese laying quails. The experiment was performed in four wind tunnels, where the continuous air temperature within each tunnel, 20 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C and 32 °C represented treatment, with 20 °C being the control treatment. Two experiments, of 21 days each, were carried out. For each experiment, we used four replicates and eight quails in each repetition, in a completely randomized design. Thermographic images of each repetition were made weekly through the Fluke Ti55 camera and analyzed using SmartView[®] software. The ST_{max} , ST_{average} and ST_{min} of each repetition were obtained by delimiting the area of the quails within the cages. Significant differences were observed between ST as the room temperature increased. The ST of quails behaved similarly from 28 °C on. Both head and feet had higher temperatures. It was possible to verify that air temperatures above 22 °C promoted an increase in the maximum, average and minimum surface temperatures. The highest surface temperatures are found in the head and foot region.

Key words: thermal comfort, quail farming, thermal image.

INTRODUCTION

Currently, quail farming in Brazil has shown rapid growth due to economic requirements in poultry. Poultry farming has been promoting competitiveness in the international food market and stimulating the emergence of small and large scale animal

production systems (IBGE, 2017), offering to the country a high profitable index, due to the growth in consumption of poultry derived food.

In recent decades farmers have sought different ways to improve and increase production. Understanding how quails react to the environment becomes an essential point to achieve efficiency and productivity in the poultry chain. An increase in the number of research regarding the control of the thermal environment was observed. This control has become extremely necessary so that the animals can express their full potential productive (Cassuce et al., 2013; Cândido, 2016). Knowledge of the environment is essential for the maintenance of bird homeothermy and a drastic change in the environment can cause significant losses in the production of these animals since it has a direct effect on their physiology. As a consequence of the elevation of the room temperature, the birds can increase the respiratory rate (Castilho et al., 2015), increase water consumption and reduce feed consumption and consequently affect their performance (Santana et al., 2018).

According to Oliveira (2014), poultry performance, nutrient consumption, weight gain and mortality are influenced by thermal comfort in the first three weeks of life. Therefore, the environmental temperature control is crucial so that hyperthermia, low productivity and mortality of these animals will not occur (Mashaly et al., 2004).

Being aware that environmental control is not only necessary but a condition for high productivity indexes, the thermographic analysis allows the monitoring of animal superficial temperature and the evaluation of variation (Ferreira, 2016; Silva et al., 2017). This variation in surface temperature can give us information about the comfort conditions of the animals.

One of the main impediments to quantifying sensible heat loss was the inability to accurately measure animal surface temperature distribution and to differentiate heat loss of different surface regions (Yahav et al., 2004).

Nowadays, thermographic analysis is one of the most accurate techniques of non-contact temperature measurement, allowing an analysis based on non-destructive tests using cameras and infrared sensors to measure temperature and heat distribution. It is a non-invasive technique to visualize the thermal profile of the animal (or object), enabling the information as infrared radiation (Carvalho et al., 2011; Nascimento et al., 2011).

Due to its positive characteristics, thermographic analysis to evaluate the surface temperature of cattle (Roberto & Souza, 2014), pigs (Pulido-Rodriguez et al., 2017) and poultry has been used (Abreu et al., 2017).

The evaluation of the animal surface temperature can be used as an index to accurately estimate the physiological state of an animal in conditions of stress, fertility, welfare, metabolism, health and disease detection. The surface temperatures are processed by computer and displayed as a thermal map over the animal, which provides a detailed analysis of the temperature profile (McManus et al., 2016).

The thermographic analysis is difficult to use in feathered animals because feathers are thermal insulators that block most of the heat emissions (Ferreira et al., 2011). However, the thermography has been applied in the study of thermal comfort of the birds with success.

The use of thermographic imaging technology allows a direct knowledge of the distribution of the surface temperature of the birds in the environment where they are created (Camerini et al., 2016), also allowing the estimation and analysis of heat dissipation (Nascimento et al., 2014).

The influence of environmental factors needs to be understood because even a small effect on infrared temperature may introduce sufficient error to alter research results when used as an alternative assessment tool or it may result in the false interpretation of an animal's state of health, and may lead diagnostically to either false positive or false negative errors (Church et al., 2014).

The purpose of this study aims to evaluate the influence of different air temperatures on the surface temperature of Japanese laying quails by thermographic analysis.

MATERIALS AND METHODS

This research was approved by the Committee on Ethics in the Use of Animals (Protocol 005-2012). Two experiments, of 21 days each, were conducted in four wind tunnels installed in an experimental laboratory applied to small animals. The laboratory was equipped with two air conditioning systems for the maintenance of the variables, air temperature (t_{air}), and relative humidity (RH), below the desired values (setpoints). They were recorded every minute.

The wind tunnels (0.8 x 5.0 m), built in steel sheets and (Polyvinyl chloride) PVC pipes, had partial air recirculation. Each tunnel had two electric heaters and two humidifiers, distributed in two stages of operation, for the most accurate control of the desired air temperature (t_{air}) and RH. Air velocity was manually controlled through potentiometers connected to exhaust fans of 0.40 m in diameter. The thermal acquisition and control system was composed of a data logger (CR1000, Campbell Scientific®), a channel multiplexer (AM16/32B, Campbell Scientific®), a relay controller (SDM-CD16AC, Campbell Scientific®) and air temperature and RH sensors (HMP45C, Vaisala®).

Twenty-eight Japanese quail (*Coturnix coturnix japonica*) were in each experiment. The birds used had the same age, 11 weeks (the beginning of peak production). The birds were selected according to body mass and egg production, to obtain a homogeneous batch and reduce possible individual effects. After the selection, the birds were housed inside the wind tunnels, where they went through an acclimatization period of ten days in t_{air} of 20 °C.

Each tunnel contained two cages (0.50 m long, 0.38 m wide and 0.21 m high, each) with a capacity of 16 birds each, and eight birds housed per compartment, obtaining 118.75 cm² bird⁻¹. Four incandescent bulbs (20 W) were installed inside each tunnel providing illuminance of 20 lux. A light program of 16 hours per day was developed (Molino et al., 2015).

Throughout the experimental period, the birds were submitted to the same feeding intake. Feeding was given *ad libitum* and feeding was carried out four times a day (7 a.m., 11 a.m., 3 p.m. and 7 p.m.). The birds were fed with a balanced diet according to (Rostagno et al., 2011).

The water was kept in a tank external to the tunnels and supplied *ad libitum* throughout the experimental period. Cage cleaning was performed daily at 7 o'clock. The experimental treatments were as follows: 20 °C (control treatment), 22 °C, 24 °C, 26 °C, 28 °C, 30 °C and 32 °C. The control treatment was repeated in each experiment. The assessed values of t_{air} were obtained from the comfort values established by Oliveira

(2004). The RH for all treatments was maintained close to 60%, according to the recommendations of Nääs (1989) and air velocity at $0.3 \pm 0.1 \text{ m s}^{-1}$. In the first experiment of 21 days, the quails were submitted to continuous t_{air} at 20 °C, 22 °C, 24 °C and 26 °C and in the second experiment at 20 °C, 28 °C, 30 °C and 32 °C. Thermographic images of each repetition were shot weekly at 9 a.m. through a Fluke Ti55camera and analyzed using SmartView® software (Fig. 1).

The quail areas within each repetition were delimited, excluding the regions belonging to the cage to identify the maximum, average and minimum surface temperatures (ST_{max} , ST_{average} and ST_{min}) later.

The design was completely randomized, with the experiments and evaluated separately with four treatments and four replications each (eight Japanese quails per replication). The variables were submitted to analysis of variance in the statistical program SISVAR 5.3 and the averages were compared through Scott Knott test at a significance of 5%.

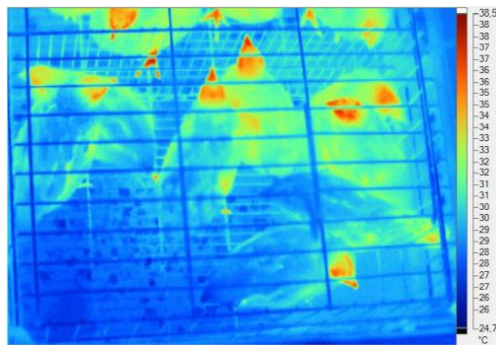


Figure 1. Thermographic image of Japanese quails inside the wind tunnel.

RESULTS AND DISCUSSION

The observed t_{air} levels were close to those desired, with a low standard deviation in both experiments (Table 1). The control treatments registered the highest standard deviations for the observed t_{air} . However, these temperatures remained between 18 °C and 22 °C, considered to be thermal comfort for Japanese quails, according to Oliveira (2004).

Table 1. Maximum, average, and minimum surface temperatures (respectively) of Japanese laying quails submitted to different air temperatures

Thermal variables	Treatments			
Experiment 1				
$t_{\text{airdesired}}$ (°C)	20	22	24	26
$t_{\text{airobserved}}$ (°C)	20.8 ± 0.5	22.2 ± 0.2	24.2 ± 0.2	26.1 ± 0.2
RH_{obs} (%)	60 ± 2	60 ± 2	60 ± 0	60 ± 1
Experiment 2				
$t_{\text{airdesired}}$ (°C)	20	28	30	32
$t_{\text{airobserved}}$ (°C)	21.3 ± 0.6	28.0 ± 0.3	29.9 ± 0.2	31.9 ± 0.3
RH_{obs} (%)	$60,7 \pm 3$	59 ± 2	60 ± 3	60 ± 1

* – $t_{\text{airobserved}}$ e $t_{\text{airdesired}}$: desired and observed air temperatures inside the heated wind tunnels; RH_{obs} : air humidity observed inside the wind tunnels, where the desired value was 60%.

Average values were recorded for the RH close to the desired ones, however in Experiment 2, higher standard deviations were observed in relation to the first one (Table 1) and they were also higher when compared to the deviations of the temperatures.

It was observed that greater deviations of the RH, compared to the deviations of the t_{air} (°C), are explained by the nature of the variable, by the precision of sensors and by the behavior of birds under conditions of heat stress. The dissipation system of heat has been characterized by hyperventilation, by evaporation of water from the lungs of birds under high air temperature (°C) (Carvalho & Fernandes, 2013) and by the evaporation of feces moisture.

Animal behavior is an important means of adapting to the physical and social environment. Based on genetically predisposed patterns, this complex instrument allows rapid reactions towards environmental and internal stimuli with high response plasticity. Different from the hair coat in mammals, birds can use their feather cover in a more flexible way. Due to the connecting net of fine muscles, rather controlled movements of groups of feathers can be exerted. Behavioural patterns play an important role as they allow modifying morphologically preformed thermal windows (Gerken et al., 2006). This type of behavior reduces thermal insulation and increases the skin's contact surface with air, helping to dissipate heat.

For Experiment 1, significant difference was observed among surface temperatures and feed intake as the ambient temperature increased. For Experiment 2, it was observed that the surface temperatures of the quails behaved similarly from 28 °C on (Table 2) and only the control treatment differed from the others by the Scott Knott test ($p < 0.05$).

The surface temperature (ST) is directly related to the t_{air} Sá Filho et al. (2011), because for the dissipation of the body heat to the environment it is necessary that there are temperature differences between them. The higher is the t_{air} , the lower is the heat dissipation in the sensitive form. From 28 °C, the thermal changes in the sensitive form decrease, so these birds have to change heat in the latent form.

Between the t_{air} of 20 °C and 22 °C, it was observed that the ST_{max} , $ST_{average}$ and ST_{min} showed no significant difference between them (Experiment 1, Table 2).

For ST_{max} , it was observed that air temperature 24 °C and 26 °C were the same in the first experiment by the Scott Knott test ($p < 0.05$). The $ST_{average}$ and ST_{min} recorded for the same experiment had differences between air temperature 22 °C, 24 °C and 26 °C.

Table 2. Maximum, average, and minimum surface temperatures (respectively)

Surface temperature			
Experiment 1			
t_{air} (°C)	ST_{max} (°C)	$ST_{average}$ (°C)	ST_{min} (°C)
20	31.9 a	27.1 a	24.2 a
22	32.2 a	28.1 a	25.0 a
24	33.1 b	29.2 b	26.1 b
26	34.0 b	30.2 c	27.7 c
CV	2.58	1.96	2.19
Experiment 2			
20	30.3 a	27.7 a	25.4 a
28	33.0 b	30.6 b	28.6 b
30	33.6 b	30.5 b	28.4 b
32	33.5 b	31.0 b	29.0 b
CV	2.23	2.01	2.14

* – Averages followed by different letters in the column differ from each other by the Scott Knott test ($p < 0.05$).

From the thermographic images, it was possible to observe that the head and feet had higher temperatures. In addition to these regions/parts, in areas with no feathers due to pecking, high surface temperatures were also observed.

The high ST found in the head and foot, according to Shinder (2007) is a genetic characteristic of birds, which have conservative regions/parts in the body, such as feathered and non-conservative regions/parts of heat such as paws, ridge, and dewlap. Burns et al. (2013) found in this species the legs take on a more prominent role in thermal balance than in other species.

Khalil et al. (2012) used infrared thermography to evaluate the adaptive reactions to short-term thermal stress (30 minutes at 35 °C) in different ages, taking as standard the leg temperature of Japanese quails. The authors concluded that increasing environmental temperature during early age will result in thermal conditioning, which can lead to increasing heat tolerance in heat stressed groups.

According to Souza Jr. et al. (2013) in the body regions/parts lacking in feathers, the thermal flow is controlled and modified altering the blood flow. These regions/parts are widely referred to as thermal windows. This term is often applied to any body surface partially or totally involved in thermal changes. They include appendages and areas with few hairs (mammals) or feathers (birds). Therefore, the measured temperatures of several regions/parts, such as the face, wattle, comb, legs, beak and unfeathered areas below the wings radiate directly, will be those on the surface (Yahav & Giloh, 2012).

This type of analysis may also indicate aggressive behavior in adult laying hens housed in cages, since feather pecking is a stress behavior and results in exposure of the skin surface, especially on the backs of birds. According to Sena et al. (2019), when the temperature increases, there is a greater flow of heat towards vasodilated extremities of birds like comb, wattle and feet, in order to exchange heat with the environment and maintain homeothermy.

According to Menuam & Richards (1975), the elevation of air temperature causes higher temperatures in the cloacal, of the epidermis, paw and temperature of the exhaled air by birds, being these artifacts to maintain homeothermy.

Pichová et al. (2017) demonstrated that infrared temperature (IRT) is an objective and feasible method for the feather cover assessment of laying hens kept in different housing systems. However, the commercial use of IRT requires further standardization/development of the methodology to obtain consistent data.

However, hens with less feather coverage could benefit from the additional heat dissipation during hot weather. The differences in sensible heat loss caused by different feather coverage remain validated by more accurate techniques such as calorimetry (Zhao et al., 2013).

CONCLUSIONS

Through thermography, it was possible to determine that air temperatures above 22 °C promote an increase in the maximum, average and minimum surface temperatures of Japanese quails and the highest surface temperatures are found in the head and feet. These areas can be used as standard for the evaluation of the surface temperature of Japanese quails. In addition, this tool can also indicate stress behaviors by identifying other non-feathered areas, which is a feature of feather pecking.

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Physicochemical properties and agglomeration parameters of biogas digestate with addition of calcium carbonate

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Abstract. The aim of the work was to determine the physical properties of digestate from biogas production - either with or without the addition of calcium carbonate and to determine the parameters of its compaction. The material for research was obtained from an agricultural biogas plant specialized in processing cattle manure, vegetable pomace, chicken manure and maize silage. The parameters of compaction of digestate were experimentally determined and its net calorific value was calculated based on the gross calorific value. Physical properties were determined according to standards. The moisture content of liquid digestate was 96%. Mechanical separation allowed to decrease the water content by 19% and addition of 20% of calcium carbonate by 30%. It was found that digestate with addition of calcium carbonate is not suitable to use for energy purposes, because of its low net calorific value (5.2–5.9 MJ kg⁻¹), however it can be used for fertilizer purposes in relation to its chemical composition. Without additives, the net calorific value was 14.9 MJ kg⁻¹, but due to the high moisture content of the raw material it is unprofitable to dry it and burn. On the other hand, it was proved that it is possible to obtain pellets of appropriate density out of the digestate using 40 mm of the die height and 0.3 g of single portion of the material.

Key words: digestate, binder, pellets, fertilizer, energy.

INTRODUCTION

The problem with the ever-increasing amount of wastes and pollution is one of the most serious problems in the world. After each production, side products are created, e.g. post-fermentation sludge in an agricultural biogas plant. In the Polish law, digestate is a waste (Czekała et al., 2012). Digestate arises from the decomposition of organic matter under anaerobic conditions caused by bacteria (Oyamoto & Kung, 1980; Pontus, 2014; Lalak et al., 2015). These are residues of the fermentation process, i.e. unfermented organic compounds, minerals and biomass of methane bacteria. The composition of post-fermentation sludge depends on the composition of the substrate to be decomposed (Alburquerque et al., 2012).

In the initial stage of fermentation, conversion to alcohols or lower organic acids takes place, followed by simple compounds such as methane and carbon dioxide. Transformation of substrates takes place in the biogas plant' digesters. This process consists of four stages, each of them takes place using a bacteria designated for it. All

stages of fermentation take place in the same fermentation chamber, and include hydrolysis, acidic phase, acetogenesis and metagenesis (Gerardi, 2003; Ziemiński & Frąc, 2012).

The unfermented fractions of substrates, most often biomass, from fermentation chambers were considered to be a waste for a very long time. Along with the development of this sector, also digestates began to be used as a full-value fertilizer used in agriculture. However, due to the inhomogeneity of the material, high moisture content and odour nuisance, the sludge was not suitable for transport, which is why it was used only within 5 km from the biogas plant. Moreover, marketing barriers include transport costs and lingering negative perceptions (Dahlin et al., 2015). Yields after using digestates are comparable to those fertilized with mineral fertilizers, and better than those fertilized with liquid manure. Due to the mineral content in the forms directly available for plants, it is characterized by fast fertilizing action (Kowalczyk-Juško & Szymańska, 2015). This was proved by investigating the effect of digestate on watermelon and cauliflower (Pontus, 2014). The size of watermelons was the same for all fertilizers, but the overall harvest was better for mineral fertilizers and digestate. After the first addition of digestate to the soil, it was noted that the concentration of nitrates and phosphorus in the soil increased rapidly. At the next doses there were no significant differences. The total organic carbon content in the soil did not increase because the supplied organic matter was degraded very quickly.

Due to the high moisture content, digestate treatment also belongs to problematic processes. This material requires dehydration and drying up to 15–20% of moisture content, because above this value processing is very difficult (Kratzeisen et al., 2010). However, there are several ways to reduce the moisture content. One of them is the drying up of the substrate under natural conditions to the moisture allowing further use (Lehtomäki, 2006). It is also possible to add a product that could absorb some of water from the material. Such a compound may be a calcium carbonate (CaCO_3) which has hygroscopic properties.

Calcium carbonate is used as a hardener and white dye, increases the yield of crops and restores the pH of the soil, which is why it is often used as a fertilizer (Ciesielska et al., 2011). However, in the production of pellets from biomass it significantly reduces moisture, but also causes decrease in calorific value (Lisowski et al., 2013; Dąbrowska et al., 2016). Chemical composition and physical properties of digestate fuel pellets depend on the blend of substrates used as feedstock for biogas production (Kratzeisen et al., 2010). Pellets made of digestate with the addition of calcium carbonate should be easier to process, transport and handling than digestate itself, due to their higher density and lower moisture content (Pedrazzi et al., 2015).

There are new technologies that have been introduced during the last years to treat biogas digestate for optimal transport and application conditions (Rehl & Muller, 2011), however there are not many studies on compaction parameters of digestate, which is an obstacle in using the material so full of potential. Therefore, tests were carried out to find out the properties of this raw material and to determine the parameters of its compaction, so that it would be possible to use the digestate as a fuel or valuable fertilizer in their best way. Thus, the aim of the work was to determine the physical properties of digestate from biogas production - either with or without the addition of calcium carbonate and to determine the parameters of its compaction. These information are crucial for large-scale technology in order to increase the efficiency of pellets production.

MATERIALS AND METHODS

Material

The material for research (Fig. 1) was obtained from an agricultural biogas plant, located in Poland, specialized in processing maize silage (40%), cattle manure (30%), vegetable pomace (10%), chicken manure (10%) and pig manure (10%). Liquid fraction was separated and a solid matters with and without addition of binder were investigated in WULS Analytical Centre according to standards to determine the main chemical compounds in the raw materials (Table 1).



Figure 1. Digestate as a raw material without binder (left) and with addition of calcium carbonate (right).

Moisture content

The moisture content of digestate was determined using drying-weighing method according to the PN-EN ISO 18134-3:2015-11 standard. Five samples of 30 g each were weighed on the scales RADWAG type WPS 600/C with an accuracy of 0.01 g. After that, samples were placed in the laboratory drier POL-EKO-APARATURA SP.J. type SLW 115 TOP+, at 105 °C for 24 hours. Dried samples were weighed once again and the moisture content was calculated using formula:

$$w = 100 \frac{m_w - m_s}{m_w} \quad (1)$$

where w – moisture content of the material, %; m_w – mass of the wet material, g; m_s – mass of the dried material, g.

Calorific value

Net calorific value was calculated based on the gross calorific value, which was determined using the calorimeter KI-10, Precyzja-BIT, Bydgoszcz, Poland according to standard PN-ISO 1928. For each material 5 repetitions were done.

$$Q_w = Q - 2454 \cdot (w + 9H) \quad (2)$$

where Q_w – net calorific value, MJ kg⁻¹; Q – gross calorific value, MJ kg⁻¹; w – moisture content, %; H – hydrogen content, %.

Agglomeration process

The parameters of digestate compaction were experimentally determined using a special designed device mounted on the universal testing machine TIRATEST (Fig. 2).

The stand was equipped with the piston and the die with heating bands on the external surface of the die. The process temperature was set using the ESM 3710i temperature controller. The die was an open cylindrical working chamber with an opening with 8 mm diameter. The changed parameters during the subsequent tests were: the dose of material and the thickness of the die, which was the height of the material layer after compaction. During test, the signals registered by the sensors of the testing

machine were processed by the Matest program, which enabled monitoring of forces acting on the piston. Tests were carried out at various doses from 0.2 to 0.4 g, and the height of the forming die was varied from 30 to 50 mm. Each dose of material was weighed on the scales RADWAG WPA 40/160/C/1 with an accuracy of 0.0001 g.

Depending on the analyzed case, the tested material were: pure digestate or digestate with addition of 10 or 20% of calcium carbonate. Initially, the outlet opening was closed with a pellet, and then the die was heated to a set temperature of 140 °C for 1 hour. When the temperature was stabilized at the entire height of the die, the study could be started. Measured samples of digestate without and with the addition of binder were poured into the working chamber using a plastic container. The next step was to insert the piston into the die opening and compact the material using the Matest strength testing program. The end of the test was determined by the limit sensor at the set height and then the die returned to its initial position. One test, with previously determined parameters, involved carrying out 40 to 60 measurements. The only exception was when the selected compaction parameters did not allow the formation of durable pellets because the material did not compact well. Then the number of repetitions decreased. After measurements, the limit sensor was moved so that the piston completely pushed out the pellets. The obtained agglomerates were then arranged in order of their obtaining, to know which were obtained in the initial phase of the test and which were in stable conditions. The work of the pressure agglomeration was calculated using the formula:



Figure 2. Testing stand for agglomeration process.

$$L_c = \int F_{max} dl \quad (3)$$

where L_c – compaction work, J; F_{max} – maximum force, N; l – piston displacement, m.

Density

To determine the density of obtained pellets an electronic caliper with an accuracy of 0.01 mm and scales RADWAG type WPA 40/160/C/1 with an accuracy of 0.00001 g were used. Two diameters on perpendicular planes and two measurements of height were made and each pellet was weighed. Then, the density was calculated using a formula:

$$\rho = \frac{m}{V} \quad (4)$$

where: ρ – pelet density, kg m⁻³; m – mass, kg; V – volume, m³.

Elemental composition and statistical analysis

A chemical analysis included determination of dry matter and elemental composition of the material were determined in Analytical Centre of WULS. Dry matter was determined using weighing method, Ca, K₂O and P₂O₅ were determined using a

spectrometry emission method (ICP-OES) and content of nitrogen N was determined using Kiejdahl method with a titration final mark.

Data analysis was carried out using Statistica v.12 computer program, with application of variance analysis procedure and Duncan test. Statistical inferences were made at the 0.05 level of probability.

RESULTS AND DISCUSSION

Received digestate was meeting the standards in the range of permissible contamination values in organic and mineral fertilizers as well as agents supporting cultivation of crops (Table 1). The main fertilizer compounds were slightly decreased after addition of calcium carbonate and the share of dry matter was significantly increased 20.3% to 64.8%. These results are also confirmed by moisture content results (Fig. 3).

Table 1. Chemical composition of digestates

Material	Dry matter, %	K ₂ O, %	P ₂ O ₅ , %	Ca, %	N, %
Digestate	20.3	1.01	1.08	-	2.26
Digestate with CaCO ₃	64.8	0.53	0.99	27.5	< 1.00

The moisture content of liquid digestate was 96%. Mechanical separation allowed to decrease the water content by 19% and addition of 20% of calcium carbonate by 30%. To verify the results also a lower addition of binder was added to the raw material and the moisture content was above 65%.

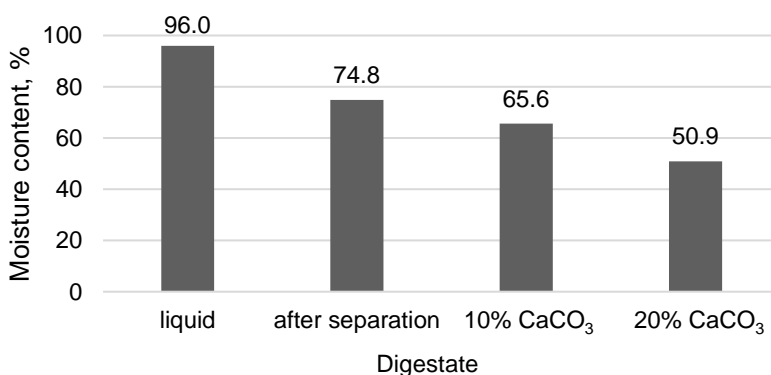


Figure 3. Moisture content for different types of digestate.

It was found that without additives, the net calorific value (Fig. 4) for tested material was 14.9 MJ kg⁻¹, but due to the high moisture content of the raw material it is unprofitable to dry it and burn. Similar results of net calorific value (15 MJ kg⁻¹) was obtained by Kratzeisen et al. (2010) however in another study (Kuligowski, 2011) this value was 11.3 MJ kg⁻¹. Differences in values of this parameter result from a different composition of digestate. High gross calorific value for pure digestate was 17.9 MJ kg⁻¹, which proves its usefulness for energy purposes. On the other hand, digestate with

addition of calcium carbonate is not suitable to use for energy purposes because of its low net calorific values 5.2–5.9 MJ kg⁻¹. Addition of 10% of calcium carbonate caused decrease in calorific value by 60% to 5.9 MJ kg⁻¹ and further addition of calcium carbonate to 20% caused smaller decrease in net calorific value nearly by 12%.

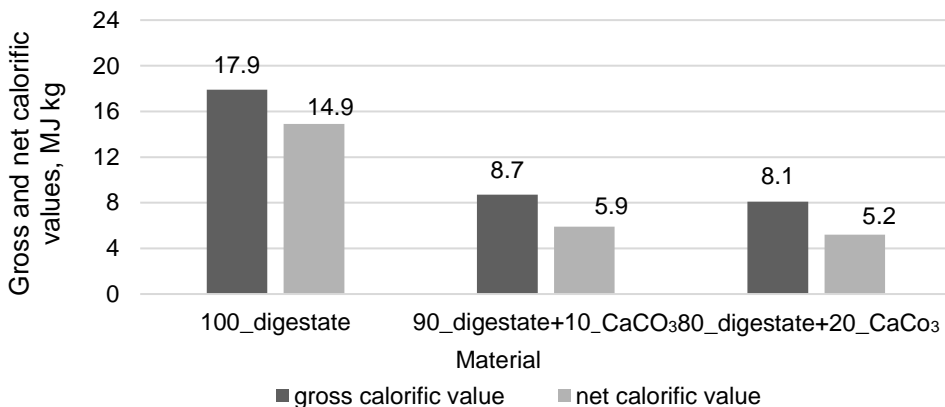


Figure 4. Gross and net calorific values of digestate with and without of binder.

Agglomeration research were carried out for various compaction parameters. Initially, for the same dose, the compaction height was changed per trial. Based on previous preliminary tests for other materials (Lisowski et al., 2017), the first trial was conducted at 40 mm and 0.3 g and durable pellets with high density (791 kg m⁻³) were obtained (Fig. 5). Then, the die height was increased to 50 mm at the same single dose of raw material and durable pellets were also made, but with lower density (536 kg m⁻³). When the die height was reduced to 30 mm at the same dose, no durable pellets were obtained, because the height was too low and the material fell out of the matrix. After determining the best height, the mass of a single dose of compacted material was changed. Both compaction tests for dose of digestate at 0.4 g and 0.2 g enabled the production of pellets with a stabilized compaction forces, but their durability was not satisfactory. Therefore it was decided, that pellets made of material with the addition of calcium carbonate should be produced for the parameters: 40 mm of the die height and 0.3 g of a single dose of compacted material as found as the best agglomeration parameters.



Figure 5. Pellets obtained at compaction parameters: 40 mm of the die height and 0.3 g of the single dose of digestate.

The obtained pellets (Figs 6, 7) were stable, with a lighter color than pellets without addition of calcium carbonate. Due to the use of calcium carbonate addition, not only the moisture of the digestate was reduced, but also the density of pellets was increased

(Table 4). Pellets created with such parameters had the smoothest surface and the most regular shape. Therefore, the quality of these pellets was the best. The displacement and compaction forces for these parameters were similar. The amount of calcium carbonate addition did not have a major impact on the pressure agglomeration process.



Figure 6. Pellets made of digestate with 10% of calcium carbonate.



Figure 7. Pellets made of digestate with 20% of calcium carbonate.

Pellets from digestate with the addition of 10% of calcium carbonate were characterized by the highest mean compaction work (1.81 J). The lowest compaction work was obtained for compaction the material without additives (Table 2).

Densities of pellets obtained under stable compaction conditions and the best parameters (40 mm and 0.3 g) were compared. The analysis of the variance of pellets density (Table 3) showed that the share of the additive statistically significantly affects the density of pellets made of digestate, which can be determined on the basis of *F*-Fisher-Snedecor test, where *F* was 13.7 for *p* < 0.001.

Table 2. The mean values of compaction work L_c , its standard deviations SD and 95% confidence intervals

Material	Binder, %	SD			
		L_c, J	L_c, J	-95% L_c, J	+95% L_c, J
digestate	0	0.32	0.2	-0.08	0.72
	10	1.81	0.2	1.41	2.21
	20	0.68	0.18	0.31	1.04

Table 3. The variance analysis results of pellets density ρ

Source	Sum of squares	Degrees of freedom	Mean square	<i>F</i> -test	<i>P</i> -value
residual	10,497,656	1	10,497,656	8,316.3	< 0.0001
binder	34,549	2	17,275	13.7	< 0.001
error	15,147	12	1,262		

Table 4. The mean values of densities ρ of pellets made of digestate with and without the calcium carbonate, their standard deviations SD and 95% confidence intervals

Material	Binder, %	$\rho, \text{kg m}^{-3}$	SD $\rho, \text{kg m}^{-3}$	-95% $\rho, \text{kg m}^{-3}$	+95% $\rho, \text{kg m}^{-3}$	<i>n</i>
digestate	0	791.23	15.89	756.61	825.84	5
	10	815.50	15.89	780.88	850.12	5
	20	902.98	15.89	868.36	937.59	5

The density of pellets with the same compaction parameters was calculated for the last five pellets, which were produced under stable conditions of the compaction process (Table 4). The increase in the share of calcium carbonate added to the digestate caused the increase in pellets density. The highest density of pellets was obtained for agglomerates from digestate with 20% addition of calcium carbonate and it was 903 kg m⁻³ and the lowest for pellets without addition equal to 791 kg m⁻³. These results were lower than those for pellets made of typical types of biomass, e.g. straw, hay and wood (Kaliyan & Morey, 2009; Larsson et al., 2008).

CONCLUSIONS

1. Digestate after separation is a difficult material to be used for energy purposes, because it is characterized by a high moisture content, reaching 75%.

2. Addition of calcium carbonate to the digestate decreased the net calorific value from 14.9 MJ kg⁻¹ to 5.9 MJ kg⁻¹ (at 10% of binder) and to 5.2 MJ kg⁻¹ (at 20% of binder).

3. The best quality of pellets were obtained at compaction parameters: die height of 40 mm and a dose of 0.3 g. With die height than 40 mm, the obtained agglomerates were of poor quality.

4. The share of calcium carbonate has contributed to the increase in compaction work.

5. Addition of 20% of calcium carbonate to the digestate reduced its moisture by almost 13%, which allowed to easier further processing.

6. Determination of agglomeration parameters for digestate are relevant information for practice in order to increase the efficiency of compaction in large-scale production.

7. Agglomerates made from the residue after the methane fermentation process should have high fertilizing properties, however, further research is needed to confirm this statement.

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Development of symbiotic interactions in the faba bean (*Vicia faba* L.) roots

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Abstract. Double-inoculation of faba bean (*Vicia faba* L.) seeds before sowing with rhizobium bacteria and mycorrhizal fungi is a known agronomic practice. It improves symbiotic nitrogen fixation and enhances legume supply with mineral elements and water. Effective symbiosis makes it possible to replace some of the required mineral fertilizer needed for plant growth with symbiotically fixed. This will ensure more environmentally friendly agricultural production. The formation of an effective symbiosis depends on a number of biotic and abiotic factors affecting the biochemical signals released by the partners. The biochemical mediator for symbiosis formation is flavonoids. The aim of this experiment was to test the effects of rhizobial and mycorrhizal inocula on symbiosis formation under different temperatures. Beans are an important source of protein for animal feed and human consumption. Four cultivars of faba beans were used - two *V. faba* var. *minor* Beck. - 'Fuego' and 'Lielplatone', and two *V. faba* var. *major* Harz. - 'Bartek' and 'Karmazyn'. The combination of microorganisms for seed inoculation influenced the frequency of root mycorrhization and abundance of arbuscules. The content of flavonoids in seed exudates correlated ($r = 0.93$) with germination temperatures. The use of mycorrhizal fungi alone or in combination with rhizobia reduced the amount of flavonoids in the bean seed exudate. In the pot experiment the amount and size of nodules significantly differed between cultivars. Use of mycorrhizal preparation mitigated the effect of inadequate germination temperature. Higher degree of mycorrhization and more intense formation of arbuscules formation was observed in the bean roots grown in vegetation pots in comparison with field ones. Local bred 'Lielplatone' had significantly better compatibility with microsymbionts in local agroclimatic conditions.

Key words: flavonoids; rhizobia; mycorrhiza; nodule; temperature, faba beans.

INTRODUCTION

Legumes usually are grown using different soil management practices; seeds are often inoculated before sowing. Sometimes seeds are inoculated with both rhizobia and mycorrhizae fungi. The reason for this double-inoculation is not only to improve the symbiotic nitrogen fixation, but also to enhance the supply of legume with mineral

elements and water. The formation of an effective symbiosis depends on several environmental factors including the interaction of microorganisms in rhizosphere. In the temperate climate zone the optimal environmental requirements of plants and microorganisms must be balanced in order to create an effective symbiosis. In Latvia agroclimatic conditions the faba beans are sown in early spring when soil temperature for microorganism activity usually is not optimal. The average air temperature during this time is between 5 and 10 °C (data obtained from the 'Latvia Environment, Geology and Meteorology Centre, <https://www.meteo.lv/>). Inappropriate temperature and thus slowed seed germination can affect the formation of symbiosis. Optimal root zone temperature for seed germination can vary between faba bean varieties, especially if seeds are inoculated with microorganisms. *Vicia faba* var. *major* seeds require higher germination temperature compared to *Vicia faba* var. *minor* (Senberga et al., 2018). In fact, faba beans are often mentioned as more sensitive to biotic and abiotic factors than other legumes, especially in the case of soil water content and temperature. Soil temperature affects not only the germination rate and the development of the primary root, but can also affect the biochemical processes in the plant. Biochemical mediators must be synthesized for both: the plant and the microorganisms. Only then a symbiotic union can be formed between symbionts (Hauggaard-Nielsen et al., 2011; Šiaudinis et al., 2011).

The biochemical mediator of symbiosis formation is flavonoids, secondary metabolites that develop in plants in low concentration. Flavonoids are synthesized and released in the rhizosphere as a protection mechanism against pathogenic bacteria and fungi. Despite the antifungal, antibacterial and antioxidant properties, flavonoids are recognized as early mediators in the rhizosphere. They exudate not only from the roots of the plant, but also from seeds (Maj et al., 2010). Qualitative and quantitative composition of flavonoids depends on used legume species, microorganisms and soil conditions. During seed germination and primary root growth, the composition of flavonoids released in the rhizosphere may differ. A variable accumulation of flavonoids in the plant roots during the formation of symbiosis has also been observed (Guenoune et al., 2001). The flavonoid composition in rhizosphere depends also on the bacterial community. It is especially important during legume – rhizobia symbiosis establishment. Flavonoids released in rhizosphere during symbiosis establishment, are important signal molecules, coordinating the actions of the genes that induce gene transcription in the microbial symbionts, as specific genes essential for the formation of nodule and N₂ fixation (Schultze & Kondorosi, 1998, Day et al., 2001).

The released flavonoids also depend on the legume species and the group of microorganisms. Qualitative and quantitative changes in the composition of flavonoids have been also identified in the mycorrhization process. Comparing the composition of flavonoids accumulated throughout the time of root colonization by mycorrhizae fungi, it was found that coumestrol and medicarpin were accumulated at the beginning of root colonization and at the end of the process. The accumulation levels of ononin and daidzein were higher at the beginning of root colonization, whereas amount of genistein was not changed (Larose et al., 2002). Guenoune et al. (2001) proved that the flavonoid medicarpin accumulated in roots with high phosphate (P) levels. Medicarpin have inhibitory effect on hyphal growth of *G. intraradices*, and is known to possess antifungal activity towards other fungi, thus possibly preventing the roots with a high P concentration from being colonized by arbuscular mycorrhiza fungi (AMF). Flavonoid accumulation in legume roots can be also promoted by rhizobia (Duc et al., 1989).

After the establishment of the symbiotic association, its effectiveness depends on environmental factors. Mycorrhizal growth depends on phosphorus availability, drought stress level and the presence of fungal pathogens (Yang et al., 2015). Plant biological features also influence mycorrhizae growth, however, the effect is poorly investigated and controversially interpreted, especially for the capacity to fix nitrogen and the C-fixation pathway (Yang et al., 2016). Hartnett et al. (1993) found that the effects of arbuscular mycorrhizae fungi can differ at the seedling stage and later development stages of plant.

Factors influencing interaction between symbionts are widely studied. Yang et al. (2015) by meta-data analysis summarised, that arbuscular mycorrhizae fungi can modify plant root morphology and architecture. Results also showed that the type of root system potentially modifies plant growth responds to colonization by AMF. For instance, plants with taproots respond better to AMF than plants with a fibrous root system. But according to Azcón et al. (1991) *Medicago sativa* L. plants with taproot system are highly mycorrhiza dependent, but *Hordeum vulgare* L. with fibrous root system does not show strong positive growth response to colonization by arbuscular mycorrhizal fungi.

Both rhizobia and mycorrhizal fungi receive approximately 4–16% of the photosynthesis products from plant to ensure their activity. In cases where the productivity of photosynthesis is not sufficient to supply all components of the symbiotic system with carbon compounds, they become competitors. In such cases the growth of symbiotic plants may be delayed compared to non-symbiotic plants (Harris et al., 1985, Bethlenfalvay & Newton, 1991).

According to Bruns et al. (2008), understanding the role and effects of mycorrhizal fungi on the nitrogen assimilation, translocation and regulation mechanism in the plant allows a better management of mycorrhizal fungi in sustainable agriculture.

Thus our hypothesis is – in suitable environmental conditions *Rhizobium* bacteria and mycorrhizae fungi may have a beneficial effect during faba beans seeds germination and root system development. The aim of this experiment was to test effects of rhizobia and mycorrhiza fungi inoculum on symbiosis formation under different temperatures. After establishment of effective symbiosis the replacement of some of the required mineral fertilizers for plant growth with symbiotically fixed ones is possible. This will ensure more environmentally friendly agricultural production.

MATERIALS AND METHODS

For the assessment of symbiosis formation, three sets of tests were organized.

Experiment 1 – Laboratory experiment in petri dishes. Determination of the amount of flavonoids in the seed exudate during germination depending on the presence of microsymbionts.

Experiment 2 – Pot experiments. Comparison of inoculation efficiency in greenhouse conditions.

Experiment 3 – Field trials. Comparison of different inoculation variants and determination of the abundance and mycorrhization intensity in faba bean roots.

The design of the experiments

Experiment 1. A laboratory experiment for assessment of seed germination rate and flavonoid content was conducted in Petri dishes at four temperatures, namely 4, 8, 12,

20 °C. Four faba bean (*Vicia faba*) cultivars were used - two *V. faba* var. *minor* Beck. - 'Fuego' (Pflanzenzucht Hans Georg Lembke KG, Germany) and 'Lielplatone' (Institute of Agricultural Resources and Economics, Latvia), and two *V. faba* var. *major* Harz. - 'Bartek' and 'Karmazyn' (Torseed®, Poland) were used. For *V. faba* var. *minor* 10 seeds were used per dish, for *V. faba* var. *major*, 5 seeds per dish. Before inoculation the seed surface was sterilized (30 sec in 70% ethanol and 3 min in 5% sodium hypochlorite). Three seed inoculation variants were chosen: mixture of rhizobia strains (R), mycorrhizae inoculum (M) and double inoculation with rhizobia and mycorrhizae (RM). For control, seeds were germinated without microsymbionts (K). The test was arranged in 4 replications (respectively 40 or 20 seeds per variant). 20 mL of sterile distilled water was added in each petri dish.

Experiment 2. At the end of seed germination experiment in Petri dishes, the experiment was continued as pot experiment. 5 seedlings from each variant were planted in pots. 5 L pots were filled up with non-sterile soil similar with field trial soil in the experiment 3. Temperature regime at night was 10 ± 5 °C and 18 ± 5 °C during the day. At the end of experiment (BBCH 50-53), plants were weighed and the ratio of root and shoot fresh weight was calculated. Fresh and dry weight of nodules, and mycorrhization parameters were established. The abbreviation **BBCH** derives from **B**iologische **B**undesanstalt, **B**undessortenamt and **C**hemical industry. BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Meier, 2001).

Experiment 3. The field trials were arranged in the experimental plots of the Study and research farm 'Pēterlauki' of Latvia University of Life Sciences and Technologies. The pre-crop of the field trial plots were cereals, and no trials with rhizobia bacteria or mycorrhizal fungi had been carried out in these plots for more than 10 years. According to WRB 2015, the used soil was an Endocalcaric Endoabruptic Luvisol (Aric, Endoclayic, Cutanic, Hypereutric, Ochric, Endoraptic, Anosiltic, Protostagnic, Epiprotovertic). The same seed inoculation variants were used. Size of each plot was 7 per 1.5 m. The faba beans were sown with a seeding-machine. The sowing rate was 45 seeds per m².

Microorganisms used for seed inoculation

Rhizobium sp. strains were obtained from the Collection of Rhizobia of the Latvia University of Life Sciences and Technology. Strains RP023 (isolated from *Pisum* sp.) and RV407 (isolated from *Vicia faba*) were mixed and used in the experiment. Both strains were isolated from Latvian soils. Seeds were inoculated before sowing by soaking in the solution with approx. 10⁶ bacteria cells per mL.

The inoculum of mycorrhizae was produced by company Symbiom[®], Czech Republic. *Glomus claroideum*, *Glomus intraradices* and *Glomus mosseae* were present in the inoculum. The mycorrhizal inoculation was performed at sowing. 20 mL of the arbuscular mycorrhiza fungi (AMF) inoculum were placed below each seed.

Determination of flavonoid concentration and composition

After seed germination, water with excreted compounds was collected. Flavonoid concentration was determined according to Robaszkiewicz et al. (2010). Germination solution (2.5 mL) was mixed with 150 µL 5% NaNO₂. After 5 min incubation at room temperature, 150 µL of 10% AlCl₃ was added, after next 5 min – 1 mL 1 M NaOH.

Absorbance of the solution was read after 15 minutes with spectrophotometer at 415 nm. Flavonoid content was expressed as catechin equivalents (CE) in 100 mL of solution.

For flavonoid composition analysis soaked seeds, primary roots and germinating seed exudate was used. Analyses were performed by HPLC at the Laboratory of Analytical Chemistry, Faculty of Food Technology, Latvia University of Life Sciences and Technology.

Each analysis were made in 4 replications.

Assessment of mycorrhizal colonization

For the assessment of mycorrhizal colonization root fragments were collected. After cleaning them with 10% KOH, root fragments were stained with black Ink (Parker Pen Company, Newhaven, UK) dissolved in 8% acetic acid. Stained root fragments were stored in glycerol:lactic acid:water (v/v/v 1:1:1) solution till microscopic examination (Vierheilig et al., 1998). From each replication 30 root fragments were selected, each approx. 1 cm long. The specimens with the root fragments were examined using a microscope with 10× objective. Parameters of mycorrhizal colonization were evaluated according to the method of Trouvelot et al. (1986) which included mycorrhizal frequency in root fragments, intensity of mycorrhizal colonisation, arbuscules abundance in the root system. The results were calculated by equations incorporated in a computer program MYCOCALC.

Frequency of mycorrhiza in the root system (F%) = (number of fragments with myco/total number)×100.

Intensity of the mycorrhizal colonisation in the root system (M%) = $(95n_5+70n_4+30n_3+5n_2+n_1)/(\text{total number})$ where n_5 = number of fragments rated 5; n_4 = number of fragments 4 etc.

Intensity of the mycorrhizal colonisation in the root fragments (m%) = $M \times (\text{total number})/(\text{number with mycorrhizae})$

Arbuscule abundance in mycorrhizal parts of root fragments (a%) = $(100m_{A3}+50m_{A2}+10m_{A1})/100$ where m_{A3} , m_{A2} , m_{A1} are the % of m, rated A3, A2, A1, respectively, with $m_{A3} = ((95n_5A_3+70n_4A_3+30n_3A_3+5n_2A_3+n_1A_3)/\text{number myco}) \times 100/m$ and the same for A2 and A1.

Arbuscule abundance in the root system (A%) = $a \times (M/100)$

(<http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download>).

Statistical analysis

Obtained data were processed using Analysis of Variance (ANOVA) with Microsoft EXCEL software. Differences were considered statistically significant when $p < 0.05$. Error bars in figures show the Least Significant Difference (LSD). Correlation coefficient was used to compare flavonoids content, seed germination temperatures and 100 nodule weights.

RESULTS AND DISCUSSION

Flavonoids in the seed exudate

Experiments in Petri dishes showed that the amount of released flavonoids depended on the germination temperature and the inoculants (Table 1). The highest amount of flavonoids in root exudates was detected at 20 °C degrees for all faba bean

varieties and inoculation variants. The amount of flavonoids in seed exudates proves a strong linear positive correlation ($r = 0.93$) with germination temperatures. The highest flavonoids content in exudates was found in variants where cv ‘Fuego’ was germinated.

Table 1. Mean amount of flavonoids (mg CE mL⁻¹) in seed exudate during faba bean seed germination

Germination temperature, °C	Inoculation variants	<i>Vicia faba</i> var. <i>major</i>		<i>Vicia faba</i> var. <i>minor</i>	
		Karmazyn	Bartek	Fuego	Lielplatone
4	K	0.202 ^b	0.165 ^b	0.314 ^a	0.264 ^b
	R	0.212 ^b	0.125 ^a	0.347 ^a	0.263 ^b
	M	0.189 ^a	0.122 ^a	0.326 ^a	0.261 ^b
	RM	0.180 ^a	0.202 ^c	0.304 ^a	0.170 ^a
8	K	0.202 ^a	0.154 ^a	0.378 ^b	0.260 ^b
	R	0.208 ^a	0.168 ^a	0.371 ^b	0.253 ^b
	M	0.214 ^a	0.227 ^b	0.306 ^a	0.313 ^c
	RM	0.230 ^b	0.155 ^a	0.359 ^b	0.225 ^a
12	K	0.305 ^c	0.215 ^b	0.466 ^b	0.337 ^b
	R	0.224 ^b	0.228 ^b	0.408 ^a	0.273 ^a
	M	0.161 ^a	0.161 ^a	0.417 ^{ab}	0.273 ^a
	RM	0.169 ^a	0.204 ^b	0.446 ^{ab}	0.254 ^a
20	K	0.441 ^c	0.341 ^c	0.644 ^a	0.444 ^b
	R	0.348 ^a	0.329 ^c	0.704 ^b	0.371 ^a
	M	0.390 ^b	0.285 ^b	0.760 ^c	0.359 ^a
	RM	0.421 ^c	0.237 ^a	0.690 ^b	0.384 ^a

Values in the same column and same germination temperature with identical letters are not significantly different ($p > 0.05$), LSD 0.075. Variants K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

The use of microsymbionts influenced the release of flavonoids. At 77.7% occasions, the use of the microsymbiont(s) significantly lowered the amount of released flavonoids. The largest effect was observed with mycorrhizal fungi preparation (M), then followed with double inoculation (RM) (Table 1). In variants where rhizobia were used, only in 9.4% occasions significant ($p < 0.05$) differences were observed. Significant decrease of the amount of flavonoids for *Vicia faba* var. *major* cultivars was detected at 12 °C.

The composition of the flavonoids differed in germinating seed, primary root and exudate. The main flavonoid in the primary roots was catechin, but rutin and luteolin were found at a lower concentration. Seed exudates contain higher concentration of catechin and rutin, but the amount of luteolin comparing to primary roots was decreased. Quercetin was detected in seed and primary root, but not in the exudate.

Experiments have shown that root development in inadequate temperature can be delayed (Senberga et al., 2018). Inadequate temperature may influence the amount and composition of flavonoids. Plants release flavonoids from small roots and root hairs, forming biochemical signals, so that hyphae grow directly on the roots. Microorganisms in the rhizosphere often induce increased release of flavonoids, thus facilitating root colonization (Frey-Klett et al., 2007). The amount of released or accumulated flavonoids depends not only on the presence of rhizobia or mycorrhizal fungi, but also on the

population of microorganisms in the rhizosphere that may affect biochemical processes and the formation of nodules, and the intensity of colonization of mycorrhiza.

Various studies (Larose et al., 2002; Petrusa et al., 2013; Wang et al., 2015) have confirmed that temperature and soil moisture content has significant effects on the flavonoid biosynthesis and expression not only in root exudate, but in different parts of the plant. Decrease of released flavonoids depended on plant variety as well as on inocula composition. Flavonoid content in the seed exudate of variety 'Bartek' and 'Karmazyn' decreased at the temperature 12 °C when inoculated with mycorrhizal fungi. This may also be due to the accumulation of flavonoids in the roots. It can influence the released amounts of flavonoids in rhizosphere. Larose et al. (2002) determined that roots colonized by mycorrhizae fungi accumulate different amount of flavonoids. In these experiments also the accumulation levels varied depending on the root-colonizing fungi. For example, flavonoid coumestrol was accumulated more in roots colonized by *G. intraradices*, but less in roots colonized by *G. mosseae*. Similarly, daidzein was more accumulated in roots colonized by *G. intraradices* and *G. mosseae*, but detected less in roots colonized by *G. rosea*. However, the statement of Schmidt et al. (1994), that flavonoid accumulation can be enhanced by rhizobia as well, was confirmed by our results.

Germinated faba bean growth at vegetation pot trial

At the end of the vegetation pot trial no statistically significant differences ($p > 0.05$) were detected for the fresh weight of plant shoots and roots. Obtained data showed only a tendency that inoculated plants can form larger aboveground part. The largest ratio of root to shoot weight was detected in germination variants with mycorrhizae fungi inoculation. In this inoculation variant average root: shoot ratio was 0.60 and varied for different cultivars between 0.49 and 0.71. The lowest ratio was in the version where seeds were inoculated only with rhizobia. Here the average ratio was 0.48, and varied between 0.42 (cv 'Bartek') and 0.53 (cv 'Lielplatone'). The largest root system was observed for cv 'Bartek' with mycorrhiza inoculation. Inoculation with only rhizobia reduced root growth in most cases.

In vegetative pot experiment, only some nodules formed on the roots of large-seeded beans (cv 'Bartek' and 'Karmazyn') and no significant differences were observed between different inoculation variants of these cultivars. *V. faba* var. *minor* 'Fuego' and 'Lielplatone' formed enough nodules and that allowed to assess effect of inoculation. The results showed that lower temperature during germination influenced nodule formation (Fig. 1). Whereas plants were grown in non-sterile soil, some nodule formation was also observed in samples grown without rhizobia inoculum (K and M). Indigenous rhizobia formed smaller nodules compared to the variants with rhizobia inoculation, especially on those plant roots which seeds were germinated in lower temperature.

Faba bean seed inoculation only with *Rhizobium* bacteria is not always a sufficiently effective agricultural practice, especially when seeds are sown in early spring, when the root zone temperature does not exceed 4 °C (Senberga et al., 2018). Though bacteria from *Rhizobiaceae* are able to survive at 4 °C (Drouin et al., 2000) and bacterial activity increases with increasing temperature, prolonged exposure of inoculated seeds to low temperatures can decrease the ability to develop symbiosis (Fyson & Sprent, 1982).

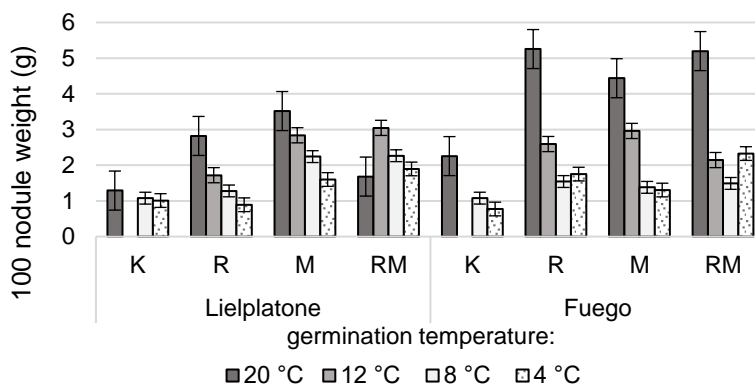


Figure 1. 100 nodule weight at the end of vegetation pot experiment: K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

The effect of germination temperature on nodule weight was observed also in the vegetation pot experiments and depended on cultivar. Nodules on the roots of ‘Fuego’ germinated in 20 °C was twice heavier as ‘Lielplatone’ ones. Such a difference may arise due to the biological nature of the varieties and the suitability for the specific agroclimatic conditions. Legume seed inoculation with mycorrhizae fungi alone or together with *Rhizobium* showed positive influence on nodule formation compared to control samples. According to the results, the decrease of released flavonoids at a low temperature and reduced root growth could have led to a formation of smaller, less effective nodules. This is consistent with the statement of Nap & Bisseling (1990) and Hirsch (1992), that the size of the nodule is related to their functional activity. Heavier nodules are considered to be more effective in N₂ fixation.

A correlation was detected between germination temperature and flavonoids exudation ($r = 0.95$), and nodule weight and flavonoid exudation ($r = 0.77$) for cultivar ‘Fuego’. For cultivar ‘Lielplatone’ a significant correlation ($r = 0.83$) was detected only between flavonoid exudation and temperature.

Abundance of mycorrhizae fungi in faba beans root

The effect of growth conditions on the frequency and intensity of mycorrhizal colonization was observed comparing vegetation pot and field trials. Frequency of mycorrhiza fungi (Fig. 2) on inoculated faba bean roots significantly differed ($p < 0.05$) between greenhouse experiment and field trials. In the field trials, plants and microorganisms were more susceptible to fluctuating environmental conditions, which is not always favourable for the formation of symbiosis. In the field cultivated faba bean roots mycorrhizae fungi structures were observed less, compared to samples grown in vegetation pots in greenhouse. Significant differences between control and inoculated variants were detected in the vegetation pot experiment, the most distinct in samples with mycorrhizae fungi inoculum. Plants inoculated with mycorrhiza fungi show different root colonization possibilities compared to samples without inoculation due to the composition of indigenous fungi species. Yang et al. (2015) concluded that plants with taproot are more responsive to symbiosis formation if AMF species richness in rhizosphere is low.

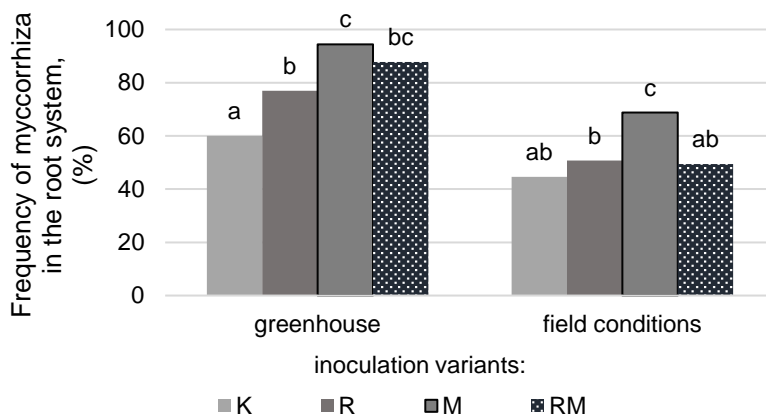


Figure 2. Comparison of the frequency of mycorrhiza fungi in the root system of cv 'Bartek' in vegetation pot experiment and in field trial. Means designated with the same letter are not significantly different ($p \leq 0.05$): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Since non-sterile soil was used in vegetation pot trial, structures of the fungus was found also in control. In vegetation pots the root colonization with mycorrhizae fungi structures was in average 3.6 times intense than in the control variant (Fig. 3). In variants where seeds were inoculated only with rhizobia, mycorrhizal colonization intensity exceeded control variants averagely 1.8 times. For samples grown on field, differences between variants inoculated only with rhizobia and control were averagely 1.3 times.

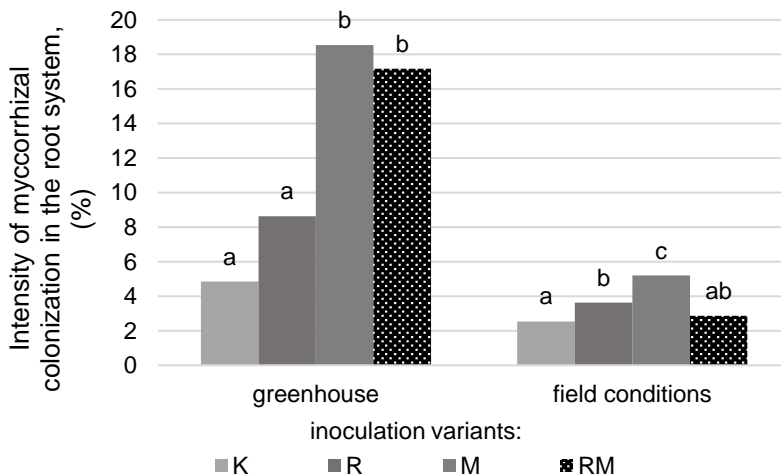


Figure 3. Comparison of the intensity of mycorrhiza fungi in the root system of cv 'Bartek' in vegetation pot experiment and in field trial. Means designated with the same letter are not significantly different ($p > 0.05$): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

In vegetation pot experiment arbuscules abundance in faba beans root system showed significant ($p < 0.05$) difference between inoculation variants (Fig. 4). The arbuscules abundance in root fragments of inoculated variants (M, RM) was 5.4, and 4.6 times higher than in control variants. Less significant differences were observed for samples grown at the field conditions. Significantly higher amount of arbuscules in roots was in the variants with mycorrhizae inoculum grown in greenhouse conditions. In contrast the field conditions did not increase abundance of arbuscules.

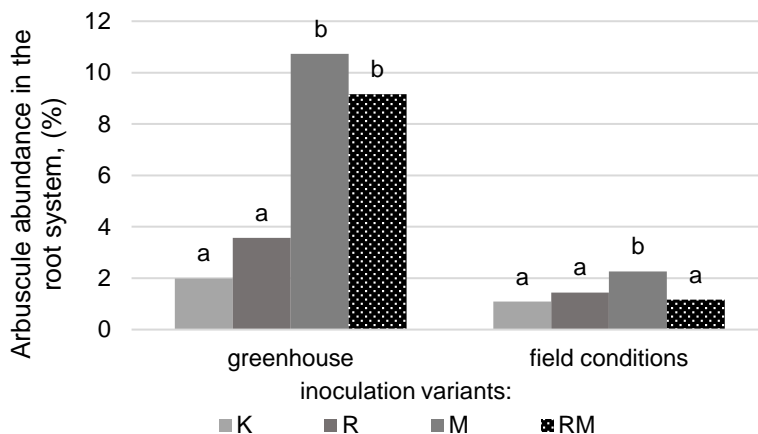


Figure 4. Comparison of arbuscules abundance in the root system of cv 'Bartek' in vegetation pot experiment and in field trial. Means designated with the same letter are not significantly different within growing conditions ($p > 0.05$): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

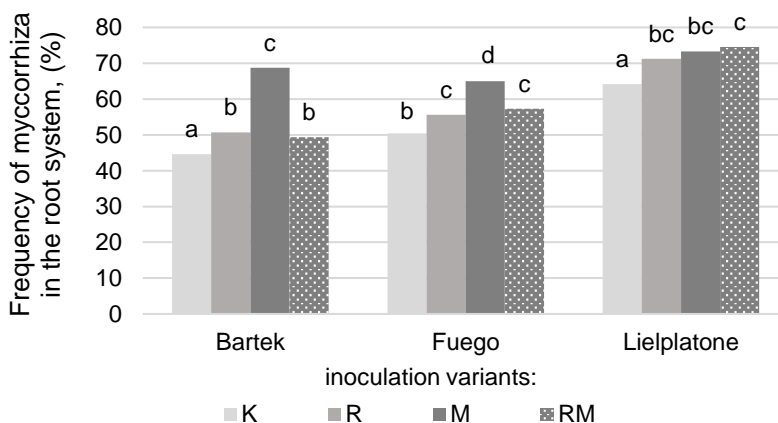


Figure 5. Frequency of mycorrhiza structures in faba bean root system in field experiment. Means designated with the same letter are not significantly different within variety ($p > 0.05$): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Observed differences between cultivars in field conditions are shown in Fig. 5. Frequency of mycorrhiza structures in all cultivars root systems were higher in inoculated variants in comparison with control, but only in cultivar ‘Lielplatone’ more fungi structure were observed in double inoculated variant compared to single inoculated samples. The degree of mycorrhization in field trials was significantly lower than in pot experiments. This can be explained by the weather conditions and their influence on plant growth, and the manifested competition of microorganisms in soil rhizosphere. Similar results are reported by Gamalero et al. (2002), where modifications of root morphogenesis and growth and mycorrhization depended on plant growth conditions and soil fertility.

Similar results between variants grown on the field were obtained for intensity of mycorrhizal colonisation (Fig. 6). For cultivar ‘Lielplatone’ more significant differences were determined between control and all three inoculation variants. If frequency of mycorrhizal structure did not show significant difference between cultivars, then mycorrhizal colonization intensity was significantly higher in cv ‘Lielplatone’ root system. It could be explained by the fact that this cultivar was bred in Latvia and is more suitable for local agroclimatic conditions. Thus, more appropriate conditions could create more favourable conditions for mycorrhizae fungi spreading in the roots.

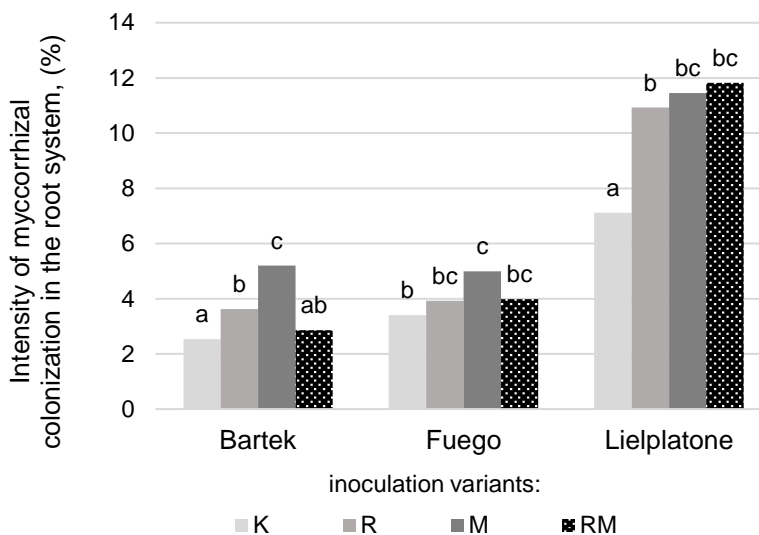


Figure 6. Intensity of mycorrhizal colonisation in the root system in faba bean root system in field experiment. Means designated with the same letter are not significantly different within variety ($p > 0.05$): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Abundance of arbuscules in the faba beans root were higher in cv ‘Lielplatone’, in comparison with cv ‘Fuego’ and ‘Bartek’ (Fig. 7). Only cv ‘Lielplatone’ in variants with double inoculation formed significantly higher amount of arbuscules in comparison with control.

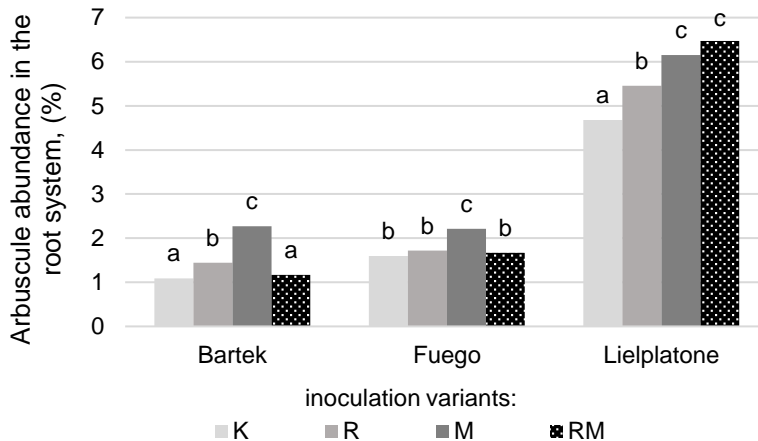


Figure 7. Comparison of arbuscules abundance in the root system of faba bean root system in field experiment. Means designated with the same letter are not significantly different within variety ($p > 0.05$): K – control; R – inoculation with rhizobia strains; M – inoculation with mycorrhiza preparation; RM – inoculation with rhizobia strains and mycorrhiza preparation.

Higher amount of arbuscules forms a wider range of possibilities for the exchange of nutrients between symbionts, thus the formation of the effective symbiosis, and that can result in the increase of bean yield and improve its quality.

CONCLUSIONS

1. The release of flavonoids from the roots was affected by the seed germination temperature and the microsymbionts used for inoculation. The correlation coefficient between temperature and released flavonoids was $r = 0.93$. The inoculation of seeds with mycorrhizal fungi resulted in a decrease in the amount of flavonoids in the exudate. The largest impact of mycorrhization was observed in 12 °C.

2. The amount and size of nodules in the pot experiment significantly differed between *V. faba* var. *minor* and *V. faba* var. *major* cultivars. The effect of germination temperature on vegetative growth of the bean was found. Use of mycorrhiza preparation mitigated the effect of inadequate germination temperature.

3. Higher degree of mycorrhization and more intense formation of arbuscules was observed in the bean roots grown in the pots in comparison with field ones. Degree of mycorrhization depended on used cultivar. Local bred ‘Lielplatone’ was significantly better responsive to microsymbionts in local agroclimatic conditions in comparison with other cultivars used in the trial.

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The production of methane from the straw pellets with addition of enzymes

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Abstract. Biogas production requires much cheaper raw materials. The use of straw, as not always the full use of agricultural residues, increases the methane yield in pelletised form compared to non-pelletised straw. Lack is the high ratio of carbon to nitrogen content of straw, which leads to a slow and incomplete breakdown of the matter, and less producing substances from which bacteria produce methane. Variety of additives can be used to improve anaerobic digestion process. This article shows the results of the study, where the enzymes alpha amylase and xylanase and catalysts Metaferm and Melafen mixture are used for the digestion process enhancement. Investigation was provided in 16 bioreactors operated in batch mode at 38 °C. Additives were filled into 14 bioreactors and only inoculum were filled into two bioreactors for control. The yield of biogas from straw pellets without additives was 0.655 L g⁻¹_{DOM} and methane 0.301 L g⁻¹_{DOM} after 34 days of anaerobic digestion. The yield of biogas from straw pellets with added alpha amylase was 0.652 L g⁻¹_{DOM} and methane 0.318 L g⁻¹_{DOM}. The yield of biogas from straw pellets with added xylanase was 0.689 L g⁻¹_{DOM} and methane 0.347 L g⁻¹_{DOM}. The yield of biogas from straw pellets with added Metaferm and Melafen mixture was 0.638 L g⁻¹_{DOM} and methane 0.254 L g⁻¹_{DOM}. The study demonstrates that the adding of enzymes increases the production of methane.

Key words: anaerobic digestion, straw pellets, methane, alpha amylase, xylanase.

INTRODUCTION

In recent years, several measures have been implemented in Latvia to reduce the support for biogas production, such as the introduction of a 9% profit margin, without taking into account large initial capital investments and high interest rates on bank loans. Raw material prices have also increased. The financial situation of the producers of biogas has deteriorated and some owners have already ceased operation of biogas plant. Therefore, the use of new, inexpensive raw biomass would be very important for them.

For the effective production of biogas from straw in typical agricultural biogas plants, it is necessary to pre-treat it. One of the rational pre-treatment methods can be granulation/briquetting which combines mechanical grinding and thermal effects. Pellets or briquettes quickly absorb moisture in the bioreactor, disintegrate, and make the biomass easily accessible to bacteria. Disgusting biomass does not float on top and does not form a floating layer as it does with chopped straw. It is known about a number of examples of successful use of briquetted/granulated straw in biogas plants, but the

experience of using significant volumes of straw for biogas production can still be considered as limited, as compared to traditional types of raw materials such as manure or maize silage (Moler & Hansen, 2014).

Straw is an abundant source of biomass that has a great potential to be used in the biogas industry, specifically in co-digestion with other substrates. Straw is poor in nitrogen and has a lignocellulosic structure giving a slow degradation. However, straw can be interesting as co-digestion material with substrates rich in easily degradable carbon and protein. One disadvantage of using straw is that it requires some kind of pretreatment, as for example reduction of particle size, prior to its use in a biogas reactor. Straw pellets and briquettes here represent an interesting alternative. These are established, easily accessible and easy-to-use products, consisting of ground and pressed straw, which can be used directly in the biogas process (Dubrovskis & Adamovics, 2012).

The results (Horwath et al., 2017) showed that the biochemical methane potential (BMP) for the straw products was $340 \pm 19 \text{ NL CH}_4 \text{ kg}^{-1}_{\text{DOM}}$. The results confirmed that, the briquetting and pelleting processes have a positive effect on the degradability of straw, higher BMP compared to virgin straw ($313 \pm 1 \text{ NL CH}_4 \text{ kg}^{-1}_{\text{DOM}}$). Equal results were obtained at the two laboratories. The BMP for food waste was however significantly higher (t-test $p < 0.05$) when the test was performed at RISE, Uppsala ($607 \text{ NL CH}_4 \text{ kg}^{-1}_{\text{DOM}}$) compared at UB, Borås ($445 \text{ NL CH}_4 \text{ kg}^{-1}_{\text{DOM}}$). The difference was likely be explained by different experimental conditions in the different laboratories (Horwath et al., 2017).

Lignocellulosic residues are relatively recalcitrant to bioconversion during anaerobic digestion (AD) for biogas production. Pre-treatments with cellulolytic enzymes or diluted alkali can facilitate biomass hydrolysis and enhance the process. Both pre-treatments require low energy and chemical inputs, without accumulation of inhibitor. Milled wheat straw (Vasmara-Ciro et al., 2017) was pre-treated with hydrolytic enzymes or with diluted NaOH before AD. The enzymatic pre-treatment only increased M_{max} by 14%. However, the same increase was observed with heat-inactivated enzymes, thus it was merely caused by the bioconversion into methane of the organic compounds contained in the enzymatic preparations. Moreover, all the pre-treatments determined a holocellulose conversion into reducing sugars lower than 4% (Vasmara-Ciro et al., 2017).

The hydrolysis of lignocellulose is assumed to be the rate-limiting step in the anaerobic fermentation process (Wellinger et al., 2013). A fungal hydrolytic enzyme mixture was used to assess the enzymatic impact on different feedstocks for biogas production. The optimal conditions for enzymatic hydrolysis of rye grain silage, maize silage, grass silage, feed residues and solid cattle manure were determined in lab-scale experiments. Finally, the effects of enhanced hydrolysis on anaerobic digestion were investigated in batch digestion tests. Enzyme treatment of substrate showed Michaelis-Menten-like behaviour and reached maximum values after 3 hours for reduced sugars as a product of hydrolysis. Methane production potential was determined for specific feedstock mixtures without enzyme, with inactivated enzyme and with active enzyme (with and without buffer). The results obtained show a clear increase in methane production after enzyme application for solid cattle manure ($165 \text{ L kg}^{-1}_{\text{DOM}}$ to $340 \text{ L kg}^{-1}_{\text{DOM}}$), grass silage ($307 \text{ L kg}^{-1}_{\text{DOM}}$ to $388 \text{ L kg}^{-1}_{\text{DOM}}$; enzyme plus buffer), feed residue ($303 \text{ L kg}^{-1}_{\text{DOM}}$ to $467 \text{ L kg}^{-1}_{\text{DOM}}$), maize silage ($370 \text{ L kg}^{-1}_{\text{DOM}}$ to $480 \text{ L kg}^{-1}_{\text{DOM}}$) and a lower increase for rye grain silage ($355 \text{ L kg}^{-1}_{\text{DOM}}$ to $413 \text{ L kg}^{-1}_{\text{DOM}}$) (Suarez et al., 2012).

One possibility to increase natural polymer degradation and concomitantly energy efficiency is the addition of exoenzymes to biogas facilities to enforce the primary degradation steps for biogas production. Only a marginal effect was obtained, when applying a tenfold higher concentration of added enzymes as proposed for practical use. The same result was achieved when commercially available enzymes were added to technical-scale fermentations using corn silage as monosubstrate. Therefore, these studies did not provide evidence that the addition of external enzymes into anaerobic degradation systems increases the methane yield in biogas facilities (Binner et al., 2011).

Metaferm and Melafen, created and produced in Latvia are substances, which induce biological processes. Metaferm contain multi enzymes, microelements and B group vitamins as well growing stimulators. Our previous studies shows that use of catalyst Metaferm has a positive effect on methane yield in anaerobic fermentation process of some biomass (Dubrovskis & Plume, 2016; Dubrovskis & Plume, 2017).

The aim of this study is to evaluate the suitability of straw pellets as substrate for biogas production and clarify whether the addition of enzymes alpha amylase and xylanase and biocatalysts Metaferm and Melafen (made in Latvia) in substrates leads to positive effect.

MATERIALS AND METHODS

The methodology described below and similar with German VDI 4630 (VDI 4630, 2006), Angelidaki et al. (2009) guideline and the German Methodenhandbuch Energetische Biomassenutzung (Thran, 2010) were used for the present study.

Average samples of wheat straw pellets were taken and it's the chemicals compositions were determined in the LUA laboratory according to the standardized methodology ISO 6496:1999. For each group of raw materials an average sample was taken and the total dry matter, organic dry matter and ashes content were measured.

The analysis were performed according to standard methods. Each group's raw material was thoroughly weighed carefully. All bioreactors (volume of 0.75 L) were filled with the same amount (500.0 g) of inoculums (digestate from a continuous working laboratory bioreactor with almost finished cows manure). Two bioreactors were filled with inoculums only as control. The others bioreactors were filled in with inoculums and biomass sample (10.0 g) with or without enzymes or catalyst Metaferm (see Table 1). Biomass sample 10 g is selected based on previous research experience so that the amount of biogas produced per day does not exceed 2 L. Biogas volume and composition analyzes are done once a day at about the same time. Gas from each bioreactor was directed into separate storage gas bag (2 L) located outside the heated chamber (see Fig. 1).

Amylase (EC 3.2.1.1) is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. The α -amylases are calcium metalloenzymes. By acting at random locations along the starch chain, α -amylase breaks down long-chain saccharides, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α -amylase tends to be faster-acting than β -amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7–7.0 (Silverman, 2002).

Xylanase (EC 3.2.1.8) is any of a class of enzymes that degrade the linear polysaccharide xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls. As such, it plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients. Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc., (mammals do not produce xylanases). However, the principal commercial source of xylanases is filamentous fungi (Polizeli et al., 2005).

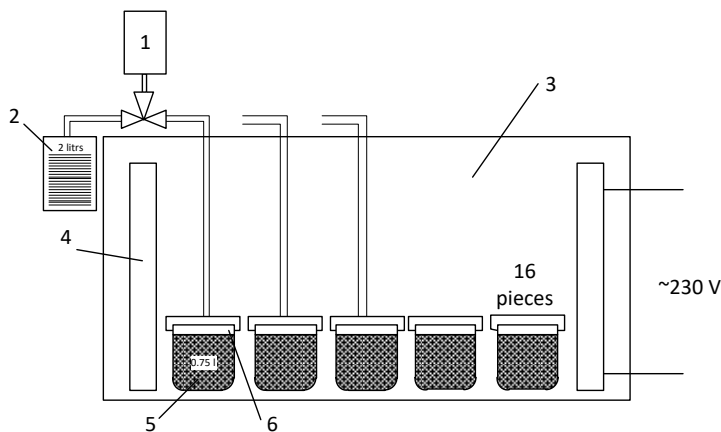


Figure 1. The schema of the experiment test bench: 1 – gas analyser; 2 – gas bag; 3 – electric stove; 4 – thermal elements; 5 – substrate; 6 – bioreactor.

Commercial applications for xylanase include the chlorine-free bleaching of wood pulp prior to the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting) (Polizeli et al., 2005).

Wheat straw pellets (10.0 g) diameter 8 mm, length 10–20 mm were filled in bioreactors R2–R15 and in bioreactors R6–R9 added 0.5 mL alpha amylase, in bioreactors R10–R12 added 0.5 mL xylanase and in bioreactors R13–R15 added 1 mL Metaferm + Melafen mixture 1:1. Bioreactors were filled with substrate and placed in a heated chamber (Memmert model). Gas from each bioreactor was directed into separate storage gas bag (2 L) located outside the heated chamber.

Dry matter (TS) and dry organic matter (DOM) was determined by investigation of initial biomass sample weight and dry weight by using scales Shimazu at 105 °C and by investigation of ashes content help by furnace (Nabertherm model) burning the samples at 550 °C according to special heating cycle. All substrates were prepared, carefully mixed, and all sealed bioreactors were put in heated chamber in same time before anaerobic digestion. Composition of gases collected in storage bag was analysed with the gas analyser (GA 2000 model). The percentage of oxygen, carbon dioxide, methane and hydrogen sulphide were registered. Substrate pH value was measured before and after finishing of anaerobic fermentation process, using pH meter (PP-50 model) with accessories. Scales (Kern KFB 16KO2 model) was used for weighting of substrate before anaerobic processing and for weighting of digestate after finishing of fermentation process.

The accuracy of the measurements was ± 0.025 L for gas volume, ± 0.1 °C for temperature and ± 0.02 for pH. Methane (CH₄), carbon dioxide (CO₂), oxygen (O₂) and hydrogen sulphide (H₂S) content in biogas was measured periodically. Weights Kern FKB 16KO2 with accuracy ± 0.2 g was used for measurement of total weight of substrates, and the unit Shimazu with accuracy ± 0.001 g was used for weighting of biomass samples to obtain total solids and dry organic matter content.

Fermentation process was provided with single filling in batch mode until biogas emission ceases (34 days). Final digestate was weighed, and dry matter and ashes were investigated to determine organic dry matter content. Total biogas and methane production values were calculated using the biogas normal volumes and quality parameters obtained from gas collected in the gas storage bag for each bioreactor (Becker et al., 2007). Experimental data were recorded in the experimental log and also stored in computer.

RESULTS AND DISCUSSION

The data on sample analysis and on amount of biogas and methane produced was estimated for all 16 bioreactors, and average results were calculated. The results of raw material analyses before anaerobic digestion are shown in Table 1.

Table 1. Results of analysis of raw materials

Bioreactor	Biomass	Weight, g	pH	TS, %	TS, g	ASH, %	DOM, %	DOM, g
R1; R16	IN	500 \pm 0.2	7.49	4.027	20.135	25.17	74.83	15.067
R2–R5	SP	10 \pm 0.001		90.38	9.038	5.79	94.21	8.515
R2–R5	SP+IN	510 \pm 0.2	7.5	5.72	29.173	19.16	80.84	23.582
R6–R9	IN+SP+AA	510.5 \pm 0.2	7.48	5.72	29.178	19.17	80.83	23.586
R10–R12	IN+SP+XA	510.5 \pm 0.2	7.47	5.72	29.177	19.16	80.84	23.586
R13–R15	IN+SP+MF1	511 \pm 0.2	7.52	5.71	29.183	19.15	80.85	23.593

Abbreviations: TS – total solids; ASH – ashes; DOM – dry organic matter; IN – inoculums; SP – straw pellets; AA – alpha amylase; XA – xylanase; MF1 – Metaferm + Melafen (1:1).

Weight of raw material in Table 1 is provided with error value depending on accuracy of respective weight measuring instrument used. Weight of total solids (TS) and dry organic matter (DOM) in Table 1 is provided with accuracy ± 0.001 g. Both inoculum substrates in control bioreactors (R1, R16) have low dry matter content as almost finished digestate were used for inoculums. As it can be seen from the raw material (Table 1) straw pellets biomass has a relatively high dry matter and organic dry matter content. This is explained due to the fact that the straw are dry and pelletized. This raw material, containing a lot of organic dry matter, is well suited for biogas production. Biogas and methane yields from straw pellets and straw pellets with added enzymes are shown in Table 2.

Table 2. Biogas and methane yields from straw pellets and straw pellets with added enzymes

Reactor	Raw material	Biogas, L	Biogas, L g ⁻¹ _{DOM}	Methane, aver. %	Methane L	Methane, L g ⁻¹ _{DOM}
R1	IN500	0.4	0.026		0.029	0.002
R16	IN500	0.2	0.013		0.0008	0.0001
R1-R16	Aver.	0.3	0.020		0.015	0.001
R2	IN500+SP10	5.4	0.634	44.79	2.415	0.284
R3	IN500+SP10	5.3	0.622	40.22	2.132	0.250
R4	IN500+SP10	5.7	0.669	48.88	2.781	0.327
R5	IN500+SP10	5.9	0.693	49.64	2.929	0.344
Aver. R2–R5		5.575	0.655	45.88	2.564	0.301
± st. dev.		± 0.29	± 0.033	± 4.39	± 0.360	± 0.042
R6	IN500+SP10+AA	5.1	0.599	48.91	2.498	0.293
R7	IN500+SP10+AA	5.5	0.646	50.77	2.800	0.328
R8	IN500+SP10+AA	5.7	0.669	47.53	2.710	0.318
R9	IN500+SP10+AA	5.9	0.693	48.05	2.843	0.333
Aver. R6–R9		5.55	0.652	48.82	2.713	0.318
± st. dev.		± 0.34	± 0.040	± 1.42	± 0.154	± 0.018
R10	IN500+SP10+XA	6.0	0.705	48.09	2.884	0.339
R11	IN500+SP10+XA	5.7	0.669	53.96	3.071	0.361
R12	IN500+SP10+XA	5.9	0.693	49.06	2.897	0.340
Aver. R10–R12		5.867	0.689	50.36	2.951	0.347
± st. dev.		± 0.15	± 0.018	± 3.15	± 0.104	± 0.012
R13	IN500+SP10+MF1	5.6	0.658	32.37	1.819	0.213
R14	IN500+SP10+MF1	6.0	0.705	40.28	2.417	0.284
R15	IN500+SP10+MF1	4.7	0.552	47.82	2.247	0.264
Aver. R12–R15		5.433	0.638	40.16	2.161	0.254
± st. dev.		± 0.67	± 0.078	± 7.73	± 0.308	± 0.037

Note: Biogas and methane values for bioreactors 2–15 with fresh source biomass are provided with already subtracted average biogas and methane values obtained from reactors 1 and 16.

Abbreviation: L g⁻¹_{DOM} – litres per 1 g dry organic matter added (added fresh organic matter into inoculum); MF1 – mixture Metaferm: Melafen 1:1.

Specific biogas and methane gases volumes obtained from bioreactors R2 – R15 are presented in Fig. 1.

The figure shows that the least methane was obtained from the R3 and R13 bioreactors. Although all bioreactors are filled with inoculum from a single bucket and thoroughly mixed, never before will all bioreactors have the same number of different bacteria. This explains the fact that different yields of methane are extracted from bioreactors. Using alpha amylase enzyme, 0.318 L g⁻¹_{DOM} methane was obtained from straw pellets. Using biocatalysts mixture MF1, 0.254 L g⁻¹_{DOM} methane was obtained from straw pellets. More than 9.12% than from alpha amylase, methane was derived from the use of the xylanase enzyme. Surprise was the deterioration of results with the use of the MF + ME mixture of biocatalysts because of positive results with other biomasses were achieved (Dubrovskis & Plume, 2016). Methane was produced less from the first days of the study, and later on its content in these bioreactors was lower. Further studies are needed to explain why methane-forming bacteria have multiplied less.

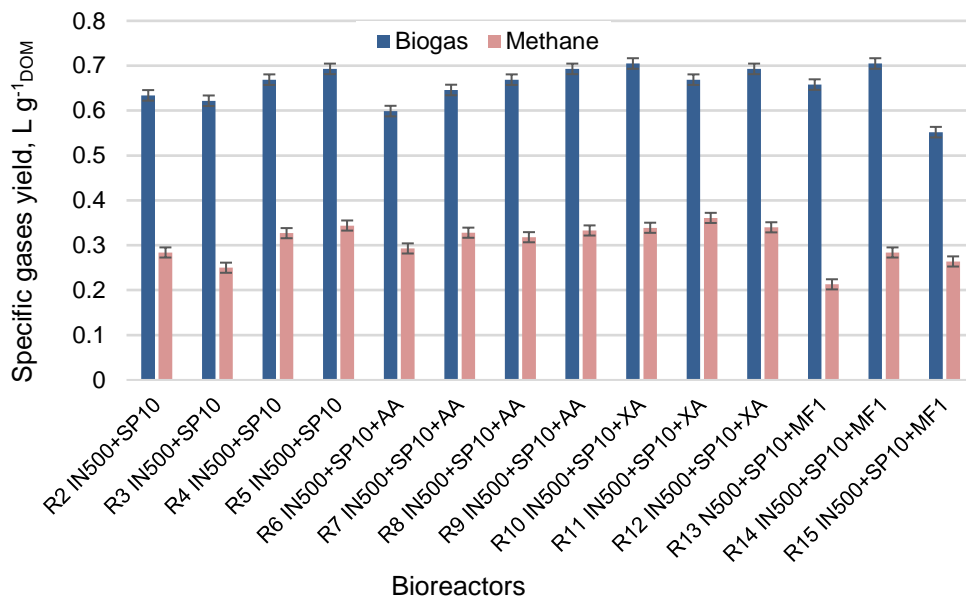


Figure 2. Specific biogas and methane gases volumes.

The relatively low average methane content in biogas can be explained by the fact that the amount of biogas was increased by the warm air and water vapour, which was released at the beginning of the process more than usual due to the use of cold inoculum. The second reason is that there is a lot of lignin and cellulose in the straw, so more CO₂ is formed. Addition of enzymes methane content increased, but MF1 decreased. MF1 contributed more to the release of CO₂.

CONCLUSIONS

The average specific methane yield from wheat straw pellets biomass was 0.301 L g⁻¹DOM. The result is good, similar that obtainable from maize silage. The average specific methane yield from straw pellets is better than from manure, but expectations in improving of high methane production were not met. The addition of alpha amylase increased the specific methane yield 5.65%.

The addition of xylanase increased the specific methane yield 15.28%. It is more advantageous to use this enzyme.

The addition of Metaferm + Melafen decreased the specific methane yield 15.57%. Using these biocatalysts (mixture 1:1) for wheat straw biomass cannot be economically. Such level of methane yield in Latvian conditions do not justify the application costs of Metaferm.

The results of the study show that wheat straw pellets can be used as raw materials for the production of methane. Addition of both enzymes improved methane yield.

In future studies, it would be desirable to clarify the effect of different pre-treatment (treatment with acids, bases, and grinding degree) methods on the anaerobic fermentation of investigated biomass.

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Potential social lifecycle impact analysis of bioenergy from household and market wastes in African cities

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Abstract. Bioenergy is touted as a viable source of stable and affordable energy in a number of remote sub-urban centres. This study evaluates the potential social lifecycle impacts of bioenergy production from household wastes and agri-wastes in some African cities. The assessment considered the use of rotten and unsold fruits, vegetables and other related agri-wastes from central open markets in Lagos and Johannesburg as case studies. The 2009 UNEP/SETAC's social lifecycle assessment (sLCA) guidelines and the associated sLCA methodological sheets are used to evaluate the potential social impacts of bioenergy production from agri-waste on operators/workers, the consumers, the value chain, and the local community. Preliminary results showed that it will provide a lot of benefits such as alternative employment opportunities, improved profits for small businesses, waste minimization, cleaner environment and improved communal health. It will also lead to improvement in energy supply, and alleviation of poverty. However, care has to be taken to protect the bio-digestion facility's neighbourhood from unpleasant odour, rodents and other organisms that may attempt to feed on the rotting agri-waste. The outcome of this study provides an insight to the necessity for the development of appropriate bioenergy policy/regulation and for the need to take preemptive steps to eliminate/minimize potential negative consequences of bioenergy production on the stakeholders.

Key words: bioenergy, household wastes, market wastes, social impact assessment, social lifecycle assessment (sLCA).

List of acronyms used:

GRCC – Grand Rapids Community College;
LCC – Lifecycle costing;
LCI – Lifecycle Inventor;
LCIA – Lifecycle Impact Assessment;
LCIO – Lifecycle Initiative Organisation;
NWIBP – Net Wellness Impact of the Bioenergy Production System;
PSI – Puget Sound Institute;
SiMSAW – A multi-level Social Impacts Aggregation Simple Additive Weighting;
UNEP – United Nations Environment Programme;
SETAC – Society of Environmental Toxicology and Chemistry;
sLCA – Social Lifecycle Assessment.

INTRODUCTION

The choice of energy supply sources is a major factor affecting our environmental sustainability. There are ongoing global efforts aimed at weaning people and organizations from dependence on fossil fuel and transitioning them to renewable energy sources. Bioenergy is widely recognized as a renewable form of energy. It has enormous potential to solving energy problems in developing countries that are dependent on fossil fuel and/or experiencing erratic electric energy supply. While significant progress has been made in many regions, Africa needs to catch up with the tempo of the ongoing changes. Currently, majority of rural dwellers in Africa, especially Nigeria depends on fuel wood/firewood while the urban dwellers depend on Kerosene (a derivative from fossil fuel) (Agunbiade, 2014). There is a need for changes in the choice of energy sources. There is a very high potential for sustainable bioenergy production from agri-industrial wastes, biodegradable municipal waste, food wastes and agri-wastes in many countries of Africa. For example, a study on the characterization of domestic and market wastes in Lagos metropolis by Oyelola & Babatunde (2008) found the putrescible fraction of the domestic waste to be 68.16% while the putrescible fraction of market waste was 68.98%. In another study, Agunbiade (2014) reported that Nigeria generate 39.1 million tonnes of Fuel wood, 11.24 million tonnes of agri-waste, 4.08 million tonnes of municipal waste, and 1.8 million tonnes of sawdust annually. In Nigeria, a lot of foods such as bananas, tomatoes, pineapples, oranges, pawpaw, mangoes and all manner of produce perish after few days of harvest due to inadequate storage capacity. Francis (2016) estimated the worth of the food waste in Nigeria to be about \$ 750 billion yearly. This incredibly large amount of food wastes in Nigeria could be a valuable source of feedstock for bioenergy generation. Quoting Danfoss, Francis (2016) stated that there is 80% food wastage in Nigeria as opposed to 33% wastage worldwide. He also hinted that 1.9 tonnes of CO₂ is emitted for every tonne of food waste generated. In the same vein, Bakare (2018) hinted that Lagos State Nigeria, with a population of over 21 million and per capita waste generation of 0.5 kg per day, generates more than 10,000 tonnes of urban waste every day. The same trend is observed in many other African countries, as Okot-Okumu (2012) revealed that 65% – 77.2% of solid waste generated in many of the East African cities is biowaste. All these are pointers to the viability of bioenergy facilities in many African cities. In addition, bioenergy has great potential to create employment, boost the economy and improve citizens' standard of living. According to Agunbiade (2014), 'Lagos state government is looking into ways of converting saw dust generated from its many saw milling plants into energy'. Realizing the potential benefits of harnessing this enormous bioenergy resource, the State government is investigating the possibility of developing bio-energy and other renewable sources for its development projects (Dunmade, 2013a, Obasiohia, 2014; Dunmade, 2016 and 2017a; Den, 2017; Tribune, 2017).

Despite all the aforementioned and other potential benefits of developing bioenergy facilities in cities with enormous bio-wastes in Africa, development of bioenergy facility have both perceptible and non-apparent social, economic and environmental implications. At a global level, most of the studies on bioenergy were focused on the technical functionality/ performance of bioenergy systems. There are only few studies on socio-economic aspects of bioenergy (Buchholz et al., 2007; Luchner et al., 2012; Dale, 2013; Segon & Domac, n.d.). Assessment of social impacts of bioenergy systems

from lifecycle perspective has not received much attention. The significance of this study therefore stems from the fact that although there are several studies on the assessment of bioenergy potentials and utilization in Africa in particular, no research report was found on the social lifecycle impact assessment of bioenergy systems in Africa (Ackom et al., 2013; Okello et al., 2013; Simonyan & Fasina, 2013; Agunbiade, 2014; Mohammed et al., 2015; Shane et al., 2016 and Arogundade, 2018). And there is a need to fully understand the pattern and effects of bioenergy production systems on various stakeholders. Hence, there is a gap regarding the social sustainability assessment of bioenergy production systems. This is particularly true about the potential social impacts evaluation of bioenergy development in Africa from a lifecycle perspective. This study is therefore focused on the social lifecycle impacts assessment of bioenergy facilities from a Nigerian context (Dunmade, 2012 and 2013a).

Social Sustainability

There are questions and several explanations on what sustainability is, on what social sustainability is and what is involved. Sutton (2000) declared that sustainability is about maintaining something. And various discussions since 1987 have mainly focused on three pillars of sustainability, namely environmental, economic and social sustainability. While environmental and economic sustainability are a bit easier to define and measure, social sustainability is not only more difficult to define and measure but it has not received as much attention as the other two (GRI, 2000; Barron & Gauntlet, 2002). Mckenzie (2004) defined social sustainability as a life-enhancing condition within communities, and a process within communities that can achieve that condition. He further explained that 'Social sustainability occurs when the formal and informal processes, systems, structures and relationships actively support the capacity of current and future generations to create healthy and liveable communities. Social sustainability refers to those social resources and processes that foster good quality of life/well-being now and in the future. It includes 'social homogeneity, equitable incomes and access to goods, services and employment'. It also includes inter and intra-generational equity, the distribution of power and resources, employment, education, health, the provision of basic infrastructure and services, freedom, justice, access to influential decision-making fora and general 'capacity-building (Littig & Grießler, 2005; Vallance et al., 2011)

MATERIALS AND METHODS

Social and socio-economic lifecycle assessment (sLCA) methodology was used to evaluate the potential social impacts of bioenergy production from household wastes and agri-wastes in some African cities. The lifecycle methodology was used because it is robust tool for evaluating various impacts of products, processes and activities from cradle to grave. The methodology facilitates an examination of the social and socio-economic aspects of products and their potential positive and negative impacts along their life cycle encompassing extraction and processing of raw materials; manufacturing; distribution; use; re-use; maintenance; recycling; and final disposal (UNEP/SETAC, 2009). sLCA technique is important for this kind of evaluation because it complements environmental LCA and Life Cycle Costing. As a toolbox, the three techniques facilitate full assessment of goods and services within the context of sustainable development.

Although UNEP and SETAC have developed guidelines for sLCA, it is still a work in progress as a socio-economic assessment tool (Jorgensen et al., 2010; Norris, 2014; Dubois-Iorgulescu, 2018; Grubert, 2018). It is progressively gaining widespread utilization for social impacts the assessment of products, systems and activities. Some of the areas of its previous applications include social impact evaluation of urban sugar production in Brazil (Du et al., 2019); an Irish dairy farm (Chen & Holden, 2017); comparison of building materials in Iran (Hosseniniyou et al., 2014), comparison of domestic water reuse alternatives (Opher, Shapira & Friedler, 2018).

The UNEP/SETAC's sLCA guidelines and the associated sLCA methodological sheets is a framework that has to be adapted to suit specific application. The framework was adapted for social impacts assessment of bioenergy industry and the African context by incorporating features such as metrics for evaluating human wellbeing in the aforementioned context. Probability of occurrence of articulated social indicators of human wellbeing and severity of their impacts are other features incorporated for the evaluation of social impacts of bioenergy industry in Africa. The aforementioned features were introduced and implemented at the lifecycle inventory analysis phase. Results obtained at the lifecycle inventory (LCI) stage was aggregated at the lifecycle impact assessment (LCIA) stage to identify hot spots along the product/system value chain. The aim was to determine the stakeholder category or categories that is/are affected and how they were affected. The conversion of linguistic qualitative ratings of probability and severity of each social indicator to numerical values and the aggregation of social indicators' impacts into groups were implemented by using SiMSAW model. Details on how SiMSAW method is used was discussed later in this paper. The compilation into various impact categories was done for each stakeholder groups assessed along the value chain. Results obtained is then diagrammatically illustrated/displayed for comparison of impacts across the stakeholders' groups. Details on the goal and scope of this study is discussed in the next section. This is followed by discussion on the lifecycle inventory (LCI). Lifecycle impact results was discussed after LCI. Interpretation of results obtained at LCI and LCIA stage were discussed in the following section before conclusion drawn from the study were finally explained.

Goal and scope definition

A. Goal of the study

According to Dunmade (2013a), the goal of a lifecycle assessment should specify the intended application, objectives of the study, and intended audience. The goal of this study therefore is to provide awareness of the potential social consequences of bioenergy production from domestic and market wastes, so that policy makers and other stakeholders can make informed decisions relating to the associated socio-economic issues.

To meet the goal of this research, the following questions will be answered:

- What are the appropriate social criteria that should be used to assess the social sustainability of household and market waste based bioenergy systems?
- What are the potential social sustainability hotspots in household and market waste based bioenergy systems?
- What are the areas of possible improvements in the sustainability of household and market waste based bioenergy systems?

B. Scope of the study

At this stage of the study, we determined the function of the system, its functional unit, the system boundaries, data averaging, limitations and exclusions (Dreyer, 2009; Dunmade, 2013b and 2015; Dunmade et al., 2016; Dunmade, 2017b; and Dunmade et al., 2018). We also identified affected stakeholder groups, impact categories/ impact measuring criteria, and social indicators to be included in the analysis based on the goal of the study. Furthermore, we articulated the metric for scoring the performance of the bioenergy system in preparation for the lifecycle inventory.

B.1 Household/Market waste based bioenergy production system's lifecycle

The bioenergy production system's lifecycle (illustrated in Fig. 1) consists of gathering and preparation of household/market wastes into a usable form as feedstock, feedstock conversion to value added commodities/ products, and the use of the produced biogas for electric energy production.

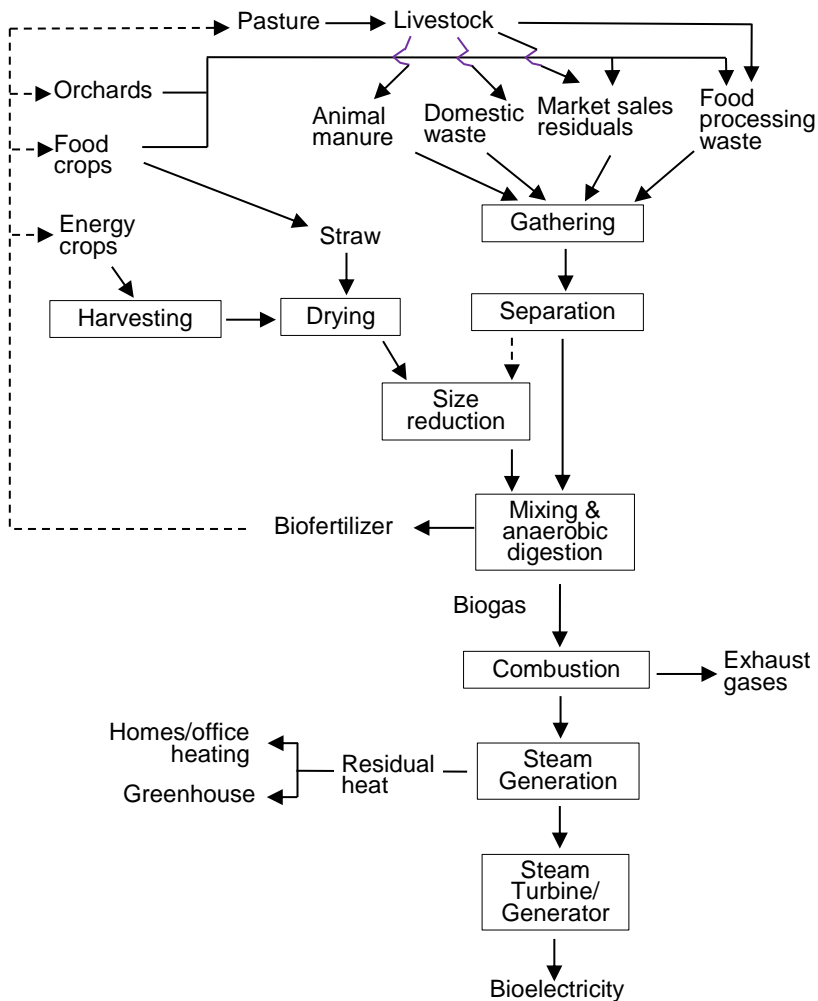


Figure 1. Household and market wastes based bioenergy production system's lifecycle.

Proper planning would involve on-site separation of the biodegradable fraction from other components of household and market wastes. On-site separation of the wastes that would be used as feedstock would be advantageous as it would ensure purity of the feedstock and ensure recovery of higher percentage of the feedstock than if the separation is done after commingling the wastes. The waste streams under consideration for this study consists of fruits, vegetables, animal manure and straw. Straw may need to be dried and subjected to dry milling to facilitate accelerated digestion. The anaerobic digester facility under study is fitted with stirring device for effective mixing to enhance biodigestion and to improve biogas yield. It also has electric heating device in case it is necessary to initialize the anaerobic digestion process. In addition, it has avenues for addition of fresh feedstock and for withdrawal of digested stock. The withdrawn old feedstock from the biodigester is then cured and eventually applied as fertilizer to grow crops or pasture. Produced biogas is then subjected to combustion to produce steam needed to drive steam turbine coupled to a generator that produces electric power. Excess biogas may be bottled for home cooking and various industrial uses.

B.2 Function and functional unit

The function of the bioenergy production system is defined in this study as the production of clean renewable energy products in a socially sustainable manner for homes, institutional, commercial and industrial use. The functional unit for measuring social impact of the bioenergy production system is defined in terms of the GRCC's seven categories of human wellness. The proposed functional unit is the 'net wellness of the impact of the bioenergy production system on each stakeholder category and customers' satisfaction resulting/derived from the product delivered' (NWIBPS). This metric was chosen because according to (Ashton & Jones, 2013), 'everyone around the world, regardless of geography, age, culture, religion or political environment, aspires to live well.' Thus, there is a need to measure how the bioenergy production systems affect various stakeholders within the circle of the system's influence.

B.2.1 Human Wellness

Human well-being includes many aspects of our everyday lives. Material well-being, relationships with family and friends, and emotional and physical health are among the components of human well-being. It also includes work and recreation, how one feels about one's community, and personal safety. Income level, individual's thoughts and feelings about how well they are doing in life, contentment with material possessions and having relationships that enable them to achieve their goals are other determinants of human wellbeing. In other words, human wellbeing is about quality of life, welfare, living well, life satisfaction, prosperity, needs fulfillment, empowerment, and happiness (McGillivray & Clarke, 2008; Dunmade et al., 2018; PSI, 2018). GRCC (2018) explained wellness as 'a full integration of physical, mental and spiritual well-being that leads to quality of life' and 'neglecting anyone dimension for a length of time has adverse effects on overall health.' This study adopted/adapted the principles of the seven dimensions of wellness described by GRCC (2018) for the evaluation of the social impacts of the bioenergy production facility.

B.3 System boundaries and exclusions

This is a cradle-to-grave sLCA involving an evaluation of household and market agri-waste feedstock gathering and associated logistics. It also include an assessment of a bioenergy facility's operations management for the production of biogas and bioelectricity. Other types of feedstock, bioenergy systems' development, bioenergy products' utilization except biogas utilization for electricity generation, and bioenergy facility's end-of-life management were not included in this study.

Lifecycle inventory (LCI)

This is the stage where requisite data for social impact assessment is collated and compiled in appropriate format for further analysis. LCI compilation for this study was in accordance with the method used by Dunmade et al., 2018. This study involved the use of locational/site specific data relating to Lagos metropolitan area. Relevant secondary data gathered from government websites, newspapers and publicly accessible corporate reports were also used where site specific data were not obtainable. The use of UNEP/SETAC methodological sheets based data collection approach focused on how bioenergy production system affect human wellbeing. The data collected was then processed in a spreadsheet format for the next stage of the study, which is social lifecycle impact assessment of the process. Tables 1 and 3 are samples of lifecycle inventory results of the bioenergy production system. They showed some of the social sustainability impact assessment criteria as they affect various stakeholder categories under consideration (Fan et al., 2015; Dunmade et al., 2016; Dunmade, 2017b; Dunmade et al., 2018).

Social lifecycle impact assessment (sLCIA)

This sLCIA study evaluated the net social effect of all/various stages of the bioenergy production system on stakeholders after considering all possible positive and negative consequences. The impact assessment process is modelled in similar pattern to the (environmental) lifecycle impact assessment. This consists of impact categories definition, classification, characterization, normalization, grouping and weighting. The adaptation of the eLCIA process for this sLCA study is as discussed below.

A. Selection of impact categories and classification

The choice of relevant impact categories (i.e. social indicators) in this sLCA is context dependent and were based on the local context (Emmanuel & Ajide, 2005; Mathe, 2014). The origin of the selected social indicators include social sustainability indicators for bioenergy developed by Oak Ridge National Laboratory and reported by Dale (2013) and Luchner et al. (2012); socio-economic sustainability issues articulated in Segon and Domac (n.d.), Johannesburg and Lagos socio-cultural observations. The classification of the social indicators was largely based on the sLCA guidelines published in 2009 by UNEP/SETAC. The guideline identified five groups of stakeholders. The focus of this study is the 'bioenergy facility workers, the neighbouring community, and the bioenergy value chain. The sLCA classification step in this study involved the mapping of relevant bioenergy systems socioeconomic impact indicators on the wellbeing of the three categories of stakeholders. The selection of impact categories and social indicators is based on (1) the principles of UNEP/SETAC methodological sheets; (2) currently relevant human wellbeing indicators of household

and market waste management practices in Nigeria, (3) projected bioenergy facility maintenance attitude by the workers, and (4) on the scope of the study. The selected social indicators are defined by a set of semi-quantitative data (Dunmade, 2001, 2012 and 2013a). Social aspects of sustainable bioenergy, according to Dale (2013) involve ‘preserving livelihoods and affordable access to nutritious food; guaranteeing the reliability of energy supply; and ensuring the safety of people, facilities, and regions. They also include using open and transparent participatory processes that actively engage stakeholders, establish obligations to respect human rights, and emplace a long-term sustainability plan with periodic monitoring.’ Social aspects of bioenergy systems, according to Segon and Domac (n.d.) can be divided into two categories, namely: (i) those that relates with standard of living, and (ii) those that contribute to increased social cohesion and stability. Standard of living in this case was related to household income, education, surrounding environment, and health care while social cohesion and stability was defined in terms of peace and communal relationship, employment, rural population stability, infrastructure and support for related industries.

The social impact assessment is focused on household and market waste based feedstock, bioenergy conversion technology operation, and the management of biogas’ utilization for electricity generation. On the positive side, development and operation of bioenergy facility or facilities in Lagos metropolis would significantly improve environmental cleanliness. Current approach to waste management mainly involves scavenging for valuables from the waste piles by private individuals and open air burning. There were incidences of explosions and plume of air emission enveloping large areas of Lagos. Such uncontrolled burnings has led to loss of properties such as public transit vehicles, houses and offices. Diversion of the municipal wastes for bioenergy production would eliminate such incidences and provide value-added commodities. It would also lead to cleaner community, improve public health, induce investment in associated businesses, create jobs, improve household income, improve energy supply, reduce social vices and crimes, enhance communal cohesion and improve government revenue. Improved revenue would ultimately make funds available for infrastructure improvement and lead to overall improved standard of living. Improved infrastructure and stable energy supply would lead to increased productivity and boost the regional economy.

Bioenergy facilities siting would necessarily have to be in the more rural areas of Lagos as the megacity is already congested. Similar suggestion is for the siting of bioenergy facility for Johannesburg. Locating such facilities in neighbouring rural areas would reduce the current tempo of high rural-urban migration to Johannesburg/Lagos, improve rural development and rural employment, and promote population stability. It would also help rural businesses to flourish and lead to regional growth. In addition, locating the bioenergy facility in the rural areas would prevent worsening the current traffic lock jam in the Lagos metropolis, lead to income and wealth creation in the adjoining rural communities, boost energy supply in the rural area, attract related industries to rural areas and even out developments in Lagos state (Haberl et al., 2011; Dunmade, 2013b and 2014; EPA, 2014; Mokraoui, n.d.; Van den Braak et al., 2016).

The main negative impact is in relation to maintenance of the bioenergy energy facilities and the related logistic systems. Inadequate maintenance could result in widespread unpleasant odour in the communities surrounding the bioenergy facility and its feedstock storage facilities. A breakdown of the facility operation could result in

spillover of the feedstock storage facilities. Improper management of the feedstock storage facilities could lead to infestation of the facilities by vermin. This could have serious implications for public health and lead to many other social-political problems.

B. Characterization and normalization

Characterization involves converting the social information obtained into interpretable indicators of a list of impacts. This study adopted a quantitative approach to characterization because it would enable us to compare the results obtained from this study with future studies. Consequently, we will be able to identify improvements that had occurred over a time period. Table 1 shows the classification of the social indicators into the various human wellness impact categories, Table 2a consists of the five probability/likelihood of indicators’ occurrence ratings, while Table 2b consists of the ratings for evaluating the extent to which the indicator would affect human wellbeing. Tables 1, 2a and 2b were used concurrently for the classification, characterization and normalization steps in sLCIA process. Column 1 and 2 of Table 1 shows the classification of the social indicators into the various human wellness impact categories. Table 2a was used to assess the probability of occurrence of each social indicator highlighted in Table 1 while Table 2(b) was used to evaluate the extent to which the indicator affected the impact category to which the indicator was mapped. The outcomes of the probability of occurrence and extent of impact ratings for each positive social indicators are shown in columns 3 and 4 of Table 1 while columns 5 and 6 of Table 1 showed the ratings for negative social indicators.

Table 1. Articulation and classification of the bioenergy production system’s social indicators into human wellness impact categories for workers’ category

Criteria	Indicator	Positives		Negatives	
		Probab.	Severity	Probab.	Severity
Economic wellbeing	Employment opportunities	Yes	EH		
	Career growth opportunities	Yes	EH		
	Household income	Yes	EH/VH		
	Food price volatility	May be	AV		
	Labour mobility	Yes	AV		
Physical wellbeing	Occupational injury			Yes	H/AV
	Risk of catastrophe			May be	AV
	Health	Yes	H/VH		
Emotional wellbeing	Socialization	Yes	H		
	Occupational injury			May be	H/AV
Intellectual wellbeing	Training opportunities	May be	H		
Social wellbeing	Socialization	May be	VH		
	Self-reliance and economic independence	May be	AV		
	Public acceptance/opinion	Yes	AV		
	Risk of catastrophe			May be	H/AV
Spiritual/cultural wellbeing	Socialization	May be	H		
Environmental wellbeing	Exposure to pollution			Yes	EH

B1. The sLCIA Calculation

Potential social lifecycle impacts of the bioenergy production system on each stakeholder group was assessed using the SIMSAW model. The first of the three steps simple additive weighting model involves multiplying the numerical values of the five probability ratings in Table 2a with the numerical seven severity of impact ratings in Table 2b that are corresponding to the linguistic scoring in Table 1. The score of each human wellness impact category is calculated by summing up the products of affecting social indicators' probability and extent of impact ratings as shown in Eq. 1. The overall (net) social impact of the bioenergy production system on each stakeholder group (i.e. workers, neighbouring community, the value chain, etc.) is the sum of the scores of the human wellness impact categories. Importance weights may be introduced by stakeholders in a participatory setting but importance weights were not used in this study. The overall social impact (score) of the bioenergy production system was calculated by adding all relevant stakeholders' scores together. According to Dunmade et al. (2018), 'the normalization at subcategories level becomes necessary to avoid certain subcategories dominating the final result.'

Table 2a. Probability of indicator occurrence

Probability	Rating
No (impossible)	0
Unlikely	2.5
May be	5
Likely	7.5
Yes (certain)	10

Table 2b. Severity of indicator effect

Severity	Rating
Extremely high (EH)	10
Very high (VH)	8.34
High (H)	6.67
Average/Moderate (AV)	5
Low (L)	3.34
Very low (VL)	1.67
Non-existent (N)	0

SiMSAW Method

SiMSAW model is a multiple levels simple additive valuation model developed by Dunmade et al. (2016) for aggregation of social indicators' impacts. The levels of aggregation of impacts could be two, three or four. This example used three levels of impacts' aggregation to illustrate the method for workers stakeholder group. A three levels of aggregation is done as follow:

1st level compilation: This involves converting the qualitative/linguistic LCI data in Table 1 into numerical values by multiplying the corresponding probability of occurrence in Table 2a with corresponding severity of the social indicator's rating in Table 2b. Results obtained for positive impact and negative impact of the indicator are then added together to obtain the net impact of the indicator. Steps taken at this point can be represented with Eq. 1 shown below.

Net social impact indicator score,

$$J_j = [P_j(d_j)]_{+ve} + [-P_j(d_j)]_{-ve} \quad (1)$$

where P_j – probability of the indicator's occurrence; d_j – severity of the indicator's effect on the stakeholder group S under the impact category K.

For example, looking at Table 1 and considering the occupational injury (a social indicator under physical impact category), the indicator has only negative impact. It does not have a positive component.

The indicator's numerical score,

$$J_j = [-P_j(d_j)]_{-ve} = -10(5.84) = -58.4 = -0.584 \quad (2)$$

The normalized value of -0.584 was obtained by dividing -58.4 with 100.

2nd level aggregation: Net impacts of social indicators in each impact category is summed up at this point. The total is then divided by the number of indicators in the impact category. The normalized value of impact category K can be mathematically represented as

$$K = \frac{1}{m} \sum_{j=1}^m J_j \quad (3)$$

where J_j – the net impact value of each social indicator under impact category K ; m – number of social indicators considered under impact category K .

For example, continuing with the physical impact category in Table 1, substituting the net values of the social indicators under the physical well-being impact category K under workers stakeholder group S into Eq. 2 would yield

$$K = 1/3[(-0.584) + (-0.25) + 0.75] = -0.028 \quad (4)$$

This is the physical impact value of the bioenergy facility on the workers (stakeholder group S). This level of aggregation appear to be the most useful level of all levels of aggregation because it enables the analyst to see how various social impact category affect different stakeholders along the value chain.

3rd level aggregation: At this point, each impact category is multiplied by its importance weight and the product of the impact categories are summed together and afterwards normalized with the sum of importance weights to obtain the overall social impact of the facility/system on a stakeholder group. The Total social impact value for stakeholder group S can be mathematically represented as

$$S_k = \frac{\sum_{i=1}^n w_i K_i}{\sum_{i=1}^n w_i} \quad (5)$$

where K – normalized impact category i value; w – importance weight of the impact category i ; n – number of social impact categories considered.

For this case study, all the impact categories were considered to be of equal importance. As a result, Eq. 5 reduces to

$$S_k = \sum_{i=1}^n K_i = K_1 + K_2 + \dots + K_n \quad (6)$$

The overall social impact score of the bioenergy production system on workers,

$$S_k = 0.7334 + (-0.028) + 0.0625 + 0.3335 + 0.30225 + 0.375 + (-1) = 0.77865 \quad (7)$$

This process is repeated for each stakeholder group in the bioenergy production system value chain.

This level of aggregation provides a summary of social impact of a facility on each stakeholder group in the value chain.

4th level aggregation: This is the level when the overall facility performance across the value chain is assessed by summing up its performance for all stakeholder groups

together and normalizing it by dividing with the number of stakeholder groups evaluated. was assessed by compiling its normalized indicators scores at sub-category level. The normalization at each level is necessary to avoid some subcategories dominating the final result. The overall social impact value of a bioenergy facility T can be expressed as

$$T = S_1 + S_2 + \dots + S_r \tag{8}$$

where S_r – normalized impact value for stakeholder group r.

This 4th level of aggregation is only useful for comparative studies. That is when the study is to compare the overall social impacts of two or more facilities or when there are two or more options being considered for selection, or when the study is to be compared with a reference. Thus, the 4th level of aggregation was not performed for this study.

RESULTS AND DISCUSSION

Tables 1 is the qualitative result of the articulation, compilation, classification and evaluation of various social indicators affecting different dimensions of human wellbeing as it pertains to bioenergy production system on workers. Table 3 is the numerical conversion and levels 1 and 2 aggregation of the results in Table 1.

Table 3. Social impact indicators’ assessment for bioenergy production system on workers

Criteria	Indicator	Indicators' normalized scores	Total per criterion
Economic wellbeing	Employment opportunities	1	0.7334
	Career growth opportunities	1	
	Household income	0.917	
	Food price volatility	0.25	
	Labour mobility	0.5	
Physical wellbeing	Occupational injury	-0.584	-0.028
	Risk of catastrophe	-0.25	
	Health	0.75	
Emotional wellbeing	Socialization	0.417	0.0625
	Occupational injury	-0.292	
Intellectual wellbeing	Training opportunities	0.3335	0.3335
Social wellbeing	Socialization	0.417	0.30225
	Self-reliance and economic independence	0.25	
	Public acceptance/opinion	0.834	
	Risk of catastrophe	-0.292	
Spiritual/cultural wellbeing	Socialization	0.375	0.375
Environmental wellbeing	Exposure to pollution	-1	-1

The aggregation involved looking at Tables 2a and 2b to replace linguistic ratings in Table 1 and implementing SiMSAW aggregation levels 1 and 2. The same procedure was used to obtain social impacts of the bioenergy production system on the community and the value chain. The overall social impact score on workers is 0.78 while the overall social impact score for the community and the value chain are 1.99 and 2.18 respectively. Fig. 2a, 2b and 2c are diagrammatic illustrations of level 2 aggregation of the bioenergy facility’s social impacts on workers, neighbouring community and the value chain

while Fig. 3 shows the overall impact of the bioenergy facility on each of the three stakeholder groups under study.

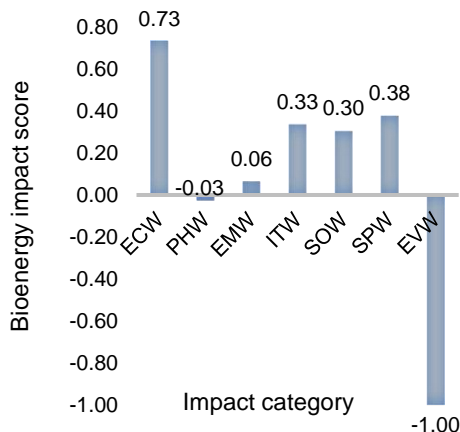


Figure 2a. The bioenergy production system’s social impacts on the workers: ECW – Economic wellbeing; PHW – Physical wellbeing; EMW – Emotional wellbeing; ITW – Intellectual wellbeing, SOW – Social wellbeing SPW – Spiritual/cultural wellbeing; EVW – Environmental wellbeing.

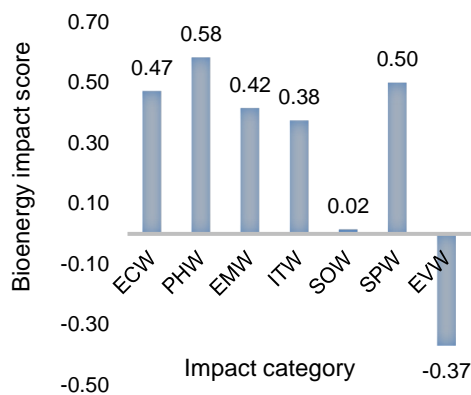


Figure 2b. The bioenergy production system’s social impacts on the neighbouring community: ECW – Economic wellbeing; PHW – Physical wellbeing; EMW –Emotional wellbeing; ITW – Intellectual wellbeing; SOW – Social wellbeing; SPW – Spiritual/cultural wellbeing; EVW – Environmental wellbeing.

The sLCA Interpretation

According to ISO 1440/44, we are to examine the results obtained from lifecycle inventory and lifecycle impact assessment for interpretation of the results. An examination of the lifecycle inventory results in Tables 1 & 3 and lifecycle impact assessment results illustrated in Fig. 2a–2c revealed economic well-being, social well-being, emotional well-being and environmental well-being as significant issues. Positive values results are indications of beneficial social impacts while results with negative values are indications of adverse effects of the bioenergy production system on the stakeholder.

Fig. 2a–2c showed that the bioenergy production system will negatively affect the environmental wellbeing of the three categories of stakeholders unless some definite steps are taken to prevent it.

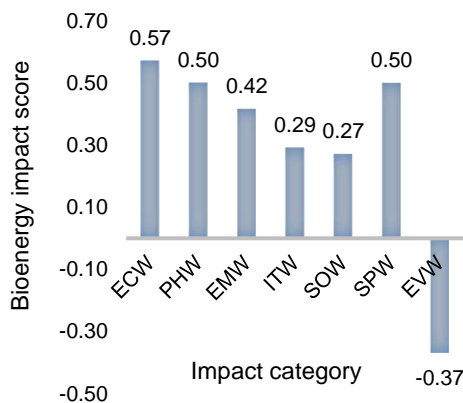


Figure 2c. The bioenergy production system’s social impacts on the value chain: ECW – Economic wellbeing; PHW – Physical wellbeing; EMW – Emotional wellbeing; ITW – Intellectual wellbeing; SOW – Social wellbeing; SPW – Spiritual/cultural wellbeing; EVW – Environmental wellbeing.

Comparison of the three figures showed that employees at the bioenergy facility will be the most negatively affected of the three groups. In addition, it could be seen that the bioenergy facility will positively affect the economic wellbeing of all the three stakeholder groups. Employee will feel the positive economic impact more than the other two groups.

Moreover, a look at the bioenergy facility's performance in other social dimensions showed that the employees will be negatively impacted physically and slightly positively impacted emotionally. Comparison of the facility's impacts on the three groups showed that apart from the environmental aspect, the neighbouring community and the value chain would benefit from the bioenergy facility in all social sustainability aspects. However, the value chain actors stand to benefit more than the neighbouring community. The implication of the overall social impact assessment score being positive is that the bioenergy production system is generally beneficial to these three categories of stakeholders in the (Lagos area) situation

under consideration. These results also showed that the value chain would benefit most by implementing the bioenergy production project. This will be followed by the community and then the employees.

Further examination of the results showed economic wellbeing as the best social benefit of the bioenergy production system on workers, the community and the value chain across the board while environmental wellbeing is the most challenging adversarial social impact of the bioenergy production system.

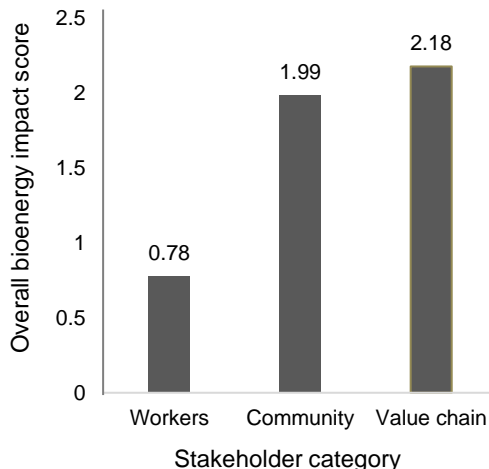


Figure 3. Overall social sustainability performance of the bioenergy system on the three categories of stakeholders.

CONCLUSIONS

This study revealed potential social impacts of bioenergy production systems in the African setting. It showed that establishment of bioenergy production systems in Africa will generally have positive social impacts on the employees, the community and the value chain. In addition it showed that environmental well-being is the only aspect of social concern that would need to be addressed to eliminate undesirable impacts of a bioenergy facility in Africa. Moreover, the study demonstrated that social lifecycle assessment is a useful technique for evaluating the social sustainability of system or an activity. The contribution of this study include its articulation of various social issues affecting bioenergy production system in a developing country. The study also provided a set of metrics for the assessment of various dimensions of human wellbeing as it pertains to bioenergy production system.

Results of this study provides some insight to social aspects of bioenergy production system that policy makers, investors and developers in that part of the world should pay close attention to, in order to eliminate or minimize unpleasant consequences of operating the bioenergy facility at the location. It is believed that the result of the study would spur responsible regulators to develop laws that would forestall the occurrence of the highlighted potential problems while promoting the positive impacts of the system. In addition, this study further increased the number of social sustainability studies carried out on products, processes and systems in Africa, especially Nigeria. This study would be a good reference for future sLCA studies on other products, processes and activities.

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Dynamics of work accidents incidence by severity, gender and lost workdays in Estonian agricultural sector and sub-sectors in 2008–2017

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Abstract. The risk of dying in a work-related accident is greatest for those employed in agricultural sector. The aim of this study is to analyse the dynamics of work accidents (WA) incidence by severity, gender and lost workdays in Estonian agriculture in 2008–2017. **Method:** The database of accidents in agriculture (2008–2017) was obtained from the Estonian Labour Inspectorate (ELI). Work accidents statistics is based on official reports of employers. **Results:** In total 1,683 non-fatal work accidents (NFA) was registered in the past decade, from which 1,235 (79.3%) were minor and 448 (20.7%) severe. In total 13 fatal work accidents (FA) have occurred, which accounts 0.8% of all the WAs ($n = 1,696$). Although the trends of minor and severe WA cases showed steady increase, the number of FA has remained low. A major part WAs (74%) was registered in farming and horticulture, with prevalence of minor accidents. A severe WA is likely to occur in forestry or very likely in fishery. The proportion of injured male and female was 51.8% and 48.2%, accordingly. In the farming and horticulture sector there were more accidents among women – of all the minor accidents ($n = 1,235$) 52.1% occurred to women. Of all the severe WAs ($n = 448$) 61.2% and all the fatal work accidents happened to male workers. About 2/3 of severe WAs caused sick leave 31–90 days and more. **Conclusion:** The number of WAs in Estonian agricultural sector show steady increase in the past decade. A major part of accidents is minor and mostly occurred in farming and horticulture, and more often with females. Severe and FAs more often occurred in forestry and fishery. Mostly the severe WAs cause long sick leave – a month or more. When to increase work accidents severity level by one step (from minor to severe), the sick leave is increasing significantly.

Key words: agriculture, injury, gender, occupational accident, severity, sick leave, work-related.

INTRODUCTION

Agriculture has traditionally been one of the most hazardous occupations for workers (Frank et al., 2004). An estimated 1.3 billion workers are engaged in agricultural production worldwide. This represents half of the total world labour force, and almost 60% of them are in developing countries (International..., 2011). Agricultural injuries are reported from all around the globe (Pickett et al., 1999; Myers et al., 2009; Yiha et al., 2010). The risk of dying in a work-related accident is greatest for those employed in agricultural sector (Thelin, 2002).

About 500 people die per year while working in agriculture, including about 100 in forestry. Moreover, there are about 150,000 reported non-fatal accidents at work in the EU agriculture, forestry and fishing sector incidence rates (IR) are generally higher than in other sectors (Thomson, 2016). For example, in 2015 the average IR of NFAs in EU-28 was 1,513 (NFAs reported in the framework of ESAW are accidents that imply at least four full calendar days of absence, they are serious accidents). If to compare economic sectors with each other, there are three sectors where the IR is higher than in agriculture (IR 1,894) – construction (IR 2,852), transportation and storage (IR 2,461) and administrative and support service activities (IR 2,274), while in all the other sectors remain lower with their IR. Even in the mining and quarrying sector the IR of NFA was 1,257 and in manufacturing sector the IR was 1,869. Moreover, on-farm accidents happen to a very wide range of ages, from the very young to the relatively old (Thomson, 2016). According to Eurostat serious problems actually lay in the ages under 25 (Non-fatal..., 2019) and also ages over 65 (Burholt & Dobbs 2012).

It is important that agricultural workers acknowledge the risks in different sub-sectors and activities. A study in Italy concluded that about 11% of agricultural workers do not consider their occupation dangerous, but in the same time they clearly perceive accident risks that cause serious injuries and long time sick leave, but not those risks that cause illness (Antonucci et al., 2012). A clear correlation was found between the attitudes of people who had received occupational safety and health (OSH) training – they considered their work more dangerous than those who had not been trained. It was also found in this study that some workers were very negatively minded of the hazards of the work environment, especially older workers who had not been trained in occupational safety. This suggests that such a critical socio-cultural attitude is difficult to change because it is an integral part of the attitudes and behavior of these employees (L'abbate et al., 2010). The second type of problem is the employees who have been trained but still do not behave as they have been taught (Stoneman et al., 2014). Changing these attitudes requires experts to be prepared to provide training that will help change attitudes from the grassroots level. However, these trainings should not be in the classical sense of training, which are often static and one-way communication from the teacher to the learner. Rather, training should be like a training program that gives the employee practical experience and develop decision-making skills (Marino et al., 2010; Cecchini et al., 2018). An emerging issue in developed country agriculture is the raising migrant working population and the difficulties in communicating during OSH mandatory training. Even though it is an issue in many European countries and also in USA but Baltic countries do not have so much migrants.

In general, WA statistics focus on non-fatal (NFA) and fatal work accidents (FAs), Eurostat has taken into account sick leave more than 3 working days due to WA. In terms of injury severity WAs are divided into three groups in Estonia: minor, severe and fatal. In other countries WAs are often divided into four or even more groups. FA are often analyzed separately from NFA. According to Estonian' Occupational Health and Safety Act 'an occupational accident which resulted in serious bodily injury to an employee or due to which an employee's life is endangered is classified as a serious occupational accident. These injuries and conditions are determined in 'Severe injury determination guide' (OHSA, 1999).

In general, if an occupational accident occurs, the employer will carry out an investigation which will establish the circumstances of the WA. The employer will

submit a report on the investigation results to the victim and the local office of the Labour Inspectorate. The report shall indicate the measures that have to be implemented by the employer to prevent a similar occupational accidents (Occupational..., 1999).

In a retrospective analysis that was made in Poland 3,791 adult patients with agriculture and forestry related injuries were analysed. To evaluate the severity of body injury, the Abbreviated Injury Scale AIS (1990 revision) was used and based on this scale an Injury Severity Score (ISS) was calculated and the results were: slight ($ISS \leq 3$) – 77.6%, moderate ($4 \leq ISS \leq 8$) – 16.9% and severe ($ISS \geq 9$) – 5.5% (Nogalski et al., 2007).

The previous studies show that in the agricultural sector there are usually more male workers and they therefore make more WAs (Solomon et al., 2007; Lower & Mitchell, 2017; Scott et al., 2017). But often the problem lies in female to whom a bigger part of accidents have happened compared to the percentage of female employees in this sector. In the study made in two states of USA from 2008 to 2010 women constituted 39.5% in Maine and 47.8% in New Hampshire of all agricultural NFA injuries ($p = 0.0002$). For these two states combined, almost half (43.8%) of those sustaining an agricultural injury were women. This percentage is significantly higher than the overall percentage of women (27.4%) in the agricultural workforce in these two states ($p < 0.0001$) (Scott et al., 2017).

In New South Wales in Australia between January of 2010 and June of 2014 a retrospective epidemiological examination was conducted of linked injury hospitalisation and mortality records. This study identified a total of 6,270 farm-related hospitalised injuries. These injuries involved a higher proportion of males (78.2%) than females (21.8%). Also, a higher proportion of males were injured during work activities compared to females (Lower & Mitchell, 2017).

On the other hand, a survey of NFA was carried out in British agriculture. This survey was restricted only to men for reasons of statistical efficiency, because the prevalence of paid work in agriculture is much higher in men than in women. Their findings cannot necessarily be extrapolated to female agricultural workers, whose occupational activities may differ substantially from those of their male counterparts. One of the most important results was that risks are particularly high in those who undertake forestry (Solomon et al., 2007).

Although NFA happen more with women compared to percentages of female employees, male workers are at higher risk of FAs. In the study in New Zealand in 1985–1994, from 159 cases of FA only 4.4% were female (Horsburgh et al., 2001).

The number of days where a NFA victim is unfit for work provides an indication on the severity of the injury (European..., 2008). According to the EU Labour Force Survey (Thomson 2016) in 2005, the average duration of absence from work (if over 3 days) was 43 days, compared to an all-sector figure of 35 days (32 in 1995), with only a few sectors (for example private household employment 53 days) having higher figures.

The aim of this study is to analyse the trends of WAs incidence by severity and gender in Estonian agriculture and its' sub-sectors (crop and animal production (CAP), forestry and fishery) in 2008–2017.

METHOD

The database of accidents in agriculture (2008–2017) was obtained from the Estonian Labour Inspectorate (ELI). WA statistics are based on official reports of

employers. Also, ELI investigates all lethal and a few severe WAs. Dynamics and trends of WAs including injury severity, gender and lost workdays are analysed in the present study. Injury severity is assessed by the doctor, who determines the injury severity by the ‘Severe injury determination guide’.

An accident at work is defined as ‘a discrete occurrence in the course of work which leads to physical or mental harm’. The data include only fatal and non-fatal accidents involving more than 3 calendar days of absence from work. If the accident does not lead to the death of the victim, it is called a ‘non-fatal’ (or ‘serious’) accident. A fatal accident at work is defined as an accident which leads to the death of a victim within one year of the accident (Accidents..., 2017).

General statistics has described as total numbers and incidence rate. The incidence rate indicates the relative importance of non-fatal or fatal accidents at work in the working population. Eurostat methodics includes NFA involving more than 3 calendar days of absence from work (Accidents..., 2017).

ELI registered all work accidents, including 0–3 days of absence, until the year 2018. The sample group consisted in 879 male and 817 female workers, in age 17–70 years. The length of work experience was 0–47 years. 62.9% of victims of WAs worked in micro- and small enterprises, 33.4% in medium and 3.7% in other. The results analysis was done using the Statistical Package for Social Sciences (SPSS25.0) and MS Excel software.

RESULTS

Estonian agricultural sector is divided into three sub-sectors: CAP (including hunting and service activities), forestry and fishery (including aquaculture). Based on the database 2008–2017, we can see, that 1,696 accidents were registered in the Estonian agricultural sector in the last decade. It constitutes 4% of all WAs registered in that period in Estonia ($n = 42,049$).

The highest WA rate 838 per 100,000 employees has shown in the CAP, where 1,465 people got injured, i.e. 86.4% of all activities in agricultural sector. In the forestry sector 197 (11.6%) and in fishery sector 34 (2.0%) employees were injured. The average incidence rate in past ten years was 308 in forestry and 300 per 100,000 employees in fishery.

The total number and incidence rate of WA in the agricultural sector have steadily been increasing over the past decade. Supposedly it is because employers and employees get more aware of the importance of registering the WA. And even if the total number of employees is decreasing, workers report more accidents. This also mean that in the past there has been underreporting of WAs. Compared to the total number of WA in all sectors of the economy, growth is modest. The main reasons of WAs are in Estonian agriculture are loss of control over an animal (usually cattle), falling and slipping, thirdly an attack of an animal.

In Fig. 1 we can see the dynamics of incidence rate in increase during the years in both agricultural and all economic sectors. If the incidence rate of occupational accidents in all economic sectors in 2009 was the lowest in the last decade ($n = 495$), then it was increased to the highest level of the decade ($n = 788$) by 2016 and it remained virtually unchanged in 2017 ($n = 787$) (Fig. 1.) The lowest WA rate in the agricultural sector was in 2012 – 543, and the highest in 2017 – 801 accidents per 100,000 employees. In

2008–2017, the average WA rate (per 100,000 employees) in agricultural sector was higher than in all areas of economy in total ($678 \pm \text{SD } 88.9$ and $672 \pm \text{SD } 97.4$ accordingly).

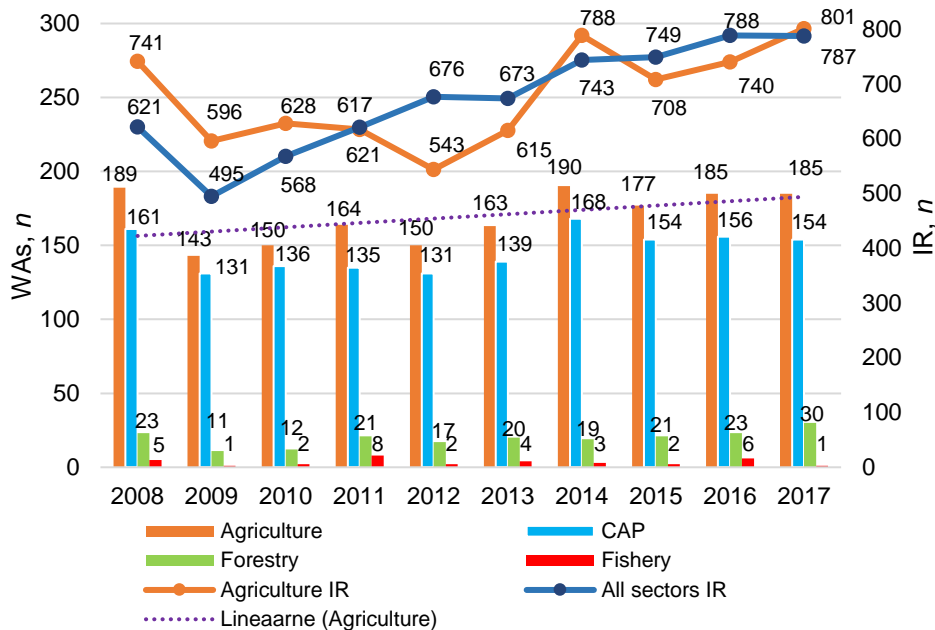


Figure 1. Dynamics of accidents at work in Estonian agriculture in 2008–2017 (absolute numbers and incidence rate per 100,000 employees).

In the past decade 1,683 NFA accidents have registered, from which 1,235 (79.3%) were minor and 448 (20.7%) severe. There were 13 FA, it counts 0.8% of all the WAs ($n = 1,696$). The dynamics of minor, severe and fatal accidents in the agricultural sector over the past decade is shown in Fig. 2.

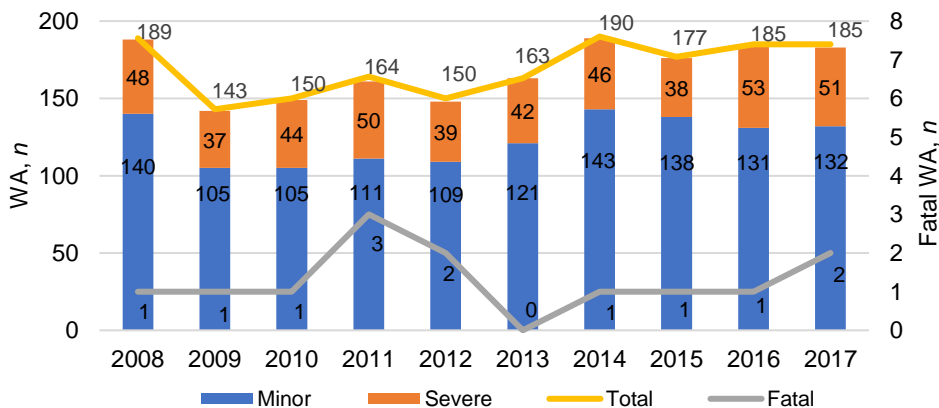


Figure 2. The dynamics by severity of WAs in agricultural sector in 2008–2017.

Fig. 2 describe the total numbers of WAs (minor, severe, fatal) in Estonian agricultural sector. We can see that there has been an increase in minor and severe WAs in the past decade, whereas the number of fatal accidents has remained low.

Table 1. Work accidents by severity – minor (M), severe (S) and fatal (F) in agriculture and it’s sub-sectors

Year	Crop and animal production				Forestry				Fishery				Agriculture all together			
	M	S	F	Total	M	S	F	Total	M	S	F	Total	M	S	F	Total
2008	123	37	1	161	14	9	0	23	3	2	0	5	140	48	1	189
2009	96	34	1	131	8	3	0	11	1	0	0	1	105	37	1	143
2010	93	42	1	136	10	2	0	12	2	0	0	2	105	44	1	150
2011	94	38	3	135	13	8	0	21	4	4	0	8	111	50	3	164
2012	98	33	0	131	11	4	2	17	0	2	0	2	109	39	2	150
2013	103	36	0	139	15	5	0	20	3	1	0	4	121	42	0	163
2014	133	34	1	168	10	9	0	19	0	3	0	3	143	46	1	190
2015	124	30	0	154	12	8	1	21	2	0	0	2	138	38	1	177
2016	110	46	0	156	18	4	1	23	3	3	0	6	131	53	1	185
2017	114	38	2	154	18	12	0	30	0	1	0	1	132	51	2	185
Total	1,088	368	9	1,465	129	64	4	197	18	16	0	34	1,235	448	13	1,696

Table 1 show that the most part of WAs (minor, severe and fatal) take place in the CAP – 1,465 (86.4%) employees have incurred into the accidents in this sub-sector. In forestry 197 (11.6%) and in fishery 34 (2.0%) employees have incurred into the accidents. The reason why CAP takes such a big part is due to the biggest part of employees who are working in this sub-sector. There are 69.9% of all the agricultural employees working in CAP, 25.7% in forestry and 4.4% in fishery sub-sector. But even if there are so much CAP workers, the portion they take from WAs is still too big. Therefore, we have to say, that CAP is the most dangerous sub-sector of agriculture in Estonia.

Of all the WAs ($n = 1,696$) the biggest part of minor accidents ($n = 1,088$; 88.1%) has taken place in CAP, 10.4% ($n = 129$) in forestry and 1.5% ($n = 18$) in fishery. Among the severe WAs, the distribution is as follows – 82.1% occurred in CAP, 14.3% in forestry and 13.6% in fishery. From all the FAs 69.2% ($n = 13$) have occurred in CAP and 30.8% in forestry.

Excluding for a moment all the FAs the ratio of the distribution of severity – minor WAs vs severe WAs, will be as follows – CAP 3:1 (74.3% and 25.1%), forestry 2:1 (65.5% and 32.5%) and in fishery almost 1:1 (52.9% and 47.1%). Compared to all economic sectors, where 79.0% of WAs were minor and 20.5% were severe, we can verify that in agriculture severe WAs occur more often. A particularly high probability (about 50%) for serious WA has detected in the fishery sector, however, no FAs have registered in this sub-sector.

The proportion of men and women who have been affected by WAs in the total sample was 51.8% M vs 48.2% F. In the CAP sub-sector there were more accidents among women (54.2% F vs 45.8% M) ($p = 0.0001$). According to Estonian Statistics female employees make 36.2% of all the employees in the CAP labour market (vs 63.8% M), which means that women make large poportion of WAs. In Estonia a big part of agriculture makes animal production and in this area, there are a lot of female workers, with whom WAs happen. In forestry sub-sector men make more WAs – 89.3% M vs

10.7% F ($p = 0.0001$). In fishery sector 94.1% of WAs have reported by the male employees. If in the CAP there were more female employees with whom WA happend but lower employment percentage compared to male workers, then in forestry and fishery it is vice versa. In forestry 12.5% and in fishery 13.6% of employees were female, accordingly.

In the forestry sub-sector 176 men and 21 women were injured in the past decade. The dynamics of WA incidence rate among the men showed steady increase in the observation period – there were injured 10 men in 2009 and 27 in 2017. In fishery 32 men and 2 women were injured. According to the ELI statistics there are 63.7% of WAs that happened to male employees in all economic sectors in Estonia. In the EU countries the number is 68.7% – therefore the incidence among men in forestry and fishery is enormously higher than in Estonia in general or also other EU countries.

When to compare minor, severe and fatal WAs among female and male employees, the results show that minor WAs happened more often to female workers. More than half (52.1%) of all the minor accidents ($n = 1,235$) were registered among women. Inversely, about two thirds (61.2%) of all the severe WAs ($n = 448$) and all (100%) the FAs ($n = 13$) have happened to male workers.

The dynamics of WAs in agricultural sector show three sharp increases of absolute numbers among the men in 2008, 2011 and 2014, and a sharp grow of female part in 2016, with overlapping F and M trendlines in 2017 (Fig. 3).

In Fig. 3 we can see that in some years female workers have reported more WAs than male with the highest number of cases ($n = 99$), and *vice versa*. At the same time WAs numbers are fluctuating quite rapidly among the men, showing the highest score ($n = 109$) in 2014.

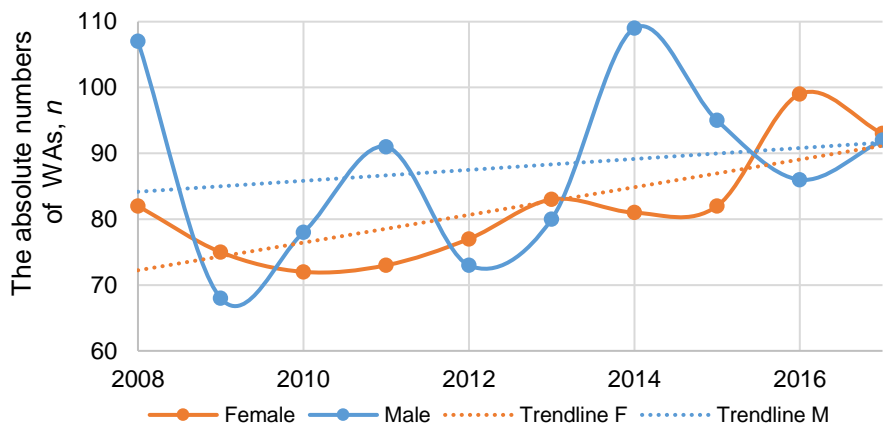


Figure 3. The dynamics and trendlines of WAs among female and male workers in the agricultural sector in 2008–2017.

From total of 179 FAs occurred in Estonia in 2008–2017 only 13 were registered in the agricultural sector, *i.e.* 7.3% of all FAs. Comparing this result with the EU – FAs accounted for 14.3% of the EU-28 in the agricultural sector, the share of FAs in Estonian agriculture is almost two times lower. Albeit, it can be confirmed that the prevalence of FAs in agriculture is twice as high as in all economic sectors in Estonia. When FAs

consists in 0.4% of total WAs in entire Estonian economic sector, in agricultural sector it was 0.8% – in the CAP 0.6% and in forestry 2.0%. It means that in forestry there is in average 5 times higher FA risk than in a whole economic sector in Estonia. To compare this number with EU-28, we can see that in 2015 FAs accounted for 0.2% or less of workplace accidents in most activities, but agricultural sector accounted 0.3%, being on the second place (after mining and quarrying with 0.7% of FAs).

Usually, when a WA has happened, the injured employee take sick leave, although our results show that 20.4% of all the WAs recorded in Estonian agriculture, the injured employee doesn't miss any working days ($n = 345$). Only 0.6% of victims have lost one to three working days. The reason is most likely wrong understanding related to the sickness benefits in Estonia. In case of injury arising from WA, the sickness benefit is paid at the rate of 100% from the 2nd day of release from work by the Health Insurance Fund in Estonia. But due to many WAs victims believe they are being funded like with the regular sick leave, they are trying to continue working after WA. The regular sick leave is as followed: in the first three days after sickness an employee does not receive benefit for health damage, in the fourth to eighth day, the benefit is paid by an employer; and from the ninth day and onwards the Health Insurance Fund pays it. The benefit is paid at the rate of 70% of the daily income.

In the Fig. 4 we can see that the biggest sick leave group is 31 to 90 days of absence with 25.6% of all the lost workdays, In this group 54.5% ($n = 237$) were minor and 45.5% ($n = 198$) were severe WAs. The second group is 15 to 30 days with 23.8%, from which major part – 85.1% minor and 14.9% severe WAs. The mean of all the sick leaves is 35.5 days when counted all sick leaves and 36.0 when counted sick leaves over 3 days. In Estonian agriculture all together the employees were 60,228 days absent from work due to WAs.

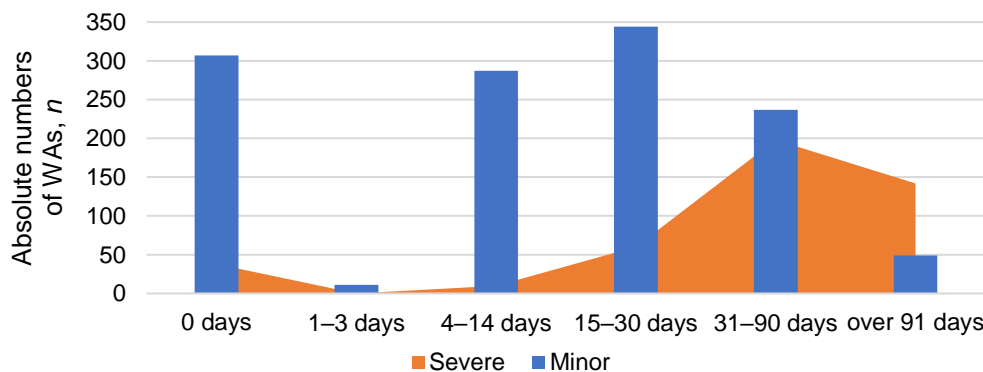


Figure 4. The absolute numbers of agricultural workers lost workdays by severity in Estonian agriculture.

Analysing sick leave by agricultural sub-sectors and severity of injury, we can see, that about 2/3 of severe WAs take more than one month and longer sick leave in all the sub-sectors. In the CAP, where in total 1,456 NFAs were registered in the past decade, the biggest sick leave group for severe WAs was 31 to 90 days of absence with 40%, the second group was 91 and more days with 31%. In the forestry, from the total of 193 NFAs, the percentages for more than 30 days and over 90 days of work absence for

severe WAs were quite similar to CAP. In the fishery, from the 34 of NFA cases, sick leave for severe WAs showed the similar picture. In all the sub-sectors, sick leave for minor WAs took most often 4 to 30 days.

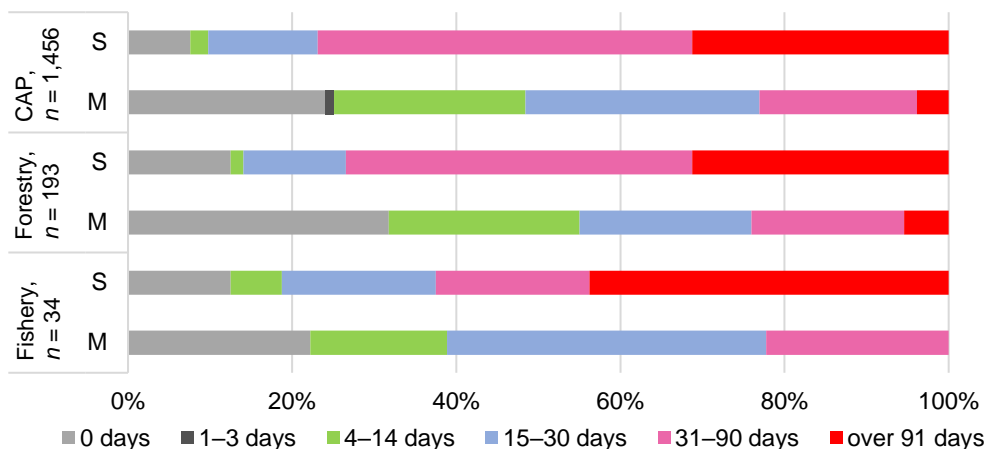


Figure 5. The shares of duration of lost workdays by severity (S – severe WAs, M – minor WAs) in the agricultural sub-sectors.

In the Fig. 5 it is shown that in the fishing sector there are much more severe WAs that cause an employee to take more than 90 days of sick leave – over 40% of all the severe WAs, while in the other two sectors the rate is lower. If to consider all the minor WAs, about 40% of sick leaves are less than two weeks of absence in fishery while in the other sectors it is about 50% or more.

We conducted a Poisson regression analysis which showed that when WA severity level is increasing by one step (from minor to severe), the days of sick leave are increasing 2.3 times. This formula does not work in cases of lost work days in accidents with lethal outcome, because no sick leave days are registered when fatal accident occurs, in spite sick leave can take days, months or even a year before death.

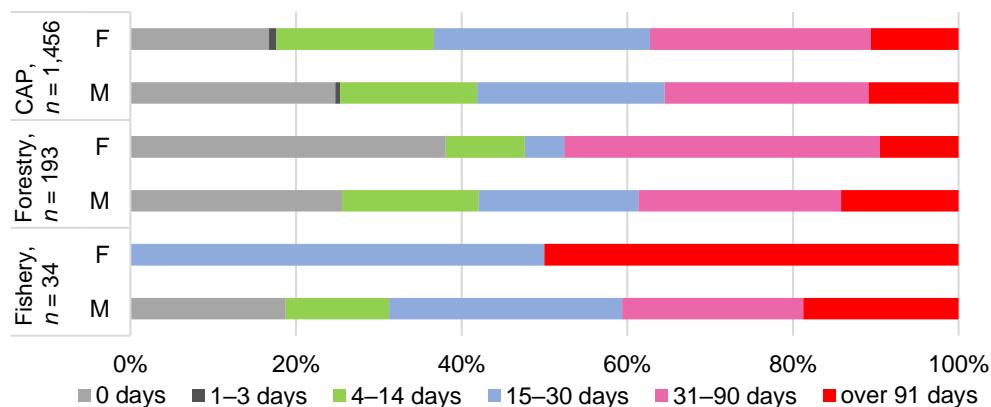


Figure 6. The shares of lost workdays by gender (F – female, M – male) in the agricultural sub-sectors in the past decade.

When to compare sick leave due to WA by gender, we can see that in general women make more minor WAs and they take longer sick leave, especially in fishery (Fig. 6). Men make more severe accidents, but often without taking sick leave. In Estonian agriculture sector all the FAs happened to male employees.

In the CAP sector there are more male employees who have never taken any sick leave days, when to compare with female employees. But in every other sick leave duration periods female employees outweigh male. In forestry there are much more female workers that have not reported sick leave compared to male. Approximately 40% of forestry and fishery male workers have taken sick leave that is longer than one month.

DISCUSSION

The risk of dying in a WA is greatest for those employed in agricultural sector. According to the EU Labour Force Survey, about 150,000 non-fatal and about 500 fatal WAs have reported in agriculture. Moreover, in the EU agricultural sector the WA incidence rates are generally higher than in other economic sectors (Thomson, 2016).

Similarly to EU statistics the prevalence of WAs in Estonian agricultural sector, in the past decade, is higher than in all economic sectors in total and in Estonia it shows a steady upward trend – there has been an increase in both minor and severe WAs. We can stress here, that severe WAs most often occur in agriculture, compared to all economic sectors in Estonia. In the fishery sector a probability for a serious accidents is particularly high, although the number of fatal accidents here has remained low. Due to the fact that in Estonia WAs by severity are divided into three groups (minor, severe and fatal) and the fact that all WAs are counted, not just the ones with 4 or more sick leave days, the comparison of WAs by severity and by the countries is not always correct.

From the Eurostat database we can compare the total numbers and the incidence rates of NFA and FA in EU countries agricultural sector and also in all economic sectors. In NFAs a particularly large increase in incidence rate was for agricultural sector comparing the years 2010 and 2016 (incidence rate in 2010 was 1,293 and in 2016 was 2,011). In Estonia there was a slight decrease in NFAs according to Eurostat in the same period of time (from 1,716 to 1,594), just like Ireland (from 1,160 to 1,035) and Sweden (from 693 to 549). These numbers show that Estonian NFAs incidence rate (IR 1,716) that was much higher than the EU average (IR 1,293) in 2010, has fallen and it was much lower in 2016 (IR 1,594). But if to compare Estonian WA statistics with many other countries, our incidence rates are still high (Non-fatal..., 2017).

Large increase in NFA was in Belgium (from 583 to 1,786), Spain (from 1,903 to 4,283), Latvia (from 85 to 314), Netherland (from 119 to 418), Portugal (from 961 to 1,819) and Finland (from 896 to 3,599) (Non-fatal..., 2017).

In Estonia, FA in agriculture accounted for more than one tenth of all FAs (14.3% in past ten years). In 2015, in EU-28, the incidence of FAs in agriculture was 5.7 per 100,000 persons employed – it is the fourth in line in the most dangerous sectors in the EU. In the period 2010–2016 the FA rate increased from 4.6 to 6.1 in agricultural sector, whereas a decrease of both NFA as FA took place in most activities in the observed period. According to Eurostat the incidence rate of FAs in Estonia in the period 2010–2016 has remained the same – 5.6. The numbers have decreased in Germany (from 2.7 to 2.3), Bulgaria (from 11.4 to 2.3), Italy (from 11 to 8.6), Slovakia (from 4.2 to 3.5),

Sweden (from 8.3 to 6.4) and United Kingdom (from 10.9 to 8.2). The incidence rate has largely increased in Denmark (from 8.2 to 15.8), Spain (from 2.3 to 7), France (from 0.4 to 14.4), Latvia (from 15.2 to 32.9) and Norway (from 8.1 to 24.3). Note that for some EU Member States some of these changes may be linked to changes in coverage of specific activities linked to the end of derogations or voluntary data collection. From these numbers we can allege, that as well as NFAs, also FAs incidence rates in Estonia are often smaller than in many other EU countries but it could be deceptive because in the last 20 to 30 years Estonian agricultural sector has dried up but the incidence rates seem to grow over time not decrease. There could be many reasons for that, for example the lack of knowledges, low risk perception and low safety culture in general in this sector. The under reporting of WA is apparent due to self-employed farmers have no duty to report WA and even their injuries are not compensated, because lack of Insurance Act on WA and occupational diseases in Estonia (Enn, 2018; Fatal..., 2019).

In the agricultural sector there are usually more male workers and based on research articles male workers therefore make more WAs (Solomon et al., 2007; Lower & Mitchell, 2017; Scott et al., 2017). And it is like that in Estonia. But in some countries (Denmark, Ireland, Estonia) female workers are making a bigger part of accidents if comparing the percentages of male and female employees (Non-fatal ..., 2017). In absolute numbers there are more male employees with whom WAs happened but in the CAP sector WA numbers show increase among female workers, taking almost half of all WAs in Estonian agriculture. The same trend is in characteristics among male workers in forestry and fishery (Enn, 2018).

Comparing minor, severe and fatal WAs among female and male employees the results show that minor WAs happened more often to female workers. Inversely about two thirds of all the severe WAs and all the FAs have happened to male workers. The Poisson regression analysis showed 2.3 times higher sick leave when WA severity level was increased from minor to severe (Enn, 2018).

The number of days where a NFA victim is unfit for work provides an indication on the severity of the injury. In 2005, the average duration of absence from work in agricultural sector in EU (if over 3 days) was 43 days, compared to an all-sector figure of 35 days (Thomson, 2016). In Estonian agriculture the average sick leave in the past decade was 36 when sick leaves over 3 days were counted. This shows that an average Estonian farmer is absent from work less time than the farmers in EU. The average number is lower due to the fact that fifth of all the workers in WAs did not take any sick leave days (Enn, 2018). At the same time in all of the economy sectors in Estonia an average 20–22 sick leave days have counted in the years 2011–2017 (ELI, 2018). The reason is most likely wrong understanding related to the sickness benefits in Estonia.

CONCLUSIONS

Based on the results we can allege that CAP accounted for a large part of accidents at work in the agricultural sector, and the most cases are minor. Severe WAs are likely occur in forestry or very likely in fishery sub-sector. The results show that in Estonia comparing agricultural sector with all the economic sectors, the risk of getting FA is much higher in the agricultural sector. But on the other hand when comparing FA rates in Estonian agricultural sector and EU-28 statistics, the rate of fatal cases in Estonia are significantly lower.

The main approaches to prevent agricultural accidents are improvement of awareness on risks and prevention strategies in agriculture. Among this machine operator manuals and safety warning systems could be improved, so that they don't contain excessive information and are readable for operators (Tebeaux, 2010). The insurance system covering work injuries and illnesses need fast implementation in Estonia. Through engineering improvements, education and training we could better prevent WAs in agriculture.

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Spatial variability of soil fertility attributes and productivity in a coffee crop farm

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Abstract. Coffee cultivation is of great importance to Brazilian agribusiness, as coffee occupies extensive production areas and is one of the most exported Brazilian products. To maintain coffee production numbers, productive techniques must be adopted that optimize productive system use. The objective of this work was to apply geostatistical techniques in the evaluation of soil fertility attributes to construct maps of variability in soil fertility parameters and the productivity of a coffee crop in the municipality of Monte Carmelo, Minas Gerais (MG), Brazil. The work was developed with coffee of the cultivar Mundo Novo 379/19, and 19 sample points were georeferenced in Universal Transverse Mercator coordinates. Spatial dependence of the fertility and productivity parameters was analysed via classic semivariogram fitting and interpolation by ordinary kriging using the statistical computer system, R. All parameters evaluated showed high degrees of spatial dependence. The attribute values varied along the sampling points, except for the sodium (Na) contents, which had similar values in all samplings. The studied parameters ranged from 80 to 200 metres. It is conclusion, the use of productivity maps linked to soil chemical attributes can be useful for determining the occurrence of variable productivity rates throughout the area, allowing the adoption of corrective practices for subsequent crops and thus making the maps very useful tools for producers.

Key words: precision coffee cultivation, geostatistics, spatial maps, semivariograms.

INTRODUCTION

Coffee cultivation plays a prominent role in Brazilian agribusiness, with Brazil being the world's largest producer and exporter of the grain. To maintain the competitiveness of Brazilian coffee production, management practices must be adopted that better utilize environmental resources and consequently optimize productivity (Embrapa, 2017).

Soil fertility is a limiting factor for coffee productivity, and fertilizer recommendation practices become necessary to supply the nutrients that coffee demands (Costa, 2011).

Applying inputs locally at variable rates by adopting precision agricultural techniques has attracted much research. Productive fields have highly variable soil attributes, and the conventional recommendation system assumes the homogeneity of a given area; the system thus recommends values that meet the needs of the crops (Tschiedel & Ferreira, 2002), which may be ineffective and may generate unnecessary expenses via purchasing fertilizers and reducing crop productivity.

This study used geostatistical techniques to correlate the soil fertility attributes with crop productivity in the municipality of Monte Carmelo, Minas Gerais (MG), Brazil, to construct spatial variability maps of these attributes.

MATERIALS AND METHODS

The experiment was conducted at the Sabana Grande farm in the municipality of Monte Carmelo, MG, Brazil, in an area covering 10 hectares cultivated with coffee (*Coffea arabica* L.) of the cultivar Mundo Novo 379/19, planted with spacing 4.5 x 0.5 meters. The area is between the geographical coordinates 18°40'05"S latitude and 47°31'48"W longitude.

According to Koppen classification, modified by Alvares et al. (2013), the climate at the site is Aw, classification of tropical climate with dry winter, with an average annual temperature of 21 °C and average rainfall of 1,444 mm. The area has an average altitude of 1,000 meters above sea level. The soil in the area is classified as Dystroferic Red Latosol, according to the Brazilian Classification of Soils - SiBCS (Embrapa, 2018), with Latosols being equivalent to Ferralsols e Oxisols, with WRB and Soil Taxonomy respectively.

To collect soil samples for the soil fertility analysis, 19 sample points were georeferenced using a global positioning system (GPS) model Garmin. At each point, 1 point per half hectare samples were collected at 0.0–0.2 m deep. The samples were analysed by the Laboratório Brasileiro de Análises Agrícolas Ltda. (LABRAS) in Monte Carmelo, MG, Brazil. The following parameters were analysed: soil pH in water, available: phosphorus (P - mg dm⁻³), sodium (Na - cmol_c dm⁻³), potassium (K - cmol_c dm⁻³), calcium (Ca - cmol_c dm⁻³), magnesium (Mg - cmol_c dm⁻³), aluminium (Al - cmol_c dm⁻³); acidity potential (H + Al - cmol_c dm⁻³), organic matter (OM – dag kg⁻¹), sum-of-bases (SB - cmol_c dm⁻³), cation exchange capacity (T - cmol_c dm⁻³), base saturation (V%) and Al saturation (m%).

To estimate coffee productivity, the fruits were harvested manually on cloths and measured in a graduated container in litres. Fruits were collected from plants around the georeferenced sampling point. From these plants, a mean was obtained for the sampling point.

Spatial dependence of the fertility and productivity parameters was analysed using classic semivariogram fitting and ordinary kriging interpolation. The classic semivariogram was estimated using the equation (1), according to Matheron (1963).

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i + h)]^2 \quad (1)$$

where $N(h)$ represents the number of pairs of values observed, $Z(xi)$ represents a value observed at a given point, and $Z(xi + h)$ represents the value observed at a 2nd point separated by a distance (h).

The semivariogram is a graph of $\hat{\gamma}(h)$ versus the corresponding values of h . The semivariogram model was fitted by ordinary least squares (OLS). Spherical, exponential, and Gaussian models were tested for all parameters. After fitting the semivariogram, data were interpolated by ordinary kriging to enable visualising the spatial distribution patterns of the plantation variables by building spatial maps.

For descriptive statistical and geostatistical analyses and mapping, the statistical computer system, R, (R Development Core Team, 2017) was used through the geoR library (Ribeiro Junior & Diggle, 2001). The maps were generated from data in Universal Transverse Mercator (UTM) coordinates in Zone 23K, which is the municipality of Monte Carmelo, MG, Brazil.

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics for the variables soil chemical analysis of the study area. The attribute values varied along the sampling points except for the Na contents, which had similar values in all samplings.

Table 1. Descriptive statistics for soil chemical attributes

	Unit	SD	Mean	Maximum	Minimum	CV (%)
pH	(water)	0.22	5.50	5.90	5.00	4.00
P resin	mg dm ⁻³	14.34	34.36	62.00	13.90	41.73
Na	cmolc.dm ⁻³	0.00	0.01	0.01	0.01	0.00
K		0.08	0.26	0.43	0.12	31.14
Ca		0.36	1.88	3.00	1.46	19.17
Mg		0.24	1.32	2.07	0.94	18.20
Al		0.03	0.01	0.11	0.00	216.17
H+Al		0.99	4.35	6.60	2.90	22.75
MO	dag kg ⁻¹	0.36	2.95	3.82	2.45	12.19
SB	cmolc dm ⁻³	0.59	3.46	5.30	2.53	6.47
T		0.88	7.81	9.56	6.47	11.27
V	%	8.13	44.68	58.20	28.60	18.19
m		0.91	0.44	3.40	0.00	205.91
Prod	L/pl	2.74	13.76	18.80	10.00	19.93

The attributes varied throughout the study area as evidenced by their high coefficients of variation (CV). As evidenced by Silva et al. (2007), the pH had a low coefficient of variation. Per Gomes & Garcia (2002), data with coefficients of variation less than 10% show homogeneity in the readings, but where this variation occurs cannot be detected spatially using only the data presented in Table 1. Geostatistics are needed to construct spatial variability maps of the area under study and interpret them.

The model most commonly used to fit semivariograms in geostatistics (Webster & Oliver, 2007) and in studies involving soil science (Grego & Vieira, 2005) is the spherical model. Silva et al. (2007, 2008, 2010) and Faulin (2010) also fitted the spherical model to their data for studying coffee productivity and soil attributes.

The nugget effect is an important parameter in semivariograms because it corresponds to the data variability not explained by the model or that occurs at random and accounts for the distance (Mcbratney & Webster, 1986). The nugget effect may also be a consequence of sampling errors or attribute variations that cannot be detected with the sampling scale used (Vieira, 2000).

The relationship between the nugget effect and the semivariogram sill determines the degree of spatial dependence (Trangmar et al., 1985). Based on the degree of dependence classified by Cambardella et al. (1994), semivariograms with nugget effects that are 25% lower than the sill are considered to have high degrees of dependence. Further, when the percentage is between 25 and 75%, dependence is moderate, and when it is greater than 75%, dependence is weak. Table 2 shows that all evaluated parameters had strong degrees of spatial dependence, similar to the results of Machado et al. (2007) for the pH in water, Mg, and base saturation variables. Corá et al. (2004) found a moderate degree of dependence for all fertility attributes evaluated in their study on sugarcane.

Table 2. Estimated models and parameters of experimental semivariograms for soil fertility components and coffee crop productivity (L plant⁻¹). C0 (nugget effect), C1 (contribution), C0+C1 (sill), a (range), $[C0/(C0+C1)] \times 100$ (degree of dependence)

Parameters	Model	C0	C1	(C0 + C1)	a (m)	$[C0/(C0+C1)] \times 100$
pH in water	spherical	0.20	0.60	0.80	200.00	25.00
P	spherical	2.00	120.00	122.00	100.00	1.64
K	spherical	0.01	120.00	120.01	105.00	0.01
Ca	spherical	2.00	120.00	122.00	105.00	1.64
Mg	spherical	2.00	120.00	122.00	100.00	1.64
Al	spherical	2.00	120.00	122.00	100.00	1.64
H + Al	spherical	2.00	120.00	122.00	100.00	1.64
SB	spherical	0.00	120.00	120.00	105.00	0.00
T	spherical	0.10	0.80	0.90	80.00	11.11
V%	spherical	0.10	0.80	0.90	80.00	11.11
m%	spherical	0.10	0.80	0.90	80.00	11.11
productivity	spherical	2.00	120.00	122.00	100.00	1.64

Another parameter of considerable importance is the range of the semivariogram for determining the spatial dependence limit, which may also serve to delimit the interval between soil mapping units, where samples collected within the maximum extent of the range are spatially correlated (Grego & Vieira, 2005). The studied parameters ranged from 80 to 200 metres, with the lowest ranges obtained for the cation exchange capacity (T), base saturation (V%) and Al saturation (m%); soil pH had the largest range (Table 2).

Silva (2007) found that the ranges reached levels of 347.82 metres for K and 60.43 metres for productivity. The other variables, such as pH, Ca and SB, reached a range of 70 metres. Faulin obtained ranges similar to those of the present study for P and K. Ferraz et al. (2010) obtained spatial dependence values greater than 200 m for coffee productivity during a 3-year study.

The spatial variability of the soil attributes might be explained by pedogenetic process and by the different management practices, providing changes in physical, chemical, biological and mineralogical attributes, and direct impacts on crop

productivity (Silva et al., 2008). Thus, the study of spatial variability of soil acts as a tool for precision agriculture, which in the case of the coffee crop, as a tool for precision coffee cultivation (Ferraz et al., 2012).

The soil attributes required for coffee according to Alvarez et al. (1999) might be characterized goods for class intervals determined by limits of 6.1–7.0 for pH; 12.1–8.0 mg dm⁻³ for P; 71–120 mg dm⁻³ for K; 2.41–4.00 cmolc dm⁻³ for Ca; 0.91–1.50 cmolc dm⁻³ for Mg; 1.01–2.00 cmolc dm⁻³ for Al; 5.01–9.00 cmolc dm⁻³ for H + Al; 4.01–7.00 dag kg⁻¹ for OM; 3.61–6.00 cmolc dm⁻³ for SB; 8.61–15.00 cmolc dm⁻³ for the T; 60.1–80.0% for V and 50.1–75.0% for m.

Spatial maps were constructed of the variability in the studied attributes as shown in Figs 1, 2 and 3 which allowed visualising the spatial patterns of the attributes throughout the area.

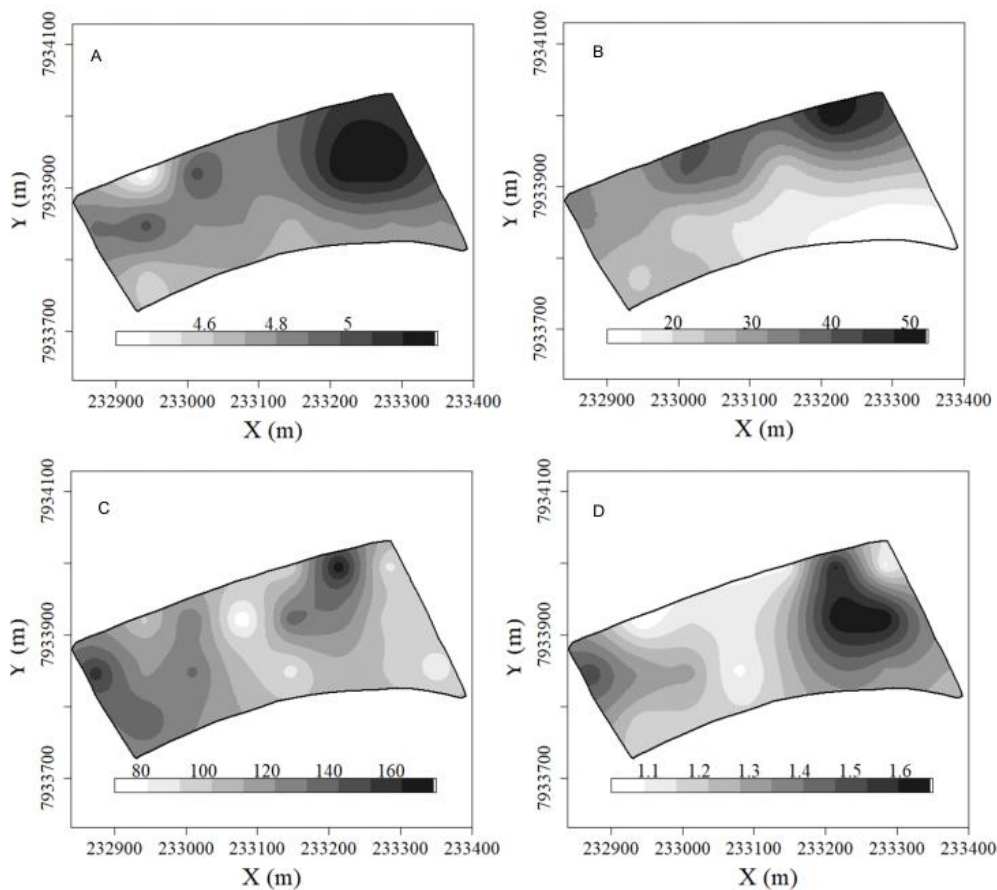


Figure 1. Maps of soil chemical attribute variability – A) pH, B) P mg dm⁻³, C) K mg dm⁻³ and D) Mg cmolc dm⁻³.

The pH plot (Fig. 1, A) shows that the east and northeast areas had the highest pH level values, which were near the ideal range for good coffee cultivation development and productivity. Soil pH is an important factor in soil fertility since the ideal range for good development of most crops is between 6.0 and 7.0; at values below this range,

nutrients are less available to plants (Luz et al., 2002). The use of conventional techniques, abstaining from the practice of precision agriculture, provides pH correction failure in this area of study with incorrect use of limestone in the implantation of the crop, which can explain the variation of the pH values.

Phosphorus is a macronutrient whose availability decreases at low pH because the phosphate anion is retained by the positively charged particles on the oxide surfaces (Zoz et al., 2009). Fig. 1, A shows that the soil pH was closest to neutral in the areas of the highest available P levels. The other nutrients were also more concentrated in the area's northeast region.

For the Al potential acidity (H + Al) (Fig. 2, C) and Al saturation (m%) (Fig. 3, C), the highest levels occurred at sites with higher soil acidity (low pH) because in acidic soil, Al is solubilized in the soil solution, thus increasing its concentration (Hartwig et al., 2007).

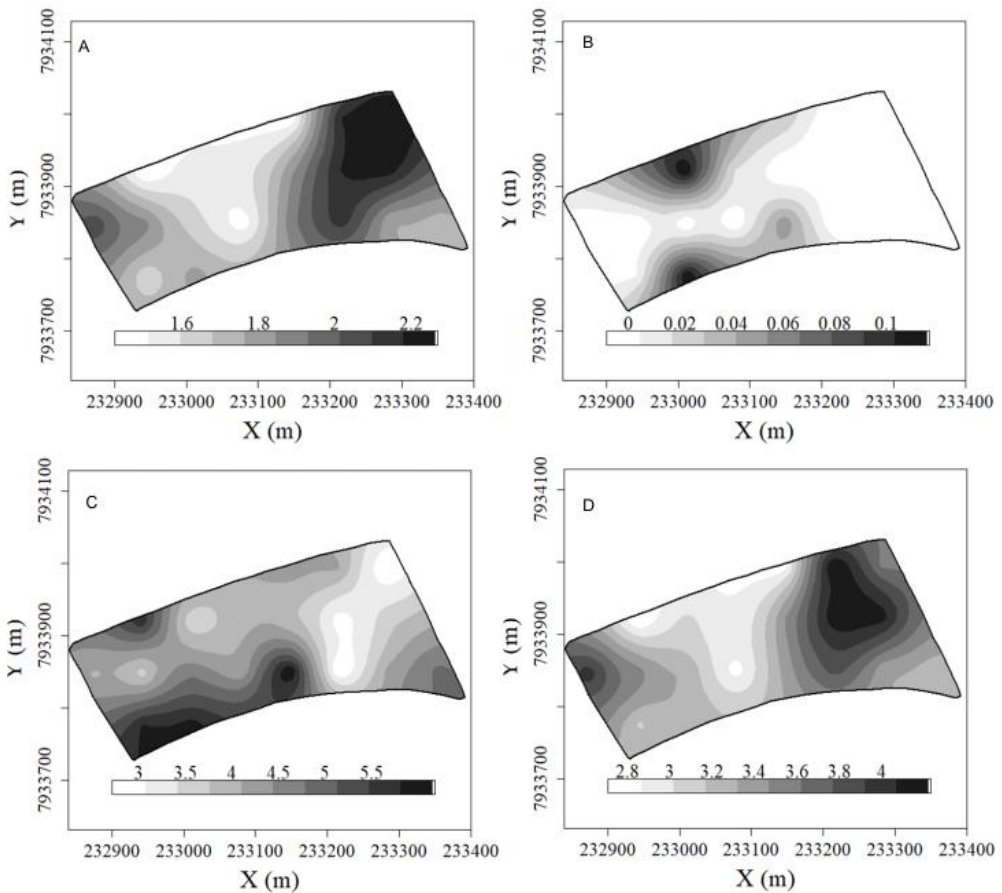


Figure 2. Maps of soil chemical attribute variability – A) Ca $\text{cmol}_c \text{dm}^{-3}$; B) Al $\text{cmol}_c \text{dm}^{-3}$; C) H+Al dag kg^{-1} and D) SB $\text{cmol}_c \text{dm}^{-3}$.

The average productivity in the area was 13.76 L plant⁻¹ (Table 1). The productivity map shows (Fig. 3, D) that the highest productivity occurred in the southeastern and southwestern areas. The other regions showed productivity values near the mean.

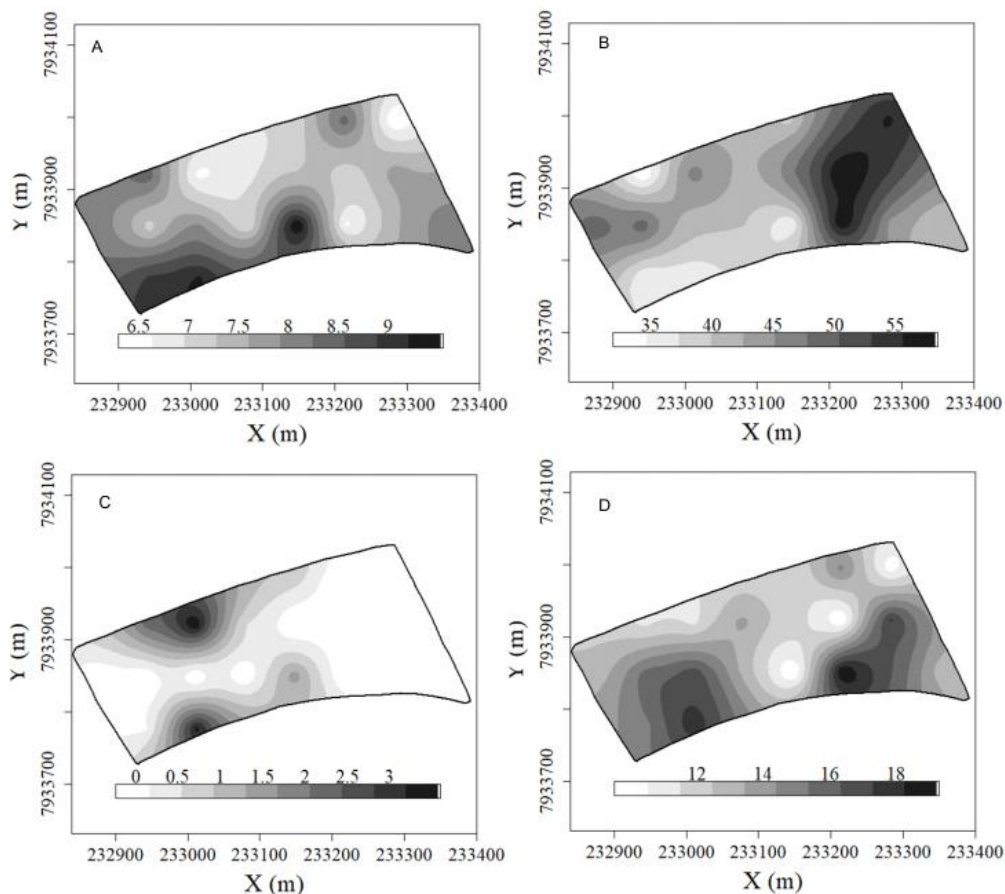


Figure 3. Maps of soil chemical attribute variability – A) T cmol_c dm⁻³; B) V (%); C) m (%) and D) coffee productivity (L pl⁻¹).

Procafé (2018) suggested that soil pH values between 5.0 and 6.0 present a medium nutritional level to the coffee. The pH variability map in Fig. 1 indicates that in every area under study, even when the observed values varied, all observations were within the medium range. Both P and Mg presented levels throughout the area that classified their nutritional levels as low, middle and high. Ca and K presented values in the low and medium nutritional ranges. Ferraz et al. (2012) also found P levels that fit all 3 fertility levels.

Productivity maps linked to soil chemical attributes may be useful for determining the occurrence of variable productivity rates throughout an area, allowing adoption of corrective practices for subsequent crops (Ferraz et al., 2017). The corrective practice most used within the context studied involved applying inputs at variable rates, which saves money for producers and allows greater application efficiency (Chang et al. 2003;

Wang et al., 2006). Ferraz et al. (2012) described the benefits of using spatial variability maps of soil fertility attributes since these maps enable easily identifying the locations where corrections and fertilizer application are necessary. These authors further described the problem of using the mean value to recommend soil fertilization.

CONCLUSIONS

Semivariograms allow characterization of the occurrence of spatial variability of the studied attributes and the variability maps are important tools for managing coffee production. The parameters that most strongly influenced coffee productivity were Ca and V in the southeast section and H + Al, SB and T in the southwest section.

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Holding the wheel in passenger cars in countries with driving on the right and left side depending on the driver's side preference

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Abstract. This paper deals with the assessment of the differences in how passenger car drivers hold a steering wheel with left and right-side steering in specific driving modes. The findings are compared to the generally-accepted optimal position in terms of active and passive safety, as well as long-term effects on the health of the driver. The research described in this work was conducted on a sample of randomly selected drivers in the Czech Republic, the UK and Australia using electronic questionnaires. The data was then subjected to a statistical evaluation, which looked primarily at the difference between the way in which the steering wheel was held in countries with driving on the right and driving on the left. Another parameter for statistical data evaluation was the used side preference of individual drivers. On the basis of a statistical evaluation of the obtained data, it was found that there is a difference in the way the steering wheel is held in the assessed traffic situations between drivers driving on the right and drivers driving on the left. The results of this work can be used in the design process of passenger car cabins, in particular in the field of adaptation of the control devices of particular models to the needs of drivers in individual countries based on the type of traffic. The results of the work point out the necessity to make innovations in the design of passenger car cabins with regard to the type of traffic in which the vehicle will be operated, which could lead to a better application of innovations, and thereby better possibilities of positively influencing traffic safety and the health of drivers.

Key words: driver, side preference, posture, hands, steering wheel, left-hand, right-hand, traffic.

INTRODUCTION

The issue of the optimal passenger car ergonomics is currently one of the main components of the construction process of new cars (Wang et al., 2007; Bhise, 2012) and an optimally-designed driver's seat plays a major role herein, particularly in terms of vehicle safety (Reed, 1998). Modern controls, such as multifunction steering wheels, provide a direct link between the driver and the machine, and the optimum design of these elements, combined with the design of various armrests, directly affects the driver's overall comfort and therefore the safety of the vehicle's operation (Chang, 2016).

The position of the driver's hand on the steering wheel is important in a number of respects, but these can often be very contradictory. Firstly, this concerns active safety requirements, a subjective sense of driver comfort, passive safety, and others. There are two groups of people in the human population that can be divided according to their side preference (laterality), or the hand they prefer as their primary hand during work.

Generally speaking, in terms of population representation, the number of people who prefer the left hand, ranges from 10–15% (Healey, 2002). Left-handedness or right-handedness can also be important in the process of driving a car where, for example, right-handed drivers driving a car with the steering wheel on the left are forced to primarily use their left hand, which is not their dominant hand, and vice versa.

The aim of this paper is to find out and assess whether there is a difference in how right-handed drivers and left-handed drivers hold the steering wheel in three basic driving modes. The paper also seeks to ascertain whether there are differences in how the steering wheel is held by drivers driving on the right or left side of the road, again in three basic driving modes.

Some of the findings from previous research are used in the paper that dealt with how drivers driving on the right held the steering wheel, but only in idle mode, which is referred to as situation A (Hruška, 2018) in this paper. As a comparison for the acquired data, the paper uses the values of holding the wheel steering labelled as optimal (Hault-Dubrulle et al., 2010). There is a relatively large consensus among experts on this issue. The most frequently cited optimum value according to the analogue clock face is the position of the left hand on the nine and the right hand on the three, while the driver holds the steering wheel with both hands (Hault-Dubrulle et al., 2010; Schiro et al., 2013). This value is considered optimal regardless of the location of the steering wheel in the vehicle.

A whole range of research deals with measurement of how passenger car drivers hold the steering wheel (Schmidt et al., 2015) and is usually focused on the impact of grip on passive safety and the subsequent type and extent of injury during an accident. Mostly, however, these are individual measurements of a small number of test persons in laboratory conditions and on special measuring stools (Schiro et al., 2013). Adversely, this paper collects data from a large group of drivers moving in a real environment so that it can be used to evaluate the results of the statistical method.

The primary objective of this paper is to assess the hypothesis that there is a statistically significant difference in the way the steering wheel is held by drivers driving in the right and by drivers driving on the left. The secondary objective of this paper is to assess the hypothesis that there is a statistically significant difference in how left-handed drivers and right-handed drivers hold the steering wheel. These measurements will be carried out in three different traffic situations in order to assess a broader range of activities that drivers perform under real conditions.

In some published studies dealing with ergonomics (Hruška & Jindra, 2016), it was found that there is a correlation between the driver's gender and his or her ability to control the vehicle and to adapt its controls. As part of the primary and secondary objectives of this paper, gender of the respondent was also used as a parameter to supplement the finding of dependency.

MATERIALS AND METHODS

Participants

A total of 160 participants (82 women and 78 men) were obtained from the Czech Republic, Great Britain and Australia, who were divided into two groups of 80 respondents. Each group of respondents comes from countries with road traffic driving on a different side. Respondents from the Czech Republic were included in the group of

drivers who drive on the right (Table 1), and respondents from the UK and Australia were included in a group of drivers who drive on the left (Table 2). The age of all participants ranged from 19 to 65 years (the average age was 33.7 years). All of the participants were given the clear condition that they must have a driver's license authorizing them to drive passenger cars. All of the participants of the survey were also strongly advised that they must be in good health and have no restrictions on their movement apparatus, which could distort the results.

Table 1. Data about test persons from the group of drivers driving on the right (CZ)

Gender	Number	Age			Side preference	
		Average	Minimum	Maximum	Right-handed drivers	Left-handed drivers
Men	40	29.7	20	52	27	11
Women	40	32.2	19	64	34	8
Total	80	30.9	19	64	61	19

Table 2. Data about test persons from the group of drivers driving on the left (UK, AUS)

Gender	Number	Age			Side preference	
		Average	Minimum	Maximum	Right-handed drivers	Left-handed drivers
Men	38	38.4	21	65	27	11
Women	42	34.7	20	56	34	8
Total	80	36.5	20	65	61	19

Tested traffic situations

Three basic traffic situations were defined for research purposes in which the interviewed drivers could find themselves and answer how they most often hold the steering wheel in the given situation. These consist of three common situations in which drivers repeatedly find themselves on the road. Each of these situations has been thoroughly verbally described and supplemented with an illustrative photo for better understanding. **Situation A** was described as highway or high speed traffic at low traffic when the driver is not subjected to psychological pressure. **Situation B** was described as driving outside the city on a secondary road with more turns, in medium traffic, when the driver is subjected to medium-intensity psychological pressure. **Situation C** was described as driving outside the city with reduced visibility and very dense traffic, with very high demands on the driver and the driver being subjected to considerable psychological pressure.

Data Collection Procedures

Basic data collection was carried out with the help of electronic questionnaires in two language versions. Their translations and semantically-identical content were verified by a professional translation agency. As part of the basic information provided in the questionnaire, respondents were advised to devote sufficient time to filling out individual questions and had schemes available to help them better imagine the situation (Fig. 1). Although the questionnaire method may not be as accurate as real-environment testing, given the set objectives and the number of subjects surveyed, testing in a real environment would be virtually impossible in organizational terms.

The questionnaire consisted of nine questions divided into two groups. The first group were questions about age (in years), gender (female, male), and side preference (right-handed, left-handed). In addition, for the period for which he or she had a driver's licence (in years), the test subject was interviewed about how often he or she drove a passenger car (every day, at least once a week, occasionally, exceptionally), with the final data being about the position in which the subject most often sits behind the steering wheel (a choice of three basic positions divided according to the subject's chest distance from the centre of the steering wheel).

In the second group of questions, the test subject was asked to gradually imagine each of the three traffic situations described above and responsibly state for each of them whether he or she held the steering wheel with one or both hands, and in which position. This was always based on the pre-selected scheme attached to each question (Fig. 1), where, according to the watch face, the range of R12-R6 was defined for the right hand, and for the left hand the analogous range of L6-L12. The overlap at 12 and 6 o'clock is selected

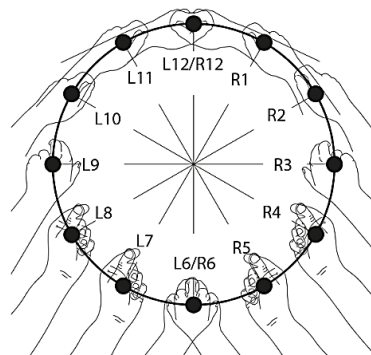


Figure 1. Scheme of positions of individual grips according to an analogue watch face.

because the test groups from left and right handed traffic were evaluated. The subject was also told to indicate the most prevalent value of the grip on the steering wheel. We dismissed extreme values, where the test subject crosses the hand and held steering wheel with, for example, the left-hand on the right, as highly unlikely in view of the objectively high degree of discomfort the driver would experience in such a position. For this reason, we completely discarded these variations.

RESULTS AND DISCUSSION

The results obtained during the measurements were statistically processed and evaluated using contingency tables, Pearson's chi-squared test and adjusted residuals methods. The percentage representation of individual hand positions on the steering wheel (regardless of whether the steering wheel is held by one hand or both) obtained from the measured data are shown in Table 3 for respondents driving on the right, and in Table 4 for drivers driving on the left.

It can be seen from the data presented in Table 3 and Table 4 that there are some differences in the positions in which the steering wheel is held by drivers driving on the right and drivers driving on the left. It is also very clear that no matter what type of traffic they are in (Table 5 and Table 6), drivers hold the steering wheel more often with both hands if the traffic situation is more complex (situation B and C). These differences are explained below based on the dependence of holding the steering wheel on the laterality of the test subjects and on the type of traffic in which they are moving.

Table 3. Percentual representation of grips for drivers driving on the right (CZ)

Item	Situation A			Situation B			Situation C		
	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)
L6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L7	8.3	8.8	7.9	2.7	0.0	5.3	0.0	0.0	0.0
L8	9.7	8.8	10.5	5.3	2.7	7.9	1.3	0.0	2.5
L9	31.9	35.3	28.9	22.7	24.3	21.1	25.0	32.5	17.5
L10	40.3	35.3	44.7	58.7	56.8	60.5	68.8	60.0	77.5
L11	5.6	8.8	2.6	5.3	8.1	2.6	3.8	5.0	2.5
L12	4.2	2.9	5.3	5.3	8.1	2.6	1.3	2.5	0.0
Σ L	100	100	100	100	100	100	100	100	100
R12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R1	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	2.6
R2	36.2	38.1	34.6	55.9	50.0	61.1	64.0	54.1	73.7
R3	25.5	28.6	23.1	30.9	37.5	25.0	30.7	40.5	21.1
R4	21.3	19.0	23.1	11.8	12.5	11.1	2.7	2.7	2.6
R5	10.6	9.5	11.5	1.5	0.0	2.8	1.3	2.7	0.0
R6	6.4	4.8	7.7	0.0	0.0	0.0	0.0	0.0	0.0
Σ R	100	100	100	100	100	100	100	100	100

Note: The percent values are calculated separately for the right and left hand and the values in this table do not take into account whether the driver holds the steering wheel with one hand or both.

Table 4. Percentual representation of grips for drivers driving on the left (UK, AUS)

Item	Situation A			Situation B			Situation C		
	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)
L6	1.6	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L7	4.8	3.4	6.1	0.0	0.0	0.0	0.0	0.0	0.0
L8	35.5	37.9	33.3	26.0	24.2	27.5	12.8	16.2	9.8
L9	17.7	20.7	15.2	26.0	27.3	25.0	29.5	27.0	31.7
L10	38.7	31.0	45.5	45.2	45.5	45.0	56.4	56.8	56.1
L11	1.6	3.4	0.0	2.7	3.0	2.5	1.3	0.0	2.4
L12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ L	100	100	100	100	100	100	100	100	100
R12	1.6	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R1	1.6	3.7	0.0	6.5	0.0	7.5	2.5	2.6	2.4
R2	35.5	25.9	42.9	49.4	50.0	60.0	52.5	50.0	54.8
R3	30.6	40.7	22.9	22.1	37.5	15.0	26.3	23.7	28.6
R4	19.4	18.5	20.0	18.2	12.5	10.0	13.8	21.1	7.1
R5	11.3	7.4	14.3	3.9	0.0	7.5	5.0	2.6	7.1
R6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ R	100	100	100	100	100	100	100	100	100

Note: The percent values are calculated separately for the right and left hand and the values in this table do not take into account whether the driver holds the steering wheel with one hand or both.

Also interesting are the differences between holding the steering wheel with one or both hands, depending on whether the respondents are men or women, as can be clearly seen in Table 5 and Table 6. It is evident that women use both hands more often than men regardless of the current traffic situation. This finding has already been published

in some earlier surveys, which can be confirmed by this finding (Hruška, 2018). This assertion is also confirmed by statistical evaluation (Table 7). The explanation for these differences can be seen both in the anthropometric parameters of men and women (Tilley 2002, Wang et al., 2007), but also at the psychological level, where women tend to approach driving more responsibly (Bergdahl, 2005) and try to observe the generally recommended position of L9R3 at the cost of lower subjective comfort.

Table 5. Representation of drivers driving on the right (CZ) according to how they hold the steering wheel

Holding the steering wheel	Situation A			Situation B			Situation C		
	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)
Left hand	41.2	47.5	35.0	15.0	20.0	10.0	5.0	7.5	2.5
Right hand	10.0	15.0	5.0	6.2	7.5	5.0	0.0	0.0	0.0
Both hands	48.8	37.5	60.0	78.8	72.5	85.0	95.0	92.5	97.5

Table 6. Representation of drivers driving on the left (UK, AUS), according to how they hold the steering wheel

Holding the steering wheel	Situation A			Situation B			Situation C		
	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)	Total (%)	Men (%)	Women (%)
Left hand	22.5	28.9	16.7	3.8	2.6	4.8	0.0	0.0	0.0
Right hand	22.5	23.7	21.4	8.8	13.2	4.8	2.5	2.6	2.4
Both hands	55.0	47.4	61.9	87.5	84.2	90.5	97.5	97.4	97.6

The claim that whether a driver holds the steering wheel with one or both hands depends on gender can only be statistically confirmed in situation A. Dependency was not demonstrated in situations B and C, which can be interpreted in that if the traffic situation is more complex and the driver may perceive it as potentially more dangerous, both men and women use both hands to drive more often. This trend is evident for all drivers regardless of whether they are driving on the left or on the right (Table 7).

Table 7. Results of the statistical evaluation of dependency of holding the steering wheel on the gender of the driver

Dependency	Degrees of freedom	X ²	Critical value	Cramer's V	Significance level	Level of dependency
Situation A	1	5.58	3.84	0.186	0.05	medium
Situation B	1	2.62	3.84	0.464	0.05	none
Situation C	1	0.80	3.84	0.071	0.05	none

Based on the data shown in Table 8, it is clear that if the driver holds the steering wheel with only one hand, there is a difference between which hand right-handed drivers use for holding the steering wheel, and which hand left-handed drivers use. This difference is statistically very significant for drivers who drive on the left (UK, AUS). For drivers driving on the right, this dependence is not as obvious.

This is mainly due to the fact that drivers driving on the right have the steering wheel on their left in their cars and are therefore indirectly forced to use their left hand as their primary hand if they are driving with only one hand. In this case, the right hand

is more often used to shift and control secondary and tertiary vehicle control systems that are located in the centre of the vehicle. In this case, therefore, all left-handed drivers use their preferred hand as the primary hand for controlling the vehicle and this group is statistically unrecognizable. For drivers who drive on the left and have the steering wheel in their vehicle on the right side, their primary hand for driving the vehicle is their right hand. Drivers whose preferred hand is their left hand primarily use their left hand when driving at the cost of possible subjective discomfort in a statistically significant number of cases, as shown in Table 8.

Table 8. Results of the statistical evaluation of dependency of according to the set hypotheses for situation A

Group of respondents	Drives with the left hand	Drives with the right hand	Drives with both hands	Σ
Left-handed (UK,(AUS)	17 (+++)	1 (0)	1 (---)	19
Right-handed (UK, AUS)	1 (---)	17 (++)	43 (+++)	61
Left-handed (CZ)	7 (0)	0 (0)	6 (0)	13
Right-handed (CZ)	26 (0)	8 (0)	33 (0)	67
Σ	51	26	83	160

Note: In the Table 8, a sign scheme is used, that expresses the standard deviation rate from the expected value; (+++) – the result very significantly exceeds the expected frequency at the significance level of 99.9; (++) – the result significantly exceeds the expected frequency at significance level 99; (+) – the result exceeds the expected frequency at significance level 95. (0) – the value does not differ significantly from the expected frequency; (-) – the result does not reach the expected frequency at significance level 95; (--) – the result does not significantly reach the expected frequency at significance level 99; (---) – the result does not very significantly reach the expected frequency at significance level 99.9.

When comparing the data between a group of drivers who drive on the left (UK, AUS) and the group of drivers driving on the right (CZ), a certain imbalance was found in the number of drivers who drive with both hands in situation A. This finding was investigated via further personal interviews on a narrower sample of respondents, and the most likely explanation is that drivers in the UK and Australia behave slightly more responsibly than drivers in the Czech Republic.

In order to confirm the goals and hypotheses defined above, it was necessary to subject the measured results to a statistical analysis. In order to test the dependency of holding the steering wheel on the other above mentioned parameters, the previously mentioned Pearson’s chi-square dependency test was used, supplemented by the method of adjusted residuals.

In Pivot Table 8, complex input data are displayed in order to evaluate the statistical significance of holding the steering wheel depending on the laterality of the tested persons driving on the right or on the left. The PivotTable (Table 8) has six degrees of freedom and distinguishes holding with the right or left hand, or both. Table 9 lists the dependency rates for the three basic combinations.

In order to evaluate the dependency of the method of holding the steering wheel on side-type traffic, the value X^2 (8.56) was calculated, which is higher than the critical value (5.99), which confirms the primary initial hypothesis that the method of holding the steering wheel depends on the side-type traffic. The dependency rate was further verified by Cramer’s V (0.231) at a significance level of 0.05 and, based on the calculated values, the dependency of the method of holding the steering wheel on side-type traffic can be labelled as medium.

Table 9. Results of the statistical evaluation of dependency according to the set hypotheses

Dependency	Degrees of freedom	X ²	Critical value	Cramer's V	Significance level	Level of dependency
Method of holding the steering wheel in side-based traffic	2	8.56	5.99	0.231	0.05	medium
Method of holding the steering wheel on the side of the driver	2	34.51	5.99	0.464	0.05	strong
Method of holding the steering wheel on the type of traffic and on the side of the driver	6	61.81	12.59	0.439	0.05	strong

In order to evaluate the dependency of the method of holding the steering wheel on the side of the driver, the value X² (34.51) was calculated, which is higher than the critical value (5.99), which confirms the primary initial hypothesis that the method of holding the steering wheel depends on the side of the driver. The dependency rate was further verified by Cramer's V (0.464) at a significance level of 0.05 and, based on the calculated values, the dependency of the method of holding the steering wheel on the side of the driver can be labelled as strong.

If both dependencies are evaluated together, in order to evaluate the dependency of the method of holding the steering wheel on the side of the driver and on the side-type traffic, the value X² (61.81) was calculated, which is higher than the critical value (12.59), which confirms the primary initial hypothesis that the method of holding the steering wheel depends on the side of the driver and on the side-type traffic. The dependency rate was further verified by Cramer's V (0.439) at a significance level of 0.05 and, based on the calculated values, this combined dependency can be labelled as strong.

When assessing the measured data, it becomes clear that any subject who does not hold the steering wheel with both hands in a lateral synchronous position will necessarily be subject to a certain degree of rotation of the upper half of the body, the rate of which will vary depending on the particular position in which the steering wheel is held. If we assess the occupational health aspects of holding the steering wheel as described above on the health of the driver, it should be noted that the worst positions for gripping the steering wheel are L11, L12, R12 and R1.

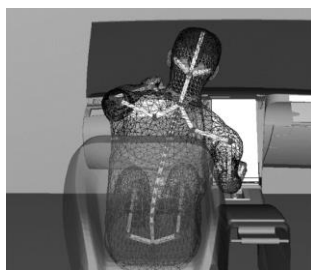


Figure 2. Driver holding the steering wheel in position L12.

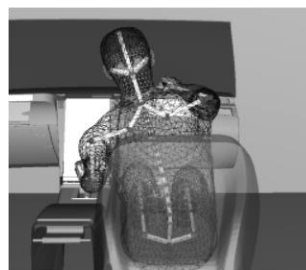


Figure 3. Driver holding the steering wheel in position R12.

The Tecnomatix Jack program was used to simply evaluate these positions, in which the L12 (Fig. 2) position for drivers with steering on the left and the R12 position

(Fig. 3) for drivers with steering on the right. Both simulations were performed using a 95-percentile man in a middle-class common sedan. In Figs 2 and 3, it is quite clear that the driver holding the steering wheel only with the left hand in the least suitable L12 position, or with the right hand in position R12, leads to significant lateroflexion with significant muscle strain, in particular *m. quadratus lumborum*, *m. obliquus externus abdominis*, *m. obliquus internus abdominis* a *m. erector spinae*. Long-term driving in such a position can lead to pain in the lumbar spine and, in the extreme, to permanent damage to the postural system (Véle, 1995, Havlíčková, 1999).

In this paper, we were able to obtain a large amount of valuable primary data from a large group of respondents from three different countries with road traffic on two sides of the road, which may be interesting in terms of possible comparisons with other statistics that could be obtained from respondents with other parameters, or from other countries. By analysing the data using contingency tables, it was found that a statistically significant group of respondents hold the steering wheel in a manner that cannot be labelled as optimal on the basis of the selected comparison parameters, regardless of whether the driver is driving on the left or on the right.

This finding could be used for the further development of passenger car cabins, whereby modelling of the interior could create conditions in which the driver of the vehicle would not be forced to find a subjectively comfortable grip on the steering wheel, but which, for the above reasons, cannot be described as optimal (Hault-Dubrulle et al., 2010; Schiro et al., 2013). This would eliminate the human factor, which is imperfect in these cases of subjective assessment.

On the basis of the above results, it can be further stated that the primary hypothesis mentioned in the introduction of the thesis has been fully confirmed. There are statistically significant differences in how a driver holds the steering wheel in countries where traffic moves on the right side of the road and in countries where traffic is on the left. This can be explained by the simple structural difference in the arrangement of the vehicle cabin with the steering wheel located on the right or on the left. It was also found that in both types of traffic there are statistically significant differences between how both men and women hold the steering wheel. The explanation of this phenomenon can be found in the generally more responsible approach of women to driving a car (Vágnerová, 2007), but also in the fact that as opposed to women, men are able to better relax in a vehicle and instinctively keep a more relaxed position (Wilson, 2001; Bergdahl, 2005).

We were also able to prove the secondary hypotheses presented in the introduction to this paper, and it can be confirmed that there is a statistically significant difference in how drivers preferring to use their left or right hands hold the steering wheel. This dependency was more strongly confirmed for drivers driving on the left.

The above hypotheses were tested in three theoretical traffic situations, but the above hypotheses have been demonstrated to be statistically significant only in situation A, which was defined as a calm ride along a highway or motorway in light traffic outside of the city. Dependency was not confirmed in situations B and C, which were defined as more complicated in terms of traffic and drivers could perceive them as more dangerous. This can also be assessed in that the more complex the situation, the more the driver tends to hold the steering wheel with both hands, regardless of gender, laterality or the side that the traffic is on.

CONCLUSIONS

The results presented in this paper could serve as a basis for further research to further refine the above findings. The data and hypotheses presented in this paper could serve as ancillary factors in the design of cars with respect to potential target customers.

The contribution of this paper can also be seen in the number of subjects tested and the involvement of the gender factor. Another benefit of this paper is the provision of valid data for further follow-up research, in which the above results could be refined or supplemented by data obtained from field studies in other countries.

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Increasing the effectiveness of aggregates for planting sugar beet stecklings to receive elite seeds

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Abstract. Planting sugar beet stecklings with planting machine is one of the stages in the technological process of producing elite seeds. The analysis of the experience of using planting units for planting stecklings showed a number of disadvantages: poor quality of planting stecklings without spacing stability and the absence of parameter control; unregulated applying nutrients and granules of water preservation to the roots negatively affect the development of plants. The aggregate having openers of the planting device was designed, and the system of supplying the spray of nutrients and growth stimulator is envisaged. The technological process is conducted in the following way: during the movement of equilateral triangles of the planting cones mechanism the root is planted in the soil. To eliminate the drawbacks while planting stecklings, the openers of the planting device for the roots having the diameter of 50–120 mm were designed. Owing to them free falling roots in the soil is ensured and their lifting together with the cone to the soil surface is prevented. The research has shown that root plant spacing depends on the angular and forward velocities of the device.

The draft resistance of furrow openers and soil compaction depend on the furrow width and depth and the distance between the axes of quadrangles and the unit rear compaction wheels.

Improving the planter, taking into account biological peculiarities of plant development, meeting the requirements of energy saving and economic expediency was proved.

Key words: accumulated energy consumption of production, growth stimulators, planter, parameters of the aggregate.

INTRODUCTION

Manufacturing ecologically safe products using energy saving technologies is a strategic direction for the economics of agrarian countries. To increase sugar production it is necessary to provide the manufacturer with high quality seeding material and highly productive technical means.

At present, about 30% of the world output of sugar is obtained from sugar beet (Balan, 2012; Gizbulin, 2012). Ukraine is among the main producers of sugar beet, however, the sowing of sugar beet with seeds grown in this country has decreased to 8% during the recent years. That is why solving the problem of seed production development in Ukraine is topical and economically expedient.

One of the important technological process operations is receiving sugar beet seed from stecklings. The existing mechanization methods do not enable to plant stecklings properly at different spacing within the row, depending on their size and agro-climatic conditions. To solve the problems, the task was set by the authors to develop and test the installation for planting sugar beet stecklings with the aim to obtain high yields of high quality seeds.

ANALYSIS OF THE LATEST PUBLICATIONS

The analysis of Ukrainian scientific sources confirms that sugar beet cultivation requires intensive technology of seed production (Kockelmann & Meyer, 2006; Method of Fertilizing..., 2007; Installation for Preparing..., 2010; Method of Determining..., 2010; Method of Choosing..., 2013). Planting stecklings of sugar beet to obtain elite seeds, which in the past was done by hand, is a responsible and labor-intensive operation.

Planting method presupposes cultivating stecklings, harvesting, and piling them for winter storage during the first stage, followed by further planting in the soil in spring, cultivating, mowing, and threshing seeds. Planting stecklings with planter is an important operation while manufacturing sugar beet by planting method (Samoilov, 1968; Fursa, 2015).

At present there are no parameters of planting stecklings depending on their size. The analysis of the existing planters is given in the following articles (Samoilov, 1968; Installation for Preparing..., 2010; Lapenko, 2011). While cultivating seed plants, the productivity and conducting operations in optimal agro-technical terms together with preserving quality indices are not ensured (Hendrick & Gill, 1971; Miller & Tolley, 1989; Anderson & Larson, 1991; Miszczak, 2005).

The experiments held in England demonstrated that using machines for planting stecklings does not ensure yield increase (Swick & Perumpral, 1988; Renner, 1990; Evenson, 1991; Larue & Ker, 1998; Fielke, 1999). According to the estimation of the leading foreign firms and companies engaged in selection and seed breeding (Germany, France, and the USA), the system of sugar beet seed production functioned in Ukraine, which enabled to mechanize its main technological processes (Thankur & Godwin, 1989; Haitiner, 1990; Sharifat & Kushwaha, 2000; Kockelmann & Meyer, 2006; Rueda-Ayala et al., 2010).

According to the methods of patent research, the proto-types of planters have been determined. The installation for planting roots having the frame with rotor planting device and bin for accumulating seed plants is known (Method of Determining..., 2010).

The installation for delivering stecklings to the planter, including pan conveyor with pusher-off of roots from cones was the following development, but there was no automatic delivery of roots to the rotor planting device in it (Installation for Preparing..., 2010).

The scientists of the State Ukrainian Scientific-Research Institute of Machinery Testing named after L. Pohorilyi (Ukraine) designed the installation for planting roots, including the bin, frame, wheels, and the device for single piece root feeding (Davydiuk, 2011).

Stecklings are delivered in the hollow of the installation, after that they are held down to immovable frames and delivered upward. Due to shifting on the extreme lower stars of draft elements, the movable frames are placed on the pin conveyor belt, making dihedral angle with immovable frames, the vertex of the angle is directed at the side

opposite to the direction of movement. When the tilt angle of the traction elements is increased, the roots, which are not held down to the immovable frames, fall into the bin. The movable frames, while turning on the upper direction stars, are placed on the pin conveyor belt, and the held down roots are freed and directed to the orientation device by the rarified stream. Further on, the process is repeated.

Planters are characterized by: a low quality at different schemes during unstable plant spacing of stecklings; the absence of parameter control of applying fertilizers and water preservation granules to the roots.

Biological peculiarities of the seed plant root system development should be taken into account during elite beet seed production.

According to the data of O.N. Sokolovskoho Institute of Soil Science and Agro-Chemistry, during the first year of sugar beet cultivation, it is necessary to apply: 7.26 mg of phosphorus, 22.5 mg of nitrogen, and 31.8 mg of potassium; and for the root – 26.8 mg, 138.3 mg, and 145.5 mg correspondingly (Fursa, 2015). During vegetation period, the roots require steady supply of nitrogen, potassium, and moisture supply. For example, during the vegetation period, the area, planted with stecklings, loses 17–24 t ha⁻¹ of moisture per day, and the area under sugar beet of the first year – 16–20 t ha⁻¹. It has been established, that one beetroot requires up to 2 dm³ of water per day. The plant root system is located in the soil at the depth of 50 cm and gets more than 90% of moisture. Beet seeds are characterized by a short vegetation period, so seed plants cannot use precipitation moisture, that is why moisture deficit during the initial period of seed-bearing plant development is undesirable and it can lead to a considerable decrease of seed yield (up to 17%) (Dankov, 2011; Fursa, 2015). Applying fertilizers during planting roots supplies the plants with nutrients and regulates the development processes resulting in obtaining maximal yields and optimal quality of seeds. At present there are no dosed applying fertilizers and growth stimulators in the root zone in the process of planting that is why it is a promising direction of research.

The conducted short analysis of the modern state of creating planters, scholars' research in this field, and also the main drawbacks enabled to determine the aim and task of the present research.

THE AIM AND TASKS OF THE RESEARCH

The aim of the research is investigating the technological aspects of raising the efficiency of producing elite seeds of sugar beet.

To achieve the set goal, the following tasks were set:

1. To conduct a short analysis of the modern state concerning the designing of planting machines for planting parent roots of sugar beet.
2. To establish the dependence of the number of injured roots on the type of the opener of planting units.
3. To substantiate the technological parameters of the aggregate and the expediency of planting sugar beet roots for seeds.
4. To investigate the rotational planting unit with planting cones for ensuring the quality of planting parent roots of sugar beet.
5. To investigate the dependence of plant spacing on the number of planter rotations and the unit forward speed.

6. To determine the dependence of the frequency of planter rotations on plant spacing on different tractor gears.
7. To establish the dependence of the density of sugar beetroot planting on the distance between compaction wheels at opening furrows by ristle for the small and medium fractions of roots.
8. To investigate the dependence of planting depth on the distance between the axes of planting tetrahedrons and compaction wheels.
9. To establish the dependence of the verticality of planting on the tilt angle of the planting cone.
10. To determine draft resistance of the furrow opener and ristles on the run depth, width of the raised furrow and parent roots' spacing of sugar beet.
11. To substantiate the dependence of the rotary moment on plant spacing: with the ristle and furrow opener.
12. To substantiate the optimal parameters and operating conditions of the planting unit and the aggregate on the whole.
13. To conduct production testing of planting aggregate in Poltava region during planting parent roots of sugar beet with different plant spacing.

MATERIALS AND CONDITIONS OF THE RESEARCH

The aggregate with planting unit opener was designed to investigate the parameters of the technological process for planting parent roots of sugar beet for elite seeds. The spray system of delivering fertilizers and growth stimulator was also envisaged (Method of Choosing..., 2013). The state of the object is characterized by the parameter control of planting roots. The aggregate conducts measuring the object parameters, memorizing and controlling of switching on the monitors while determining angular and forward velocities, the frequency of the planting unit rotations, the density of and depth of planting parent roots, and draft resistance of furrow openers. The obtained information is transmitted to the computer, equipped with the corresponding software for processing the results (Lapenko, 2011).

Parent root of sugar beet undergoes harvesting, transporting, calibrating, putting to storage, removing from piles, loading, transporting, and planting the following year. The cycle of mechanized work leads to injuring the roots, which negatively influences the germination energy, germination capacity, and results in decreasing the yield of seeds. The results of testing confirm that injuring the beet crown during planting in the existing planting machines exceeds the established indices of agro-technical requirements.

To eliminate the drawbacks the openers in planting units have been designed. The sizes of roots to be planted according to the existing agro-technical requirements (Lapenko, 2011) (having the diameter of 50–120 mm and weight 100–800 g (Dobrotvortseva, 1986)) were taken as a basis for the testing. The injuries of planted beet crowns, which had not to exceed 1.5% were also taken into account. The size of the opener diameter excludes the probability of root sticking in the planting cone and ensures opening of the back fold at the moment, when forced movement of the root along the axis of the cone finishes. It ensures the free falling of roots into the soil and prevents their elevation with the cone on field surface. In order to prevent root injure, the opener is made like depressed semi-sphere with a fixing of porous rubber, the opener grips the beet crown and centers it in the cone. The porous fixing of the opener, having the diameter of 70 mm,

excludes injuring the beet crown. Planting unit openers, designed by the authors, are presented in Fig. 1. The first one has the whole semi-sphere with porous fixing, and the second one – the central opening.

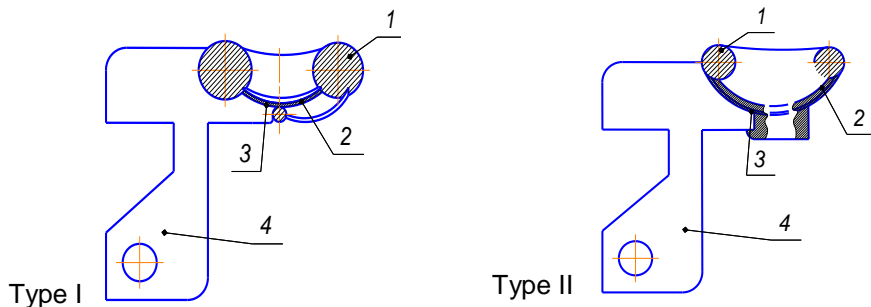


Figure 1. The designed openers of planting unit: 1 – metal ring; 2 – porous fixing; 3 – semi-sphere; 4 – hanger.

Laboratory and field testing of the designed openers, installed on planting units of SP-4M planter (modernized sugar planter), was conducted. The opener of the planting unit USP-4 (universal sugar planter) was also tested for comparison. The obtained results of testing are presented in Fig. 2, and they show, that the opener of the first type injures the roots less.

The opener of type I is made in the form of a metal ring with a porous fixing, and it decreases the number of injured roots in comparison with USP-4 machine (universal sugar planter). The opener of type I, made like a semi-sphere with a porous fixing, decreasing root injuring to 1.0–1.5%, was the most efficient. The conditions of conducting trials corresponded to agro-technical requirements. The number of injured roots (in the crown zone) was determined visually according to the standard methods for each type of the opener. The observation of the root injuries of 120 roots was conducted visually for each type of the opener.

The suggested opener of type I installed on SP-4M planter (modernized sugar planter), which had state trials, ensures the sufficient quality of planting parent roots at different spacing and practically excludes root injuring, resulting in raising the yield of sugar beet.

The system of spray delivery of nutrients and growth stimulators through the cup to the roots having the diameter of 50–120 mm, the length 150–250 mm, and the average weight 2.5–4.3 kg is envisaged. The liquid comes to the measuring device by changing

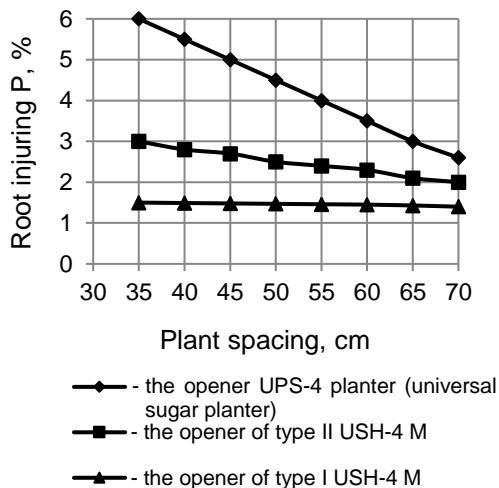


Figure 2. The dependence of root injure on the type of the opener.

the movement of the star with the flute, causing the measuring device lever move, and it enables to ration the liquid depending on the speed of the mechanism movement and deliver the liquid from the reservoir through rubber pipeline. Mineral fertilizers and water preservation granules are delivered in the device having a double-section bin with the pipeline rationing mechanism, directing top and is delivered in the furrow having the depth of fluffed soil 300 mm, moisture 25–40%, beginning from upper layers, and field relief sloping no more than 10°. At the same time, nutrients and growth stimulators are delivered to the cup with a cover and owing to the internal spiral-like surface of the injector the mixture is sprayed on the root (Medvedovskyi, 1988; Balan, 2012; Method of Choosing..., 2013).

The technological process is conducted in the following way: furrow is made by the furrow opener, and planting cones enter there. During the movement of equilateral triangles of the planting cones' mechanism and interaction with the opener, the root is planted in the soil, and the gauge compaction wheel fixes the root in vertical position. The speed of the triangles' movement per one rotation and planting cones in the process of planting is not constant and it changes in a wide range (0.1–0.7 m sec⁻¹) with the help of eccentric star which is set on the shaft with the given eccentricity. It slows down the movement, decreases the speed to a complete stop of the planting cone at the moment of filling the root in it. Creating the planting unit having equilateral triangles presupposes the contact of the cones with soil at the moment of filling. The system of spray delivery of nutrients and growth stimulators directly to roots is envisaged on the installation (Dobrotvortseva, 1986).

The research was conducted on the installation for planting root crops. The area of the plot was 0.5 ha; the type of soil – ordinary, low-humic black soil; the depth of the preliminary soil tillage – 300 mm. The depth and soil wetness content were determined by the standard methods (Lapenko, 2011). The surface slope of the fluffed layer was determined with the help of a metal ruler in three points diagonally. Soil firmness was defined relatively across the field 3–5°, with Reviakin penetrometer (State technical requirements 5096:2008. Soil quality. Determining soil firmness with Reviakin penetrometer). The length, diameter, and mass of roots were determined with standard accuracy and recorded with data significance of 95%. In accordance with agro-technical requirements, the roots having the diameter of 50–120 mm and length 150–250 mm were used. While investigating the quality of planting roots the following diameters were chosen: small – 50–70 mm; medium – 71–100 mm; large – 101–120 mm; having the length of: 150–180 mm, 181–210 mm, and 211–240 mm; and the weight 50–150 g, 151–300 g, and 301–500 g.

THE RESULTS OF INVESTIGATING THE PARAMETERS OF AGGREGATES FOR PLANTING SUGAR BEET STECKLINGS FOR ELITE SEEDS

At simultaneous planting stecklings and applying liquid fertilizers, the optimal rate of the substance consumption is 50 L ha⁻¹. Water preservation granules which can absorb and keep 90% of moisture in its volume were used in our research. The granules keep melted and rain moisture without changing its properties and 95% of it is used rationally during plant vegetation period.

After disintegration the granules are degraded into nitrogen compounds – carbon dioxide and water, which enriches the soil with nutrients.

Theoretical research (Samoilov, 1968; Davydiuk, 2011; Lapenko, 2011) has shown, that plant spacing of roots is set by measuring the angular and forward velocities of the unit (Fig. 3), which is achieved by:

- a) changing the star with different number of tines on the reducing gear of the drive shaft;
- b) changing the forward (operating) speed of the tractor.

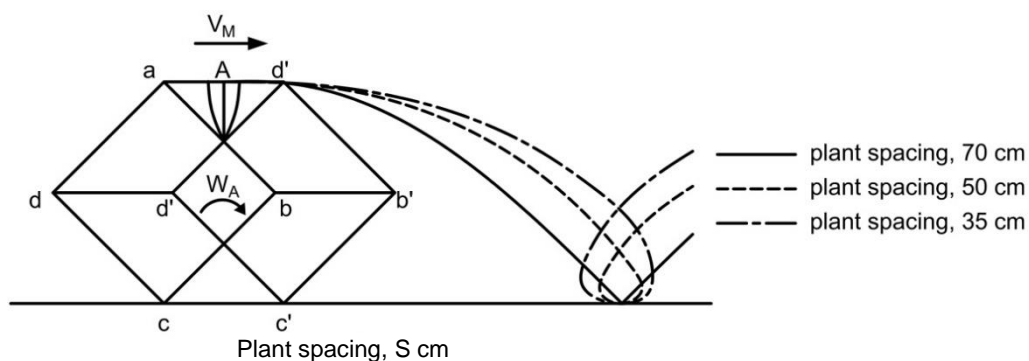


Figure 3. The trajectory of the planting cone movement at different plant spacing while tractor’s moving on the second gear with speed reducing gear.

As it is seen from Fig. 3 the value of the ‘loop’ made by the cone in the process of planting increases together with decreasing plant spacing and reaches the maximum at plant spacing 35 cm (curve V).

The experiments were conducted according to the standard methods using medium size roots. The real speed and the number of experimental planting unit rotations at each gear were determined by the stop-watch at passing the definite way (Fig. 4). The graph was made according to the results (Fig. 5).

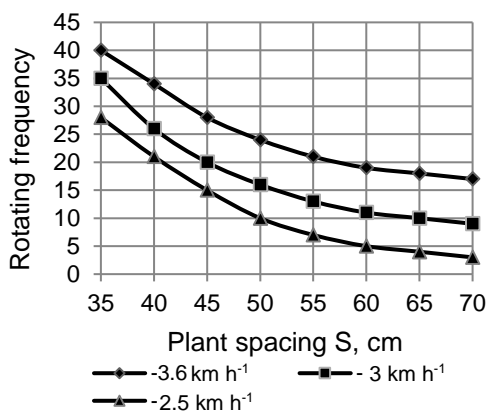


Figure 4. The dependence of the planting unit rotating frequency on plant spacing at different tractor gears.

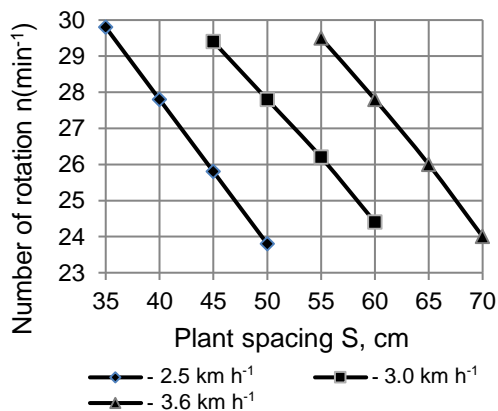


Figure 5. The dependence of plant spacing on the number of the planting unit rotations.

The dependence of plant spacing on the planting unit kinematic parameters is expressed by the dependence

$$S = \frac{k \cdot V_m}{Z_x \cdot n_r} K_s \quad (1)$$

in which S is plant spacing; V is the speed of the machine movement; Z_x is the number of tines in the changeable star of the reduction gear; n is the number of rotations of the tractor engine power take-off shaft; K_s is the experiment coefficient which takes into account the deviations of the real plant spacing from the calculated one; K is the experiment coefficient.

$$K = \frac{60 \cdot Z_{np}}{Z_x \cdot i_1 \cdot i_2} \quad (2)$$

in which Z_{np} is the number of tines in the star of intermediate shaft; Z_x is the number of planting; i_1, i_2 is the gear box ratio and the ratio of the intermediate valve to the planting unit.

At decreasing plant spacing as a result of the considerable discrepancy of the forward and angular velocities of the planting unit, different loadings (17.3–19.2 kH) on the planting cone occur. That is why the necessity to solve this problem constructively by changing planting unit and developing furrow opener arises. Such furrow opener would provide the furrow of the corresponding configuration and sizes for planting cones. While choosing the main design parameters of furrow opener we decided that that furrow depth had to be not less, than the root length, the width – not less than the root diameter, and the tilt angle had to be equal the angle of the planting cone.

The task of the research was establishing the optimal sizes of the raised furrow and the distance between compaction wheels for conducting the planting with the given density, decreasing the loading on the planting mechanism, decreasing energy consumption. At first, the compaction and fixation of roots by gauge wheels ($D = 400$ mm, $B = 10$ mm; $\beta = 15^\circ$) of the planting mechanism were conducted. The following stage of soil compaction after planting roots was conducted by compaction wheels ($D = 700$ mm, $B = 150$ mm; $\beta = 7^\circ$).

The influence of the distance between the rear compaction wheels and the density of planting roots was studied. The range of changing every 20 mm from 100 to 180 cm was made by the adjusting screw. The analysis of dependencies (Fig. 6) showed, that during furrow raising with ristle the density of planting increases and exceeds the upper limit of agro-technical requirements by 18.7%. That is why the improved furrow opener for raising furrow of the operational width was used. This furrow opener has the ristle with movable and immovable folds assisting to regulate furrow width with the screw and such design excludes overloading of the planting unit arising at discrepancy of angular and forward velocities. Using the improved design of the furrow opener ensured the following of agro-technical requirements concerning the density of planting by the compaction wheels' screw.

In case of water-logging soil the quality of planting deteriorates because of adhesion between the wheels that is why the distance between the rear compaction wheels 140 ± 5 mm should be considered optimal. The estimation of soil compaction quality was defined around the planted root by the reference portable dynamometer of the 3d category. The results of the research are presented in Fig. 7.

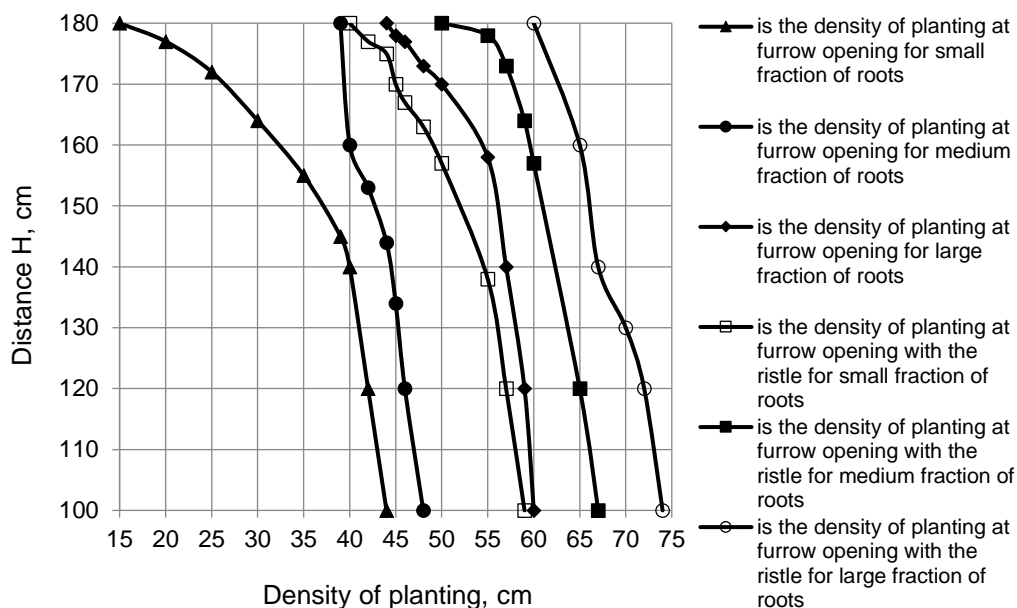


Figure 6. The dependence of the density of root planting on the distance between the rear compaction wheels.

The necessary density of planting small and medium roots is ensured at furrow width 110–140 mm.

The depth of planting roots was changed owing to: changing the distance between the axes of planting tetrahedrons of the planting unit compaction wheels, which was achieved by a special wheel regulation screw within 380–460 mm. Graduation was made on the movable and immovable frames of the compaction wheels. The motion depth of the furrow opener was constant – 250 mm. The results of the research are shown on the graph (Fig. 8).

The analysis of Fig. 8 showed that planting depth grows in proportion to increasing the distance between the axis of trihedrons and compaction wheels and according to agro-technical requirements it is ensured at 405–440 mm, which is determined at test planting.

Increasing the planting depth depending on the motion depth of the furrow opener is noticed. The planting depth of roots is affected by furrow depth. Changing the motion depth is achieved by the reinstallation of the furrow opener vertically on fixing frames. The depth range with the interval of 30 mm from 200 to 290 mm was changed at constant

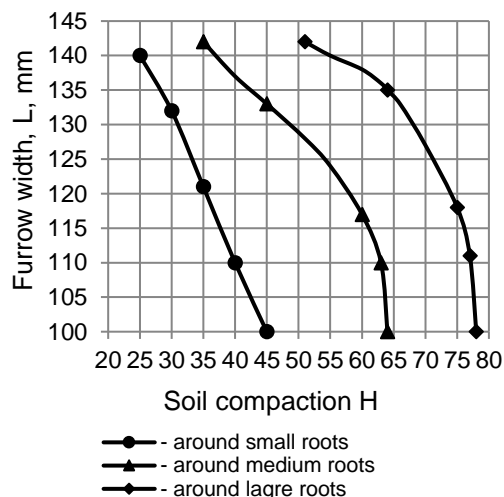


Figure 7. The dependence of soil compaction on the width of the raised furrow.

inter-axis distance (420 mm) of the planting unit. Increasing furrow depth for small roots affects the quality of planting.

During the research the tilt angle was regulated with the chaser of the planting cone within 10–20° and the interval of 2.5° using angle meter in case of discrepancy of the forward and angular velocities of the planting unit. Other parameters remained optimal. Plant spacing for the roots having the diameter of 70, 60, 50, and 40 mm was investigated.

It was established by the results of the research, that root sizes practically do not affect the verticality of planting. The optimal conditions for every plant spacing were chosen provided that agro-technical requirements were followed – not less than 90% of roots with the tilt up to 10°. The results of the research are presented in Fig. 9 by the curves of vertical root distribution depending on the tilt angle of the planting cone for large roots with spacing 70 cm. The tilt angle of the planting cone for the specified spacing is equal –2.5°.

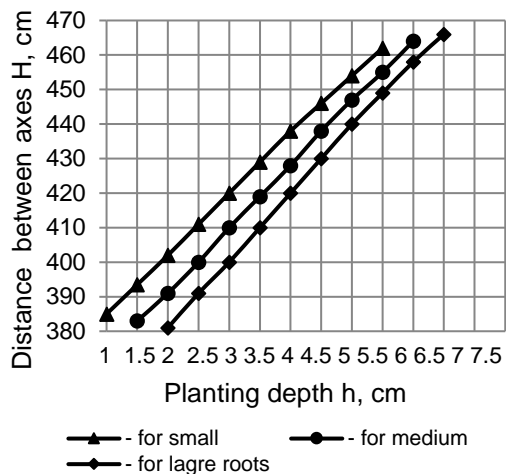


Figure 8. The dependence of planting depth on the distance between the axes of planting trihedrons and compaction wheels correspondingly.

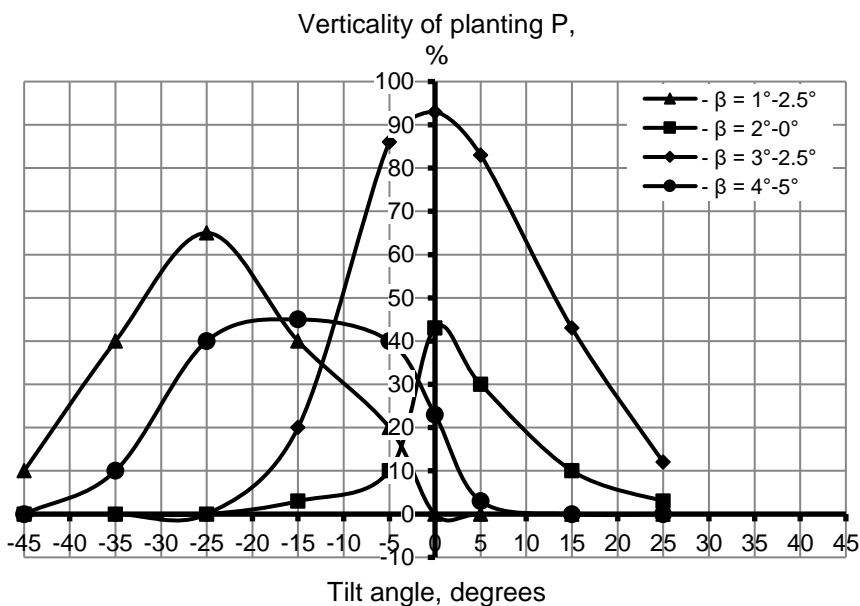


Figure 9. The dependence of planting verticality on the tilt angle of the planting cone β .

Openers with porous fixing, central opening and enlarged diameter were used in the experiments. Optimal indices were achieved in openers with porous filling: the beet crown and flank surface of the root may be injured up to 15%.

During energy testing the following information was determined in succession:

- 1) draft resistance;
- 2) moving the investigated sample on idle run;
- 3) ristles and furrow openers;
- 4) rotary moment on driving shaft;
- 5) the real speed of the investigated sample.

Draft resistance of the furrow opener was determined by the dynamometer of the 3d category at different values of running depth and the width of the raised furrow (Fig. 10, 11). The impact of the furrow width on draft resistance was investigated while changing its value by the adjusting screw, with spacing of 10 mm within 100–140 mm. The curve is presented in Fig. 7. The parameters are chosen depending on the conditions and sizes of roots.

As it is seen in Fig. 10, draft resistance grows by 2.7–4.6% while increasing the running depth of the furrow opener.

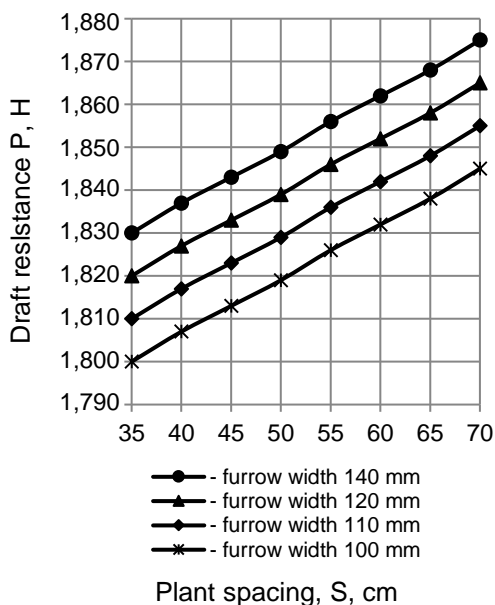


Figure 10. The dependence of draft resistance of furrow openers on plant spacing.

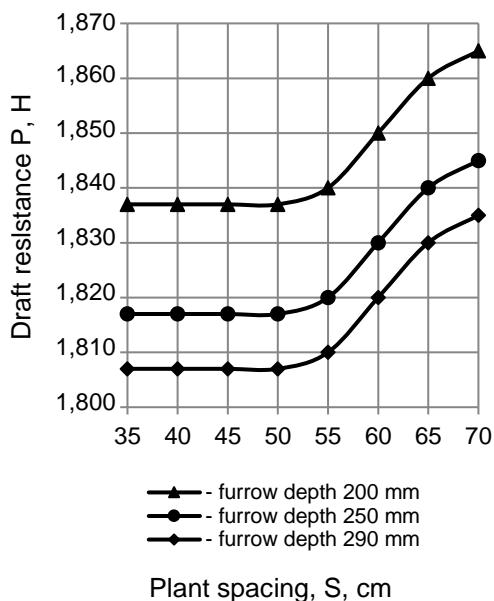


Figure 11. The dependence of draft resistance of furrow openers on plant spacing.

The dependencies of draft resistance of ristle and furrow opener are presented in Fig. 12.

During furrow opener operating draft resistance is not much higher than 2.9–4.7%, than with the ristles.

The dependence of the rotary moment on the machine driving shaft upon plant spacing and operating mode is presented in Fig. 13.

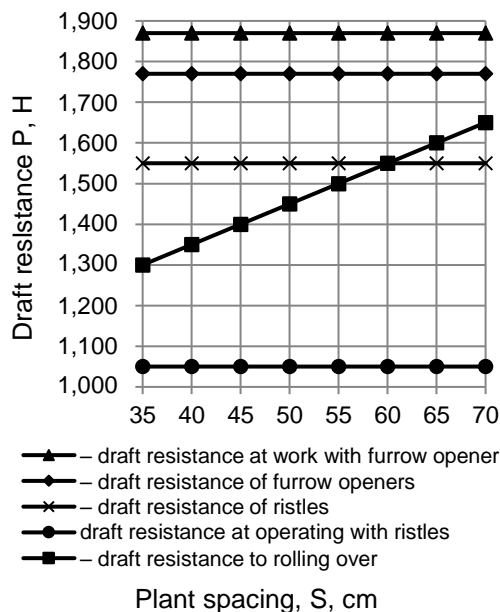


Figure 12. The dependence of draft resistance on plant spacing.

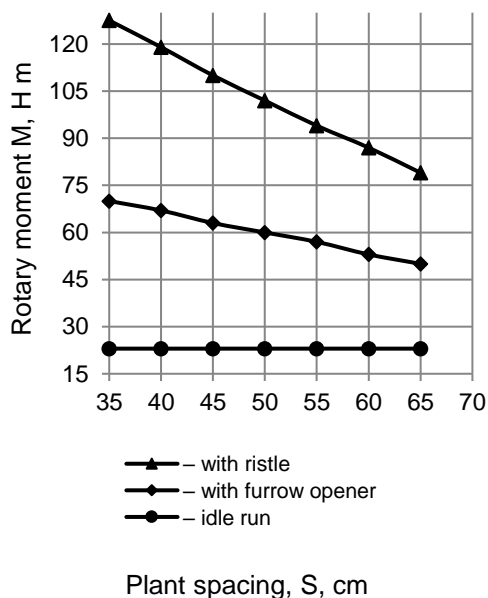


Figure 13. The dependence of the rotary moment on plant spacing.

At raising furrow with the ristle, the rotary moment on the driving shaft of the experimental planter sample grows from 70 to 120 Hm with decreasing plant spacing from 70–35 cm correspondingly. During using furrow opener, the rotary moment decreased considerably (by 10–50 Hm).

As a result of conducted research, the optimal parameters and operating modes of the planter at different planting patterns were established (Table 1).

Table 1. Optimal parameters and operating modes of planter at different planting patterns

Indices	Planting pattern, cm			
	70×40	70×50	70×60	70×70
Root diameter, mm	50–70	71–100	71–100	101–120
Root length, mm	150–180	181–200	181–200	201–220
Planter rotation frequency, min ⁻¹	20–35	10–25	7–22	5–20
Furrow width, mm	100	130	160	180
Soil compaction, Hm	105–135	115–140	110–140	100–130
Sugar beet seed yield (bunker weight), t ha ⁻¹	3.45	3.63	3.35	3.70

The indices of planting quality were determined by the standard methods (Dankov, 2011; Davydiuk, 2011; Lapenko, 2011). The experimental sample of the installation can change plant spacing together with ensuring the necessary quality and enables to use

optimally the area of additional fertilizing depending on root sizes and specific agro-technical conditions. It enables to raise the seed yield, which is confirmed by the experimental data, entered in Table 2.

The production trials of the installation have shown that the following factors are ensured: plant spacing of planting roots is 40–70 cm, which corresponds to agro-technical requirements; the evenness and dynamics of plantlets, the development of seed-bearing plants with their simultaneous ripening; favorable conditions for mechanized seed harvesting. The optimal root planting pattern for Poltava region 70×50 cm was determined. Moreover, the yield increase is 3.9–5.4 hundred weights ha⁻¹ comparing to the classical scheme 70×70 cm. Planting quality indices were determined according to the State Standard System 6053:2008.

It has been established that at applying nitrogen, phosphorus and potassium directly in the bunch the yield was 5.4 hundredweight/ha higher, and it replaced the double amount of fertilizers at tilling with simultaneous applying and decreased the percentage of injuries on roots at considerable concentration and unevenness of fertilizers' application.

Soil moisture content was determined with moisture indicator RIXEN MTR-732 at the depth of 100–150 mm 12, 20, and 40 days after planting and the indices were the following: 81%, 78%, and 69% correspondingly, which is optimal for the development of these crops. According to the results of the research energy saving technology of cultivating sugar beet for seeds was developed, including the sequence of operations, chosen in such a way, that the resource spending (plants, fertilizers, herbicides, hand labor, and investments) was minimal for ensuring the maximal harvest.

DISCUSSING THE RESEARCH RESULTS OF THE PARAMETERS OF AGGREGATES FOR PLANTING SUGAR BEET STECKLINGS FOR ELITE SEEDS

The aggregate with planting unit opener was designed to investigate the parameters of the technological process for planting parent roots of sugar beet for elite seeds. The spray system of delivering nutrients and growth stimulator was also envisaged (Method of Determining..., 2010; Lapenko, 2011).

The opener excludes the probability of root sticking in planting cone. It ensures the free falling of roots into the soil, root fixation, and minimizes parent beet crown injuring. The opener is made like depressed semi-sphere with a fixing of porous rubber with the possibility of centering the root in the cone. The opener ensures the sufficient quality of planting parent roots at different spacing (from 40 to 70 cm depending on the size of stecklings) and practically excludes injuring the roots. The suggested aggregate with the openers of the planting unit has quality control and parameter registration, which sufficiently ensures applying different fertilizers and water preservation granules to the roots.

Table 2. The yield of sugar beet seeds (bunker weight), t ha⁻¹

Year of research	Planting pattern			
	70×40	70×50	70×60	70×70
2013	2.14	2.27	2.43	1.54
2015	3.45	3.63	3.35	3.17
2016	3.24	3.54	3.82	3.36

The problem of mechanized root delivery to the planting cone has not been solved yet. Its positive solution will result in decreasing the number of workers.

It is expedient to conduct trials of the unit in different zones with different roots under the conditions of long-lasting maintenance, which will also enable to determine the reliability of assemblies and mechanisms. Wear-resistant materials (manganese steels) should be used for making the planting cones of the aggregate. The biological peculiarities of crop root system have not been studied sufficiently in connection with receiving elite seeds of sugar beet. Matrix experiment planning to broaden the field of research should be envisaged taking into account the determined new factors.

The results of the research can be used while modernizing the existing and designing new planting machines for planting sugar beet stecklings to obtain elite seeds in agro-industrial complexes of Ukraine.

CONCLUSIONS

1. The analysis of the scientific-technical information concerning the designing of planting machines for planting sugar beet stecklings to receive seeds has been made. The analysis has revealed the drawbacks and advantages of such information.

2. The dependence of the number of injured roots on the type of the opener has been established. It is recommended to use the opener of the first type.

3. The technological parameters of the aggregate and the expediency of planting sugar beet roots for seeds with the following spacing have been substantiated: 40–70 cm and 50–60 cm.

4. The rotational planting unit with planting cones for ensuring the quality of planting sugar beet stecklings has been adopted.

5. It has been confirmed by the research, that plant spacing of roots is regulated by changing angular and forward velocities of the unit, which is envisaged by:

- changing the star with different number of tines on the reducing gear of drive shaft;
- changing forward (operating) velocity of tractor.

6. The dependence of the frequency of planting unit rotations on plant spacing at different tractor gears and the aggregate speeds was determined: 2.5 km h⁻¹, spacing 35–40 cm; 3.0 km h⁻¹, spacing 50–55 cm; 3.6 km h⁻¹ and spacing 35–70 cm.

7. The dependence of the density of root planting on the distance between the rear compaction wheels at raising furrows by the furrow opener and ristle for the small and medium fraction of roots is confirmed at furrow width of 110–140 mm with the constant running depth of the furrow opener 250 mm.

8. The dependence of planting depth on the distance between the axes of planting tetrahedrons and compaction wheels has been investigated for roots. The distance grows in proportion and according to agro-technical requirements and is ensured at 405–440 mm. The depth range with the interval of 30 mm from 200 to 290 mm was changed at constant inter-axis distance (420 mm) of the planting unit.

9. The dependence of vertical planting on the tilt angle of the planting cone has been established β : -2.5° ; 0° ; -2.5° ; 4.5° , which was regulated by the chaser within 10° – 20° with the interval of 2.5° . Root sizes do not affect the verticality of planting at optimal conditions – not less than 90% of roots with the tilt of 10° .

10. Draft resistance of the furrow opener and ristles depending on the motion depth within 200mm, 290 mm, the width of the raised furrow within 100–140 mm, and plant spacing of sugar beet parent roots 40–70 cm has been determined.
11. The dependence of the rotary moment on plant spacing: with the ristle from 40 to 120 Hm; and furrow opener from 40 to 60 has been substantiated.
12. As a result of the conducted research the optimal parameters and operating modes of the planting unit and the aggregate on the whole have been determined.
13. The results of laboratory testing have been confirmed by production trials of the planting aggregate in Poltava region during planting sugar beet stecklings with plant spacing from 50–70 cm and yield growth 5.4 hundredweight/ha in comparison with the classical scheme 70×70 cm.

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Modelling of the bioeconomy system using interpretive structural modelling

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Abstract. Due to European and global resource efficiency efforts, the bioeconomy research and the search for new bioresource valorisation alternatives has become topical. Bioeconomy directly concerns such major sectors of the economy as agriculture, forestry, fishery, as well as other indirect bioeconomy sectors. However, the practical implementation of bioeconomy has had quite low implementation rate, which is partly caused by the multitude and variety of factors that affect the bioeconomy system. This paper evaluates seven bioeconomy affecting factors (particularly related to biotechnology concept) and links between them in order to promote successful implementation of bioeconomy. To evaluate these factors interpretive structural modelling method (ISM) is used. The application of ISM method allows to not only identify the factor interaction links, but also to graphically represent their directed structure. The results show that three out of seven factors have the strongest interrelation, namely, climate change, bioresources and technologies. This research can be complimented by further adding other factors that could be influencing for bioeconomy development, for example, financial resources, human health, well-being, and so on; therefore, to reach better understanding about influential factors and bioeconomy dependency on them; also, system dynamics approach could be used in order to fully uncover the factor interaction links.

Key words: bioeconomy, interpretive structural modelling, bioenergy, bioresources, climate change, production, pollution, biotechnologies, natural environment, infrastructure.

INTRODUCTION

Driven by the concerns of our major dependence on fossil fuels, their foreseen depletion and the search for alternatives, as well as such societal challenges as climate change, resource depletion and scarcity, environmental pollution and its negative impact on human health and lifestyle, the transition from current fossil-based economy to a knowledge-based bioeconomy (also known as bio-based economy) has become even more topical and important in recent years (European Commission, 2012, McCormick & Kautto, 2013).

Bioeconomy aims to manage bioresources in a way that allows to turn them into energy, goods, fuel, food and feed in a sustainable manner (European Commission, 2012). Within the bioeconomy concept, large attention is also given to valorisation of wasted bioresources (industrial co-products, by-products and waste) so that they can be used for production of other products or energy instead of treating them as wastes. Successful implementation of bioeconomy would result in reduction of CO₂ emissions

released in the atmosphere, more sustainable resource management, increased food safety, reduction of waste and pollution as well as increased employment rate in bioeconomy sector (European Commission, 2011).

The Bioeconomy sector is advancing fast – the data shows a growing number of annually published bioeconomy related research papers, especially regarding biotechnology and applied microbiology, energy and fuels and environmental sciences (Bugge et al., 2016). In 2012, the European Union (EU) launched their Bioeconomy Strategy, followed by its member countries – Latvia, Finland, Germany, France, Spain, and Italy – to frame their national bioeconomy strategies (Lier et al., 2018). This fast-growing field is predicted to peak by 2030 (Koukios & Sacio-Szymańska, 2018), however the results so far show low development rate in the bio-product and chemical production sectors (Carus et al., 2016). This could be related to the deficient approach of practical bioeconomy implementation strategies despite the rapidly growing scientific research on bioeconomy. There is a lack of research accounting for the complex interrelated nature of the bioeconomy system and other factors related to it (Muizniece et al., 2018). Bioeconomy is affected by many multifaceted factors, therefore, one of the reasons for its slow development rate could be the lack of considering all those factors and the links between them (European Commission, 2011). Similarly, McCormick & Kautto (2013) stress the necessity to examine the key factors that influence bioeconomy development. Therefore, a research into those factors that affect the bioeconomy and the identification of their interlinkages and their quality would promote faster implementation of bioeconomy and increase sustainable use of bioresources.

In our previous study, the Nexus approach (i.e. identification and analysis of interaction links) has been suggested for the analysis of the multi-faceted factors that influence bioeconomy development (Muizniece et al., 2018). In this research, 22 factors were considered as selected from literature and by logical analysis: land, waste, welfare, climate change, bioresource, fossil resource, human resources/population, research and innovation, energy, education/knowledge, policy, health, behaviour, technologies, water, natural environment, consumption, financial resources, economic growth, food, production, and pollution.

To initiate an in-depth analysis of the interlinkages of the bioeconomy system, first, it is beneficial to reduce the number of factors for the initial analysis (due to time and resource constraints, as well as for more successful testing of the initial research concept). Bioeconomy researchers have reported various factors and their subsets that are assumed to be the most influential for further development of bioeconomy. Sillanpää & Nicbi (2017) identify biomass as the core of the bioeconomy; Gatzweiler & von Braun (2016) predict that agriculture will be the main constituent of bioeconomy. In another study, Finnish future environment professionals named climate change as the main driver towards the bioeconomy (Vainio, 2019). Koukios & Sacio-Szymańska (2018) researched bioeconomy value-based demand factors. Based on expert assessment and application of the radical technology inquirer tool, they named following factors as the ‘hard core’ of bioeconomy value chain: food, health, life, materials, goods, energy, governance, eco-systems. In their study, these factors accounted for 60% of the total weight of the bioeconomy value relevance.

Therefore, based on literature analysis, two sub-groups of factors are selected that are related to environmental and technological aspects of bioeconomy. Specifically, we focus on the biotechnomy or technology based bioeconomy concept by analysing

following factors: bioresources, technologies, production, pollution, infrastructure, natural environment and climate change.

However, the mere identification of factor interaction links would not allow to explain the full extent and relationships of their impact on bioeconomy development. Therefore, the aim of this paper is to design a graphical representation of the structure of this biotechnomy subsystem by indicating its interlinked factors and the direction of their relationships (causal links). The methodology used in this research paper is supplemented with the use of Interpretive Structural Modelling (ISM) method to build a directed graphical description of this complex system.

MATERIALS AND METHODS

Interpretive structural modelling

The previous study (Muizniece et al., 2018) uses a simple graphical representation to describe the interlinkages between the factors that are influencing bioeconomy system. However, this approach gives only an initial insight into the structure of the system. After a more in-depth literature analysis of the links between each pair of bioeconomy influencing factors, the need for the use of structural modelling was evident.

ISM method, created by Warfield in 1973 (Azevedo et al., 2019), has been applied in wide variety of research to hierarchically represent the structure of complex systems to aid decision-making process (Sajid et al., 2017). Lately, Azevedo et al. (2019) performed analysis of countries' biomass related sustainability; Zhao et al. (2019) have used ISM to structure factors representing the development of renewable energy projects; Sajid et al. (2017) applied ISM to model risk factors in biodiesel systems; while Lim et al. (2017) applied it to investigate sustainable supply chain management. However, to the authors' best knowledge there is no previous study regarding the ISM of biotechnomy factors.

ISM is a theoretic causal mapping approach that is used to analyse the impact of one variable on another variable (Azevedo et al., 2019). Thus, ISM allows to identify the contextual relationships between analysed factors and organize those complex relationships in a directed structure (Wu et al., 2015). The inputs for the ISM model are the factor relationships that are identified through literature analysis, as well as expert judgement of the bioeconomy research team. Wu et al. (2015) note the ability to systematically incorporate expert knowledge as one of the advantages of ISM method.

The implementation of ISM method includes five sequential steps that are summarized in Fig. 1.

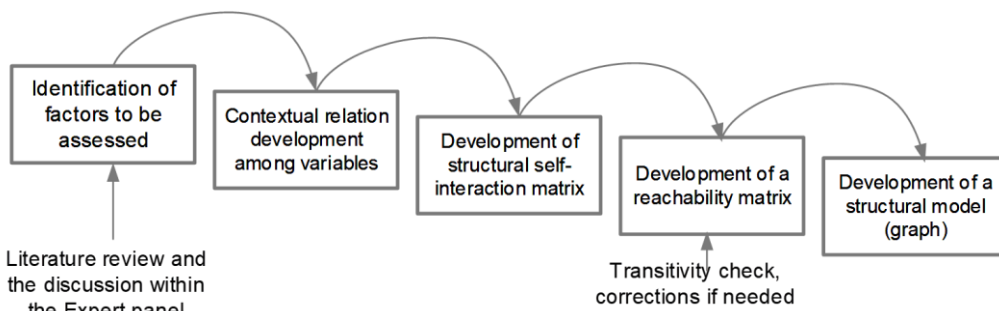


Figure 1. The sequence for implementation of interpretive structural modelling.

First, specific factors characterizing the structure of the studied system may be selected either by an expert panel or by literature review. In the current study, the most significant biotechnology related factors are identified, defined and described based on literature analysis. After, the pair-wise contextual relationships between the studied factors are evaluated as neutral, influential or comparative, if there is a relation between the pair of factors it is designated with Y, while in case there is no relation between two factors, then it is designated with N (Sajid et al., 2017). Sequentially, within the third step of ISM method, the previously identified relations are further assessed regarding the contextual direction of the relationship. For a binary (adjacent) matrix ($i \times j$) four different symbols are used to denote the type of relationship (Sajid et al., 2017):

V – factor i is linked to j but j does not link to i ;

A – factor j is linked to i but i does not link to j ;

X – when both factors are linked to each other;

O – when neither factor is linked to the other.

The fourth step includes transforming the structural self-interaction matrix into a binary reachability matrix (RM) and checking its transitivity. To create a RM following rules are applied (Majumdar & Sinha, 2019):

if (i,j) entry is designated with V, in RM this entry is designated with 1 and the (j,i) entry with 0;

and vice versa, for each relation designated with A, the (i,j) entry in RM is designated with 0 and the (j,i) entry with 1;

in case of X then both entries (i,j) and (j,i) are substituted with 1;

and for O – both entries become 0.

Simultaneously the ISM transitivity is checked by applying the rule that if a factor A is related to factor B and if factor B is related to another factor C, then factor A is also related to factor C (Sajid et al., 2017). Within the fifth step of ISM, the transitive links are removed and the reachability matrix is converted into a structural model, i.e., a directed graph (Azevedo et al., 2019).

RESULTS AND DISCUSSION

In order to build the structural model, the links between all the factors need to be identified, which was done through analysis of scientific literature and by considering experts' opinions to characterize of each particular link.

Prior to further evaluation, each factor is defined and clearly described to avoid any misinterpretation:

bioresources are renewable biological resources that can be obtained from water, land, air, as well as waste and co-products from industry (Blumberga et al., 2016);

technologies are methods, systems and equipment that have been created based on the knowledge and are being used for practical purposes (Collins Dictionary);

climate change is a change in the climate that is directly or indirectly linked to human actions that cause changes in the atmosphere and that is additional to natural change in the climate within the certain time of period (Kyoto Protocol, 1997);

production is rational, sequential, purposeful action system in order to provide products or certain services (Saksonova, 2010);

pollution – water, air, soil pollution that has negative impact on living organisms and surrounding environment. Pollution can present as chemical leakages, heat

discharge, and physical pollution – radiation, noise, vibration, electromagnetic pollution (Harrison, 2006);

natural environment – all natural or by human affected living or non-living environment (Melecis, 2011);

infrastructure – simple physical and organisational structures and facilities (e.g. buildings, roads, power supplies) required to ensure the operation of a society or enterprise (Oxford Dictionary).

Interlinkages of Climate change factor

On one hand climate change is forcing people towards implementation of climate change mitigation measures, e.g., replacing fossil resources with bioresources, and thus increasing **bioresource** use. However, climate change also has a negative impact on bioresources, as changes in temperature or humidity are crucial for the growth of bioresources and the environment in which they grow. Therefore, if these parameters change, the bioresource distribution region may change (Gibbons et al., 2000). Climate change has contributed to the development and use of alternative **technologies** that are more environmentally friendly. These technologies – biotechnologies and climate technologies – have been designed to reduce the causes of climate change – greenhouse gas emissions. Biotechnologies are considered to be more environmentally friendly as they generate lower emissions (Hedenus et al., 2014). The use of such alternative technologies would lead to Climate change mitigation, whereas the technology lock-in, i.e., use of older technologies that are usually tied-up to fossil resource use would enhance Climate change. Climate change also affects the **natural environment**, where the natural development of bioresources is ensured, including food production. The greatest impact on the natural environment caused by climate change is the increase in the average ambient temperature; more frequent natural disasters (such as fires, storms, floods). Climate change and its consequences directly affect the natural environment (Liu, 2016), (EPA, 2016). On-going climate change is forcing **manufacturers** to improve their production technologies or evaluate production processes and their efficiency. Directives, as the directive on industrial emissions (integrated pollution prevention and control) are designed to reduce the environmental impact of industry (European Union, 2010). Climate change also has an impact on **production** through the raw materials needed in the production process. For example, climate change is predicted to reduce coffee bean productivity (Bunn et al., 2015). Climate change has a direct negative impact on the infrastructure stability, longevity and appropriateness to local conditions, i.e. climate change is responsible for floods and other disasters that affect infrastructure. The most affected would be the less developed areas, rural areas, coastal and mountainous regions (European Commission, 2013). The improvement of infrastructure resilience reduces its vulnerability to climate change effects (European Commission, 2013). Climate change does not directly affect pollution; it is however the consequence of environmental pollution.

Interlinkages of Bioresource factor

Bioresource use has an inverse effect (presented as an opposite direction link) on **Climate change** increase. Thus, the more fossil resources are substituted by bioresources, the lower are the society's generated non-renewable greenhouse gas (GHG) emissions because by replacing fossil resources with natural resources, the

climate change will be decreasing (Gaurav et al., 2017). The demand for bioresource-based products promotes the need for **technologies** that can process those bioresources into a wide range of products. The European Union directs significant resources directly into research and innovation to promote the development of new biotechnologies (European Commission, 2018). The demand for bioresources contributes to the development of greener technologies (Engelmann, 2011) and the properties of the bioresources impact the complexity of the technologies. Bioresources are one of the most important products of the **natural environment**. Bioresource production provides oxygen, food and other primary and secondary important products for the society. The increase in bioresource demand would also increase the amount of oxygen produced and the amount of CO₂ attracted within the biomass, thus improving the natural environment (Rubene, 2011). The local bioresource availability, as well as, bioproduct manufacturing know-how, significantly impact the development of **production** facilities. The manufacturing industry has to become sophisticated in order to deliver as its core function the bioresource conversion into necessary bioproducts (European Commission, 2018). Various alternatives for replacing fossil resources by bio-renewable sources for the production of such products as various types of chemicals (Reddy et al., 2016), fuel (Behera & Ray 2019) and plastic alternatives, have already been invented (Sagnelli et al., 2017). Bioresource use may be the culprit for some environmental **pollution**, e.g., the use of biomass for energy production leads to emissions in air. Thus, increase of bioresource use would lead to pollution increase. On the other hand, the increased use of bioresources substitutes GHG emissions from non-renewable sources with ones that are from renewable sources, so the link between bioresource use and pollution is quite versatile. Bioresources do not have a direct impact on infrastructure as a whole.

Interlinkages of Technology factor

Technologies, especially their efficiency, have an impact on **climate change** (Salar-García et al., 2019). Technology improvements reduce environmental impact, and hence climate change. Technologies also have an indirect positive effect climate change through innovation and knowledge, as through the development of innovative technologies, bioresource use and substitution of fossil resource use can be increased. Technologies are used to turn raw **bioresources** into finished bioproducts. This is a very important and strong link (Loeffler et al., 2017). The impact of technology on the **natural environment** is indirect and exhibits through technology's link to pollution (Fernández-Dacosta et al., 2019). Technologies are an important part of the manufacturing process – it is a strong direct link. Technologies affect the amount of **pollution** generated – improving the efficiency of the technologies reduces their impact on environmental, thus this is an opposite direction link. Technologies can also be used to detect contaminants that are not easily detectable by the eye. For example, modern technologies allow to detect ozone pollution (Ripoll et al., 2019), thus leading to better environmental research and detection and monitoring of pollution. Technologies are required to ensure public technological **infrastructure**, as transport systems or sewerage, and relieve societal problems as environmental pollution (Aichholzer & Schienstock, 1994).

Interlinkages of the Natural environment factor

The environment is responsible for the natural regulation of **climate change**. However, as a result of human economic activity, those natural processes are hindered. One of the pathways to mitigate climate change is to increase the area of forests, especially because young trees grow faster and attract carbon dioxide to a greater extent than the old trees can (Latvian State Forests). The natural environment has a strong direct impact on bioresources. The natural environment determines which **bioresources** can be grown and extracted in a particular area. The quality of bioresources is affected by a set of environmental conditions such as water regime, soil quality, rocks, climate, etc. Improper bioresource management (depletion of land, changes in water regime, reduction of biodiversity) can change the natural environment, which in turn affects the quality and quantity of potential bio-resources. By sustainably managing the natural environment, its quality will not be lost and, if necessary, nature will be able to self-clean and regenerate. The natural environment does not directly affect **technology**. However, some indirect effect can be transferred through the linkages between natural environment and bioresources and bioresource linkage to technologies. The natural environment affects **production** indirectly, for example because of the demand for resources (including bio-resources) whose production depends on the natural environment. However, in the current model this link is depicted with zero, as the explained connection is depicted by the natural environment and bioresource positive link. Natural environment has a strong direct connection to **pollution**. As the natural environment is the medium through which air, water and other pollution may be degraded (e.g., by microorganisms) or captured, thus the pollution level may be reduced. The natural environment has a direct impact on infrastructure, as the natural environment (e.g. terrain, climate, special nature areas) can be a limiting factor as to whether an infrastructure can or should not be realized.

Interlinkages of the Production factor

Similarly, as the applied technologies, the production has a significant impact on **climate change**. The production processes can be understood as a process where the raw materials are turned into the goods, energy or food and feed by using various processing methods. Most of the **pollution** that contributes to climate change comes from the production process, such as the processing of iron and the extraction and use of non-renewable resources. Renewable energy and bioresources are the environmentally friendly alternative that reduces production's impact on climate change (Handayani et al., 2019). **Bioresources** constitute an essential raw resource for production, especially in the context of sustainable development and bioeconomy. Considering current national and EU and global level legislation, it is envisaged that the use of bioresources for production will increase (European Commission, 2018). Production volumes, the used raw materials and legislation determine which **technologies** should be used in the particular production process (BREF). Production efficiency can determine how large and how dangerous the **pollution** will be (Ghaly et al., 2004). The manufacturing of bioproducts indirectly affects the **natural environment**, as it enhances the demand for bioresources (but this is conveyed by production and bioresource positive connection). With constantly increasing number of population, larger amount of food and goods are required for the society, which means increased load on land and natural environment (The Conversation, 2015).

Interlinkages of the Pollution factor

Climate change is most affected by pollution resulting from agricultural activity and energy production. The intensification of agriculture has led to an increase in the use of synthetic fertilizers, pesticides, tractor equipment and energy (mostly produced from fossil resources) thus contributing to **climate change** (Landrigan et al., 2019). Pollutants such as nitrogen oxide (NO₂), sulphur dioxide (SO₂), ozone and particulate matter (PM) affect **bioresource** growth by impairing photosynthesis, altering plant structure and functions, and lowering production yields. Excessive heavy metal concentrations worsen seed germination and plant growth, resulting in reduced agricultural production (Sun et al., 2017). This indicates an opposite direction link between pollution and bioresources, larger pollution levels reduce bioresource production yields. Air pollution may be transferred to the **natural environment** through settling or precipitation. For example, acidous emissions containing sulphur and nitrogen can bond with water molecules and can be transferred to the earth through precipitation, sequentially leading to acidification of the soil and affecting plant growth (Sun et al., 2017). Agricultural activities (especially intensive agriculture) may lead to diffuse environmental pollution, e.g., when pesticide residues get into surface waters, or to point source pollution, e.g., when untreated sewage is introduced into the environment. Therefore, pollution has direct impact on the natural environment – the higher the pollution, the worse the condition of the environment will become. The direct effects of pollution and **technology** interaction are related to damage that the pollution can cause to agricultural and transport equipment, e.g., the acid rain causes corrosion of various metals, resulting in accelerated equipment failure (Sun et al., 2017). The additional connection of these factors is related to the fact that increased pollution levels and the problems they cause lead to development of new pollution treatment technologies. Therefore, this connection has two sub-links, a positive and a negative direction. The effects that the pollution has on infrastructure are reflected through its impact on technologies, and similarly, the impact of pollution on production reflects through the impact on bio-resources. No direct interaction of pollution on infrastructure and on bioresources was identified.

Interlinkages of the Infrastructure factor

Much of the infrastructure is energy intensive, thus impacting the demand for energy sources (including **bioenergy sources**) and generating pollution, that affects **climate change**. The efforts to reduce greenhouse gas emissions should also apply to infrastructure, especially energy and transport infrastructure. Thus, infrastructure improvements (and adjustment towards bioenergy use) would lead to reduction of the causes of climate change (Ingram & Brandt, 2005). The availability or lack of infrastructure can affect the development of **technological** innovations and, consequently, economic productivity (National Research Council, 1995). Infrastructure availability is an important aspect when choosing where to place or implement an economic activity, as water, wastewater and energy infrastructures are needed for production processes (Ingram & Brandt, 2005). The availability of infrastructure (both transport and utility) contributes to the development of **production** facilities in a particular area, while the lack of infrastructure hinders it, indicating a similar direction link. This applies to both the traditional industries and the development of the bioeconomy. Vice versa, the industrial development in a specific area attracts development of the necessary infrastructure. The infrastructure and **pollution** link is

significantly related to infrastructure construction period, when both air, water and other emissions are produced (Moretti et al., 2018). On the other hand, some types of infrastructure are directed specifically towards pollution reduction, i.e., sewerage and wastewater treatment plants. For the structural model this factor is subdivided into two parts to show its dual nature. Infrastructure competes with the natural environment for land resources, but there is no direct link between the factors. The introduction of sustainable construction practices among other things, for infrastructure projects would lead to fewer disturbances to the **natural environment**, however this would manifest through reduction of primary resource and fossil-based energy consumption and through lowering the pollution (Georgopoulos et al., 2014).

Modelling of the identified links

According to the described ISM methodology, first, the structural self-interaction matrix is developed for all assessed factors (see Table 1). The information of factor interactions is based on previous in-depth literature analysis identifying the interactions and the direction between each pair of factors.

After, the structural self-interaction matrix is transformed into the reachability matrix; as well, the driver and dependence power is determined for each factor. The result can be seen in Table 2. In complement to the common ISM approach of denoting interactions in the reachability matrix with 0 and 1, we indicate the similar and opposite

Table 1. The structural self-interaction matrix

	1	2	3	4	5	6	7
1 Climate change							
2 Bioresources	X						
3 Technologies	X	X					
4 Natural environment	X	X	O				
5 Production	X	X	X	O			
6 Pollution	V	X	X	X	A		
7 Infrastructure	X	V	X	A	X	V	

direction of the link, by also using value -1 (for opposite direction links).

Furthermore, this allows to also account for the different direction sub-links of factor interaction, and this information may be further transferred to the graphical representation. However, our approach does not affect the ISM calculation, as the absolute value of each interaction is considered for those calculations.

Table 2. Reachability matrix

	1	2	3	4	5	6	7	Driver power
1 Climate change	1	±1	1	-1	1	0	-1	6
2 Bioresources	-1	1	1	1	1	±1	0	6
3 Technologies	1	1	1	0	1	-1	1	6
4 Natural environment	1	1	0	1	0	1	-1	5
5 Production	1	1	1	0	1	1	1	6
6 Pollution	1	-1	±1	1	0	1	0	5
7 Infrastructure	1	1	1	0	1	±1	1	6
Dependence power	7	7	6	4	5	6	5	

The highest dependence power is for climate change and bioresources, but the lowest for natural environment. In addition, the evaluation for driver power divides

factors in two groups – five factors has the highest driver power, but the rest of the factors – natural environment and pollution – have the lowest.

Table 3. Determination of levels

	Reachability set R(si)					Antecedent set A(si)					R(si) ∩ A (si)					Level				
Climate change	1	2	3	4	5	7	1	2	3	4	5	6	7	1	2	3	4	5	7	1
Bioresources	1	2	3	4	5	6	1	2	3	4	5	6	7	1	2	3	4	5	6	1
Technologies	1	2	3	5	6	7	1	2	3	5	6	7	1	2	3	5	6	7	1	
Natural environment	1	2	4	6	7	1	2	4	6	7	1	2	4	6	7	1	2	4	6	4
Production	1	2	3	5	6	7	1	2	3	5	7	1	2	3	5	7	3			
Pollution	1	2	3	4	6	2	3	4	5	6	7	2	3	4	6	2				
Infrastructure	1	2	3	5	6	7	1	3	4	5	7	1	3	5	7	3				

Based on the developed reachability matrix, the reachability and antecedent factor sets are derived and after iteration, the factors can be assigned to various levels accordingly to its characteristic (see Table 3). The results divide assessed factors into four levels: three factors at the first level, one factor at the second level, two factors at the third level, and one factor at the fourth level. Lastly, by considering all the previously mentioned results, the structural model is designed by graphically representing the interaction links between all factors (see Fig. 2).

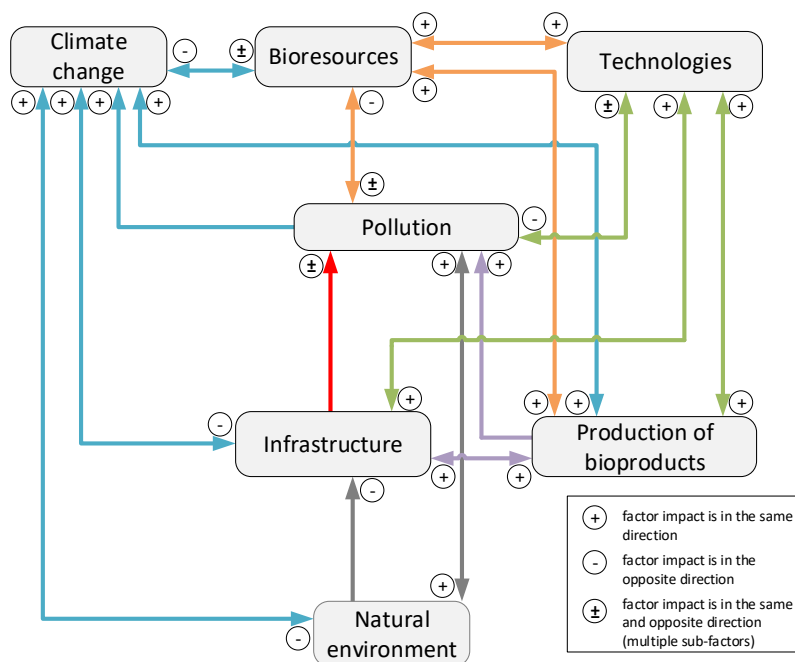


Figure 2. The levelized structure with links.

The obtained results show that the factors that are most connected to others are climate change, bioresources and technologies. These factors are also the main parts of biotechnology itself, and are essential for bioeconomy’s development. At the next level

is pollution, which has high influence on other factors because of its effects on climate change; however, the reciprocal effect of how climate change is influencing pollution is an open question.

In addition, the plus and minus signs have been added to each link in the directed graph, in order to indicate whether the factor impact is in the same direction or opposite direction, e.g. bioresource use has an inverse effect on climate change increase (depicted by minus sign). This approach extends the current ISM practice and allows to indicate not only the direction of the links, but also cases when the impact may be in both directions (direct and opposite). However, due to the complex nature of the bioeconomy concept and interrelations between assessed factors, even with the foundation of literature analysis, some of the identified linkages are not unequivocal (including contrary effects as well as double effects in the same direction), thus leading to a need for further research that could account for this multifaceted nature of factors that affect the bioeconomy.

CONCLUSIONS

Stakeholders and decision makers could gain from a structured model that accounts for the multi-faceted and interrelated aspects that affect bioeconomy study field. To complement the bioeconomy research field, authors propose using ISM method to develop a directed graphical description of this complex system. The results obtained from this pilot study assessing seven important factors affecting biotechnology development (e.g. bioresources, technology, infrastructure, climate change, production, natural environment, pollution) uncover the hidden levels of interaction between those factors and promotes further research into the modelling of the bioeconomy system.

This paper presents initial research regarding bioeconomy development, and can be further used as a carcass for the future researches where the wider list of essential factors within bioeconomy will be assessed. The additional factors in the future research would represent also social and economic factors, for example, behaviour, consumption, health, financial resources etc. Therefore, together with environmental and technological factors (that have been viewed in this paper) would cover the idea and requirements of sustainable development and would give comprehensive look at bioeconomy and related factors. This study proves that ISM approach is a valuable tool for designing the structure of the bioeconomy system. However, several limitations were recognized that affect the full uncovering of the structure and especially the subsystems and sub-connections between the bioeconomy influencing factors. We therefore propose that system dynamics modelling method could be used in further research to indicate positive and negative direction between the factor links and better explain the impacts of potential sub-factors. The results obtained within this study can be used by stakeholders for planning and evolving practical bioeconomy implementation strategies within the regional and national planning documents in order to accelerate the development of bioeconomy within the region.

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Adoption and advantages of eco-friendly technology application at the Shallot farming system in Indonesia

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Abstract. This study aimed to evaluate the benefits farmers obtained by adopting eco-friendly technologies on local shallot cultivation in Central Sulawesi, Indonesia. The technology applied includes; trap crops, biofertilizers (bokashi and mycorrhiza arbuscular) biopesticides (*Beauveria bassiana* and *Trichoderma* sp.) and plastic mulch. Ninety-nine shallot farmers were selected, consisting of 35 farmers who applied the technology (adopters) and 64 farmers who did not (non adopters). Characteristics, rates of adoption, and benefits of both groups of farmers were measured and analyzed. The results showed that the level of technology adopted by the farmers was high and supported by farmer characteristics, particularly their age and education. The application of technology increases the production of shallots and the total profits of farmers by 29.5% and 79.1% respectively. In addition, adopters can reduce the costs of chemical inputs by around 69.5%. Therefore, the technology introduced provides multiple benefits for farmers; economically and environmentally.

Key words: adoption, sustainable farming system, shallot cultivation, farmer's benefits.

INTRODUCTION

Shallot is one of the horticultural commodities that has a significant contribution to farmer's income in Indonesia. The farmers in Central Sulawesi Province of Indonesia have grown a local variety of shallot called 'Lembah Palu Shallot' or 'Palu Fried Shallot' as one of the leading commodities potentially to be registered as a geographical indication of this area (Nur et al., 2015). The productivity of this local shallot at the farmer's scale varies from 3 and 5 tons per hectare which are still below from its biotic potential of 11.10 tons per hectare (Christoporus et al., 2016).

The productivity of the shallot cultivation in Palu valley is limited by several factors such as low soil fertility, lack of water availability as well as an infestation of pests and plant diseases (Lasmini et al., 2015; Saleh et al., 2018). In addition to the low productivity, the local shallot is also faced with food safety and environmental problems.

Recent studies reported that the presence of chlorpyrifos residues on local shallot tubers has exceeded the maximum limit the plant can tolerate according to ISO 7313: 2008 (Jamaluddin et al., 2015). This chemical compound is very hazardous for human health (Saunders et al., 2012).

To overcome environmental problems due to the high application of pesticides by the farmers, FAO recommends the integrated pest management system that prioritizes the use of bio-pesticides and bio-control (FAO, 2017). This is in line with the principles of sustainable agriculture which emphasizes the management of agroecosystems and optimizes ecosystem services of the biological control agents to manage the pests (Sullivan, 2003). Some techniques that can be applied for this purpose are the use of trap crops, mulch, biofertilizers such as bokashi and mycorrhiza, and biopesticides like *Beauveria* and *Trichoderma*. These techniques could suppress the plant pests and diseases (Ownley et al., 2004; Shelton & Badenez-Perez, 2006; Shahabuddin et al., 2015, Sharma et al., 2017, Saleh et al., 2018) as well as increase crop yields (Hart & Trevors, 2005; Vinale et al., 2008; Lalitha et al., 2010; Álvarez-Solís et al., 2016).

The successful implementation of the above-mentioned technology is strongly dependent upon the production factors including characteristic of the farmers (Christoporus, 2017). These factors along with the technology characteristics such as relative advantages, compatibility, simplicity, and trialability are important to be considered to ensure that the adoption of the technology by the farmers (Rogers, 2003). This study aims to evaluate the contribution of eco-friendly technology introduced to the shallot farmers for supporting sustainable farming systems. Particularly, the study examines the adoption level of the technology introduced to the shallot farmers, and the effects of the introduced technology on shallot production, farmer income and budget for chemicals.

MATERIALS AND METHODS

The study was conducted in the Guntarano village, Tanantovea sub-district, Donggala district, one of the central areas of shallot production in Central Sulawesi, Indonesia. The study was carried out from February to September 2018. The shallot farmer population in the study area was 216 people. A census method was used to determine the number of adopters so that all populations were selected as respondents. Of all respondents, 35 farmers were practicing the technology (adopters) and 181 farmers were not (non adopters). A simple random sampling method was used to select the number of non adopters. Based on Slovin's formula (Ryan, 2013) and a sampling error of 10%, thus the sample size of non adopters farmers was 64 respondents.

Data collection was done by interview method using a structured questionnaire (Fig. 1). The data collected is then calculated to analyze the adoption level of technology by the farmers and farm profitability. The level of adoption was measured by means of scoring techniques based on the score weight and the percentage of each technology component applied by the farmers according to the following formula (Santoso et al., 2005).

$$\text{Adoption score} = \frac{P}{\sum S} \times S \quad (1)$$

where P – Percentage of farmers who apply technology; S – Score weight; $\sum S$ – Total score weight.

The adoption level of above technology is categorized as follow: **low** if the score is between 0–33.33%, **medium** if the score is between 33.34–66.66, and **high** if the score is between 66.67–100%. Data on the characteristics of respondents that can influence the adoption level particularly their age, education, and cultivated land was collected. Farmer profit is calculated using a budgetary technique which involves the cost and returns analysis. The model specification is given below:



Figure 1. The interview process with the shallot farmer based on the prepared questionnaire.

$$\pi = TR - TC \tag{2}$$

$$TR = Pq \times Q \tag{3}$$

$$TC = \text{Total Cost (VC + FC)}$$

where π – Total profit; TR – Total Revenue; Pq – Unit price of output; Q – Total quantity of output (production), VC – variable cost, and FC – fixed cost.

Differences in the total revenue of the adopters and non adopters were analyzed by using t-test of the independent sample, $\alpha = 0.05$ (Sokal & Rohlf, 2012).

The technologies applied were (1) trap crops, (2) biofertilizer consisted of bokashi composting and mycorrhizal arbuscular and (3) biopesticides consisted of *Trichoderma* sp., and *Beauveria bassiana* and (4) plastic mulch.

Twenty-four of the 3×2 m² plots were used to grow shallots. Plastic mulch was installed at each plot and 76 holes (7 cm in diameter) with 15×15 cm² in spacing was made for sowing the shallot seeds (Fig. 2). The cucumbers used as the trap crop were planted 2 weeks before the shallot planting with a spacing of 40×40 cm². Bokashi (5 t ha⁻¹) was applied one day before the shallot planting. Bokashi was made from chicken, cow and goat manure (50:25:25 m m⁻¹) and enriched with effective microorganism solutions (EM4, PT. Songgololangit Persada, Indonesia). Subsequent fertilization was implemented 14 days after planting using 400 kg ha⁻¹ of NPK. Shallot seeds dipped for 5 minutes into *Trichoderma* liquid before planting and then mycorrhiza (5 g shallot⁻¹) was applied. *Beauveria* (10 g L⁻¹) was applied once a week after planting from week 2 to 9.



Figure 2. Some experimental plots showing the installed mulch with holes in similar size and space for shallot seed sowing and the trap crop (cucumbers) grown between two shallot plots.

RESULTS AND DISCUSSION

The results showed that farmers who applied the technology (the adopters) at the research location had different adoption rates. Most of the adopters (80%) applied the technology package as a high category and only 2.86% of them are considered as low adopters (Table 1). Majority of the respondents (94.29%) are categorized as productive labor or in the level of active work participation (an average of 42.3 years). In the context of education, 65.71% of the respondents experienced ranging from six to nine years of formal education or graduated from junior high school. The land area size cultivated by the respondent farmers is relatively low varied between 0.35 ha – 1.35 ha or 0.78 ha on average.

Table 1. Adoption level of introduced technology by the adopters

No.	Adoption level	Number of adopters	Percentage
1	Low	1	2.86
2	Middle	6	17.14
3	High	28	80.00
	Total	35	100.00

The high level of technology adoption was supported by the characteristics of the farmers particularly their age, level of formal education as well as the farm field size (Table 2). The farmers are mostly in their productive age and therefore they have a high capability to adopt the new technology. Similar results were also shown by Zhou & Yang (2010) who analyzed the factors affecting the decisions of farmers on using fertilizer and found that the young farmers had higher adaptability toward the change and more responsive to new information than the old farmers.

The level of education of the shallot farmers also plays an important role in adopting the new technology. Individuals with a good level of education are more open-minded in adopting new technology into their farming activities. They also have

Table 2. Farmer characteristics at the study area

No.	Farmer characteristics	Mean ± Sd
1	Age (year)	42.3 ± 5
2	Formal education (year)	6 ± 3
3	Cultivated area size (ha)	0.78 ± 0.2

sufficient capability in response to new technologies, as an effort to increase farm production and income. This situation is expected to improve their ability to manage a business related to the farm, thereby giving a positive impact on an individual or societal economic growth. Previous research emphasized the significance of the farmers' education in increasing technical efficiency on the shallots cultivation (Christoporus, 2017). However, the adoption of new technology in farming activity is not necessarily affected by the farmer's education level (e.g. Arshad et al., 2007).

Another factor influencing the farmer's productivity is the size of the cultivated area. Keskin & Sekerli (2016), Christoporus (2017) and Ahmad et al. (2018) noted that the size of farm field has a significant positive correlation with the adopted technology and farmer's productivity. The more extensive of the land cultivated, the higher the production if it is supported by good farming techniques (Anik et al., 2017). The farmers with the larger size of land usually have sufficient resources to manage their crops so that they will be more quickly in adopting new technology than smaller land holdings (Arshad et al., 2007, Ahmad et al., 2018). Therefore, an increase in the shallot area size in our study area presumably can lead to greater farmers' productivity and profitability.

In addition to the characteristics of the farmers that support technology adoption, the high adoption of the eco-friendly technology also indicates that it is quite easy to implement and can be combined with the existing conventional cultivation techniques. Therefore the introduced technology fulfills the elements of adoption factors particularly the compatibility and simplicity of technology as stated by Rogers (2003).

Economic benefits of the introduced technologies

The results of the farm profit analysis showed that the shallot productivity generated by the adopters was greater than those by the non adopters. Consequently, the adopters have a greater net income (Table 3). The study denotes that with the application of the technology, the productivity and the total income of the adopters were higher about 1.3 and 1.8 times than the non adopters, respectively. On the contrary, the budget for chemicals spent by the adopters decreased 3.3 times lower than the non adopters (Table 4).

Table 3. Farm profit analysis of the respondents

No.	Description*	Adopters (n = 35)	Nonadopters (n = 64)
1	Yields (kg ha ⁻¹) :		
	a. Shallots	3,917.75	3,024.79
	b. Cucumber	1,120.55	0
2	Price (IDR ha ⁻¹) :		
	a. Shallots	27,450.50	26,510.93
	b. Cucumber	3,500.00	0
3	Gross income (IDR ha ⁻¹) :		
	a. Shallots	107,544,196.38	80,190,000.00
	b. Cucumber	3,921,925.00	0
	Total Gross Income :	111,466,121.38	80,190,000.00
4	Cost :		
	a. Fixed cost (IDR GS ⁻¹):		
	- Equipment maintenance	245,335.55	203,237.06
	- Land rent	666,666.67	666,666.67
	- Others (tax, ceremonial, etc.)	586,976.74	586,976.74
	Sub Total a : (IDR)	1,498,978.98	1,456,880.47
	b. Variable cost (IDR)		
	- Labor	13,095,125.75	12,292,182.09
	- Shallot seed	22,250,765.50	23,295,833.33
	- Cucumber seed	750,155.55	0
	- Chemical fertilizers	5,500,000.00	15,969,383.26
	- Chemical pesticides	0	2,067,797.36
	- Bio-fertilizer (Bokashi manure)	4,708,333.00	0
	- Biopesticides		
	(Mycorrhiza, Trichoderma, Beauveria)	14,683,484.00	0
	- Plastic mulch	4,000,000.00	0
	Sub Total b: (IDR)	64,987,863.80	53,625,196.04
5	Total cost (4a + 4b) (IDR ha ⁻¹ GS ⁻¹)	66,486,842.78	55,082,076.51
6	Total profit (IDR ha ⁻¹ GS ⁻¹)	44,979,278.60	25,107,923.49
7	RC ratio	1.67	1.45

Note: * GS: Grown Season.

Table 4. Economic advantages of the technology introduced to shallot farmers

Variables	Adopters (a)	Nonadopters(b)	Differences (%)*
Shallot yield (kg ha ⁻¹)	3,917.75	3,024.79	29.5
Cucumber yield (kg ha ⁻¹)	1,120.55	0	100
Gross income (IDR ha ⁻¹)	111,466,121.38	80,190,000.00	39.0
Total profit(IDR ha ⁻¹)	44,979,278.60	25,107,923.49	79.1
Chemicals cost (IDR ha ⁻¹)	5,500,00.00	18,037,181.00	69.5

Note: *Differences between both groups was obtained by using formulae; (a-b/b) x100.

In accordance with the results of the t-test of independent sample (p -level < 0.05), there is a significant difference in income between the adopters and the non adopter's farmer. The total profit differences between both groups were IDR. 19,871,354.51. The additional income of 3.65% of the adopters was due to the contribution of income generated from cucumber plants used as trap crops. This clearly shows that the intercropping of shallots with cucumber plants provides economic benefits for farmers. Meanwhile, the RC ratio of the adopters is higher than that of the non adopters (1.67 vs. 1.45). This indicates an increase of 0.22 in the RC ratio if the farmer implements the introduced technology or there is an increase of IDR 220.00 in revenue for every IDR 1,000 spent. This higher RC ratio is achieved as a consequence of higher production and production prices.

The shallot farmers have generated relatively high economic benefit by adopting the novel technology introduced in our study area. It has been known that the relative advantages of technology determine the level of technology adopted by the farmers (Rogers, 2003; Foster & Rosenzweig, 2010; Obayelu et al., 2017).

An extensive study in Africa found that the application of trap cropping and intercropping system at maize and sorghum fields, tremendously increasing the farmer's income (Khan et al., 2014). In addition, intercropping system combined with bokashi significantly favored the yield and quality of pepper and shallot in Mexico (Álvarez-Solís et al., 2016).

Environmental benefits of the implemented technology

In addition to the economic benefits, the novel technology implemented in this study also provides some environmental benefits. The trap crops protect the main crops from being attacked by pests (Shelton & Badenez-Perez, 2006) and increase the diversity of natural enemies (Shahabuddin et al., 2015, Saleh et al., 2018). The plastic mulch replaces the herbicides for controlling the weeds, ameliorates the microclimate and contributes to pest control (Frank & Liburd, 2005; Lalitha et al., 2010). The use of bokashi enhance the fertility and nutrients availability of the soil as well as the crop yields (Xiaohou et al., 2008, Álvarez-Solís et al., 2016; Lasmini et al., 2018). Mycorrhizae improves nutrient uptake and induces plant resistance against pathogen and herbivore (Cardoso & Kuyper, 2006; Sharma et al., 2017). While Trichoderma and Beauveria act as biological control agents against pests and pathogens of the crops (Ownley et al., 2004; Vinale et al., 2008).

Integrating all eco-friendly technologies has successfully reduced the use of chemical fertilizers and pesticides by the farmers (see Table 4) and replaced them with habitat management, bio-fertilizer, and bio-pesticides. Thus, the novel technology applied in this study provides both economic and environmental benefits to the shallot

farmers. Previous study in our study area has been documented the enhancing of the shallot yield by application the bokashi as biofertilizer (Lasmini et al. 2018) and increasing of the farmers income by participating in good agricultural program for shallot (Christophorus et al. 2016). However, by combining the agronomical and socioeconomical approach, this study was succesfull to show the economic and environmental benefits simultaneously by implementing of the eco-friendly technology.

This study was conducted only for one growing season when the weather is quite dry so it is quite normal that the effect of the biotic factor particularly the pest infestation was relatively low (see Saleh et al., 2018), however it is expected that the pest infestation will be higher during wetter season and presumably decreased the shallot production. So it is suggested to conduct a longer study period covering several planting season to evaluate the effect of weather fluctuation. It is also important to search the best crop management to mitigate the detrimental effect of the climate change on the agriculture and food security (see Tubiello et al., 2008). The application of biological control in papaya farming in Pakistan had succeeded in providing benefits from economic and environmental aspects (Bajwa et al., 2018). Accordingly, the eco-friendly technology could solve problems faced by the farmings that intensively use chemical inputs leading to tremendously decreased productivity in the global scale (FAO, 2017).

The agricultural system in the last decade has been a shift from a traditional to a sustainable agricultural system. In this new paradigm, the driving factor of the farming system is not only farmers' tradition or habits that put the emphasis more on the economic aspects (higher, faster and easier production) but has highly considered the environmental and human safety factors. The user preferences, information, and the market has become the main driving factors, accordingly. The consumers prefer to buy safe products, free from pesticide residues (Shinohara, 2011). Shortly, in a sustainable farming system, economic, environmental and social goals must be achieved simultaneously (Sullivan, 2003). In this context, the technology introduced to the shallot farmers at the research location has good prospects to be developed and applied to other commodities in a wider area to support sustainable farming systems.

CONCLUSIONS

Based on the results of the study, it was concluded that the adoption level of the shallot farmers to the technology introduced is high and it was supported by the age and education of the farmers. Implementation of this eco-friendly technology elevates the shallot production and the farmers' total profit by 29.5% and 79.1%, respectively. Moreover, the adopters could reduce the chemicals input cost about 69.5%. The application of such eco-friendly technologies as trap crops, biofertilizers, biopesticides and plastic mulch provides a high advantage to the adopters compared to non adopters. The additional benefits of the technology introduced in terms of the environmental aspects are able to minimize the farmers' dependency on the chemicals and to produce shallot safer to consume.

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Measurement Robotic Arm (MRA) for the evaluation of localization sensors properties

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Abstract. The purpose of this longitudinal project is to verify the dynamic properties of the Real Time Kinematic receivers ('RTK'). For this purpose, a verification method using Measurement Robotic Arm ('MRA') described in this paper has been developed. This device can be moved along a circular trajectory in a horizontal surface. Using the methodology described in this paper, the absolute position of the MRA trolley in absolute time can be defined with absolute accuracy and can serve as a reference for the verification of RTK receivers positioning. Its movement, including its breaking, can be controlled via a PC app and various sensor properties can be monitored. The position of the trolley is determined by the encoder. A Hall sensor indicates the absolute start position of one full turn. The absolute time marks of the measurement and the time synchronization of the microprocessor based on Pulse Per Second ('PPS') were obtained from the standard GNSS receiver. This study provides information about a proposal solution of the MRA reference system in terms of the frame construction description, the design of electronic equipment and the design of a software solution for processing and logging of messages. This paper also presents the results of three tests performed to verify the functionality and reliability of the MRA system: PPS time accuracy verification, the quantity and correctness of sent messages according to the arm instantaneous speed, and a real RTK verification test. The MRA also can be used to verify the dynamic properties of other localization devices.

Key words: localization, accuracy, precision, dynamic properties, reference system, reference trajectory, time synchronization, PPS, RTK, GNSS, agriculture, etc.

INTRODUCTION

The purpose of this study was to build and verify the equipment for evaluating the dynamic properties of localization systems. The main task was to develop a new method to evaluate the dynamic properties of the Real Time Kinematic ('RTK') receivers during the 'fix' state when the RTK receivers can measure most accurately. The RTK is the most accurate localization technology among Global Positioning Systems (GPS) that is available on the market. This method uses a correction signal from reference stations for more accurate positioning (Feng & Wang, 2007).

Thanks to its accuracy of around 20 mm, this localization method can be applied in many industries, e.g. in precision agriculture for tractor guidance. Tests of evaluating the

dynamic properties of the accuracy of localization systems have already been performed. In one study, tractors were moved in a defined path at speeds ranging from 0.83 to 1.94 m s⁻¹ and the deviation ranged from 20 to 30 mm (Gan-Mor, 2007; Carballido, 2014). Other studies tested field robots. For instance Bakker et al. (2011) has shown that during a field test, where a robot followed a straight path at speed of 2 km h⁻¹, the lateral error was from 16 to 45 mm. An extremely successful measurement by Jilek (2015) has shown that lateral errors were less than 10 mm when the robot followed a sequence of waypoints. Unfortunately, in any of these measurements, time synchronization was not taken into account. In other studies, where the time deviation was defined, the precision of more than 10 mm s⁻¹ was obtained for the instantaneous speed estimate provided by a consumer-grade GNSS (Global Navigation Satellite System) receiver (Boffi, Gilgien & Wieser, 2016). In this research, a downhill coaster track with a set of photocells and antennas placed on a coaster car was used as reference system.

Another method for evaluating the receiver's accuracy is processing the data from localization sensors as deviation from a reference point, RMS error or a number of satellites. This data has been evaluated in several studies through static or dynamic measurements for evaluating the accuracy and precision of localization sensors (Feng & Wang, 2008; Garrido et al., 2011; Berber et al., 2012; Kabir et al., 2016). This methodology was also used in authors' previous research where the RTK receiver's capability to determine the accurate position was evaluated through static measurements (Kadeřábek et al., 2018). However, the aforementioned method is more suitable for static measurements and, in case of dynamic movement, the determination of reference points changing in time during the movement is problematic. For dynamic measurements, a precision device in range of mm capable to provide location data at certain time points is needed as reference equipment. The purpose of this research was to build such equipment. Longitudinal tests had been conducted and a prototype of the equipment was built. It was then decided to build a Measurement Robotic Arm ('MRA') that would move on a circular trajectory which is easier to monitor in time positions. This method was chosen because of its high expected accuracy and minimal technical requirements (on the mechanical construction as well as on the conduct of measurements themselves).

This study focuses on the design and development of the methodology for future validation of RTK receivers. The MRA can also be used in future to validate other localization systems, such as incremental systems (systems using data fuse of Inertial Measurement Unit sensors, vehicle odometer systems, the Correvit laser sensor, the mouse-based camera sensor, etc.) and absolute measuring systems (based on the method of triangulation or trilateration). Furthermore, it also allows for the verification of systems that are used for object tracking based on a machine vision technology: laser scanner systems, camera systems, ultrasonic systems, infrared sensors or the fusion of them all). Moreover, it is also possible to use the MRA during the development and tuning of localization algorithms. Raskaliyev et al. (2017) used similar reference system for the development and debugging of algorithms that process information from IMU. The MRA can also be used as a reference system for machine learning development annotation process.

This study provides information about a proposal solution of the MRA reference system in terms of the frame construction description, the design of electronic equipment (namely its variant for RTK tests) and the design of a software solution for processing and logging of messages. This paper also presents the results of three tests performed to

verify the functionality and reliability of the MRA system: Pulse per Second (PPS) time accuracy verification, the quantity and correctness of sent messages according to the arm instantaneous speed, and a real RTK verification test demonstrating the future benefits of this longitudinal project.

MATERIALS AND METHODS

The system of Measuring Robotic Arm ('MRA') consists of three main parts: the frame construction with sensors, motors and brake system, the hardware part with all main electronics (microprocessors, data loggers, operation centre, voltage source, etc.) and the software part providing for a distant controlling of the MRA and processing of sensors signals. The parts are described in the following chapters.

MRA construction

The MRA (Fig. 1) was made of aluminium prototype extrusion profiles 30 mm and 40 mm wide that are widely used in different industries. The centre part of the rotation was static and consists of a cross with main axis of the rotation. The aluminium cross was attached to the ground to provide stable and repeatable placement of the MRA during all measurements and at all tested speeds. A rotary slip ring KS 54-D12-12x5A was placed on the main axis to supply electricity for all systems during the rotation. The axis was connected to the arm with industrial bearing. This bearing connected a movable part of a trolley and a beam with the axis. A cogged pulley was attached to the axis to transfer the rotary movement via a cogged belt to Solid-Shaft Incremental Encoder ZSP5208-001G-2500BZ3-5E that was placed on the beam.

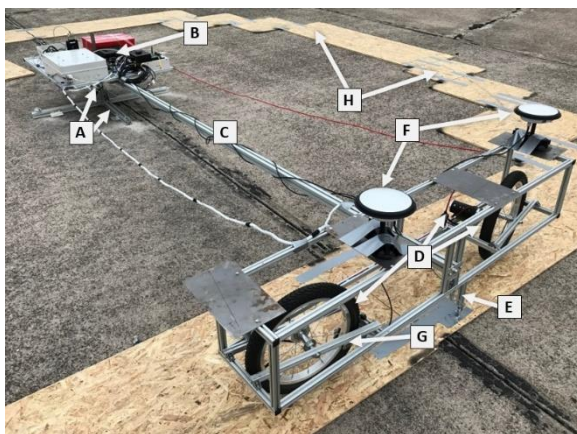


Figure 1. The Measuring Robotic Arm set during measurement. (A – cross with the rotation axis; B – board with electronics attached to the beam; C – beam; D – trolley with wheels and motors; E – Hall sensor; F – two antennas; G – brake; H – OSB boards).

The board with electronics was placed above the static axis and attached to the beam. It was placed in the centre of rotation of the construction. The main purpose was to concentrate all the heavy electronics in the centre of rotation to limit a rapid increase of the centrifugal force. This force can affect the accuracy of measurements or

destabilize the construction at a high speed which could result in a collision and damage the expensive equipment.

The trolley, connected to the beam, enabled to assemble four antennas in 0.5 m distance from each other and at 3 m distance from the axle, the centre of rotation, along a circle line. The trolley was moving on two 16-inch wheels that placed on opposite sides at 1.22 m distance of to ensure better stability. These wheels helped to move the trolley smoothly and reduced vibrations that can affect measurements. Both wheels are driven by DC motors with 100 W power each. The motion power system (motor driven wheels) was placed on the trolley with the goal to eliminate small bending or deformation of the construction during acceleration, deceleration or movement. These drive forces did not burden the construction on the beam, axis or trolley during the movement, and thus, an overall better accuracy of the system was ensured.

The cuboid shape of the 0.44 m high trolley construction including wheels enabled to place the antennas above the construction in order to eliminate possible signal disturbances. The trolley was equipped with one V-brake type brake driven by a linear DC motor for the purposes of evaluating the properties of sensors during the deceleration or stopping the movement after each measurement. The Hall sensor was placed on the bottom side of the trolley, close to a magnet fixed on the wooden OSB boards. The aim of was to ensure that the sensor records each full turn of the MRA.

MRA electronic equipment

The electronic equipment consists of several components that, as a whole, provide required functionality of the MRA and communication between each component. Fig. 2 describes a component linking model, namely a variant for verifying the dynamic properties of up to four RTK receivers using one channel of the VRS correction via the Internet. However, the MRA was developed as a variable system, which means that its components can be exchanged to verify the dynamic properties of other localization systems. The core of the platform is the MRA embedded system, which consists of the Sensor Board ('SB'), Motor Control Board ('MCB') and Break Control Board ('BCB').

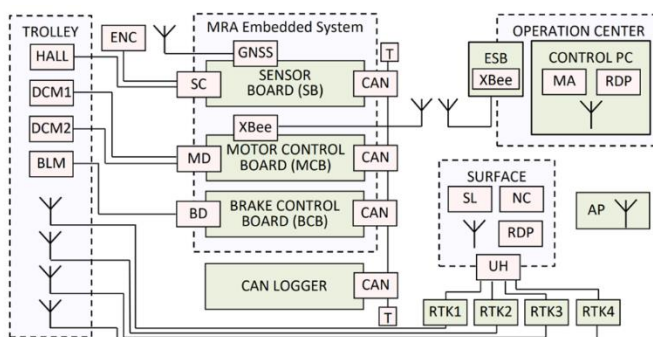


Figure 2. The connections scheme. (MD – motor driver; BD – brake driver; DCM1 and DCM2 – DC motors; BLM – brake linear motor; SC – sensors connector; CAN – CAN bus module; T – terminator resistor; UH – UART/USB hub; AP – cellular modem with WiFi connection; MA – MRA Application; RDP – software using Remote Desktop Protocol; SL – serial logger (Putty bash script); NC – Lefebure software as NTRIP client; ESX – Emergency Stop Box).

The SB unit is used to process signals from the MRA sensors (according to the algorithm described in the subchapter below). It creates records in the form of messages sent over the CAN bus. The hardware of this unit is based on the Arduino DUE single-board computer platform with the ATMEL SAM3U microprocessor. The SB is directly connected to the Adafruit Ultimate GPS Breakout GNSS receiver via the UART interface, and the SC sensor connector is the output. The Proximity NJK-5002C Hall Sensor ('HALL') and Encoder ZSP5208-001G-2500BZ3-5E ('ENC') are connected via the SC to the digital inputs of the SB. The power supply for the HALL and the ENC is also powered via the SC. As an interface for the CAN bus, the MCP2515 is connected directly to the SB via the SPI interface. The MCU is based on the Arduino DUE platform (as well as the SB) and serves primarily to control the MRA engines. Two 100W/24V DC motors (DCM1 and DCM2) are controlled by the Pololu Dual VNH5019 driver shield motor module and are located on the MRA trolley. In addition, this unit serves as the communication interface of the CAN bus (again via the MCP2515 module) and the wireless connected Operation Centre. This part is a kind of 'communication node' of the entire MRA system. The Xbee-S2C module with 2,4 GHz wireless communication via the UART interface was used to communicate with the Operation Centre. The BCB is only used for controlling the braking system. It is based on the Arduino UNO single-board computer with ATmega328P microcontroller. The MCP2515 module in the CAN-BUS Shield V1.2 variant was used to communicate with the CAN bus BCB. The braking mechanism is controlled by a 4-channel relay of the DSP AVR MSP430 which operates a 12 V DC linear motor with an axial thrust of 1,300 N.

Two logging devices were used for recording the measurement process. The first logger ('CAN LOGGER') was designed to read and store CAN bus messages sent by the SB. For this purpose, a high-end automotive logger Vector (GIN) GL4000 was used. However, the MRA platform was designed in a way that enables to replace this logger in future by any comparable CAN bus logging device. The second logging device ('SURFACE') was designed to record the output communication of the RTK receivers (in this case up to four). These outputs were assumed to be the UART communication standard via which the measured positions and time stamps were sent from RTK receivers as NMEA messages. SURFACE was realized by the Microsoft Surface Pro 4 laptop (CPU Intel i5 7300U, RAM 8GB, USB 3.1, Windows 10x64). The AXAGON HUE-S2B-4 USB 3.0 ('UH') was connected to the SURFACE USB interface and individual RTK receivers were connected to the UH. However, SURFACE served not only as a logging device but also for the purpose of assuring the Networked Transport of RTCM via Internet Protocol ('NTRIP') client. By this Trimble, VRS Now correction signal ('VRS') can be distributed to all RTK receivers via the UART interface. As an Access Point ('AP'), mobile TP-LINK M7350 Wi-Fi Modem was used with the support of 3G/LTE (800, 900, 1800, 2100, 2600 MHz) and Wi-Fi 802.11a/b/g/n standards with 2 Ah battery.

The MRA can be controlled from the Operation Centre which was implemented in a laptop Lenovo IdeaPad 720s-13IKBR (CPU Intel i7 7300U, 8GB RAM, SSD 256GB, USB 3.0, Wi-Fi 802.11ac, Windows 10x64). The Emergency Stop Box ('ESB') was connected to this laptop via the USB/UART converter. The EBS includes the Xbee-S2C wireless communication module. A shredding button was placed on the UART communication interface cable and directed from the Operation Centre to the MRA. This button can be used to stop the movement of the operating MRA. The Operation Centre

was connected to SURFACE via the AP with using Remote Desktop Protocol ('RDP'). This connection was used for the purpose of managing the RTK receivers, including logging and setting up a NTRIP client.

MRA software solution

The software solution is based on several separate mutually cooperating units that provide the control of the MRA and processing of its sensor signals (GNSS, Hall sensor and encoder). The most important software unit is the firmware installed on the Sensor Board ('SB'), which is based on the Arduino DUE development platform. The code for this unit was written in the Wiring programming language version 1.0. It was optimized in order to assure the highest possible quality and signal processing speed for three sensors using Arduino DUE board hardware interrupts. The optimization was supported by the DueTimer.h library version 1.4.7. Some message losses happen at higher sensor pulse quantity due to the speed of rotation of the encoder. These message losses did not however cause the loss of the arm position data because this information was stored in the SB internal memory. The validation of this and the verification of the quantity of sent messages by the SB unit according to the arm's rotation speed will be described below.

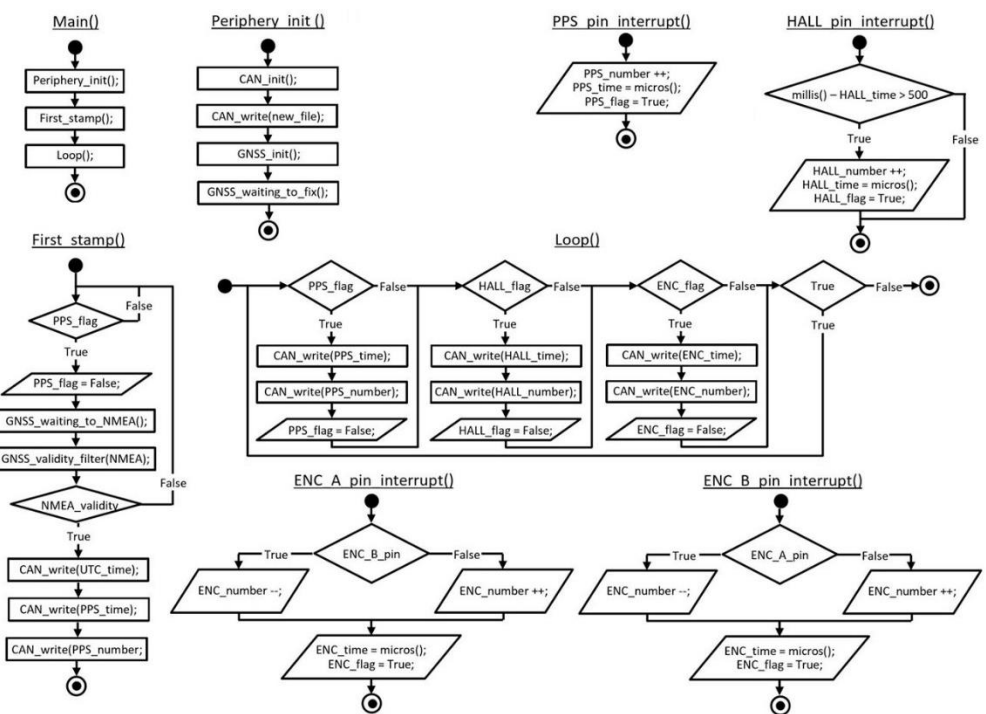


Figure 3. UML Activity Diagram of SB algorithm including interrupt headers of three sensors.

The simple principle of signal processing method of the SB unit is described in the UML (Unified Modeling Language) Activity Diagram (Fig. 3). The process begins with the Periphery_init() method in base branch Main(). In the CAN_init() method, CAN bus communication is initialized using the mcp_can.h library version 1.1.1 at the speed of

500 kb s⁻¹ with 11-bit identifier. Next, the CAN command is sent as a message for generating of a new measurement file at the CAN LOGGER. Next, the GNSS_init() method initializes the initial state of the measurement. An internal GNSS is set up in a proper communication mode on the serial line (115,200 kb s⁻¹, 8, 1, N, GPGGA, 1 Hz). Then, the algorithm goes into a loop in method GNSS_waiting_to_fix() until the signal state of the GNSS 'FIX_state' is indicated. The goal of next main method First_stamp() is to determine the absolute alignment of the starting time of measurements. In the first loop, the algorithm waits for the interrupt flag set in PPS_pin_interrupt(). If this signal occurs, a NMEA message on the serial port is expected by method GNSS_waiting_to_NMEA() and its validity is subsequently tested by the filter GNSS_validity_filter(NMEA). The filter must fulfil two conditions: the NMEA report meets its checksum and the time stamp contains an undamaged value. If the filter is valid, the time stamp in UTC format, included in a valid NMEA message, is sent to the CAN bus. Also both values obtained in interruption are sent to the CAN bus. In the method Loop(), it is checked whether there an interrupt flag was set by at least one of the four interrupts. Four interrupts secure three sensors (PPS, Hall sensor and encoder) because the encoder must be secured by two interrupts. In the positive case of at least one set flag, the obtained information (microprocessor time and the iterator of the interrupt) are sent to the CAN bus. Each type of message of each sensor which is sent on CAN bus has its own unique address. Individual interrupt processes are also shown in Fig. 3.

The MRA Application ('MA') was developed in the C# programming language using the .NET framework version 4.6.1. This application allows to control and to check the individual functions of the MRA via wireless transmission. Its primary function is to control the MRU motion with the MCB unit. It is possible to set parameters for the MRA instantaneous speed and acceleration. The MA also allows to restart the SB unit for start of a new measurement file in CAN LOGGER, as described Fig.3 It also allows to control the brake by the BD unit when it is necessary to influence the measuring trajectory or to stop the trolley movement in emergency. Furthermore, the MA allows the operator to read the interruption indications from the individual sensors and to show the approximate angular speed of the trolley.

The firmware for the MCB and BCB units, which is the same as for SB unit, was written in the programming language Wiring version 1.0. The task of the MCB was to ensure a two-way communication with the MA and a two-way communication with the devices connected to the CAN bus. The MCB enables to control the motor driver, including a watchdog feedback protection (signal sending from the MA to the MCB) against dangers caused by a failure of wireless signal. The BCB firmware enables to control the trolley's brake mechanism.

Verification of the time accuracy of the PPS signal

The first test was based on a general verification of the time accuracy of the PPS signal (Pulse per Second). A similar work is discussed in the study of Niu et al. (2014), which takes other types of GNSS receivers into account, and uses the reference system in the form of atomic clocks to verify the time accuracy. Our study looks at this verification from the point of view of mutual verification between PPS signals of three GNSS receivers using statistical verification method. For this to be done, the selection of the GNSS receiver is essential to align the time base of the SB unit. If the microcontroller clock of the SB unit is checked regularly (one time per one second), a correction of

deviations can be made in the subsequent post-processing. Thanks to using of GNSS in the MRA it is subsequently also possible to provide accurate absolute time alignment of two signals (from the encoder and the Hall sensor) that ultimately determine the position of the arm during the whole measurement period. The following GNSS receivers have been tested: Adafruit Ultimate GPS Breakout, U-blox C94-M8P and Garmin 18x LVC.

The logic analyser Saleae Logic 8 was used to log the PPS output signal from three GNSS receivers, with a set sampling frequency of 25 MHz. To log the data in Lenovo E540 (Intel i3-4000M CPU, 8GB RAM, USB 2.0, Windows 10x64 OS), the Saleae Logic logging software version 1.2.18 was used. The GNSS receivers were connected to power supply and three connected identical GNSS antennas FURUNO AU-15 were placed on a metal plate of the trolley in a stable position with an open access to the orbit. The GNSS receivers were set up to receive only GPS signals. Measurements took place at the location of 50°04'32.8"N, 14°31'10.3"E and took 3,600 s. The screenshots (Fig. 4) from Saleae Logic logging software show the cut of the measurement of three PPS signals.

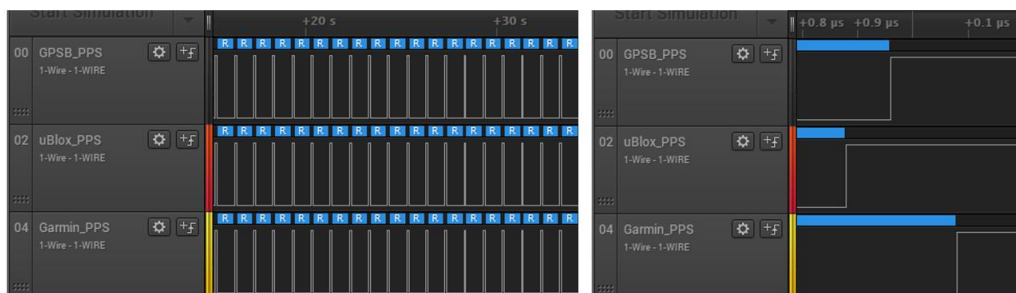


Figure 4. Screenshot of Saleae Logic logging software from the measurement of three PPS signals.

The measured data was processed in the Spyder software version 3.2.8 as part of Anaconda distribution version 5.2 that used the Python programming language version 3.6.5. The goal was to calculate the deviations of the individual upcoming edge of PPS from the GNSS receivers against the clock of the logic analyser. This calculation is described by formula (1):

1. PPS time errors (Δt_{seleae}) – data-set of time deviations of PPS opposite to logic analyser clock:

$$\Delta t_{seleae_i} = t_i - t_{i-1} - 1 \quad (1)$$

where: t_{pps} – value of measured PPS sample by logic analyzer; i – iterator of PPSs DataFrame, $i \in \langle 1; 3,599 \rangle, i \in N$.

Verification of the quantity and correctness of the sent messages according to the arm instantaneous speed

The second verification aimed at verifying the ability of the MRA to process signals from the sensors and to send them to the CAN bus. This test was performed with the help of the MRA device. The RTK receiver Tersus BX-305 was also attached to the MRA, but the verification of this sensor is not taken into account in this study. The MRA was assembled, anchored to a flat concrete surface, and OSB boards were installed under

the trolley's trajectory. Also, the MRA sensors (encoder using a cogged belt to both pulleys, Hall sensor with a magnet and SB internal GNSS receiver antenna) were installed, calibrated and verified. During the measurement, fifteen tests were performed with an approximate duration of 30 seconds at different speeds (with max. speed 8.3 m s^{-1}). Before each measurement, the SB was reset in order to create a new measurement file. Each measurement ended by stopping the trolley by brake.

These measurements were evaluated by using the developed Python script (using the same components described in the previous chapter). Because of the largeness, complexity and variability of the calculations, all individual steps will not be described. The important calculations of time corrections are specified below. Other calculations are based on basic knowledge of physics, geometry and data processing methods.

The first task of the script was to parse the messages in the CAN messages file stored on the CompactFlash card. The messages are sorted into variables of DataFrame data structure from the Pandas library version 0.23.4 according to the sensor type (PPS, Hall sensor, encoder). From these structures, the values of the microprocessor time t_{fix} with the t_{nmea} were extracted. The t_{fix} value represents the last stored value of the microprocessor time of the first interruption triggered by the leading edge of the PPS signal when the valid NMEA message passed through the filter (see the MRA software solution chapter). The t_{nmea} value represents this valid NMEA message converted from the UTC HH:MM:SS.sss time format to the float format of the running total of seconds in a day starting at midnight (time of day in seconds).

This pair of values guarantees the measurements data in absolute time. It was necessary to subtract the value t_{fix} from all the measured times of all sensors t_{pps} , t_{hall} and t_{enc} , as at this time the SB unit started the measurement. In addition, the clock deviations of the SB microprocessor from the time of the measurement were calculated by the following formula (2):

2. Sensor board time error difference (Δt_{err_i}) – data-set of time deviations differences of Sensor Board clock opposite to the PPS signal:

$$\Delta t_{err_i} = t_{pps_i} - t_{pps_{i-1}} - 1 \quad (2)$$

where t_{pps} – saved values of microprocessor-time by Sensor Board to CAN messages in times of PPS interrupts with offset of start of measurement; i – iterator of PPSs DataFrame, $i \in \langle 1; \text{number of PPS messages} \rangle, i \in N$.

The set of obtained deviations Δt_{err} from one selected measurement (which is further used as example) was shown in Fig. 5. Furthermore, this set of errors Δt_{err_i} for time marks correction used the Hall sensor t_{hall} and the encoder signal t_{enc} with the help of relations (3), (4):

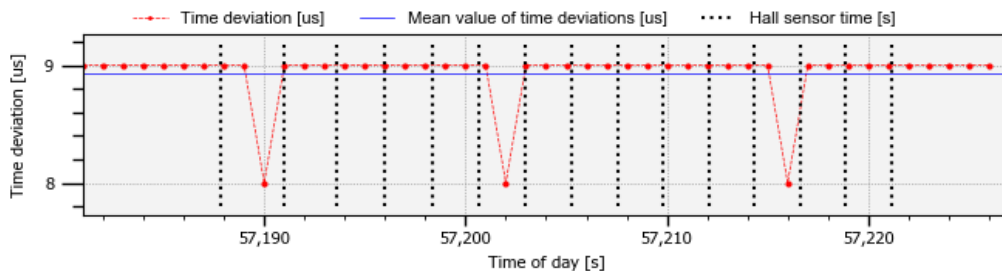


Figure 5. System Board time deviation in time.

3. Sensor board time error (t_{err}) – data-set of time deviations of Sensor Boards clock opposite to the PPS pulse:

$$t_{err_i} = \sum_{j=1}^i \Delta t_{err_j} \quad (3)$$

where Δt_{err} – data-set of time deviations differences of Sensor Boards clock opposite to the PPS signal; i – iterator of PPSs DataFrame, $i \in \langle 1; \text{number of PPS messages} \rangle, i \subset N$; j – iterator of summation, $j \in \langle 1; i \rangle, i \in N$.

4. Hall sensor or encoder corrected time (t_{enc_corr}) – data-set of time corrected time-stamps of the Hall sensor or encoder signals:

$$t_{enc_corr_i} = t_{enc_i} + t_{err_{int(t_{enc_i})-1}} + \Delta t_{err_{int(t_{enc_i})}} * (t_{enc_i} - int(t_{enc_i})) + t_{nmea} \quad (4)$$

where t_{enc} – saved values of microprocessor-time by the Sensor Board to CAN messages in times of encoder interruptions with the offset of the start of the measurement; t_{nmea} – value of the first valid string of NMEA message providing absolute placement in time; i – iterator of encoders DataFrame, $i \in \langle 1; \text{number of encoder messages} \rangle, i \in N$.

For the interpretation of formula (4), an encoder corrected time formula was selected. This code was also used for calculating t_{hall_corr} (Hall sensor corrected time). Now, when all times from both sensors were corrected, it was possible to start off assigning individual messages from the encoder’s appropriate angle of the MRA. This was performed by algorithmically scanning the encoder times t_{enc_corr} and assigning them the value of a particular angle of turn according to the Hall sensor time t_{hall_corr} , which was used for this purpose as a boundary value. The value of the interrupts order of the encoder then conveys, together with the knowledge of the number of encoder pulses per turn, the specific angle of the arm rotation. Next, it is necessary to calculate the offset of the angle from the first turn before the first pulse of the Hall sensor. With the knowledge of the angles and the knowledge of the initial absolute position of the MRA (given from two surveyed points), it is possible to calculate with simple goniometric functions the relative coordinates of the MRA trajectory in the Cartesian coordinate system (for example East-North-Up or East-North-Down) (see next subchapter). It is also possible to obtain the measured trajectory in an absolute representation in a geographic coordinate system (such as WGS84). The calculated positions, for data analysis purposes, can be limited to two-dimensional view depending on the time (Fig. 6). These positions can be used as the final reference points forming the reference trajectory.

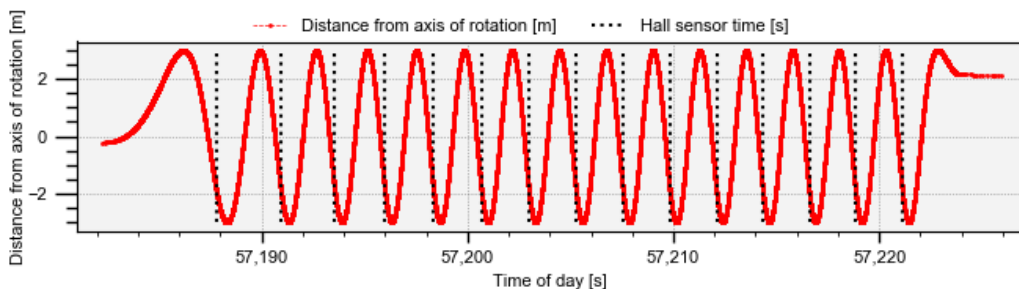


Figure 6. The relative positions of ‘x’ axis of the MRA trolley in time.

For further analytical processing of the results, such as verifying the quantity of sent messages according to the instantaneous speed, it was necessary to know the instant angular velocity of the MRA. This was obtained from the difference of angular distance and time between the two calculated points of trajectory (Fig. 7). However, since some messages were omitted (see the results in this chapter), it was necessary to eliminate this destroyed signal by deploying the de-noising technique Savitzky-Golay smoothing and median filtering as used by Mishra, (2019). Since we can assume that the trolley has a high value of inertia at the tested speeds (to 8.3 m s^{-1}), we can consider the distortion of this filter to be minimal.

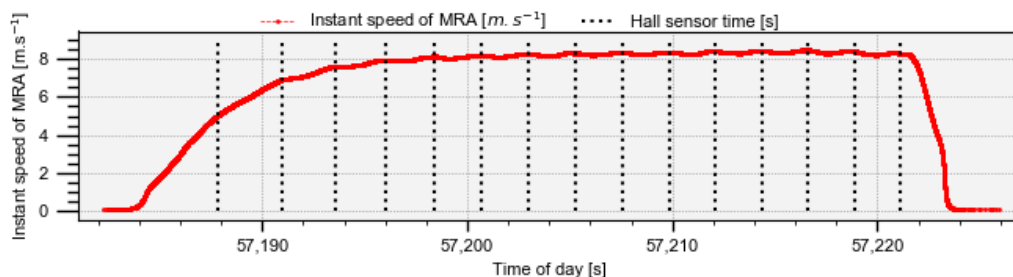


Figure 7. The instant speed of the MRA trolley in time.

Measurement of the RTK system

The Tersus BX-305 RTK system was measured to demonstrate the benefits of this project. The processing of the results in this paper did not include the evaluation of specific values of the RTK receiver. The aim was, with the help of visualization, to show the ability of the system to determine the exact position in different modes.

The measurement took place at an old military airport runway in Milovice in Nymburk District, Czech Republic on the 1st of November 2018 ($50^{\circ}14'9.1''\text{N}$, $14^{\circ}55'22.4''\text{E}$). This location was chosen because of the flat horizontal concrete surface without high buildings, trees or other obstacles in the near surroundings. The measurements were part of the measurement set used in the evaluations of the previous subchapter. As a reference signal, the VRS signal was chosen. A virtual base station was located at the centre of the MRA. The measurements were made in three RTK receiver modes. The first was a non-correction mode, the second used the VRS correction, and the third used the VRS correction with the built in fusion of internal IMU with this RTK receiver. Each measurement was carried out by rotating the MRA at three different speeds (2.7 , 5.5 and 8.3 m s^{-1}) for about 30 seconds and then the movement was stopped by brake. The last measurement at the speed of 8.3 m s^{-1} with the activated internal IMU and VRS correction were shown in the subchapter above (Figs 6 and 7).

For future location of the reference trajectory to the absolute space, it is necessary to determine the location of the centre of the axis of the MRA and its inception angle of rotation. This will be done by one of the RTK receivers that will survey two points using the geodetic method as in the case of study of Gao (2011). The first point will be measured at the point of the axis placement, the centre of rotation of the MRA (after MRA dismantle). The second point will be measured anywhere on a straight line that intersects the centre of rotation (axis) and a centre point of the trolley at a distance of

30–50 m. At this MRA position the Hall sensor pulse detection point will be located. To determine the surface tilt of the OSB boards 8 more points will be carried out at the circular trajectory of the trolley.

RESULTS AND DISCUSSION

Verification of the time accuracy of the PPS signal

Three sets of 3,600 samples of data were obtained and processed according to formula (1) from the three GNSS receivers. These three sets of deviations in microseconds were plotted in Fig. 8, where the horizontal axis represents the order of the samples and the vertical axis represents their deviation Δt_{seleae} against the clock of the logic analyser in microseconds. It was clear that these deviations of all three GNSS receivers have a very close relation, as it was evident from one of the represented segments of the measurement (Fig. 8). The time deviations of all three PPS signals in this measurement showed very similar values in maximum and average values. In both cases were these deviations in order of microseconds, concretely the maximal value $\Delta t_{\text{seleae}_{max}} = 5.7201 \mu\text{s}$ and the mean value $\Delta t_{\text{seleae}_{mean}} = 4.693 \mu\text{s}$.

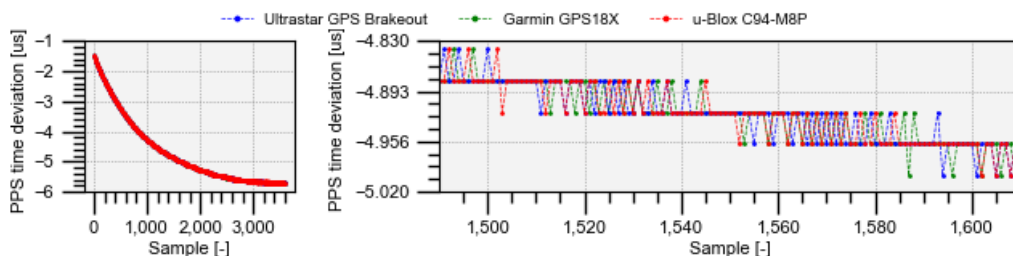


Figure 8. The time deviation of three PPS signals against the logic analyzer clock.

Since the paper of Niu et al. (2014) showed the deviations of the PPS signal of GNSS receivers against the measuring device with atomic clocks as the reference time source (working with systematic error of 0.25 ns) in tens of nanoseconds, it was assumed that the error occurred in our measurement method. Furthermore, it was evident from the deviations of the three PPS signals that the error has the same gradually decreasing non-linear trend. This may be caused by the deviation of the time base (i.e. the internal oscillator of the logic analyser), for example due to the temperature in the outside measurement area or many other negative influences on this equipment (Zhou et al., 2008).

The objective of this study was to verify whether a particular type of GNSS receiver can be used as an undivided component in order to assure a time base functionality of the MRA. Since insufficient time stability of the logic analyser clock was detected and it was not possible to bring originally intended results, it was useful to at least verify the PPS signal and in particular to detect whether the three sets of time deviations of the PPS were statistically significantly different or not. The histogram (Fig. 9) showed the distribution of the measured PPS signals deviations against the logic analyser clock in microseconds. Since the data of the PPS time deviations were not distributed normally, it was not possible to use the Analysis of Variance parameter test. Therefore,

nonparametric Kruskal-Wallis test was chosen. The p-value = 0.999 of this test was significantly higher than 0.05, so the zero hypothesis was not rejected. Scattering of all three compared groups was not statistically significant and it can thus be confirmed that the choice of the GNSS receiver did not affect the accuracy of the PPS signal.

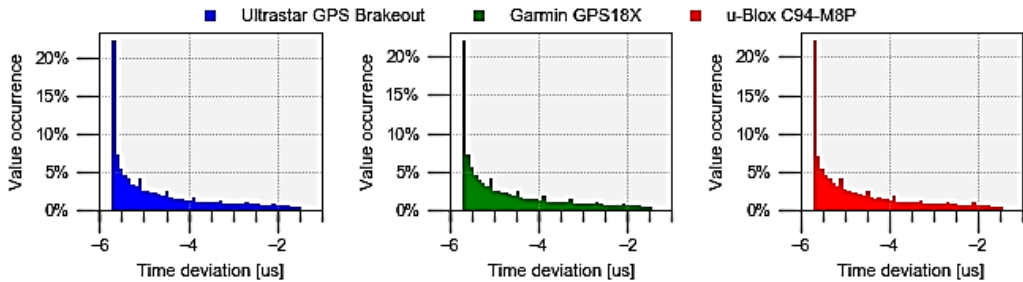


Figure 9. The distribution of time deviations of three PPS signals against the analyzer clock.

Verification of the quantity and correctness of the sent messages according to the arm instantaneous speed

The verification of the correctness of the times and iterators messages was gradually conducted in the intermediate stages during the data processing of sample measurements (Figs 5, 6, 7). The trajectory could be calculated by using the sets of these message pairs. The data was successfully logged at a reasonable density to ensure a future reference trajectory creation. The density as well as the angular distance of points of the reference trajectory of the MRA was defined by the number of received pulses from the encoder between two points and by the length of the arm. In the ideal case of all messages sent and logged, it would get the position information every 3.77 mm.

The SB unit was not fully successful in sending all messages as shown in Fig. 10. This figure indicated a one-dimensional focused view of a trajectory formed from points of the same measurement as in Figs 5, 6 and 7. This section showed twenty milliseconds cut out, when the MRA was moving at a speed causing the most varied values of omitted messages. The vertical axis represents the relative distance of the ‘x’ coordinates of the MRA from the centre of the axis of rotation of the MRA (to the East). The information about the number of unsent messages between two points was plotted in vertical axis in the graph.

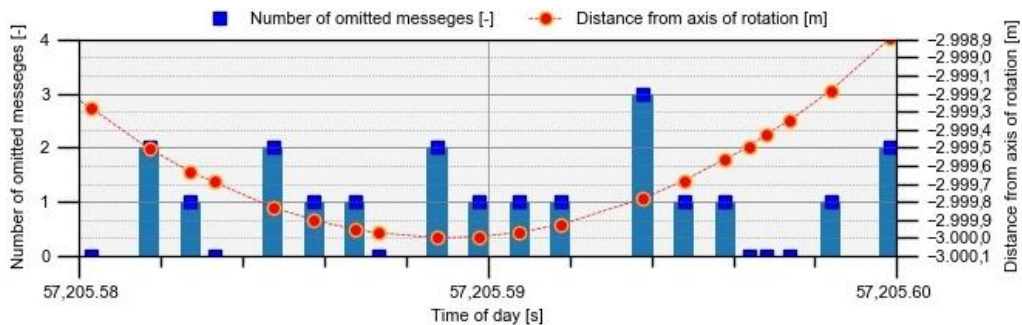


Figure 10. Verification of the quantity of omitted messages.

The verification of the quantity of omitted messages was carried out on a larger dataset of samples in the total number of 271,830 obtained points. Fig. 11 shows the dependency of the number of omitted messages between two measured points (on the vertical axis) on the instantaneous MRA speed (horizontal axis).

During the development phase, it was assumed that the most time-consuming operation for the SB will be the sending of messages on the CAN bus. For these purposes, the algorithm of the SB unit (Fig. 3) was designed to store the location information in the SB memory and to send the correct position of the MRA in the following message. As shown Fig. 10, in case of unsent messages, the position of the points shifts over time and the trajectory doesn't get deformed. To smooth the final reference trajectory, interpolation methods can be used.

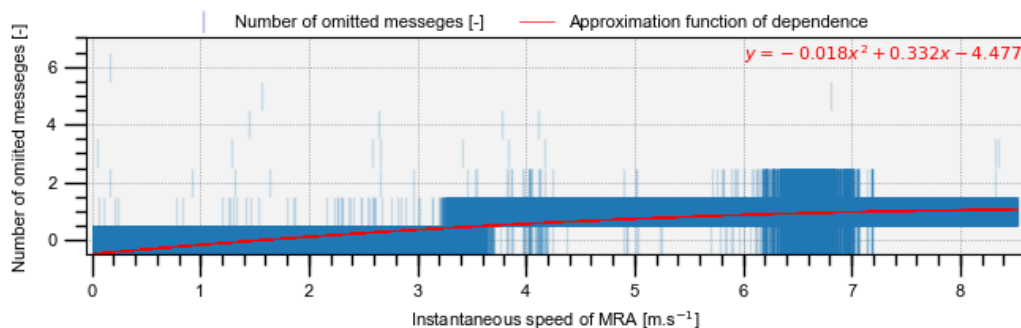


Figure 11. The number of omitted messages between two measured points according to the instantaneous MRA trolley speed.

As shown above, the number of omitted messages had an increasing tendency with the increasing instantaneous angular velocities of the arm. In extreme situations of six omitted messages, the maximum value of the angular distance between two points could be 22.619 mm. Nevertheless, on this large sample of data, such situation occurred only once. Unfortunately, the average value is unsuitable for this evaluating method because it would be burdened by the unevenly distributed number of samples depending on the instantaneous speed.

The real measurement of RTK systems

Since the planned verification of RTK systems implies the introduction of conditions that are difficult to implement and will be object of future authors' studies, only figures expressing the character of the positioning accuracy in the used mode were given. Fig.12 shows the measured RTK points to the Tersus BX-305 receiver whose antenna was placed on the MRA trolley.

Fig. 12, (1) indicates the measurement with no corrections (the RTK receiver worked as a separate GNSS). It shows that the measured points in the short-term periods tracked the circle of the reference trajectory, but from a long-term point of view, the precision of the positioning is unpredictably displaced by the impacts described in the previous authors' study (Kadeřábek et al., 2018). Fig. 12, (2) depicts the measurement of the RTK receiver in 'RTK fix' mode. Better ability to track the reference trajectory can be observed, but the scattering of the deviations of measured points in direction from the

centre of the circle is also evident. This was due to the acceleration and deceleration effects during the measurement process. Fig. 12, (3) finally shows the points measured by the RTK receiver in ‘RTK fix’ mode together with the activated signal fusion with the sensors of integrated IMU. A very good tracking of the reference trajectory was observed. This was possible probably thanks to a well-designed signal fusion of RTK and integrated IMU in this unit.

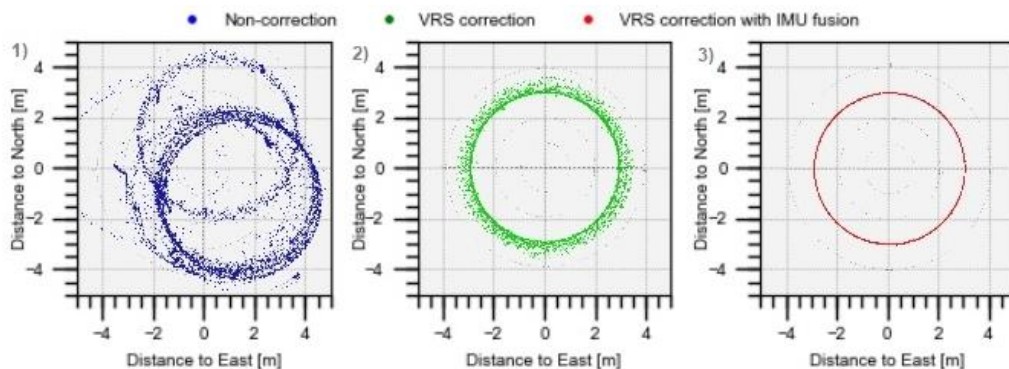


Figure 12. The points measured by Tersus BX-305 occurring in three modes.

As stated above, this rating was not compared with the MRA reference trajectories in given specific values. However, this figurative evaluating indicates that using of the MRA equipment as method to validate RTK receivers described in this paper is useful. The authors assume that further work on evaluations using MRA is needed since it can be beneficial not only in case of RTK receivers, but also for validating other localization systems.

This study has gradually demonstrated that the method using the MRA for the measurement enabled to log the data into the desired form. The data was successfully processed into the trajectory form which could thus serve as a reference system for verifying the dynamic properties of RTK or other localization systems. Previous studies have not so far identified any similar system for a RTK receiver dynamic evaluation. The dynamic evaluation of the RTK positioning receiver properties has already been discussed by some researches (Gan-Mor et al., 2007; Bakker et al., 2011; Carballido et al., 2014; Jilek, 2015; Kabir et al., 2016) where for example tractors or robots were moved in defined or straight paths that were used as reference system. The research of (Boffi, Gilgien & Wieser (2016)) used a downhill coaster track as part of reference unit, but the results focused primarily on velocity estimation, not position estimation. From our literature review it was evident that no study has used such equipment or methods as precise as the method described in this study.

CONCLUSIONS

The goal of this study was to describe the design of the method using the MRA device to verify the accuracy of localization systems on a reference circular trajectory created with absolute coordinates and labeled with absolute time stamps of one day. The design of this method was presented by the description of the mechanical design of the

MRA, a block diagram of electronic interconnection, a process diagram of the data acquisition from MRA sensors and a description of data processing with the aim of calculating the points of the final reference trajectory.

The verification of the present method was performed by several evaluations. First of all, the time accuracy of a selected type of GNSS receiver for the MRA was confirmed based on the examination of the accuracy of its PPS signal for the purposes of the MRA time base. Secondly, the method of data processing was verified together with the demonstration of the influence of the quantity messages sent by the SB according to the instantaneous speed of MRA. Finally, the contribution of this study was presented in the form of graphical representation of obtained results of measurements of the RTK receiver (Tersus BX-305) placed on the trolley on the MRA. In future research, the authors aim to present the findings of the evaluation of the RTK receivers' ability to determine the accurate position during their movement. Moreover, the employment of the method using the MRA device for validations of other localization systems will be verified.

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Impacts of some cultivated crops on water erosion in the Central Bohemia Region

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Abstract. This paper aims at assessing the impact of crops on water erosion parameters. Water erosion is the most common cause of damaging agricultural land in the Czech Republic. This is due to the large average slope of land and the prevailing soil types. The field trial was based on a site with an average slope of 5.4°. The primary objective was to assess the effect of the crop on the surface runoff and soil loss. The crops cultivated in the experiment were winter wheat, rape, potatoes, corn and oats (conventional tillage for all variants). Black fallow was used as the comparative variant (without vegetation). There is a light cambisol on the experimental field. To assess erosion, the method of microplots was used. The physical properties of the soil were also evaluated. The results show the risk of growing wide-rows crops (potatoes, corn). The soil loss in these crops was similar to the variant without vegetation.

Key words: water erosion, wide rows crops, surface runoff, erosion wash-out.

INTRODUCTION

One of the most important input parameters with a great influence on erosion processes is organic matter (Franzluebbers, 2002). This effect can take many forms, but the two basic ones are the soil vegetation cover formed by plants and also the soil cover formed by organic plant residues (Kovář et al., 2016).

A sufficient vegetation cover affects the course and intensity of erosion processes (Hangen et al., 2002). Its aim is to protect the soil against the impact of rain drops, to improve soil consolidation through the root system of plants especially in subsurface layers, to increase the infiltration capacity of the soil due to the growth of the root system and to improve the physical, chemical and biological properties of the soil (Šařec & Novák, 2017). The roots of some plants can destroy the compacted layer of soil created especially by the technogenic compaction (Kroulík et al., 2011). The choice of the appropriate crop is the most important issue for each plot. The study by Morgan et al. (2005) is based on data collected from the authors around the world. Morgan also states that soil losses caused by water erosion can be reduced up to 4.7 times by soil fertilization, by adjusting the microrelief up to 30 times and choosing a suitable crop to 37 times.

The greatest danger for the soil consists in the inappropriately created stands of broad-leaved crops, especially in the early stage of development, as well as in newly

established vineyards. The above mentioned problem under the conditions in the Czech Republic will mainly touch the corn crops sown on sloping land. The crops that are mainly threatened are grains and temporary grasslands, while permanent grasslands are less threatened (Zhang et al., 2014).

The initial hypothesis based on the assumed reduction of surface runoff and subsequent soil erosion wash-out in crops that have a higher cover of soil by plant cover at the time of intense rainfall. Furthermore, what is anticipated is higher surface runoff and soil erosion washout in wide-row crops.

MATERIALS AND METHODS

A field trial was established for the needs of measurements in the village Nesperská Lhota (see Fig. 1). The plot is situated on the border of the Vlašimská pahorkatina Hills (typical annual rainfall 700 mm). The experiment was based on light, sandy loam soil at an altitude of 450 m a.s.l. The average slope of the land is 5.4°. The soil contains particles smaller than 0.01 mm – 32.3% and organic C of 3.8%.

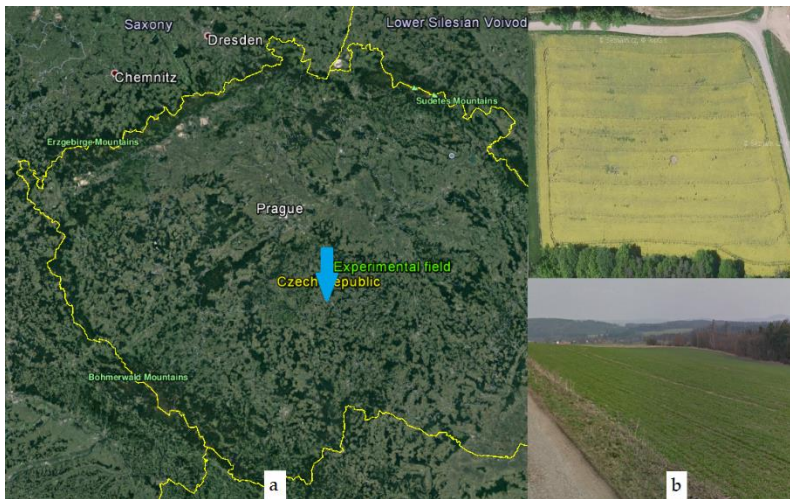


Figure 1. Experimental location (a), field photo (b),

The field experiment consists of five basic variants and one comparative variant. Each variant has an area of 300 m² with dimensions of 6 x 50 m. The long side of each variant is oriented to the slope. Each version represents a different crop growing typical for local conditions.

Variants of the experiment:

1. Winter rape (slope 5.6°): Conventional soil tillage cultivation technology. Primary and secondary tillage (a plough and seedbed cultivator) was done before sowing; sowing was carried out on August 17th, 2017.

2. Winter wheat (slope 5.4°): Conventional soil tillage cultivation technology. Primary and secondary tillage during autumn; sowing was carried out on October 2nd, 2017.

3. Oats (slope 5.2°): Conventional soil tillage cultivation technology. In autumn – plowing was carried out and a rough furrow was left during winter; in spring - secondary tillage (using a combinator) was effected, after which sowing took place on April 6th, 2018.

4. Corn (slope 5.3°): Conventional soil tillage. In autumn - plowing was carried out and a rough furrow was left during winter; in spring - secondary tillage and sowing corn took place on April 28th, 2018).

5. Potatoes (slope 5.5°): Conventional soil tillage technology. In autumn - ploughing was carried out and a rough furrow was left during winter; in spring - pre-seed preparation using a tine tiller was followed by potato planting on 29th April 2018.

6. The last variant is black fallow and it is comparative variant for whole experiment (slope 5.4°): the soil was treated by implementing conventional technology (the same as in the case of the previous variants), a non-systemic herbicide (glyphosate) was used to destruct plants; this eradication with the above mentioned herbicide was repeated several times during the 2018 season.

For each of the described variants, four outflow microplots were installed after sowing. The microplots were surrounded by 1.5 mm thick steel sheet. The walls of the microplots were pushed into the soil to the depth of 0.08 m (see Fig. 2). The collector is located at the bottom of each micro plot. It transports water into a plastic container buried below the catching microplots. The area of each microplots is 0.4 m x 0.4 m.



Figure 2. Microplot with a plastic container for measurement.

For the purpose of measuring the volume and intensity of precipitation weather station Vantage Vue was placed near the experiment. The surface runoff was measured after every heavy rainfall. The surface runoff was detected by measuring the volume, the amount of soil washed by filtering runoff (erosion wash-out values) and subsequent soil drying at 105°C and weighing the soil on a laboratory scale. The measurement and evaluation method used in the study was published by Bagarello & Ferro (2007).

The data obtained from the measurements were evaluated in the STATISTICA 12 program. Chart graphs were used to illustrate field trial data. The data were further evaluated by the ANOVA analysis using the Tukey HSD test.

RESULTS AND DISCUSSION

Table 1 shows the results of the measurements of physical properties of soil. In general, the values are very similar. At the time of measurement, the effect of spring secondary tillage in the spring variants could be observed. The data reveal similar physical soil conditions. Thus, a similar effect on water infiltration into the soil can be assumed. There is no significant compacted layer in the measurement range.

The first soil erosion event occurred as a result of two storms from May 10th and 13th. Total precipitation in this period was 33 mm. The precipitation intensity ranged from 80–100 mm h⁻¹. The results in Figs 3 and 4 show that the lowest surface runoff and erosion wash-out were recorded in the first three variants. The lowest surface runoff and erosion wash-out is in winter rape, which is already involved in this period and begins to bloom. On the contrary, the largest surface runoff and wash-out was expected in the case of the conventional soil cultivation for corn and potatoes (variants 4 and 5). In these variants, the soil is still insufficiently protected by the growth of plants. Variant No. 6 also showed a high surface runoff and erosion wash-out. The highest surface runoff was reached by variant No. 5 (potatoes), but the soil was a bit lower than the comparative variant (black fallow).

Table 1. Soil bulk density and total porosity

Variant	Depth [m]	Porosity [%]	Bulk density [g cm ⁻³]
1	0.05–0.1	37.50	1.62
	0.1–0.15	39.48	1.57
	0.15–0.2	40.84	1.53
2	0.05–0.1	36.31	1.63
	0.1–0.15	38.76	1.57
	0.15–0.2	38.51	1.61
3	0.05–0.1	40.21	1.49
	0.1–0.15	40.62	1.53
	0.15–0.2	39.78	1.56
4	0.05–0.1	37.04	1.57
	0.1–0.15	38.51	1.47
	0.15–0.2	42.41	1.61
5	0.05–0.1	40.99	1.59
	0.1–0.15	37.90	1.58
	0.15–0.2	40.86	1.51
6	0.05–0.1	38.63	1.54
	0.1–0.15	41.23	1.48
	0.15–0.2	40.97	1.52

The difference in soil loss weight between rape, wheat and oat variants is below statistical significance. On the contrary, the soil erosion wash-out in the corn, potatoes and control variants, is statistically significantly higher than in the first three variants (Fig. 3 and Fig. 4).

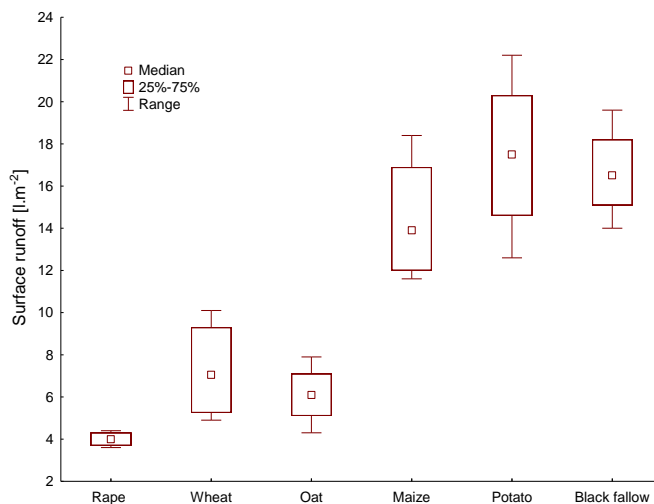


Figure 3. Surface runoff after two storms in May 2018.

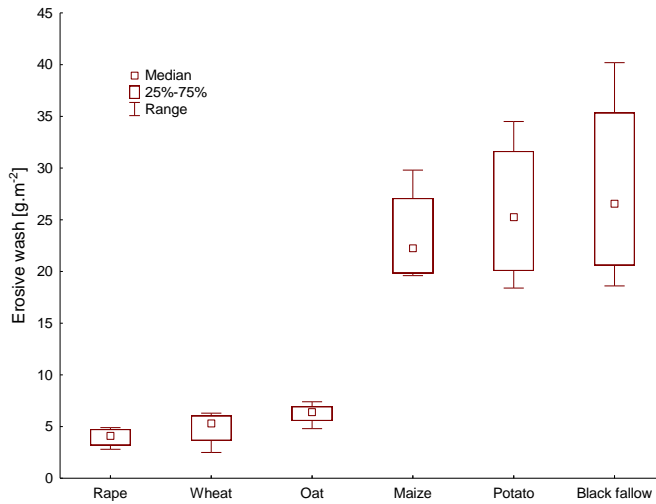


Figure 4. Erosion wash-out after two storms in May 2018.

Two more significant erosion events occurred during June. The first event occurred between June 10th and 14th, when the rain and two storms with a rainfall of 34 mm were recorded. The precipitation intensity ranged from 80–100 mm h⁻¹. The graphs in Figures 5 and 6 show that again the lowest surface runoff and erosion wash-out were recorded in the first three variants. Conversely, the highest surface runoff and erosion wash-out was measured for variant 5. High values were observed in the corn variant whose surface runoff was higher than in the comparative variant, but the soil wash-out was smaller than in the case of the potato variant and comparative one. The surface runoff of the comparative variant was lower than that of the corn variant and the potato, but the erosion wash-out reached similar values here (Fig. 5).

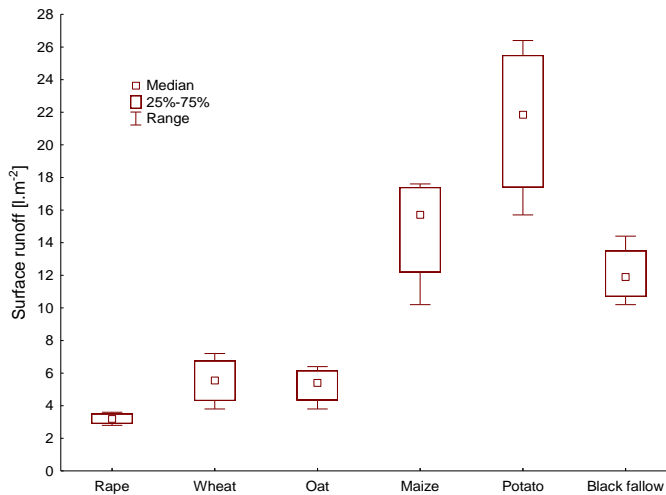


Figure 5. Surface runoff after two storms in the period 10th to 14th June 2018.

The difference in erosion wash-out between rape, wheat and oat variants is below statistical significance. On the contrary, the soil loss in the corn, potato and control variants is statistically significantly higher than in the first three variants (Fig. 6).

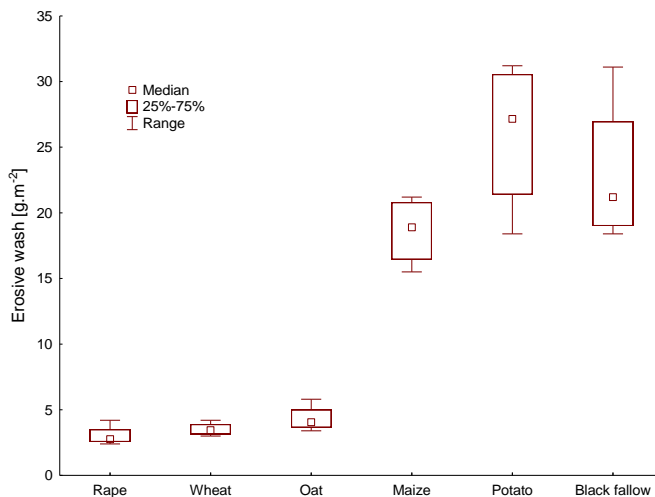


Figure 6. Erosion wash-out after two storms in the period 10th to 14th June 2018.

The second significant event was occurred between June 26th and 28th. There were two storms with a total precipitation of 44 mm. The intensity of these precipitation reaches up to 200 mm h⁻¹ for a short time. The graphs of Figs 7 and 8 show that the lowest surface runoff and erosion wash-out were recorded in the first three variants. In these variants the plants are already involved and the soil is well protected against erosion hazardous rainfalls. The lowest surface runoff and erosion wash-out were observed in winter rape. On the other hand, the highest surface runoff and wash-out were achieved by variant 5. Similarly, high values were achieved with the corn and comparative variants, but erosion wash-out was slightly lower than in the potato variant.

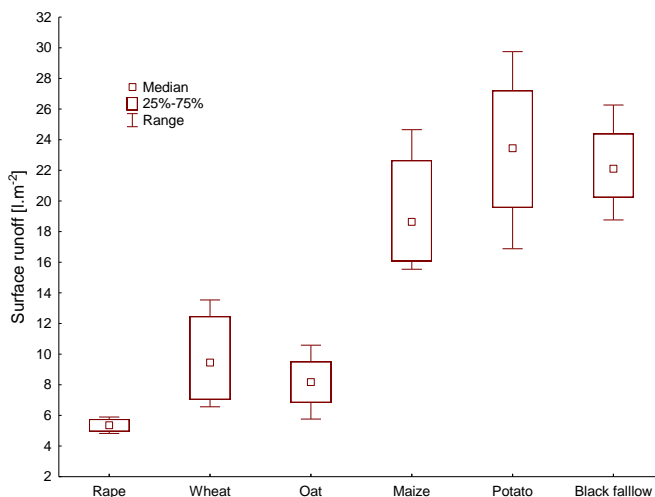


Figure 7. Surface runoff after two storms in the period 26th - 28th June 2018.

Again, there is a problem of poorly protected soil by plant residues. Another problem can be seen in wide rows crops production. This measurement reveals the most extreme values of erosion wash-out measured during the season 2018. The difference in erosion wash-out weight between rape, wheat and oat variants is below statistical significance. On the contrary, the soil runoff in the corn, potato and comparative variants is statistically significantly higher than in the first three variants (Fig. 7 and Fig. 8).

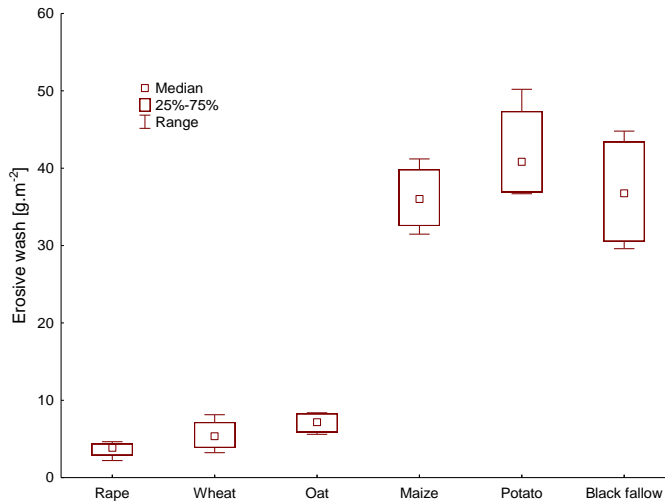


Figure 8. Erosion wash-out after two storms in the period 26th – 28th June 2018.

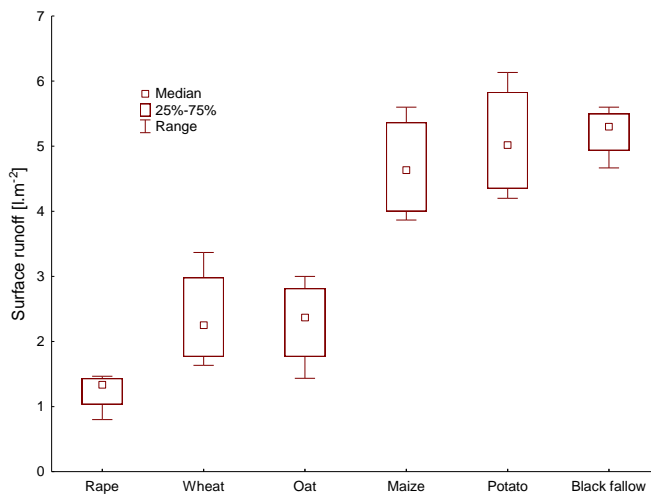


Figure 9. Surface runoff after a storm on July 6th, 2018.

Last erosion event occurred on July 6th during a short storm, when the rainfall was 9 mm and the intensity of the rain reached up to 80 mm h⁻¹. The graphs in Fig. 9 and Fig. 10 show that the lowest surface runoff and erosion wash-out were recorded in variant No. 1. In the case of wheat and oats, a slightly higher surface runoff was recorded than in the case of the rape variant. Higher values of surface runoff were captured in the

potato and comparative variants, where the highest value was reached by the black fallow variant compared with the corn and potatoes variants. The highest values of surface contours were achieved by variant 4 compared with the comparative and potatoes variants.

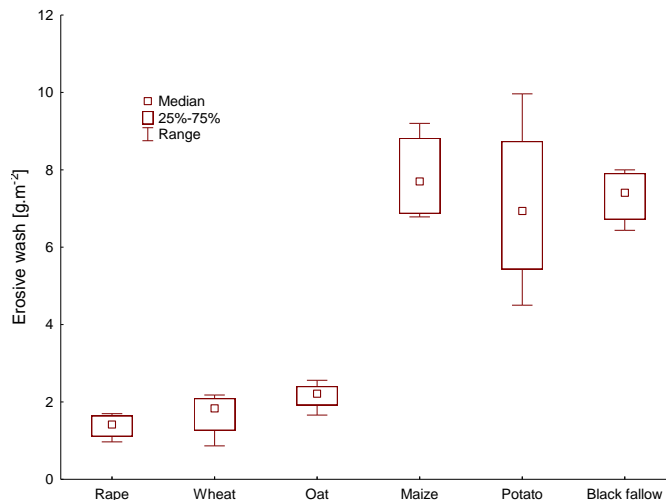


Figure 10. Erosion wash-out after a storm on July 6th, 2018.

The difference in the erosion wash-out weight between rape, wheat and oat variants is below statistical significance. On the contrary, the soil loss in the corn, potato and comparative variants is statistically significantly higher than in the first three variants.

The highest surface runoff and erosion wash-out were in variants with broad-leaved crops, in this case corn and potatoes. This has also been confirmed by Karlen et al. (1994). The soil is not well protected by the associated crop and the large kinetic energy of the falling droplets results in increased surface drainage and consequently an undesirable loss of the soil. The negative aspect for comparative variant (No. 6) was leaving the soil without any surface coverage by plant residues. In this case as well as in the other cases, there was also an increased surface drainage and consequent soil loss down the slope.

Brown et al. (1989) measured that for all monitored soil conditions (soil with plants or fallow) the amount of erosion of discrete particles decreased with increasing time since the beginning of the experiments. Generally, freshly treated soil is more inclined to soil erosion than the soil that has undergone several cycles of drying and rewetting. This phenomenon may be due to differences in cohesive forces between soil particles. This assertion has also been confirmed in the case of the above mentioned assessment, in which the differences between experimental variants of surface water drainage values during intensive rains events in the case of soil erosion have diminished. This is confirmed by Bradford et al. (1994).

Microplots were used to evaluate erosion parameters by Bagarello & Ferro (2007). This study confirmed the different behavior of the soil surface at a different cover. Most similar studies have focused on the impact of soil tillage. Novák et al. (2012) found a significant reduction in surface runoff by implementing reduced tillage technologies. The crop effect both on surface runoff and soil loss is evident and approved by many authors.

CONCLUSIONS

The hypothesis stated in the introduction has been confirmed by the field experiment. Clearly, a beneficial effect of vegetation soil cover during erosion hazardous precipitation has been demonstrated as a protection of the soil against the large kinetic energy of the falling droplets. It has also been proved that the infiltration of water into the soil has increased, which has led to minimum surface runoff and soil loss. On the contrary, it has been confirmed that broad-line or wide-rows crops considerably suffer from surface runoff and soil loss, which are much higher than in narrow rows sown crops. These crops are under risk of creating soil crust, which has a negative effect on the infiltration of water into the soil and the consequent increased surface drainage and soil loss.

The results of this study cover only for one year, one site and one soil type. The results obtained for different soil types might differ from these results.

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Robotic electromechanical object control by means of variable structure system

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Abstract. The practical purpose of robot design is to transfer types of complex human activities that require much effort, are monotonous and harmful. The robotic systems differ from traditional automation measures in terms of their universality and the possibility to reconstruct them quickly which enables them to create flexible automation production measures on the basis of universal equipment. Therefore, the subject matter of the present article is constituted by manipulator robot control system methods (semi-continuous control method, coordinator parameter control method and adaptive control method etc.) and the aim of the present study is to cover the said manipulator robot control system methods in order to assess the problems relating to their application and to provide the potential solutions. In analysing studies by other authors and assessing the results based on them, the following results of the present article were obtained: having regard to the peculiarities of control object model, due to their universality, theoretical methods of systems with semi-continuous control are the most attractive. The approach of other studies is also improper as it is claimed that the dynamics of electric executive equipment may be neglected and control moments can be formed in the same way as breakage functions and the problem which occurred may partly be solved, by using the advantages of the system with semi-continuous control in the pre-limiting situation which occurs by approximating semi-continuous control by means of continuous functions. The fundamental gap of the majority of electromechanical object control studies is, first of all, related with the fact that the phase variables are considered measurable, so the necessity arises to note that the entire complex of measurement equipment may lead to a significantly more expensive control system; moreover, measurement equipment adds additional dynamics to the control system and makes the synthesis procedure even more complex.

Key words: manipulative robot chains, slip mode, coordinate parametric control, adaptive control, approximating semi-continuous control.

INTRODUCTION

Robotics has recently turned into a large-scale scientific and technological field developing rapidly which encompasses issues of kinematics, dynamics, strategy planning, programming languages and artificial intelligence. In the present paper robot manipulators are analysed as a control object, i.e. mechanisms characterised by several degrees of mobility intended the motion of objects and orientation in working environment (Fig. 1). The multi-chain manipulator construction ends at a gripper or a

replaceable tool which are intended to grasp objects of a particular shape and to perform particular operations.

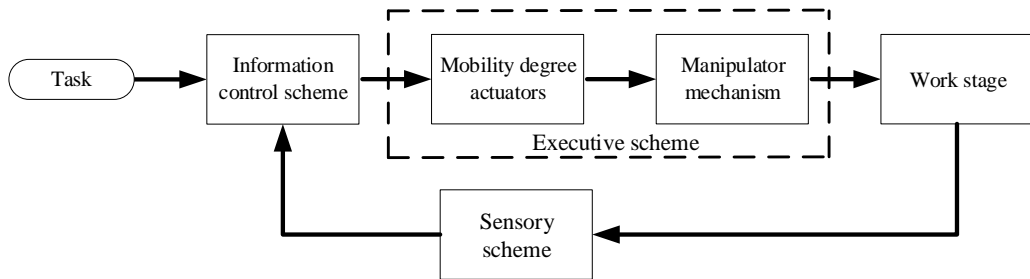


Figure 1. Functional robot control scheme.

The manipulator chains are interconnected by means of joints. The engines which make the chains move can be distributed in these joints or the respective powers and torques can be transmitted by means of movement mechanisms which do not change manipulator kinematic systems (Boujnah & Knani, 2015).

The engines of one model or another are usually produced as a module containing a movement transmission mechanism (reductor), feedback sensors (potentiometers, tachometers, rotary transformers (resolvers) etc.), whose signals are processed by microprocessors or analogue devices creating the control effect to the engine (Sassi & Abdelkrim, 2015). This system is referred to as the manipulator motion degree actuator (see Fig. 1). The executive system (see Fig. 1) is a control system consisting of actuators affecting the general mechanic load and ensuring the necessary movement of the manipulator and working organ respectively.

The first tactical manipulator control stage is trajectory planning (Jazar, 2010; Bazylev et al., 2014), i. e. setting the movement of manipulator chains or movement organ programmed movement at a certain period in off-line mode. The robot movement shall be modelled considering the limitations at the working zone. It is determined that the following aspects are known at the movement planning level: the purpose of movement, the description of the working space with the existing limitations (obstacles) and the capabilities of the robot capabilities. Moreover, when forming a continuous movement trajectory, the following requirements are considered: acceleration and braking mode, implementation of critical conditions etc. In the systems containing programmed control the entire trajectory (in the general case, movement and the existing working organ orientation) shall be programmed in advance. Provided that this is not possible, the technical vision system is applied (the entirety of visual information system), whose signals are used to either correct robot movement trajectory or to calculate it (Boujnah & Knani, 2015). The planning of industrial robot manipulator movement trajectory shall be carried out by means of control algorithms. These control algorithms shall be analysed as non-linear command movement designation algorithms according to the manipulator motion degrees. Their actualisation is related with the solution of non-linear equations describing the configuration of the mechanism upon the determined position of industrial robot working organs. Either ESM or imitation modelling shall be employed for such non-linear tasks, by also using actual manipulator

system models. The second strategic phase is the performance of the determined programmed trajectory with actuators joints at the online mode.

MATERIALS AND METHODS

In analysing the tasks of manipulator robot control, it is, first of all, necessary to determine the aim of the control. The aim in the most common solutions is to ensure the movement from one given point to another in terms of its sufficient speed and accuracy. Another task is to ensure the ‘practical stability’ of manipulator gripper movement along the determined special trajectory which stands for a requirement that actual deviations from the given (nominal) trajectory shall be limited. It shall be noted that a high positioning accuracy (i. e. statistical accuracy) manipulator robot, for instance, PUMA–560, may also be not characterised by sufficient dynamic accuracy. The control task is to form a required manipulator actuator functioning algorithm based on the respective manipulator control methods (Dong et al., 2018).

The essence of the speed vector control method is to determine the speeds of the robot system working objects as a six-dimensional vector representing the projections of the working object angular velocity and the speed vectors of its certain point in any coordinate system which are the planned control algorithms so that it is possible to determine the speed of the working object in the current trajectory point. The direct application of this solution is limited by degenerate configurations of the mechanism which shall be considered by the control algorithm. The above-mentioned method which is actualised in a relatively complicated manner is effective in order to quickly move the working object from one position to another if necessary when there are no high positioning requirements.

The successive situation adjustment method is used most widely in different numerical control systems. In this case the control algorithm according to the speed vector shall be formed as a manipulator coordinate variation during one algorithm calculation cycle. The disadvantage of the method is a frequent selection of nodal points in a complex trajectory so that the transitions from one point to another do not alter the necessary picture of the movement essentially when planning the movement trajectory.

In performing the synthesis of manipulator control system, the approximative methods are employed, which originate from the setting of limited coordinate values. Three values are usually taken: two outermost and one middle value; then the inverse Jacobian matrix is calculated on the basis of them which depends on the manipulator configuration, while for all other coordinate values (including the nodal ones, the necessary points of the trajectory) the inverse matrix is calculated by means of interpolation. In many cases, especially in the case of feedback according to the position, this is absolutely enough in order to achieve the final control goal. The disadvantage of the method is the errors which occur during the interpolation which become significant in case there is no feedback according to the position (Jazar, 2010).

The following shall also be classified as a disadvantage of the power vector control method: it is impossible to guarantee the formalisation of robot working object movement approaching the given direction provided that the given trajectory contains points at which the manipulator configuration matrix is degenerate. The trend of control process simplification and its resulting calculations led to the power vector control method where the idea of control on the basis of the given direction is simulated. In

reality, no power is directed/added to the manipulator, but the tracking actuators create the entirety of generalised powers which are dynamically equivalent to the given powers simulating their adding/directing to the manipulator. As the calculator receives the set signal at the entrance, it sets the generalised powers attributed to the coordinates which are directly controlled by actuators. The actuators, on their turn, constantly create such generalised powers which are obtained at the exit of the calculator. These generalised powers can be clarified automatically by regulators and compensators.

The main task of manipulator control is to generate external moment in such a way that the robot movement takes place in the chosen trajectory. The robot movement usually is implemented in two different controllable phases. During the first control phase (approximate movement) the robot shall move from the initial position to the environment of the location of the set goal alongside it from the pre-determined trajectory. The second control phase is a precise movement phase where the robot working object is dynamically interacting with the object, by also using the external sensor feedback information channel in order to perform the task. In case the traditional tracking system is used in the robots, the non-linearity and interaction which depend on the manipulator dynamics cannot be compensated at the approximate movement phase. As the requirements for industrial robot performance characteristics are increasing, it is necessary to consider the above-mentioned dynamic effects. Therefore, many improvements have recently been proposed to the schemes and algorithms of industrial robot direct control (Han et al., 2017).

Some researchers (Bazylev et al., 2014; Lee et al., 2018) have suggested that linear system models should be used as a basis for further actualisation of regulation dissociation. It is understood that the regulation system based on the linear model may seem to be unacceptable if the actual working conditions are different from the conditions determined during linearisation.

One of the first methods where the robot is analysed as a non-linear interrelated system with a variety of entrances and exits was the torque calculation methodology (Bazylev et al., 2014). Moreover, it was believed that inert reaction powers can be precisely calculated, Korol powers inert powers and gravity powers. Therefore, the operational characteristics of this regulation system basically depend on the accuracy of the model used. The moment identification method requires a high number of calculations which is often regarded as a disadvantage.

The regulation of the motion designed is the identification methodology of joint movement speed variables necessary for ensuring the robot terminal point movement at the given direction. In the case of such control scheme, all the trajectories set are expressed in Descartes coordinates. It is a particular advantage because for many users it is much simpler to determine the sequence of movement in Descartes coordinates as compared to joint position variable coordinates. The regulatory methods of torques and the designed movement speed are reconciled by means of the designed movement acceleration control method (Lee et al., 2018). This method provides that the user shall determine the necessary positions, speeds and accelerations of the arm of the determined. The above-listed methods are also notable for the disadvantages: it is necessary to have a detail dynamic model and to delegate much time for machinery calculations.

Another group of methods is focused on the compensation of the existing indefinite uncertainties. These are the following methods: coordinate parametric control (Shakibjoo & Shakibjoo, 2015), methods of adaptation to unknown parameters (Huang

& Liu, 2017). In the case where external disturbances are described by the known dynamic model with unknown initial conditions, the external disturbance dynamic compensation principles are applied (From et al., 2014; Mustafa et al., 2017).

The main reason for the interest in the adaptive robot control methods is that some of the practical robototechnics solutions for the technological tasks require very precise restitution of the determined trajectory (for instance, the pilot stand robots, assemble robots, low flight robototechnical root simulators, laser technological stand robots, emergency robots, space robots etc.). In order to perform these tasks, it is impossible to achieve the required accuracy by means of linear feedback due to the fundamentally non-linear nature of manipulator equations as well as due to the dependence of these equations on the load transferred.

In the adaptive control with a benchmark model a model corresponding to the benchmark is chosen and the adaptation algorithm modifies the strengthening coefficients in the controller feedback channels. The adaptation algorithm is determined by the difference between benchmark model output signals and the actual parameters of the robot. The robot is controlled when the strengthening coefficients are regulated in the feedback channels with regard to the position and the speed so that the close contour characteristics are close to the benchmark model. Currently quite many different universally stable manipulator adaptive control algorithms are known already, the majority of which may be received by applying the standard procedures of speed gradient method, by also properly selecting the target functionality and phenomena for an error. It is necessary to note that the majority of the existing adaptive cannot fully solve the problem of designing the manipulator automatic control systems intended for particular application (including the industrial one).

First, the fact that it is complicated to perform as many calculations at the real time as it is needed in order to actualise at least the simplest Slotine-Li algorithm (Zhang & Wei, 2016) intended for six-degree anthropomorphic manipulator PUMA-560 constitutes a considerable obstacle for the actualisation of universally stable adaptive manipulator control algorithms. The second reason has to do with the fact that all the universally stable adaptive algorithms are focused on the so-called object equation parametric indeterminacy, i. e. on the mathematical object description, which would be precise until the final number of stable parameters. Upon such access, all the components of the non-linear description of the object shall be repeated in the control principle which means that these components need to be known exactly. It is quite difficult and sometimes even impossible to design an object model which is precise enough in practice. In other words, universally stable adaptive algorithms are calculated with regard to lower-degree indeterminacy as compared to the one which is usually encountered in practical tasks. Therefore, it is reasonable to base the design of control adaptive systems of manipulators which are intended to be applied in real conditions on totally different principles which do not require the restoration of object non-linearity and the calculated higher degree indeterminacy as well.

The modern trend of mechanical object control is to design multi-regime multi-functional widely applicable system control principles which are universal enough which should not be cumbersome, and which should not require enormous calculation costs. For this purpose, it is necessary to reduce the dependence of control principles on the dynamic parameters of the control object.

RESULTS AND DISCUSSION

Another trend in designing robot systems is basically related with the theory of variable system structure. In the systems containing the variable structure, the slip mode occurs on the surface of the switch (Han et al., 2017; Jung & Jeon, 2017). When the system is working at the slip mode, the system remains insensitive to variations of parameters and disturbances. In order to achieve control indicating the slip mode no precise modelling is needed; it is enough to know the ranges of model parameter measurements. This access provides that control principles need to be established in the breakage function class, by also assuming that the execution equipment dynamics is low, and it cannot basically affect the mechanic system movement. Some studies will be mentioned where the methods of systems with semi-continuous control were used in robot systems. In the studies by V. Utkin external powers affecting the mechanical system are considered unknown but limited (Liu et al., 2017a). It is assumed that control impacts are dominating. The control principles obtained (by also having regard to the common limitations of phase variables and control impacts) determine the tasks of terminal control upon the application of game access, i. e. uncontrollable disturbances are treated as manifestation of the opponent's actions. In the studies by A. Mustafa the unknown also refers to mechanical inert characteristics (Liu et al., 2017b). Only the interval where the energy matrix real values may vary is known. The presumptions submitted contain the sectionally continuous principle. J. Juang has formulated the task of the black box control of mechanical nature has been formulated in his works (Xiao et al., 2017). Critical situations were analysed where information on the dynamic parameters of the system was basically unavailable were analysed. The principle of division has been set which is one of the accesses in order to solve a control task under indeterminacy conditions. The principle of division has been elaborated on by B.T. Kulakowski in his works, where not only the very fact of the presence of final intervals of the change of the inertial characteristics of external powers and the mechanical system is known. Universal control principles which stabilise any potential movement of the mechanical system in practice were obtained (Raj et al., 2016; Yagur & Belov, 2018).

Manipulators as control objects are multi-chain non-linear interrelated systems. Although, in order to facilitate control tasks, it is required to somehow dissociate manipulator motion degrees to make them controllable independently, such access is not the best one from the control quality perspective in general. Two groups of motion degree interrelate influence compensation methods exist:

Constructive, based on the alignment of chain mass of the manipulator;

Algorithmic, actualised control systems, the above-mentioned distribution principle in particular.

The general measure intended for the reduction of self-imposed influence is the increment of manipulator motion degree control system functioning increases the efficiency of retraction of the disturbances affecting these motion degrees (including their mutual influence).

CONCLUSIONS

Considering the peculiarities of control object model, a conclusion can be made that, due to their universality system, semi-continuous control theory methods are the most attractive. These methods enable suppressing of a wide class of both parameter and external disruptions and ensuring durable properties of a closed system, especially because they are much simpler to realise as they do not need a detailed dynamic model and much time for performing machinery calculations.

The majority of the above-mentioned studies have proven that the dynamics of electric executive equipment may be neglected and control moments may be formed in the same way as breakage functions. However, these results are impossible to actualise these results in practice directly due to the powers evolved by executive equipment and physical limitations of the moments. However, having employed the advantages of the system with semi-continuous control in the pre-limiting situation which occurs upon the approximation of semi-continuous control by means of continuous functions one can partly overcome this problem.

The main gap of electromechanical object management research is related with the fact that phase variables (generalised coordinates, their speeds, acceleration, actuator variable states) are considered measurable, i.e. no objective to observe is set and solved. It shall be noted that the entire complex of measurement equipment may lead to a significantly more expensive control system; moreover, measurement equipment adds additional dynamics to the control system and makes the synthesis procedure even more complex.

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Effect of densification variables on water resistance of corn cob briquettes

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Abstract. Solid biofuels can be used in heat and power generation applications. The utilization of agricultural residues for this purpose would be of immense benefit to rural communities of developing countries where the resource is being produced. Water resistance is a crucial property for transport and storage of biomass briquettes under moist climate conditions. In this study, the effect of process and material variables on the water resistance property of corn cob briquettes was investigated. The water resistance of briquettes produced ranged between 32.6 and 94.8% for die temperature between 90 °C and 120 °C, hold time from 7.5 to 15 minutes and die pressures between 9 and 15 MPa. A higher die temperature resulted in an increase in the water resistance of the biomass briquettes. Also, increasing the hold time improved the water resistance of the briquettes. Using a particle size less than 2.5 mm resulted in higher briquette water resistance property compared to briquettes produced from particle sizes greater than 2.5 mm. It was also shown that the effect of the interaction of the temperature with particle size on the water resistance of corn cob briquettes was statistically significant ($p < 0.05$).

Key words: biomass briquette, temperature, particle size, pressure, hold time, uniaxial compaction.

INTRODUCTION

Biofuels are being considered for replacement of fossil fuels in existing energy and power technologies due to the ease of finding, low price, carbon neutral feature and very high regional and global potentials (Yokoyama et al., 2000; Kaygusuz & Türker, 2002; Omer, 2005; Moreira, 2006; Haykiri-Acma & Yaman, 2010; Ojolo et al., 2012). The huge potential is yet to be fully utilized for the generation of energy particularly in the rural areas of the developing countries where there is an abundance of agricultural residues (Jekayinfa & Scholz, 2009). Solid biofuels are mainly used for the production of heat and/or electricity (FAO, 2009; Voytenko, 2010). Combustion technologies produce energy from biomass, converting biomass fuels into several forms of useful energy, e.g. hot air, hot water, steam and electricity. Biomass combustion covers basic

energy requirements for cooking in rural households. It also provides heat for processes in a variety of traditional industries in the developing countries. Commercial and industrial combustion plants can burn many types of biomass ranging from woody biomass to municipal solid waste. One of the primary determinants in the selection and design of any biomass combustion system is the characteristics of the fuel. Conversion of biomass into briquettes improves the fuel characteristics and allows for automating its feeding into combustion systems (Bhattacharya, 2003; Rosillo-Calle, 2007; Ojolo & Orisaleye, 2010).

Quality attributes of densified biomass play a major role in the end-user applications (Tumuluru et al., 2010). Quality parameters include the moisture content, unit and bulk density, durability index, compressive strength, percent fines and calorific values. Furthermore, storability and transport behaviour are important characteristics of solid biomass fuels for its availability all over the year. Water resistance of briquettes shows the resistance to absorption of water during transportation and storage. The water resistance property is crucial for biomass briquettes which must be transported through high humidity environment.

Some studies have determined the resistance to water penetration based on the quantity of water absorbed within a specified period when the briquettes are fully immersed in water. Sengar et al. (2012) stated that the resistance to water penetration of cashew shell briquettes was better when compared with grass and rice husk briquettes. Saha et al. (2014) determined the water resistance of coconut coir dust briquettes and rice husk briquettes and stated that the briquettes made from mixed coconut coir dust and rice husk had desirable properties when compared to rice husk briquette. Lindley & Vossoughi (1989) noted that briquettes made from sunflower stalk, wheat straw, and flax straw had average weight gains of 9.9, 32.3 and 38.1% after immersion for 30 seconds in water at room temperature. Davies & Davies (2013a) stated that water hyacinth briquettes produced with small particle sizes had higher water resistance characteristic compared to briquettes produced from larger particle sizes. Davies & Davies (2013b) obtained results between $52 \pm 2.42\%$ to $97.1 \pm 3.39\%$ for the water resistance property of water hyacinth briquettes for five binder levels studied.

Other studies, however, determined the water resistance quality as the time taken for the briquette to disperse in water at room temperature. Križan (2007) stated that when the briquette disperses in water in less than 5 minutes, the briquette is of low quality while high quality briquettes should have dispersion times up to 20 minutes. Mitchual (2015) observed that water resistance quality of briquettes produced from different biomass materials used ranged between 1.01 to 6.63 minutes. Li & Liu (2000) reported that briquettes produced from oak sawdust, pine sawdust, and cottonwood sawdust disintegrated in less than 5 minutes after immersion in water at room temperature.

Pilipchuk et al. (1975) have also stated that high temperature pressing of wood increases the reaction rate and the accumulation of high molecular products, mostly lignin. It was further stated that the accumulation of partly melted and insoluble high-molecular substances in the voids of capillary and submicrocapillary systems will prevent the soaking of water into the cell walls. This inhibits the swelling and secures the water resistance of the wood base laminate (Zandersons et al., 2004).

Some studies have been carried out on the investigation of properties of briquettes produced from corn cobs, but they have either been limited to the use of binders (Oladeji & Enweremadu, 2012; Muazu & Stegemann, 2015; Oyelaran & Tudunwada, 2015), or

composite briquetting with other materials (Ikelle & Ivoms, 2014; Muazu & Stegemann, 2015; Oyelaran & Tudunwada, 2015; Nurhayati et al., 2016). Studies on production of binderless briquettes did not consider the water resistance property of the briquettes (Kaliyan & Morey, 2010a; Okot et al., 2018; Orisaleye et al., 2018). Studies which consider the effects of interactions of densification variables on water resistance of briquettes are also rare. This study, therefore, aims to determine the effects of pressure, temperature, hold time and particle size and their interactions on the water resistance property of binderless briquettes produced from corn cobs.

MATERIALS AND METHODS

Materials

Corn cobs were collected from a corn processing firm in Lagos, Nigeria. The corn cobs were then air-dried for three weeks to remove excess moisture within the cobs. Afterwards, the corn cobs were milled in a commercial hammer milling machine. The milled corn cobs were sieved using a 2.5 mm mesh sieve into two particle sizes – less than 2.5 mm and greater than 2.5 mm.

Preliminary experiments for the determination of moisture content were carried out by following procedures outlined in ASTM D 4442-92 standard test methods. The difference in the masses, Δm , was determined and the moisture content was computed using:

$$\text{Moisture content (\%)} = \frac{\Delta m}{m_i} \times 100 \quad (1)$$

The initial mass of corn cob sample is m_i ; Δm is the change in mass. The moisture content in the milled air-dried corn cobs was determined to be 9.8%.

The briquetting press

The uniaxial briquetting press used for the production of briquettes was locally developed and is shown in Fig. 1. The press produces one briquette in a setting and the briquette diameter is 50 mm. The components of the briquetting press are a 5 tonnes hydraulic jack, die, heating band rated at 1,000 W, tie bar, pressure gauge (Econosto, EN 837-1, Germany), cover plate and bottom plate. The hydraulic jack is used to apply the required pressure to the raw biomass and the heating band which is controlled by a temperature controller (Jetec, JTC-903, China) supplies the heat required to raise the temperature of the biomass.

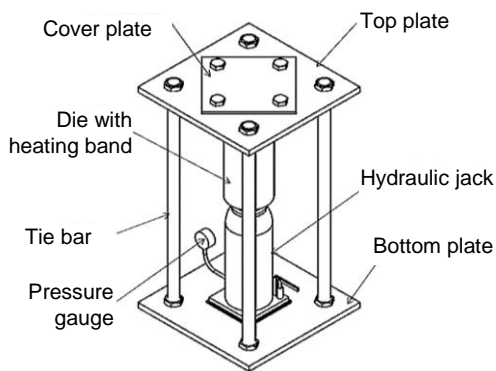


Figure 1. The developed experimental biomass briquetting Press.

During operation, the top of the hydraulic jack was set to the bottom dead centre of the die. The electric heater, which surrounds the die, was then powered on and set to the required temperature. At this position of the hydraulic jack, biomass material was loaded

into the die until it was filled up. The top plate was then fastened to the die. The hydraulic jack was operated until the required pressure is reached. At this pressure, the apparatus was left to stand for the required hold time. At the end of the period, the top plate was removed from the die and the hydraulic jack operated until the briquette reached the top dead centre of the die. The briquette formed was picked away from the top of the piston of the hydraulic jack and allowed to cool and stabilize for one hour.

Design of experiments

The effects of temperature, pressure, hold time and particle size on the density of biomass briquettes were determined during the experiments. The temperatures used were 90 °C and 120 °C. The pressures used were 9 MPa, 12 MPa and 15 MPa. The hold time used were 7.5 minutes and 15 minutes. All possible combinations of values of pressure, temperature, hold time and particle sizes (< 2.5 mm and > 2.5 mm) were utilized during the experiments. The variables were coded using Table 1. A full factorial experimental design was used which considers all possible combinations of variables being studied. The experimental design is shown in Table 2.

Determination of water resistance

The water resistance is determined from the percentage of water absorbed by a briquette when immersed in water. The briquettes formed from each experimental run in Table 2 were immersed in water at room temperature for 30 seconds. The procedure follows studies of Birwatkar et al. (2014), Sengar et al. (2012) and Saha et al. (2014). The column of water in bucket used was 150 mm high at room temperature. The resistance to water penetration is determined from:

$$\%WR = 100 - \left[\frac{M_{wet\ briquette} - M_{initial}}{M_{initial}} \times 100 \right] \quad (2)$$

where % WR – water resistance in percentage; $M_{wet\ briquette}$ – mass of wet briquette; $M_{initial}$ – initial mass of briquette.

Table 1. Coded values of densification variables

Variable	-1 (Low)	0 (Medium)	1 (High)
Pressure	9 MPa	12 MPa	15 Mpa
Temperature	90 °C	-	120 °C
Hold time	7.5 mins.	-	15 mins.
Particle size	< 2.5 mm	-	> 2.5 mm

Table 2. Full factorial experimental design for the study of water resistance of corn cob briquettes using coded variables

Exp. No.	Pressure	Temp.	Hold time	Particle size
1	-1	-1	-1	-1
2	0	-1	-1	-1
3	1	-1	-1	-1
4	-1	1	-1	-1
5	0	1	-1	-1
6	1	1	-1	-1
7	-1	-1	1	-1
8	0	-1	1	-1
9	1	-1	1	-1
10	-1	1	1	-1
11	0	1	1	-1
12	1	1	1	-1
13	-1	-1	-1	1
14	0	-1	-1	1
15	1	-1	-1	1
16	-1	1	-1	1
17	0	1	-1	1
18	1	1	-1	1
19	-1	-1	1	1
20	0	-1	1	1
21	1	-1	1	1
22	-1	1	1	1
23	0	1	1	1
24	1	1	1	1

RESULTS AND DISCUSSION

The briquettes produced using the experimental runs in Table 2 had densities ranging between 570 and 1,300 kg m⁻³. This depended on the operating conditions specified in the experimental runs. The results of the graphical and statistical analysis of the effects of the temperature, pressure, hold time and particle size on the water resistance of briquettes are presented in this section.

Effect of temperature on the water resistance of corn cob briquettes

Figs 2–5 show the effect of temperature on the water resistance of biomass briquettes produced from corn cobs. It is observed that the water resistance, which ranges from 32.6% to 94.8%, increases for all particle sizes, die pressures and hold times with an increase in the temperature from 90 °C to 120 °C. It has been noted from previous work (Kaliyan & Morey, 2009) that increased temperature is required to activate the natural binding components such as lignin, protein, starch and water-soluble carbohydrates. The increased water resistance may likely be the result of improved bonding between particles due to better flow or plastic properties of the binding components at higher temperature. Lignin, which is the primary binding agent, has been identified to be hydrophobic in nature (Anglés et al., 2001; Kaliyan & Morey, 2010b) which would increase the water resistance of the briquettes. Pilipchuk et al. (1975) and Zandersons et al. (2004) have stated that melted lignin prevents soaking of water into cell walls.

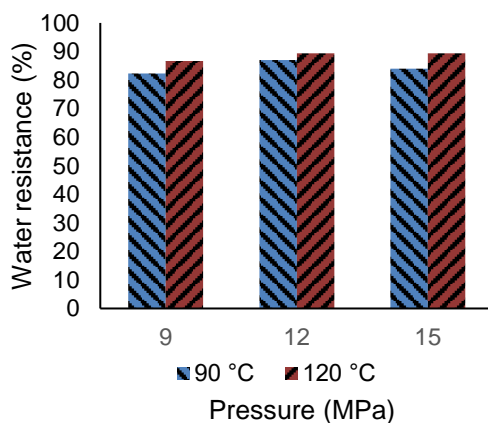


Figure 2. Water resistance of corn cob briquettes produced at different die temperature with a hold time of 7.5 minutes and particle size less than 2.5 mm.

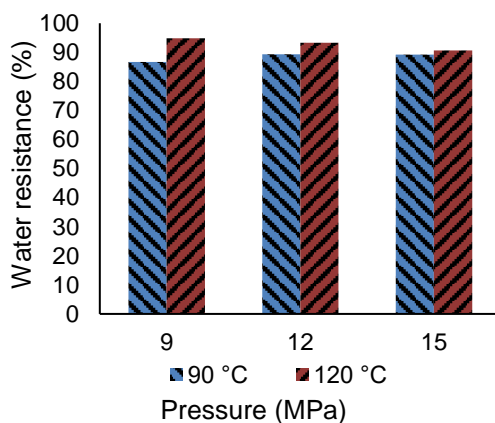


Figure 3. Water resistance of corn cob briquettes produced at different die temperature with a hold time of 15 minutes and particle size less than 2.5 mm.

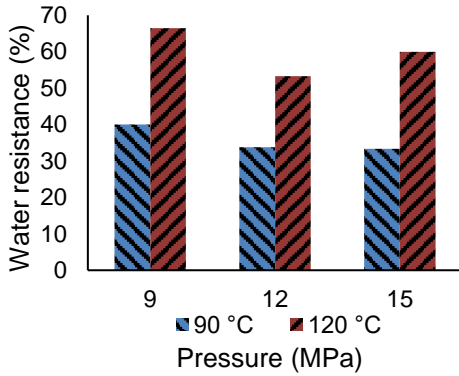


Figure 4. Water resistance of corn cob briquettes produced at different die temperature with a hold time of 7.5 minutes and particle size greater than 2.5 mm.

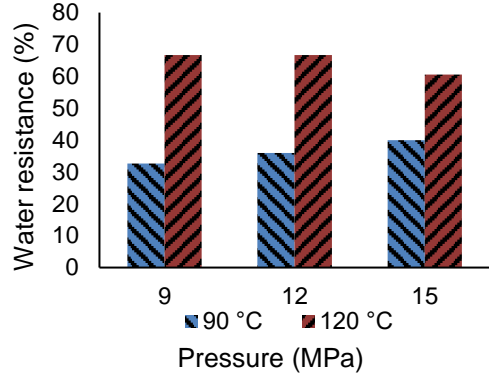


Figure 5. Water resistance of corn cob briquettes produced at different die temperature with a hold time of 15 minutes and particle size greater than 2.5 mm.

Effect of hold time on the water resistance of corn cob briquettes

The influence of hold time on water resistance of the corn cob briquettes is shown in Figs 6–9. It is seen that the water resistance using a longer hold time of 15 minutes was mostly higher compared to when a hold time of 7.5 minutes was used.

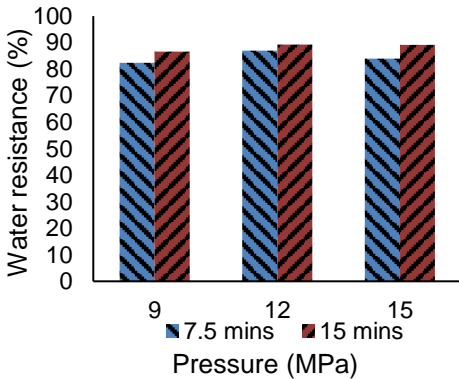


Figure 6. Water resistance of corn cob briquettes produced at different hold times with die temperature of 90 °C and particle size less than 2.5 mm.

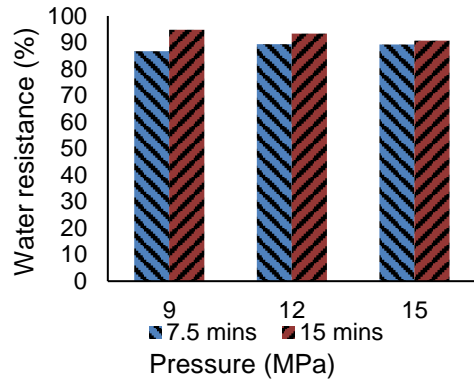


Figure 7. Water resistance of corn cob briquettes produced at different hold times with die temperature of 120 °C and particle size less than 2.5 mm.

Fig. 8, however showed that for particle size greater than 2.5 mm, die pressure of 9 MPa and temperature of 90 °C respectively, the water resistance for the hold time of 15 minutes is lower than that obtained using 7.5 minutes. The increase of water resistance with increased hold time is probably due to the lignin and other natural binding materials having time to coat particles and ensure stronger bonds between the particles. He et al. (2011), have however, stated that a longer holding time did not guarantee better water resistance when lignite briquettes had already gained water resistance through the heat treatment process.

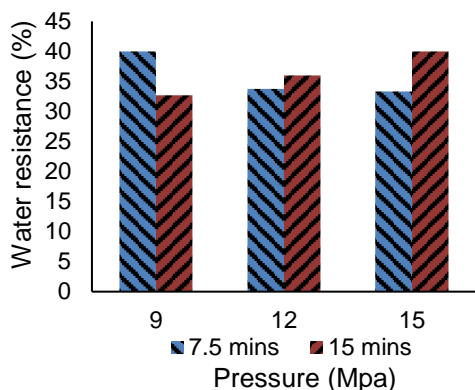


Figure 8. Water resistance of corn cob briquettes produced at different hold times with die temperature of 90 °C and particle size greater than 2.5 mm.

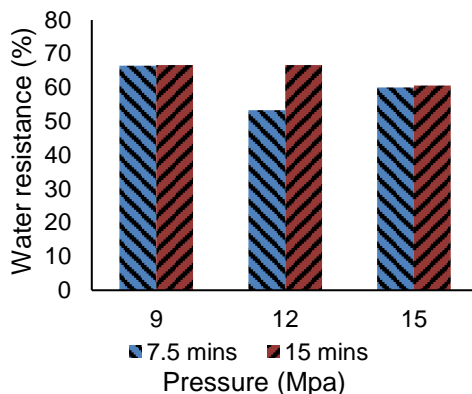


Figure 9. Water resistance of corn cob briquettes produced at different hold times with die temperature of 120 °C and particle size greater than 2.5 mm.

Effect of pressure on water resistance of corn cob briquettes

The effect of pressure on the water resistance of corn cob briquettes is shown in Figs 2–9 and Figs 10–13. The figures show that there is no clear pattern for the effect of pressure on water resistance. This indicates that pressure at the level applied during these experiments is not a significant factor in determining the water resistance of corn cob briquettes. Contrary to results obtained, Mitchual (2015) stated that compacting pressure between 20 and 50 MPa, together with interactions with biomass materials, had significant effect on quality of briquettes produced from sawdust of *Piptadenia Africana* and *Ceiba pentandra* at room temperature. Mitchual (2015) noted that water resistance quality of briquettes produced from the using low compacting pressure was low. Zanjani et al. (2014) also stated that the increase of briquetting pressure showed positive but insignificant effects on water resistance index.

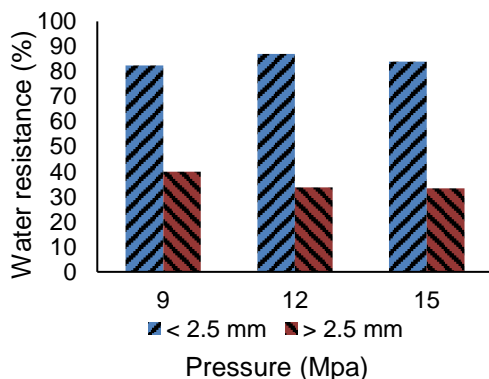


Figure 10. Water resistance of corn cob briquettes produced at different particle sizes with die temperature of 90 °C and hold time of 7.5 minutes.

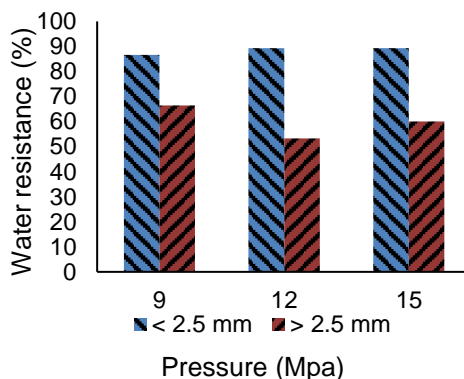


Figure 11. Water resistance of corn cob briquettes produced at different particle sizes with die temperature of 120 °C and hold time of 7.5 minutes.

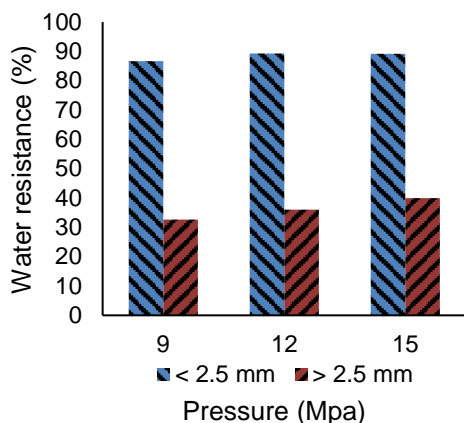


Figure 12. Water resistance of corn cob briquettes produced at different particle sizes with die temperature of 90 °C and hold time of 15 minutes.

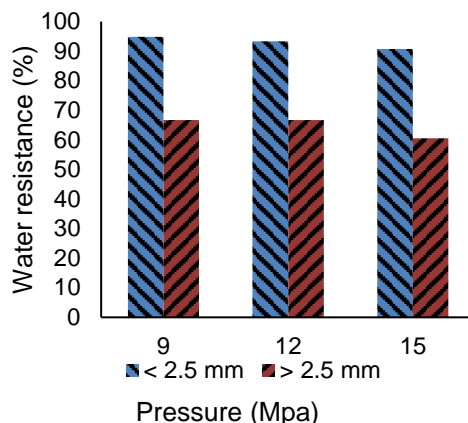


Figure 13. Water resistance of corn cob briquettes produced at different particle sizes with die temperature of 120 °C and hold time of 15 minutes.

Effect of particle size on water resistance of corn cob briquettes

The effect of particle size on the water resistance of corn cob briquettes is indicated in Figs 10–13. It is shown that for all combination of variables, the water resistance of briquettes produced with smaller particle sizes of corn cob (< 2.5 mm) was higher than those produced with larger sized particles (> 2.5 mm). This is likely due to the better bonding which occurs with smaller particle sizes which results from the increase in the contact points for inter-particle bonding. For briquettes produced with larger particle sized materials, there are wider inter-particle voids which limits the bonding of the particles, thereby reducing the water resistance of the briquettes. In addition, the surface of the briquettes produced using smaller particle sizes was smoother than those produced using the larger particle sizes. This indicates that the pores on the surface of the briquettes produced with larger particle sizes was larger and allowed easier penetration of water into the briquettes. Križan et al. (2018) also observed that there is a positive relationship between particle size and porosity. Davies & Davies (2013a) also observed that the water resistance capacity of briquettes progressively improved with decrease in particle sizes. Some of the briquettes produced using particle sizes greater than 2.5 mm are shown in Fig. 14, (a) while Fig. 14, (b) shows briquettes produced with particle sizes less than 2.5 mm.

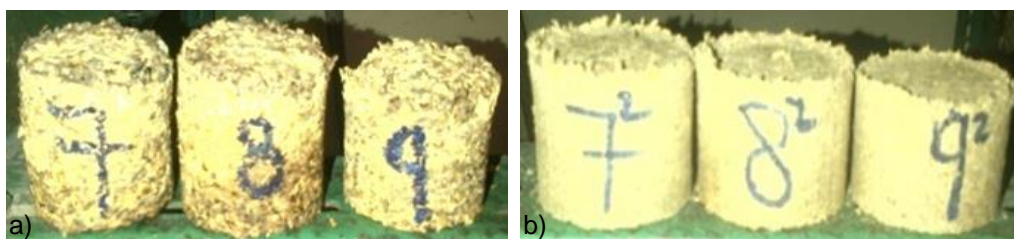


Figure 14. Briquettes produced with (a) particle sizes greater than 2.5 mm (b) particle sizes less than 2.5 mm.

Statistical analysis

The effects of the densification have also been investigated using statistical methods of analysing factorial experimental designs using the analysis of variance (ANOVA). The assumptions made in ANOVA include normality, constant variance and independence. Normality of the ANOVA implies that the population in each treatment from which the sample is drawn is normally distributed. Constant variance implies that the variance of observations in each treatment should be equal. Independence is the assumption requiring that the observations should be randomly selected from the treatment population. These assumptions need to be checked for the ANOVA before deductions are made from the analysis.

Fig. 15 shows the normality probability plot of residuals for water resistance of corn cob briquettes. The plot shows that the distribution of residuals is normal. Fig. 16 shows the plot of residuals versus fitted means. It is observed that the plot has a random pattern and shows no recognizable pattern which satisfies the constant variance assumption of ANOVA. The independence assumption is checked with the plot of residual versus observation order shown in Fig. 17. The plot shows that the assumption of independence is met since there is no defined pattern for the plot.

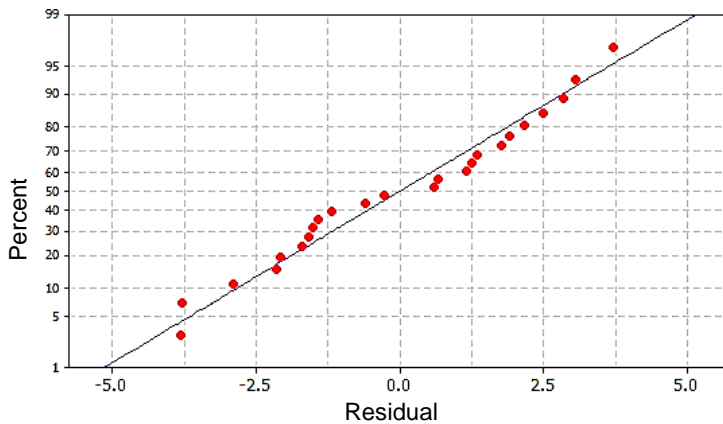


Figure 15. Normal probability plot of residual for water resistance of corn cob briquettes.

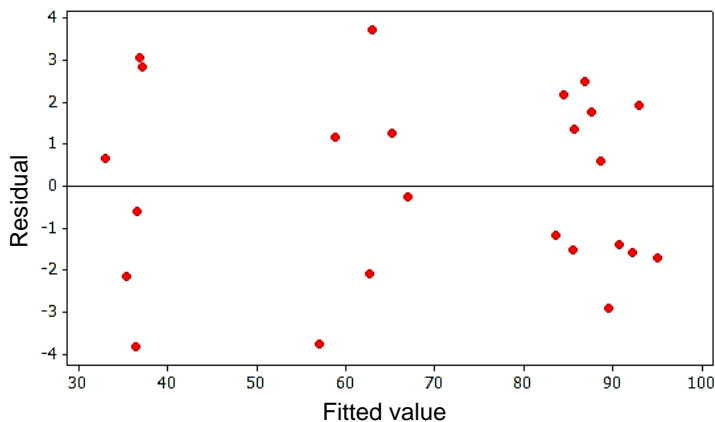


Figure 16. Plot of residual versus fits for water resistance of corn cob briquettes.

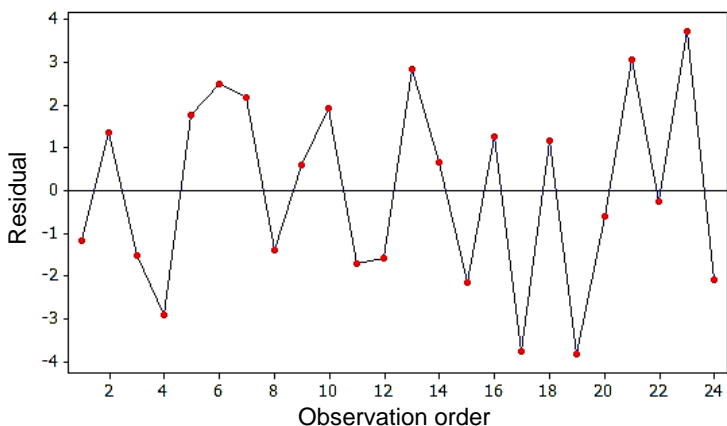


Figure 17. Plot of residuals versus fits for water resistance of corn cob briquettes.

Table 3 shows the ANOVA table for the water resistance of corn cob briquettes. It is shown from the tables that the temperature, hold time and particle size have P-values less than a significance level of 0.05. This implies that their effects on the water resistance of corn cob briquettes are statistically significant. It is also observed that the interaction of temperature with particle size also has a statistically significant effect on the water resistance of the corn cob briquettes.

Table 3. Analysis of variance (ANOVA) table for water resistance of corn cob briquettes

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	2	6.11	6.11	3.05	0.24	0.788
Temperature	1	1,403.62	1,403.62	1403.62	112.47	0.000**
Hold time	1	70.11	70.11	70.11	5.62	0.042**
Particle size	1	9,326.77	9,326.77	9,326.77	747.36	0.000**
Pressure *Temperature	2	26.39	26.39	13.19	1.06	0.387
Pressure *Hold time	2	17.25	17.25	8.62	0.69	0.526
Pressure *Particle size	2	38.00	38.00	19.00	1.52	0.269
Temperature *Hold time	1	8.35	8.35	8.35	0.67	0.434
Temperature *Particle size	1	729.74	729.74	729.74	58.48	0.000**
Hold time *Particle size	1	3.95	3.95	3.95	0.32	0.587
Error	9	112.32	112.32	12.48		
Total	23	11,742.62				

**Statistically significant.

The main effects plot for the water resistance is shown in Fig. 18. It shows that the temperature and hold time improve the water resistance of the corn cob briquettes when a higher value is used for each of them. However, a higher particle size will have a negative effect on the water resistance of the briquettes. The interaction plot for the water resistance is shown in Fig. 19 which shows that briquettes with the highest water resistance are produced with higher temperature and lower particle size.

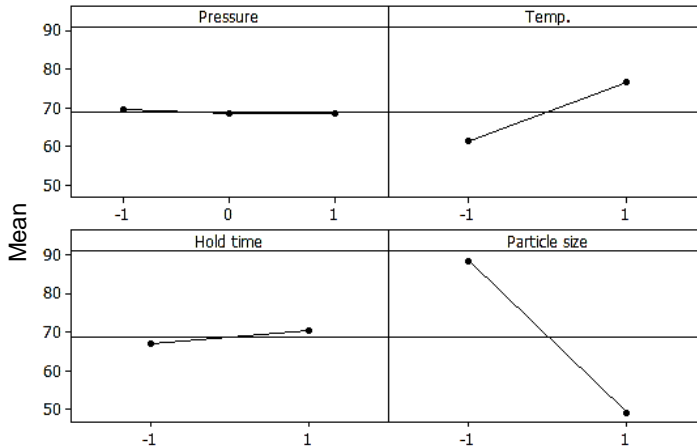


Figure 18. Main effects plot using data means for water resistance of corn cob briquettes.

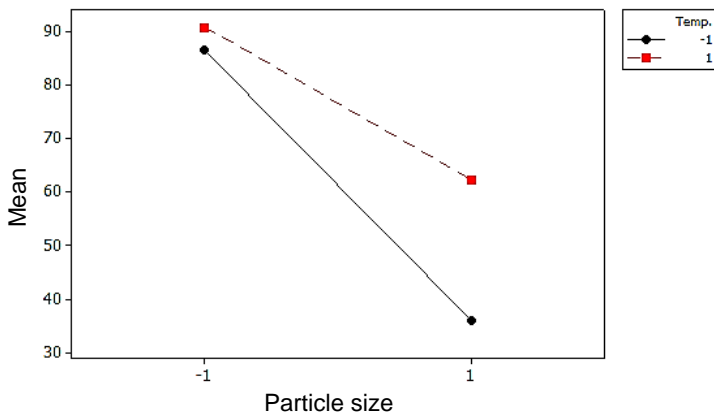


Figure 19. Interaction plot using data means for water resistance of corn cob briquettes.

CONCLUSIONS

In this study, the effect of pressure, temperature, hold time and particle size on the water resistance property of briquettes produced from corn cob was investigated. The water resistance of briquettes produced ranged between 32.6% and 94.8%. Graphical analysis showed that increasing the die temperature from 90 °C to 120 °C resulted in an increase in the water resistance of the biomass briquettes. Also, increasing the hold time from 7.5 minutes to 15 minutes increased the water resistance of the briquettes. Using a particle size less than 2.5 mm resulted in higher briquette water resistance property compared to briquettes produced from particle sizes greater than 2.5 mm. The pressure had no definite effect on the water resistance of the briquettes. Hence, water resistance of corn cob briquettes is largely determined by the temperature, hold time and particle size of the particles. Statistical analysis using ANOVA also showed that the effects of temperature, hold time and particle size on the water resistance of corn cob briquettes were statistically significant. It was also shown that the effect of the interaction of temperature with particle size on water resistance of the briquettes was statistically significant. It can

be concluded that high quality briquettes which have good water resistance can be produced from corn cobs. This is achieved by compacting under high temperature and with small particle sizes although more energy will be required to mill the materials to finer particles.

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Effects of salicylic acid and spermine foliar application on some morphological and physiological characteristics of isabgol (*Plantago ovata* Forsk) under water stress

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Abstract. Yield as well as concentration of relevant component in many medical plants are influenced by growing condition water stress. Field experiment was done based on a randomized complete block design with 18 treatments and three replications, at Gonbad Kavous University, Golestan, Iran in winter 2016. Irrigation treatment with three levels (normal irrigation, water stress imposed at flowering stage or at seed filling stage) was placed in main plot. Salicylic acid (SA) with three levels (control, 0.4 and 0.8 mM) and spermine (Spm) with two levels (control and 0.02 mM) were assigned in sub-plots. Result showed the foliar application SA and Spm treatments under normal irrigation and cutoff irrigation at seed filling stage have significantly affected relative water content, electrolyte leakage and membrane stability index, but under cutoff irrigation at flowering stage did not significantly affected measured traits. Exogenous applications of SA and Spm applied increased the plant height, 1,000 grain weight and biological yield in compared to the non-use product at normal irrigation regime. Foliar spraying SA and Spm under cutoff irrigation at flowering stage did not significantly affected measured traits. Application product especially SA_{0.8} mM and Spm_{0.02} mM under cutoff irrigation at seed filling stage increased the most characteristics in compared to control. Foliar application of phenolic compounds such as salicylic acid and polyamines like spermine can be considered as an effective measure to reduce the adverse effects of water stress and ultimately increase the yield and yield components of isabgol.

Key words: isabgol, membrane stability index, relative water content, phenolic compounds, water deficit.

INTRODUCTION

Climate change is a matter of concern in the twenty-first century that warns of rising temperature, unprecedented drought, flood, desertification, radiation, cyclones, forest fires, extreme low temperature that can adversely affect agriculture and human life (Reynolds & Ortiz, 2010). The responses of crops to these abiotic stresses have a number of similarities, although the genetic basis is not necessarily the same (Reynolds & Ortiz, 2010). Plants are challenged by a variety of biotic or/and abiotic stresses, which can affect their growth and development, productivity and geographic distribution (Liu et al., 2015). Abiotic stresses due to physical factors can occur in all stages of a plants lifespan (Venkateswarlu et al., 2012). Growth rate is accelerated due to increased

temperature, which reduces photosynthesis since the life cycle is shortened, while both heat and drought stress may also inhibit growth directly at the metabolic level. Furthermore, harvest index may be reduced if reproductive processes are impaired by stress that occurs at critical developmental stages (Reynolds & Ortiz, 2010). In order to survive adverse environmental conditions, plants have evolved various adaptive strategies, among which is the accumulation of secondary metabolites that play protective roles (Liu et al., 2015).

Increasing the crop production under irrigation has several limitations such as irregular rainfall distribution and non-availability of permanent irrigation. An alternative approach is to apply exogenous phyto-hormones (polyamines, salicylic acid and gibberellic acid), plant growth promoting rhizobacteria or other effective components application that can plant protection and maintain under limited moisture (Hara et al., 2012).

Salicylic acid (SA) is an important phytohormone that plays a role in response to biotic stresses. Apart from this role, recent studies have demonstrated that SA also participates in the signaling of abiotic stress responses, such as drought, high and low temperature, salinity, ozone, UV radiation, and heavy metals (Hara et al., 2012). In addition, abiotic stresses also induce endogenous SA accumulation. The appropriate application of SA could provide protection against several types of environmental stresses (Hara et al., 2012). According to several reports salicylic acid has a significant role in reducing injuries abiotic stresses in plants. The use of salicylic acid under stress condition decreased the lipid peroxidation in basil plant (Delavari et al., 2010). Bayat et al. (2012) reported that application of salicylic acid under salt stress conditions greatly reduced the amount of electrolyte leakage in calendula. In addition, salicylic acid plays a vital role in various physiological processes, e.g. growth, plant development, ion absorption, photosynthesis and germination (Shekofteh et al., 2015).

Polyamines (PA) are a group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure and present in almost all living organisms including plants. They are involved in many physiological processes, such as cell growth and development and respond to stress tolerance to various environmental factors (Gill & Tuteja, 2010). Recently several reports showed that application polyamines (putrescine (Put), spermidine (Spd) and spermine (Spm)) also improved tolerance of plants against several abiotic stresses including german chamomile (Sharafzadeh et al., 2012), wheat (Gupta et al., 2012), citrus (Shi et al., 2010), pistachio (Kamiab et al., 2013), clerodendrum (Mukherjee & Bandyopadhyay, 2014), jojoba (Lobna et al., 2015), cotton (Loka et al., 2015), valerian (Mustafavi et al., 2015), tomato (Nowruzi Givi et al., 2015) and sweet basil (Pazoki, 2017). Darvizheh et al. (2018a) stated that foliar application of SA (75, 150 mg L⁻¹) and Spm (70 mg L⁻¹) on purple coneflower, increased secondary metabolites and physiological characteristics in plant under drought stress condition.

Isabgol (*Plantago ovata* Forsk) is an annual herbaceous plant belonging to the Plantaginaceae group. The origin of this plant is the Mediterranean region, North Africa, Southwest Asia, including Iran (Omidbeygi, 2005). The seeds of *Plantago ovata* are commercially known as white or blonde psyllium, Indian plantago or isabgol. India dominates the world market in the production and export of psyllium (Bokaeian et al., 2014). Mucilages are generally polysaccharides, which are polymeric in nature and are obtained from seed, husk and leaves isabgol. Decomposition of isabgol seeds has shown

some sugars and polysaccharide compounds in grain mucilage, including galactose, glucose, xylose, arabinose, rhamnose and galacturonic acid. (Omidbeygi, 2005). Koochaki et al. (2007) reported that water stress had a negligible negative effect on most parameters evaluated except length of spike and seed yield of isabgol were affected negatively by increasing the length of irrigation intervals. In addition, they stated the highest yield was obtained from 10 and 20 day intervals irrigations. Rahimi et al. (2010) also found the significant decrease in leaf chlorophyll *plantago ovata* under drought stress conditions. The aim in the study was to determine first if these products have an ameliorative effects and then to determine the best dose.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the Gonbad Kavous University, located in Golestan province, Iran in 55°21'E, 37°26'N, 45 m above sea level with 450 mm mean 10 years precipitation. The precipitation, average temperature and relative humidity during the experiment were 119.1 mm, 61.7 °C and 300% respectively. The soil had a silt-loam texture. Chemical characteristics of the upper soil layer (30 cm) at the start experimental were: pH (7.92), electrical conductance (1.2 dS m⁻¹), bulk density (1.5 g cm⁻³), field slope (≤ 0.2), organic carbon (1.11%), total N (0.11%), available P (21.2 mg kg⁻¹) and K (504 mg kg⁻¹).

The experiment was arranged as a split plot factorial base on randomized complete block design with 18 treatments and three replications with the following treatments: three irrigation levels (control (non-stress), irrigation cutoff at flowering stage and irrigation cutoff at seed filling stage), three salicylic acid level (SA0= No salicylic acid (water spray), SA2= spraying 0.4 mM of salicylic acid, SA3= spraying 0.8 mM of salicylic acid) and two spermine levels (Spm1= not using spermine (water spray), Spm2= spraying 0.02 mM of spermine). Irrigation was used as main-plot, salicylic acid application and spermine spraying were as sub -plot. The unit plot size was 5 m×1 m; with 5 rows (row spacing and plant distance were 20 and 10 cm, respectively). Blocks distances were considered 3 meters and the distance of main plots and sub plots inside the blocks was 3 and 0.5 meter, respectively. Isabgol was hand sown at a 0.5–1 cm depth soil on March 2016. Isabgol seeds (98% viability and seed purity) were obtained from Sabz Rooyeshe Mahallat Company, Iran.

In this experiment nitrogen and phosphorus fertilizers were added respectively with a dose of 100 kg ha⁻¹ urea (46% N) and 150 kg ha⁻¹ triple super phosphate (19/8% P), based on soil test and fertilizer recommendations for isabgol.

Soil moisture content at field capacity and permanent wilting point were 0.9 and 0.7% (equivalent to a weigh moisture of 16.8 to 21.6), respectively (Walter & Gardener, 1986). The depth of irrigation was determined based on the average soil water content that calculated by following equation (Allen et al., 1998):

$$dw = \frac{(\theta_{m1} - \theta_{m2})}{100} \cdot \rho_b \cdot ds \quad (1)$$

In this equation; dw (cm): depth of irrigation; θ_{m1} : initial weight moisture (FC) (%); θ_{m2} : secondary weighs moisture (WP) (%); ρ_b : bulk density (g cm⁻³) and ds: depth of soil (cm).

Irrigation (Furrow irrigation system) was carried out in all plots until the complete plant establishment (four-leaf stage) as needed. Then, soil moisture content was maintained before the application of stress treatments in all experimental plots in the same range as mentioned above.

The exogenous salicylic acid (molecular weight 138.1, Sigma) and spermine (molecular weight 202.3, Sigma) were applied on the budding process (flowering stem production), flowering and seed filling in the required treatments.

Measurement of morphological and physiological characters

After biological maturity 10 plants were randomly sampled from each plot to measure plant height, spikes per plant, spike length, seed per spike, membrane stability, electrolyte leakage and relative water content. We harvested two square meters of three central rows were from each plot to determine the seed yield, biological yield, harvest index, 1,000 seed weight, seed mucilage percent and yield mucilage yield.

Harvest index (HI) is calculated using the formula (2):

$$HI = \text{Seed yield} / \text{Biological yield} \cdot 100 \quad (2)$$

Mucilage contents in the seed were determined according to Kalyanasundaram et al. (1984). Grain mucilage yield (GMY) was calculated by the following equation:

$$GMY = \text{Seed yield} \cdot \text{Content of grain mucilage} \quad (3)$$

Sairam et al. (1994) method was followed for analysis of membrane stability index (MSI).

$$MSI \text{ was calculated as } MSI = [1 - (EC1/EC2)] \cdot 100. \quad (4)$$

Electrolyte leakage (EL) percentage was calculated by the following equation proposed by Tas & Basar (2009).

$$EL = EC1/EC2 \cdot 100 \quad (5)$$

Leaf relative water content (RWC) was estimated gravimetrically according to the method of Andersen et al. (2004). In this method; leaf relative water content was calculated from the following equation.

$$RWC = \frac{fw - dw}{ftw - dw} \cdot 100 \quad (6)$$

In this equation; dw: drought weight, fw: fresh weight and ftw: fully turgid weight.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the SAS package (version 9.1). The LSD test was applied to test significance of treatment means at 0.05 and 0.01 levels of probability. The regression was estimated amount the parameters of the SPSS package (version 22.0).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed that the 2way interactive effects of irrigation and salicylic acid were statistically significant ($P \leq 0.01$ and $P \leq 0.05$). The 2way interactive irrigation and spermine had significant effect on measure treat except

for plant height, number of spike per plant, number of seed per spike, 1,000-seed weight, seed mucilage percent, membrane stability index and relative water content. Salicylic acid and spermine had significant effect on plant height, spike length, biological yield, seed yield and seed mucilage yield, but no had significant effect on number of spike per plant, number of seed per spike, 1,000-seed weight, harvest index, seed mucilage percent, membrane stability index, electrolyte leakage and relative water content) on other traits were significant ($P \leq 0.01$ and $P \leq 0.05$). Analysis of variance (ANOVA) revealed that the 3way interactive effects of irrigation and salicylic acid and spermine was statistically significant on all characteristics ($P \leq 0.01$ and $P \leq 0.05$) (Table 1).

Plant height

Comparison of means showed that the plant height increased on foliar application of SA_{0.8} mM and Spm_{0.02} mM under normal irrigation (with 4.42% in compared to control). The plant height was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage (Table 2). In this regard, Shekofteh et al. (2015) reported that maximum plant height in isabgol was obtained with irrigation based on 100% FC (field capacity) and use of 0.5 mM salicylic acid, whereas the minimum was achieved through irrigation based on 25% FC and non-use of this acid. Also foliar application of SA and Spm increased the plant height (except SA₀ mM and Spm_{0.02} mM treatment) under cutoff irrigation condition at seed filling stage, although they did not significant different with each other (Table 2). Reducing the plant height in response to drought stress can be due to the blockage of xylem and phloem vessels, after which the transfer of materials and assimilate from the plant is reduced (Khalil et al., 2010). In a study to investigate the use of putrescine and spermidine on vegetative traits of basil herb under drought stress conditions, the application of 0.8 mM putrescine and 0.8 mM spermidine had the highest plant height (Pazoki, 2017). Darvizheh et al. (2018b) evaluated the effects of SA and Spm application at different concentrations on purple coneflower and reported that the growth indices increased in treated plants with SA150 + Spm 70 mg L⁻¹.

Spikes number per plant

Comparison of mean of treatments showed that highest spikes number per plant observed in the normal irrigation and non-use of SA and Spm treatment (18.60 ± 0.60). Exogenous application SA_{0.8} mM and Spm_{0.02} mM under cutoff irrigation at flowering stage increased of spikes number per plant (16.53 ± 1.50) in compared other treatments. Also, under cutoff irrigation condition at seed filling stage, SA_{0.8} mM treatment had a better effect on this trait than other treatments and the highest of spikes number per plant obtained at 17.73 ± 0.90 (Table 2). Mousavinick (2012) reported that the number of spikes per plant was reduced under stress due to reduced irrigation frequencies. In a study conducted on isabgol the highest spikes number stemmed from irrigation based on 100% FC and application of 1 mM salicylic acid and the lowest from 25% FC and without using salicylic acid or at lower concentrations (0.01 and 0.5 mM) (Shekofteh et al., 2015).

Table 1. Variance Analysis effect of cutoff irrigation, salicylic acid and spermine foliar application on some morphological and physiological traits of isabgol

Source of variation	Rep.	Irrigation (IR)	Error (Ea)	Salicylic acid (SA)	Spermine (Spm)	IR×SA	IR×Spm	SA×Spm	IR×SA×Spm	Error (Ebc)	C.V (%)
df	2	2	4	2	1	4	2	2	4	30	-
Plant height	Ns	**	2.42	**	Ns	*	Ns	*	*	1.013	3.54
Number of spike per plant	Ns	**	1.37	**	Ns	**	Ns	Ns	**	0.67	5.41
Number of seed per spike	Ns	**	5.08	*	*	**	Ns	Ns	**	15.67	5.46
Spike length	*	**	0.03	**	**	**	**	**	**	0.01	2.49
1,000-seed weight	*	**	0.001	Ns	Ns	**	Ns	Ns	**	0.001	2.13
Biological yield	Ns	**	26,594	*	Ns	*	*	**	**	33,289	7.75
Seed yield	*	**	964	**	**	**	**	**	**	855.5	7.26
Harvest index	Ns	**	3.16	**	**	**	**	Ns	**	3.1	10.18
Seed mucilage percent	Ns	**	0.10	**	Ns	**	Ns	Ns	**	1.37	6.90
Seed mucilage yield	Ns	**	25.63	**	**	**	**	**	**	41.44	9.39
Membrane stability index	Ns	**	0.56	**	Ns	**	Ns	Ns	*	0.65	0.86
Electrolyte leakage	Ns	**	0.49	**	Ns	**	Ns	Ns	*	0.65	12.65
Relative water content	Ns	**	13.51	**	Ns	**	Ns	Ns	**	9.91	4.38

Ns, * and ** are Non-Significance and Significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2. Comparison Mean effect of cutoff irrigation × salicylic acid × spermine foliar application on some morphological characteristic of isabgol

Irrigation regime	Salicylic acid	Spermine	Plant height (cm)	Number of spike plant ⁻¹	Number of seed spike ⁻¹	Spike length (cm)	1,000 seed weight (gr)	Biological yield (kg.ha ⁻¹)	Seed yield (kg.ha ⁻¹)	Harvest index (%)
Normal	control	control	29.20 ± 0.40bcd	18.60 ± 0.60a	85 ± 4.36a	3.40 ± 0.02e-h	1.66 ± 0.04c-f	1,820 ± 50g	513.50 ± 10ab	28.21 ± 1.2a
		0.02 Mm	28.37 ± 1.16cde	15.73 ± 0.11c-f	76.67 ± 4.04bc	3.53 ± 0.40cde	1.61 ± 0.05fg	2,255 ± 82ef	321.83 ± 39e	14.30 ± 2.01fgh
	0.4 mM	control	28.25 ± 1.85cde	12.60 ± 0.40i	55.33 ± 4.93g	3.43 ± 0.11d-g	1.67 ± 0.01b-e	2,315 ± 180c-f	360.67 ± 19de	15.63 ± 1.15e-h
		0.02 Mm	28.10 ± 2.10cde	14.93 ± 0.61d-g	72.00 ± 7.00cde	3.45 ± 0.02d-g	1.68 ± 0.03bcd	2,151.7 ± 245f	343.83 ± 56e	15.96 ± 1.42efg
	0.8 mM	control	29.20 ± 1.81bcd	13.60 ± 0.72ghi	66.67 ± 6.66ef	3.54 ± 0.19cde	1.71 ± 0.03bc	2,613.3 ± 73abc	332.50 ± 9e	12.73 ± 0.60hi
		0.02 Mm	30.55 ± 0.25ab	13.27 ± 50hi	63.67 ± 3.21f	3.00 ± 0.08k	1.66 ± 0.04c-f	1,601.7 ± 45g	214.33 ± 6f	13.39 ± 0.24gh
Irrigation cutoff at flowering stage	control	control	25.70 ± 1.75f	13.07 ± 0.83hi	62.67 ± 2.08f	3.16j ± 0.09jk	1.65 ± 0.03def	2,451.7 ± 148b-e	367.83 ± 21de	15.05 ± 1.50fgh
		0.02 Mm	26.04 ± 0.55f	14.13 ± 0.58gh	67.67 ± 2.08def	3.25 ± 0.07hij	1.58 ± 0.04g	2,398.3 ± 253b-f	433.50 ± 51c	18.17 ± 2.42cde
	0.4 mM	control	25.67 ± 0.83f	14.47 ± 1.30fgh	68.33 ± 4.73def	3.18 ± 0.16ij	1.64 ± 0.02d-g	2,386.7 ± 5.77b-f	319.50 ± 8e	13.39 ± 0.37gh
		0.02 Mm	27.42 ± 1.12def	13.82 ± 0.42ghi	67.67 ± 4.04def	3.35 ± 0.17fgh	1.66 ± 0.04c-f	2,513.3 ± 291a-e	342.83 ± 54e	13.75 ± 2.64gh
	0.8 mM	control	27.02 ± 0.89ef	16.23 ± 0.93cd	68.33 ± 1.53def	3.50 ± 0.12c-f	1.62 ± 0.03efg	2,453.3 ± 281b-e	405.67 ± 16cd	16.72 ± 2.46def
		0.02 Mm	26.70 ± 1.13ef	16.53 ± 1.50bc	71.67 ± 3.21cde	3.33 ± 0.09ghi	1.65 ± 0.03def	2,578.3 ± 103a-d	256 ± 35f	9.90 ± 0.97i
Irrigation cutoff at seed filling stage	control	control	29.72 ± 0.85abc	15.00 ± 1.11d-g	73.67 ± 1.15cd	3.58 ± 0.07cd	1.68 ± 0.07bcd	2,311.7 ± 173def	497.33 ± 48ab	21.58 ± 2.43b
		0.02 Mm	26.87 ± 0.64ef	14.87 ± 0.94d-g	80.33 ± 2.08ab	3.24 ± 0.06hij	1.79 ± 0.01a	2,285 ± 121def	493 ± 31ab	21.64 ± 2.09b
	0.4 mM	control	29.82 ± 0.02abc	16.00 ± 1.00cde	80.00 ± 2.00ab	3.63 ± 0.12bc	1.73 ± 0.04b	2,226.7 ± 178ef	485.83 ± 45b	21.94 ± 2.94b
		0.02 Mm	31.37 ± 0.70a	14.73 ± 1.17efg	80.67 ± 1.53ab	3.74 ± 0.01ab	1.67 ± 0.06b-e	2,578.3 ± 211a-d	494.33 ± 22ab	19.30 ± 2.35bcd
	0.8 mM	control	31.13 ± 0.40a	17.73 ± 0.90ab	82.00 ± 1.00ab	3.91 ± 0.06a	1.72 ± 0.04b	2,795 ± 263a	540.83 ± 3a	19.47 ± 1.97bcd
		0.02 Mm	29.90 ± 0.87abc	16.20 ± 1.40cd	81.67 ± 3.21ab	3.53 ± 0.13cde	1.63 ± 0.03d-g	2,631.7 ± 25ab	528 ± 5ab	20.07 ± 0.32bc

Means within each column followed by the same letter are not statistically different $\alpha = 0.05$ by LSD test.

Number of seed per spike

The comparison of mean of treatments showed that highest number of seed per spike observed in the normal irrigation and non-use of SA and Spm treatment (18.60 ± 0.60) (Table 2). Hayat et al. (2010) reported that at higher concentrations, SA itself may cause a high level of stress in plants. The number of seed per spike was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Also foliar application of SA and Spm increased the number of seed per spike (except non-use of SA and Spm treatment) under cutoff irrigation condition at seed filling stage, although they were not significantly different from each other (Table 2). Ramroudi et al. (2011) reported that the number of spikes per plant and the number of seeds per spike on isabgol decreased in the irrigation delay at flowering stage. It is inferred that salicylic acid and spermine spraying in different concentrations were able to compensate part of the negative effects of moderate drought stress on yield and yield components.

Spikes length

Based on the comparison of means, the spikes length (except SA_{0.8} mM and Spm_{0.02} mM treatment) increased under normal irrigation in compared to control (Table 2). We can infer that, the reduction of the spike length under SA_{0.8} mM and Spm₀ mM treatment is probably due to the high concentrations of phenolic acid salicylic compound. The spikes length (except SA_{0.8} mM and Spm₀ mM treatment with 3.50 ± 0.12) was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Also the spikes length (except SA₀ mM and Spm_{0.02} mM treatment) increased by spray treatments under cutoff irrigation condition at seed filling stage. The longest spike length observed in SA_{0.8}+Spm₀ mM and SA₀+Spm_{0.02} mM treatments (with 3.74 ± 0.01 and 3.91 ± 0.06 , respectively) (Table 2). The reduction of spike length under stress conditions can be as a result of reduced photosynthesis and subsequently reduced production and transfer of assimilates for plant growth (Pirasteh-Anosheh et al., 2012). It is inferred that the use of salicylic acid and spermine, as a result of increased water use efficiency (Kumar et al., 2000; Fariduddin et al., 2003; Farooq et al., 2009) and reduction of oxidative stress (Mittler, 2002; Todorova et al., 2015), could prevent reduced spike lengths under interrupting irrigation condition.

1,000 seed weight

Comparison of mean of treatments showed that Spm_{0.02} spraying treatment under cutoff irrigation condition at seed filling and flowering stage had the highest and lowest seed weight with respectively 1.79 ± 0.01 and 1.58 ± 0.04 grams (Table 2). Mousavinick (2012) stated that under drought stress conditions, due to the reduction of photosynthesis, the amount of dry matter production and accumulation, thereby reducing the amount of dry matter transferred to the isabgol seeds and the weight of the seeds decreased.

Biological yield

The biological yield in some treatments (except SA_{0.8} mM and Spm_{0.02} mM treatment) increased in compared to control under normal irrigation and spraying salicylic acid and spermine (Table 2). The biological yield was not significantly affected by foliar application of SA and Spm under cutoff irrigation condition at flowering stage. Increasing the concentrations of salicylic acid and spermine under cutoff irrigation at

seed filling stage significantly increased the biological yield and the heaviest biological yield was related to SA_{0.8}+Spm₀ treatment with 2,795 ± 263 kg ha⁻¹ respectively. However, this treatment had no significant difference with some treatments (Table 2). Rahimi et al. (2011) showed that water stress (irrigation terminated at the start of flowering) increased the biological yield of isabgol and psyllium. These compounds reduce the adverse effects of drought stress preventing the reduction of plant height and leaf loss as a result of and strongly affects on photosynthetic apparatus and photochemical enhancement of light energy is absorbed.

Seed yield

The highest seed yield observed in cutoff irrigation at seed filling stage and SA_{0.8}+Spm₀ treatment with 540.83 ± 3 kg ha⁻¹. However, no significant difference observed between irrigation cuttings at seed filling stage at different levels of salicylic acid and spermine spraying. The lowest seed yield was allocated to foliar application SA_{0.8}+Spm_{0.02} at normal irrigation and cutoff irrigation at flowering stage treatments (214.33 ± 6 and 256 ± 35 kg ha⁻¹, respectively) (Table 2). We can infer that salicylic acid and spermine spraying could increase the grain yield of isabgol by stimulating the physiological processes that cause active transfer of photosynthetic products from source to sink. Rezaichianah & Pirzad (2014) reported a 13% increase in black cumin grain yield under drought stress condition with 0.5 mM salicylic acid application.

Harvest index

Comparison of mean between treatments showed that normal irrigation and non-use of SA and Spm treatment had the highest harvest index (28.21 ± 1.2). In this regard, no significant difference was obtained between irrigation cuttings in the seed filling stage at different levels of salicylic acid and spermine spraying. Under intense stress conditions, the seed yield decreased by 35% in compared to the control (Table 2). The results are in agreement with the observations of Ramroudi et al. (2011) on isabgol. The results showed that both grain yield and biological yield were reduced under the integrated salicylic acid and spermine treatments at cutoff irrigation condition at flowering stage, which led to a reduction in the harvest index. In contrast, the treatments under irrigation condition at seed filling stage, despite the high level of grain yield; the harvest index was reduced due to the high biological yield function.

Seed mucilage percentage

Exogenous application of SA and Spm increased the seed mucilage percentage (except SA_{0.8} and Spm_{0.02} mM treatment) under normal irrigation, although they were not significantly different with each other treatments. The highest amount of seed mucilage percent observed in SA_{0.8}+Spm_{0.02} mM with 19.67 ± 1.53% (Table 3). In some treatments under moderate and intense stress, spraying salicylic acid and spermine increased the seed mucilage percent, but had no effect on other treatments. The lowest amount of seed mucilage percent was obtained from SA_{0.8}+Spm_{0.02} mM with 13 ± 1% (Table 3). The results are in agreement with the observations of Koocheki et al. (2011) on isabgol and psyllium. They reported that the maximum amount of mucilage and swelling factor were obtained for both species were obtained at irrigation level of 2,000 m³ ha⁻¹.

Table 3. Comparison Mean effect of cutoff irrigation × salicylic acid × spermine foliar application on some physiological characteristics of isabgol

Irrigation regime	Salicylic acid	Spermine	Seed mucilage (%)	Seed mucilage yield (kg. ha ⁻¹)	Membrane stability index (%)	Electrolyte leakage (%)	Relative water content (%)
Normal	control	control	18 ± 1.50ab	92 ± 5.20ab	92.97 ± 0.89fg	7.03 ± 0.89bc	77.95 ± 1.64bc
		0.02 Mm	18.67 ± 1.53ab	60 ± 8.18g	92.08 ± 0.15gh	7.92 ± 0.15ab	75.50 ± 1.28cd
	0.4 mM	control	18 ± 1.00ab	65 ± 3.46fg	93.85 ± 0.67def	6.15 ± 0.67cde	71.13 ± 3.01de
		0.02 Mm	19.67 ± 1.53a	67.33 ± 5.86efg	94.82 ± 0.58cd	5.18 ± 0.58ef	75.37 ± 5.01cd
	0.8 mM	control	18 ± 1.00ab	60 ± 3.46g	96.57 ± 1.17ab	3.43 ± 1.17gh	87.69 ± 3.01a
		0.02 Mm	16 ± 1.50cd	34.33 ± 0.58i	95.40 ± 0.87bc	4.60 ± 0.87fg	81.37 ± 1.46b
Irrigation cutoff at flowering stage	control	control	18 ± 1.00ab	66.33 ± 3.51fg	92.04 ± 0.52gh	7.96 ± 0.52ab	69.56 ± 4.00e
		0.02 Mm	15 ± 1.00d	65.33 ± 10.97fg	91.44 ± 0.604h	8.56 ± 0.60a	63.26 ± 2.33f
	0.4 mM	control	14.33 ± 1.15de	45.33 ± 4.04h	91.42 ± 0.85h	8.58 ± 0.85a	59.82 ± 2.52f
		0.02 Mm	18 ± 2.00ab	61.67 ± 11.59g	91.44 ± 0.94h	8.56 ± 0.94a	59.66 ± 3.87f
	0.8 mM	control	19 ± 1.00ab	77.33 ± 6.66cde	91.89 ± 0.64gh	8.11 ± 0.64ab	61.50 ± 2.34f
		0.02 Mm	17.67 ± 0.58bc	45 ± 5.57h	92.78 ± 0.72fg	7.22 ± 0.72bc	69.39 ± 4.63e
Irrigation cutoff at seed filling stage	control	control	16 ± 2.00cd	78.67 ± 4.73cd	92.62 ± 1.11fgh	7.38 ± 1.11abc	69.11 ± 5.32e
		0.02 Mm	17.67 ± 0.58bc	86.33 ± 8.08bc	93.51 ± 0.50def	6.45 ± 0.50cde	72.03 ± 3.24de
	0.4 mM	control	15 ± 1.00d	73 ± 7.00def	94.71 ± 0.72cde	5.29 ± 0.72def	70.31 ± 2.77de
		0.02 Mm	13 ± 1.00e	64.33 ± 7.50fg	93.46 ± 1.11ef	6.54 ± 1.11cd	72.13 ± 2.56de
	0.8 mM	control	17.67 ± 1.53bc	94.67 ± 8.50ab	97.63 ± 1.31a	2.37 ± 1.31h	80.06 ± 3.42bc
		0.02 Mm	18.67 ± 0.58ab	97.67 ± 3.51a	96.56 ± 0.75ab	3.44 ± 0.75gh	77.52 ± 3.33bc

Means within each column followed by the same letter are not statistically different $\alpha = 0.05$ by LSD test.

Seed mucilage yield

The highest seed mucilage yield observed in the normal irrigation and non-use of SA and Spm treatment ($92 \pm 5.20 \text{ kg ha}^{-1}$). The seed mucilage yield (except SA_{0.8} mM and Spm₀ mM treatment with $77.33 \pm 6.66 \text{ kg ha}^{-1}$) was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Also the seed mucilage yield increased at foliar application of SA_{0.8} mM and with/without Spm under cutoff irrigation condition at seed filling stage (with 94.67 ± 8.50 and $97.67 \pm 3.51 \text{ kg ha}^{-1}$, respectively) in compared to control (Table 3). In the research conducted by Ramroudi et al. (2011), the highest yield of mucilage of isabgol was related to irrigation regime, and the least was obtained from discontinuation of irrigation after flowering. Shekofteh et al. (2015) reported that with an application of 1 mM salicylic acid and without it, the highest and lowest percentages of mucilage of isabgol were 41.16 and 41.03, respectively.

Membrane stability index (MSI)

The interactions between irrigation levels and spray treatments showed that SA_{0.08} and Spm_{0.02} application increased membrane cell stability in plants under normal irrigation and cutoff irrigation at seed filling stage. The highest amount of MSI demonstrated in SA_{0.8}+Spm₀ mM with $97.63 \pm 1.31\%$. Also there was not significantly different in MSI between SA and Spm spraying under cutoff irrigation condition at flowering stage (Table 3). In a study Naghashzadeh (2014); MSI as affected by different irrigation regimes was decreased by increasing drought stress. Researcher reported that well-watered had the highest MSI of all irrigation regimes and severe drought stress was 28% lower than well-watered conditions. A similar result was reported that exogenous salicylic acid and spermine was effective in enhancing the cell membrane stability under drought stress. Bandurska & Stroinski (2005) reported that plant treatment with SA before drought stress reduced a damaging action of water deficit on cell membrane in leaves. The increase of cell membrane stability with 300 ppm salicylic acid under drought stress conditions was reported by Sibi et al. (2012). Application of spermine and putrescine increased drought tolerance through reducing the electrolyte leakage, increasing compatibility osmolytes and antioxidant enzyme activity (Amraee et al., 2016).

Relative water content (RWC)

The highest relative water content was related to SA_{0.8}+Spm₀ treatment with 38.97 ± 1.33 percent under normal irrigation. The RWC in leaves was not significantly affected by spray treatments under cutoff irrigation condition at flowering stage. Foliar application of SA and Spm increased the relative water content in leaves in compared to control under cutoff irrigation condition at seed filling stage (Table 3). Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance (Barahuyi Nikju, 2017). A similar result Naghashzadeh (2014) reported that there was not significantly different in RWC between well-watered (70% field capacity) and moderate drought stress (50% field capacity). Severe drought stress (30% field capacity) was 26% lower than well-watered conditions. When osmotic stress occurs, the solution metabolites on cells increase to prevent water deficit and turgor pressure reduction, which include nitrogen ingredients, such as proline and other amino acids, poly amines and ammonium (Tamura et al., 2003; Naghashzadeh, 2014). In a study, Kumar et al. (2000) reported an increase in water use efficiency, transpiration rate and internal CO₂ concentration in

response to foliar application of salicylic acid. Also Hayat et al. (2008) reported that the treatment of under stressed plants with lower concentrations of salicylic acid significantly enhanced the photosynthetic parameters, membrane stability index, leaf water potential and relative water contents thereby improved tolerance of the plants to drought stress. In fact, foliar spraying of salicylic acid moderated the damaging effects of water deficit on cell membranes plant through increased the ABA content in leaves, which might have contributed to the enhanced tolerance of plants to drought stress (Bandurska & Stroinski, 2005).

Electrolyte leakage (EL)

Comparison of means between treatments showed that the Electrolyte leakage percentage (except SA₀mM and Spm_{0.02} mM treatment) under normal irrigation and spraying salicylic acid and spermine decreased in compared to control (Table 3). Also the Electrolyte leakage percentage was not significantly affected by foliar application of SA and Spm under cutoff irrigation condition at flowering stage (Table 3). The highest amount of EL observed in this stage however, had no significant difference with some treatments. Exogenous application of salicylic acid and spermine decreased the Electrolyte leakage percentage in leaves in compared to control under cutoff irrigation condition at seed filling stage. The lowest amount of EL obtained in SA_{0.8}+Spm₀ mM with 2.37 ± 1.31% (Table 3).

Masoumi et al. (2010) stated drought stress causes a significant decrease RWC in the *Kochia Scoparia* leaves and increase electrolyte leakage compare with control.

Regression

Based on the results of stepwise regression analysis, the number of spikes per plant was the first trait that entered the model and justified 0.36 of grain yield changes. In the resulting model, the number of seed per spike (x1) and spike length (x2) remained at the end. The above-mentioned traits explained 0.44% of the total variation (Table 4), therefore, it can be stated that these traits are the most important traits affecting the yield of isabgol (*Plantago ovata* Forssk.) in the studied treatments and possibly selecting to increase grain yield through these traits, will be effective; therefore, the final model of yield with the dependent traits was obtained as follows:

$$\text{Yield} = -457.23 + 5.22 (x1) + 140.43(x2) \tag{7}$$

Table 4. Stepwise regression analysis for grain yield per unit area as the dependent variable and the other independent variables

Variables added to the model	Constants	Regression coefficients		Coefficient of Determination (R ²)
		b1	b2	
Number of seed per spike	-106.98ns	7.038**		0.357
Spike length	-457.23**	5.22**	140.43**	0.439

CONCLUSION

We can conclude, the application of salicylic acid and spermine moderate the drought stress and it lead to increase plant height, number of seed per spike, biological yield, seed yield, seed mucilage percent, membrane stability index, electrolyte leakage

and relative water content. The best dose for salicylic acid and spermine was it 0.08 mM and 0.02 mM, respectively. They were more effect when we applied in cutoff irrigation at seed filling stage condition.

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Development of an efficient regeneration system for bombarded calli from immature embryos of Moroccan durum wheat varieties

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Abstract. One of the biggest obstacles limiting genetic transformation of durum wheat is the lack of an efficient regeneration system for bombarded tissues. Our study aims to optimize culture conditions for regenerating bombarded calli from immature embryos of four durum wheat varieties ‘Amria’, ‘Chaoui’, ‘Isly’ and ‘Marouane’, through comparing the effects of phytohormones (IAA, zeatin and their interaction) and nitrogen amount and sources on callus induction and plant regeneration. Both tested induction media induced approximately the same rate of induced calli for all the tested varieties. However, the interaction of the induction and the regeneration media showed a highly significant effect on plantlet regeneration for all tested varieties. After bombardment, IM1/RM2 combination proved to be the favourable medium with up to 200% and 120% plantlets regenerated for ‘Chaoui’ and ‘Isly’ varieties respectively. Encouraging results obtained in this study will help to promote the research in genetic transformation and its improvement.

Key words: bombarded calli, callus induction, durum wheat, immature embryos, plantlets regeneration.

INTRODUCTION

Durum wheat (*Triticum turgidum* subsp. *durum*), also called pasta or macaroni wheat, is a tetraploid species. It is the second most cultivated species of wheat after bread wheat, although it only represents around 5% of global wheat production (Arzani & Ashraf, 2017). It is grown mainly in countries in the Mediterranean basin, North America and Australia (Ranieri, 2015). It is a major staple food crop in North Africa and West Asia, and contributes to food and nutrition security of these countries. Commercially produced dry pasta, or pasta secca, is made almost exclusively from durum semolina. The coarse semolina is used to produce couscous in Morocco and other North African countries. Several recent reports have revealed the negative impact of climate change on

durum wheat production, especially for African regions which already have low productivity levels (Ouraich & Tyner, 2014). Natural variability for adaptation to climate change and various biotic and abiotic stresses are limited. Genetic transformation is an alternative to conventional breeding for the development of stress tolerant cultivars of durum wheat (Semenov et al., 2014).

Genetic transformation of durum wheat has been challenging due to its recalcitrant nature for *in vitro* regeneration (Moghaieb et al., 2010; Bouiamrine et al., 2012) and its large genome size (approximately 17,000 Mb). The biolistic method has proven to be an effective method of genetic modification for many crop species such as wheat, but it is essential to target cells that are competent for both transformation and regeneration. Yamashita et al. (1991) and Hunold et al. (1994) independently showed that more than 90% of bombarded cells integrate DNA in their nucleus and express the *gus* gene, but the limiting step is cell division and plant regeneration.

Based on many previous reports, there is no unique method to achieve efficient culture conditions after bombardment. The induction of direct shoot regeneration depends on several factors, e. g., genotype (Ozgen et al., 1996; Vendruscolo et al., 2008), type and concentration of auxin (Mendoza & Kaeppler, 2002; Filippov et al., 2006), medium components (Redway et al., 1990; Mendoza & Kaeppler, 2002; Greer et al., 2009), and the nature and the stage of the plant organ from which the explant was derived (Ozias-Akins & Vasil, 1982; Redway et al., 1990; Hess & Carman, 1998). Most transformation protocols are developed for single genotypes and can't be easily extrapolated to other genotypes with different abilities to form embryogenic callus and regenerate plants. Cultivar plays the greatest role in the competency for somatic embryogenesis, outweighing other known factors such as explant source (Li et al., 2003), donor plant conditions (Maës et al., 1996) and even medium composition (Mathias & Simpson, 1986). Although cultivar properties are fixed, other factors can be altered to improve somatic embryogenesis, especially constituents of the media used throughout the process.

Several studies have shown that modification of ammonium nitrate concentration (NH_4NO_3) is important for induction of somatic embryogenesis in wheat (Greer et al., 2009), rice (Grimes & Hodges, 1990), barley (Mordhorst & Lörz, 1993) and other species (Choi et al., 1998; Kothari et al., 2004). Greer et al. (2009) showed that increasing the nitrogen content by 3- and 6-fold and modifying the nitrogen sources resulted in a two-fold increase in the regeneration of primary embryos and a seven-fold increase in the number of regenerated transgenic wheat plants for the cultivar 'Superb'.

Plant growth regulators are also important factors influencing *in vitro* plant regeneration from embryos (Brown et al., 1989). Auxins and cytokinins are the most common and important plant growth regulators for regulating growth and morphogenesis in plant tissue and organ cultures (George et al., 2008). Concentrations of auxins and cytokinins and their combinations play a major role in promoting regeneration in wheat (Malik et al., 2004). The purpose of this study was to optimize culture conditions for regenerating bombarded calli from immature embryos of four Moroccan durum wheat cultivars, through comparing the effects of plant growth regulators and nitrogen source and concentration on callus induction and plant regeneration.

MATERIAL AND METHODS

Plant materials

Seeds of Moroccan durum wheat varieties ‘Amria’, ‘Chaoui’, ‘Isly’, and ‘Marouane’ were supplied by INRA Experimental Station, Marchouch, Morocco. Immature embryo explants were collected 12–16 days post-anthesis, from greenhouse grown plants.

Seed Sterilization and Embryo Culture

Immature seeds were surface-sterilized by washing in 70% ethanol (v/v) for 3 min, followed by 2.4% sodium hypochlorite plus a drop of Tween 20 for 10 min with agitation. Thereafter, they were rinsed three times in sterile distilled water. Immature embryos were aseptically dissected away from the caryopses and the remaining endosperm and radical removed to prevent early germination. The embryos were then placed on two induction media: IM1 (Murashige & Skoog, 1962) with 20.6 mM NH₄NO₃ and 18.8 mM KNO₃; and IM2, a modified MS containing 62.5 mM NH₄NO₃ as the sole nitrogen source (and double total amount of nitrogen as compared to IM1). Both media were supplemented with 20 g L⁻¹ sucrose, 2 mg L⁻¹ picloram, 100 mg L⁻¹ myo-inositol, 150 mg L⁻¹ L-asparagine and 2.5 g L⁻¹ Phytigel™. pH was adjusted to 5.8. The immature embryos were cultured for 4 to 5 days in the dark at 25 °C. Following bombardment, embryos were incubated on the same induction medium for 40 days. The studied induction parameters for each genotype were:

$$PCIBB = \frac{NIC}{TNEC} \times 100 \quad (1)$$

$$PSCAB = \frac{NICAB}{TNBC} \times 100 \quad (2)$$

where PCIBB – percentage of callus induction before bombardment; NIC – number of induced calli; TNEC – total number of explants cultured; PSCAB – percentage of survived calli after bombardment; NICAB – number of induced calli after bombardment; TNBC – total number of bombarded calli.

Rooting and elongation of in vitro regenerated shoots

After 40 days, embryogenic calli from immature embryos were transferred to two different regeneration media: RM1 as described by Iraqi et al. (2005) composed of MS medium supplemented with 100 mg L⁻¹ myo-inositol, 2 mg L⁻¹ IAA (indole-3-acetic acid) and 30 g L⁻¹ sucrose; and RM2, with the same components of RM1 except 1 mg L⁻¹ zeatin was used instead of IAA; and RM3, with 0.5 mg L⁻¹ zeatin and 0.1 mg L⁻¹ IAA. Calli were incubated in 16/8 h light/dark cycle at 25 °C. The media were solidified using 3 g L⁻¹ Phytigel™. pH was adjusted to 5.7 before sterilization at 120 °C for 20 min. IAA, zeatin and MS vitamins were filter-sterilized and added to the medium after autoclaving. The studied regeneration parameters, calculated eight weeks after transfer of callus to regeneration medium, were:

$$PCR = \frac{NCRP}{NCTR} \times 100 \quad (3)$$

$$PPR = \frac{NPR}{NCTR} \times 100 \quad (4)$$

$$\text{NPPRC} = \frac{\text{NRP}}{\text{NCRP}} \times 100 \quad (5)$$

where PCR – percentage of callus regeneration; NCRP – number of calli with regenerated plantlets; NCTR – number of calli transferred to regeneration; PPR – percentage of plantlets regeneration; NPPRC – number of plantlets per regenerating callus; NPR – number of plantlets regenerated; NPPRC – number of plantlets per regenerating callus.

Experimental design and statistical analysis

The treatments consisted of 10 replications of each medium for each variety; each replication contained 20 explants (immature embryos). In addition, 3 replications of non-bombarded calli for each medium and each variety were done. Analysis of variance (ANOVA) was performed using the *General Linear Model (GLM)* procedure in SAS (SAS Institute 1985). Means of treatments were compared using the *Least Significant Difference (LSD)* test. *Student's t-test* was applied at a probability level of $p = 0.05$ to find significant differences between the means.

RESULTS

Callus induction

Two induction media (IM1 and IM2) were tested. Callus was induced 3 to 5 days after plating of the immature embryos.

Prior to bombardment, no significant differences were observed between varieties, induction media, or their interaction, since callus formation was induced from 100% of the immature embryo explants (Table 1).

Table 1. Callus induction, survival, and plant regeneration from immature embryos of four durum wheat varieties on two induction media after 4 weeks of culture

	Callus induction (%)		Callus survival after bombardment (%)		Callus regeneration (%)		Plantlet regeneration (%)		Number of plantlets per regenerating callus	
	IM1	IM2	IM1	IM2	IM1	IM2	IM1	IM2	IM1	IM2
Variety										
Amria	100a	100a	91.4b	87.3b	18.4bc	5.8b	55.0bc	16.1b	2.0bc	0.8bc
Chaoui	100a	100a	99.1a	96.7a	44.7a	22.4a	181.5a	59.1a	3.7a	2.0a
Isly	100a	100a	94.5b	93.0a	27.2b	20.5a	104.4b	53.7a	2.8ab	1.7ab
Marouane	100a	100a	94.0b	88.0b	13.7c	4.9b	29.8c	5.9b	1.3c	0.4c
LSD	0	0	4.2	4.8	9.6	8.4	57.7	31.5	1.0	1.0
IM1	100a		95.1a		28.8a		106.9a		2.7a	
IM2	100a		92.0b		15.1b		39.0b		1.4b	
LSD	0		2.1		4.2		22.0		0.5	

Effect of bombardment

a. Callus survival

The cultivars varied in response to bombardment pressure. The highest percentage of survived calli was observed for 'Chaoui' at 99.1% and the lowest was for 'Amria' at

87.3%. After bombardment, the average frequency of callus survival was reduced for all genotypes; however, this reduction wasn't statistically significant for 'Chaoui' and 'Isly' (Fig. 1, a). Across all cultivars, post-bombardment survival was better for callus induced on IM1 than on IM2 (Table 1; Fig. 2, a).

b. Regeneration

The efficiency of regeneration was also affected by bombardment. 'Amria', and 'Marouane' exhibited lower callus regeneration (Fig. 1, b), plantlet regeneration frequency (Fig. 1, c) and number of plantlets per regenerating callus (Fig. 1, d) following bombardment compared to non-bombarded calli. However, non-significant differences were observed in plantlet regeneration frequency (Fig. 1, c) and number of plantlets per regenerating callus (Fig. 1, d) between bombarded and non-bombarded calli for 'Chaoui' and 'Isly'.

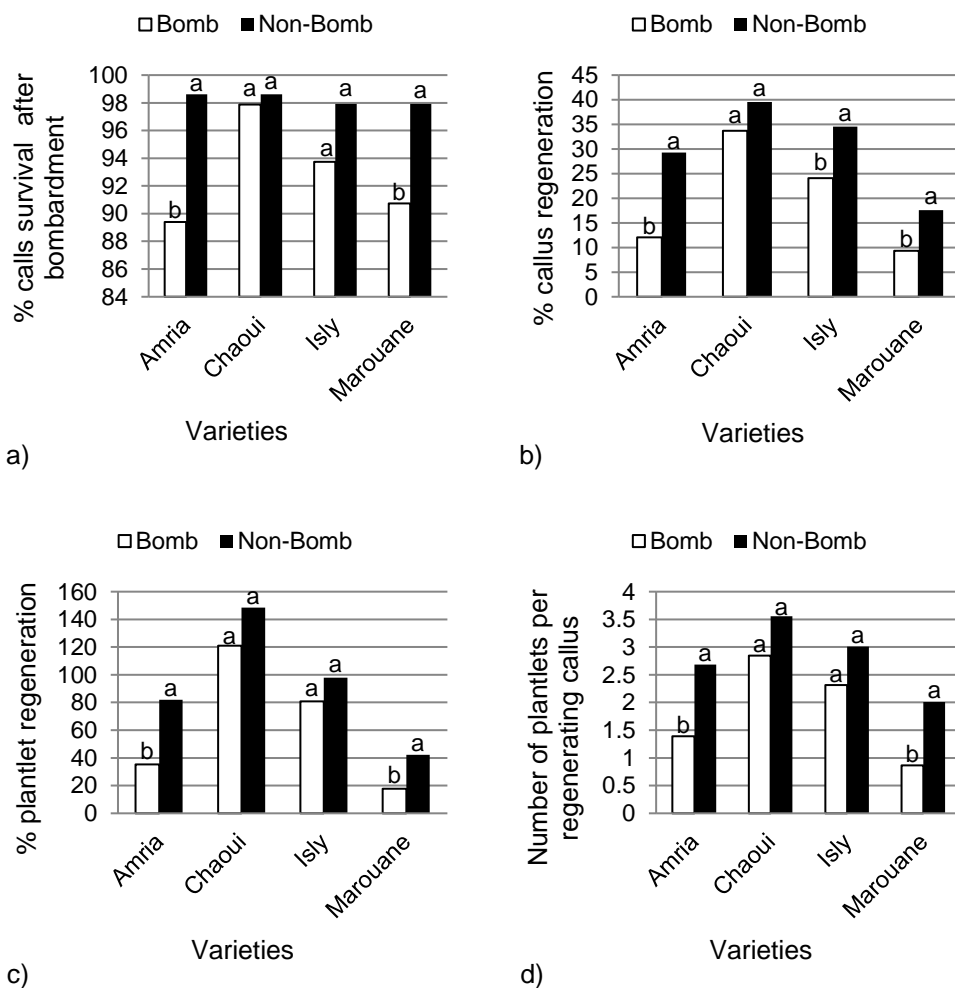


Figure 1. Effect of bombardment on % callus survival after bombardment (a), % callus regeneration (b), % plantlet regeneration (c) and number of plantlets per regenerating callus (d).

Effect of media on regeneration after bombardment

After 40 days of culturing on the induction media, embryogenic calli were transferred to the regeneration media. After an additional 8 weeks, the percentage callus regeneration (Fig. 2, b), plantlet regeneration (Fig. 2, c) and the number of plantlets per regenerating callus (Fig. 2, d) were recorded. The medium used for callus induction (IM) had a significant effect ($p < 0.001$) on all regeneration parameters. The use of different regeneration media (RM) did not have any significant effect. However, the interaction effect (IM*RM) was highly significant ($p < 0.001$) for all regeneration parameters (Table 2). Therefore, the analysis of the different regeneration parameters will be done through comparing different combinations of IM and RM.

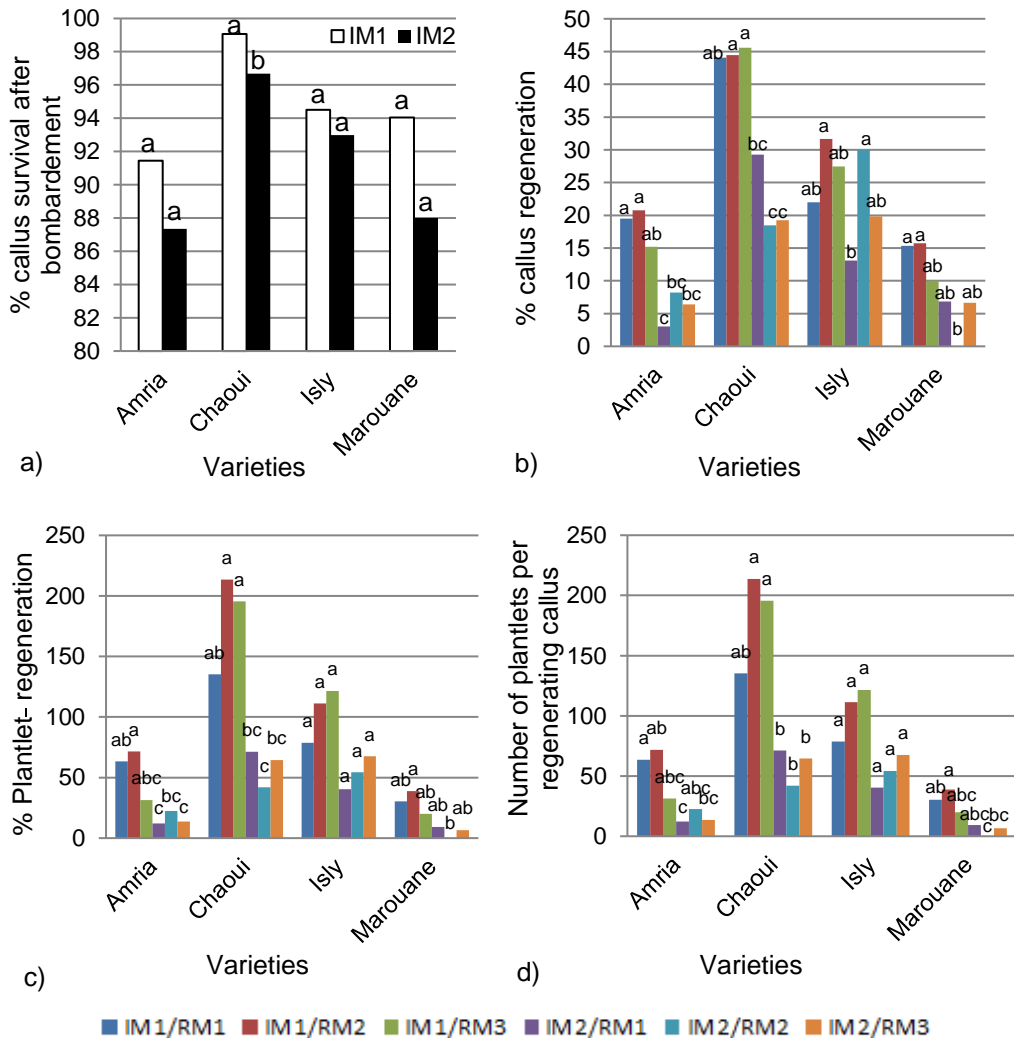


Figure 2. Effect of induction medium on % callus survival after bombardment (a) for the tested varieties. Effect of induction and regeneration medium combinations on: % callus regeneration (b), % plantlet regeneration (c) and number of plantlets per regenerating callus (d), for the tested varieties.

Table 2. Analysis of variance for the effects of variety, induction medium, and regeneration medium, and their interactions on callus and plantlet regeneration (%) and number of plantlets per regenerating callus

	Callus regeneration (%)	Plantlet regeneration (%)	Number of plantlets per regenerating callus
Variety	26.64***	16.10***	11.85***
IM	39.76***	35.91***	28.74***
Variety*IM	3.00*	3.98**	0.42
RM	0.65	0.88	0.03
Var*RM	1.35	0.45	0.52
Combination (IM/RM)	8.21***	8.01***	5.88***
Var* Combination	1.4	1.20	0.61

*Significant at $p < 0.05$; **Significant at $p < 0.01$; ***Significant at $p < 0.001$.

a. Percentage of callus regeneration

Percentage of regenerated calli was significantly affected by the genotype and the media combination used (Table 2). The efficiency of callus regeneration was assessed by counting the number of callus producing plantlets for each combination tested and all studied genotypes. The highest callus regeneration rates were observed on IM1 combined with the different regeneration media (Table 1). As illustrated in ‘Fig. 2, b’, IM1 combined with either RM1 or RM2 was best for both ‘Amria’ and ‘Marouane’. IM1 combined with either RM2 or RM3 gave the best results for ‘Chaoui’. IM1 or IM2 combined with RM2 performed best for ‘Isly’. Based on these observations, the IM1/RM2 combination is the best common media regime for all tested varieties.

b. Plantlets regeneration

There was a significant difference ($p < 0.001$) in plantlet regeneration capacity between different media combinations and cultivars (Table 2). The highest rate (213.6%) was observed for ‘Chaoui’, and the lowest was recorded for ‘Marouane’ (Fig. 2, c). The IM1/RM2 combination produced the highest percentage plantlet regeneration for all varieties except ‘Isly’, which showed a non-significant improvement with IM1/RM3 (Fig. 2, c). For the varieties less affected by bombardment stress (‘Chaoui’ and ‘Isly’), the IM1/RM2 combination enhanced regeneration by 28% compared to unbombarded controls with the IM1/RM1 combination used previously in our research laboratory (data not shown).

c. Number of plantlets per regenerating callus

Number of plantlets per regenerating callus was also affected by genotype, the induction medium, and the combination (Table 2). The IM1/RM2 combination showed the highest number of plantlets per regenerating callus for ‘Chaoui’ and ‘Marouane’ varieties. The best combination for ‘Amria’ was IM1/RM1. No significant differences among all combinations were observed for ‘Isly’ (Table 1; Fig. 2, d).

DISCUSSION

The first key requirement for a successful transformation system is a highly regenerable target tissue. It is well known that the production of embryogenic calli and regeneration capacities are genotype dependent in many cereal species such as maize (Manivannan et al., 2010), barley (Sharma et al., 2005), rice (Khanna & Raina, 1998)

and wheat (Bennici et al., 1988; Ozgen et al., 1996; Mzouri et al., 2001; Filippov et al., 2006; Vendruscolo et al., 2008).

In the present study, we have compared four Moroccan durum wheat varieties for their ability to produce embryogenic calli and regenerate plantlets after bombardment. Our results show that the 'Chaoui' and 'Isly' varieties have higher regeneration rates than 'Amria' and 'Marouane'. Mathias & Simpson (1986) and Li et al. (2003) also showed that genotype was the most important factor controlling callus formation and plant regeneration in wheat. Those results can be explained by the variability of genetic components between genotypes and specifically due to the endogenous auxins/cytokinins balance (Carman et al., 1987).

Non-bombarded callus gave higher percentages of regeneration than bombarded callus for all tested varieties. These results are in accordance with several studies which stated that the regeneration frequency of bombarded calluses was always lower in comparison to non-bombarded calluses in rice (Alfonso-rubi et al., 1999), in the eight best 'Bobwhite' accessions (Pellegrineschi et al., 2002) and in Moroccan bread wheat varieties (Ekoum et al., 2014). The 'Isly' and 'Chaoui' varieties were only slightly affected by bombardment compared to 'Amria' and 'Marouane'. The comparison of bombarded and non-bombarded explants is important; the results of this study will be helpful for wheat genetic transformation.

According to many previous reports, callus induction and regeneration capacity of wheat are not only influenced by genotype, but also by other factors such as explant source, culture medium, physiological status of the donor plants, and the interactions between these factors (Ozgen et al., 1996).

In this study, two callus induction media (IM1 and IM2, with different sources and concentrations of nitrogen and different plant growth regulators in the regeneration medium (IAA, zeatin and their combination) were tested for their effects on callus formation and plant regeneration from immature embryo explants of four Moroccan durum wheat varieties ('Amria', 'Chaoui', 'Isly' and 'Marouane').

No significant differences were observed for callus and plantlet regeneration rates between the three regeneration media tested for the same induction medium (Table 2), although a slight improvement using zeatin in RM2 regeneration medium was seen for most varieties. However, the interaction of induction and regeneration media showed a highly significant effect on all regeneration parameters due to the prevailing role of the induction medium on embryogenic callus formation and the ultimate relationship between the two stages.

IM2 induction medium decreased the average percentage of survived calli and plantlet regeneration for both bombarded and non-bombarded calli. Our results are in accordance with the study of Abdollah et al. (2014) who reported that for wheat somatic embryogenesis, when the concentration of ammonium nitrate is more than the normal concentration used in MS medium, the formation of green nodules decreased from 3.4 ± 0.2 to 1.9 ± 0.2 and percentage of nodulated callus from 80% to 50%. It was suggested that the high concentration of NH_4^+ might be toxic (Mengel & Kirkby, 1982). Indeed, Greer et al. (2009) found that increasing the nitrogen content was ideal for regenerating the wheat cultivar 'Superb', but was minimal for regenerating other cultivars. It was suggested that each species, cultivar and even tissue has its own unique preference for different salt concentrations (He et al., 1989; Maës et al., 1996). In our study, the reduction of plantlet regeneration rates with higher nitrogen was more

significant for bombarded than for non-bombarded callus (from 106% to 38% and from 124% to 59% respectively). This could be explained by the suggestion that after the physical stress of bombardment and the high osmotic treatment of mannitol, cells already plasmolyzed were more sensitive to the high concentration of ammonium nitrate and were unable to regulate their turgor pressure to survive.

CONCLUSION

An efficient and reliable plant regeneration protocol for immature embryos of four Moroccan durum wheat varieties after bombardment has been established. The present study demonstrated significant effects of genotype, medium and bombardment on embryogenic callus formation and plant regeneration. An induction medium with a high concentration of nitrogen proved to be toxic for the tested Moroccan durum wheat varieties. Bombardment decreased the regenerative capacity of all tested varieties, with less damage for 'Chaoui' and 'Isly' than for 'Amria' and 'Marouane'. The interaction of IM1 induction medium with RM2 regeneration medium enhanced the plantlet regeneration rate for most tested varieties from both bombarded and non-bombarded calli. The results of our study may be beneficial to future applications of durum wheat immature embryo culture for transformation and other biotechnological objectives.

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Blends of unrefined vegetable oils for functional nutrition

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Abstract. The unbalanced ratio of ω -3/ ω -6 of polyunsaturated fatty acids (PUFAs) causes a number of alimentary-dependent diseases, and the intake of capsulated forms as biologically active additives does not always take into account the established eating behavior of the population and the hedonic aspect of food consumption in general.

The Saratov region is one of the leading agricultural regions in the Russian Federation, one of the leaders in the cultivation and processing of low-used but valuable oily raw material, such as seeds of mustard, milkthistle, camelina, safflower.

The object of the study were the above listed oils and their food compositions obtained by blending. The functionality and biological efficiency of the initial oils were investigated by gas-liquid chromatography. Applying the methods of mathematical modeling, new food systems with the specified characteristics were designed (achieving the optimal ratio of ω -3: ω -6 acids). The most promising samples were selected through sensory analysis. Functional and sensory properties were taken as reference points for selection. The developed mathematical model is applicable to this food system, which was proved by the study conducted empirically. As a result of the work performed, blends of elite unrefined vegetable oils with health-promoting properties were obtained; their use in nutrition is designed to contribute to the formation of a healthy and active longevity in general, as well as to the minimizing the deficit of essential factors of nutrition in the child's body, athletes in the popular sports and sports of records.

Key words: blends of unrefined vegetable oils, sources of polyunsaturated fatty acids, safflower oil, camelina oil, milkthistle oil.

INTRODUCTION

Edible fats are the necessary part of human diet, making up about one third of its total calorie content, they occupy the second place in solving the world food problem and are the most important factor in the food security of any country (Teesalu, 2006; Lenihan-Geels et al., 2013; Zaytseva & Nechaev, 2014; Sanguansri et al., 2015; Pitsi et al., 2017). The consumption of fats in the diet of contemporary man has increased dramatically, mainly in the latent form.

Studies of late years, which revealed the role of lipids in humans and animals, allowed evaluating and specifying the effect of these substances on the metabolic processes at all levels of the organization of living matter. Fats are characterized by a wide range of functions due to the high diversity of their structures, although initially

they were considered only as a powerful source of energy. In addition, lipids are the part of the information framework of the body, they form and organize information flows and fill them, providing active interaction with the environment. Disorders in the lipid system of the body are observed at different pathological disturbances. A while ago it became known that essential fatty acids enhance the protective functions of the body (Filipovic et al., 2016; Grant & Guest, 2016; Andrieu et al., 2017; Arnold et al., 2017). Their role in the regulation of signal information is great. PUFAs are able to interact with ion channels, thereby initiating a rapid response of the cell to the information received. Due to the peculiarities of its structure, PUFAs can undergo oxidative transformations of the carbon skeleton with the formation of several hundreds biologically highly active metabolites, united by the common term ‘oxylipins’ (Arnold et al., 2017; De Oliveira et al., 2017; Kerdiles et al., 2017; Okonenko & Shakhabutdinova, 2017; Saini & Keum, 2018; Mazahery et al., 2019). The two main groups of PUFAs are the acids of the ω -6 group and ω -3 group; ω -6 acids are found in almost all vegetable oils. The body's need for polyunsaturated fatty acids is not permanent, it may vary depending on age, nature of labor activity, living conditions, in particular climatic conditions, health condition and other factors.

Recommendations on the fatty acid composition of edible fats for the nutrition of a healthy body, approved by the Research Institute of Nutrition of the Russian Academy of Medical Sciences, are given in Table 1.

Table 1. Composition of fat products for healthy human nutrition

Degree of unsaturation of fatty acids	The ratio of fatty acids, in % from total calorie value of the daily diet
Saturated	Not more than 10
Monounsaturated	10
Polyunsaturated	From 6 to 10
The ratio of polyunsaturated acids ω 6/ ω 3	From 5 to 15

These standard rates for the total consumption of polyunsaturated fatty acids are close to the recommendations of FAO/WHO, but significantly lower than the level of consumption of ω -3 acids recommended by the International Society of Nutrigenetics/Nutrigenomics (Zaytseva & Nechaev, 2014). Instructional guidelines of Rospotrebnadzor of the Russian Federation (Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing of the Russian Federation) determine the optimal ratio in the daily diet of ω -6: ω -3 fatty acids as 5–10:1 (Tutelyan, et al., 2009). Each fat taken separately (from used in food) both vegetable, and animal origin does not fully meet all those requirements which are imposed to edible fat now. Therefore, to create complete diets, it is necessary to use fats and oils in certain combinations that are favorable for the human body (O'Brien, 2008).

The Saratov region is one of the leading agricultural regions in the Russian Federation, one of the leaders in the cultivation and processing of low-used but valuable oily raw material, such as seeds of mustard, milkthistle, camelina, safflower. These types of oils, unfortunately, have not yet found wide application in food industry.

The presented types of oils have functional potential determining their antitumor and bactericidal properties, stimulation of digestive, cardiovascular, endocrine and respiratory systems, improving the immune status of the body (Zilmer, et al., 2010; Obukhova, 2013; Aung et al., 2018).

The aim of this work was to create blends of these unrefined vegetable oils obtained by cold pressing, balanced by ω -3/ ω -6 acids from the regional raw materials of the Saratov region for functional nutrition.

MATERIALS AND METHODS

The work was performed in 2017–2018 in the Russian Federation hosted by Saratov State Vavilov Agrarian University at the Department of Food Technology in the university training and research laboratory for determining the quality of food and agricultural products.

The object of the study were:

- safflower oil, milkthistle oil, camelina oil, mustard oil. Their fatty acid composition is shown in Table 2.
- food compositions of the above listed oils in various combinations obtained by blending.

The research methods used and their brief characteristics are presented in Fig. 1.

RESULTS AND DISCUSSION

At the first stage of the research the fatty acid composition of the used oils produced from the seeds grown in the Lower and Middle Volga region was determined, it is indicated in Table 2. Method error: at the content of the desired substances: less than 5%–0.28%; equal to or more than 5%–1.42%.

Table 2. Fatty acid composition of the studied oils (averaged)

Acid name	Fatty acid composition, %			
	Camelina oil	Safflower oil	Mustard oil	Milkthistle oil
C14:0 (myristic)	0.09	0.20	0.07	0.13
C16:0 (palmitic)	5.36	7.50	3.70	9.90
C16:1 (palmitoleic)	0.09	7.20	0.16	0.11
C18:0 (stearic)	2.26	4.00	2.35	11.40
C18:1 (oleic)	14.83	21.00	43.71	25.70
C18:2 (linoleic)	17.37	75.00	32.09	34.80
C18:3 (linolenic)	37.85	0.20	11.19	2.00
C20:0 (arachidic)	1.06	0.40	0.61	6.90
C20:1 (gondoinic)	12.72	0.40	2.53	1.60
C20:2 (eicosadienoic)	1.88	1.60	0.20	2.10
C22:0 (behenic)	0.22	0.40	0.30	3.80
C22:1 (erucidic)	2.35	1.90	2.30	1.70

The imbalance of fatty acid composition of the presented vegetable oils taken separately proves the necessity of their blending. It should be noted that safflower oil is characterized by a significant content of linoleic acid isomers 18:2 with conjugated double bonds, which has anticarcinogenic activity.

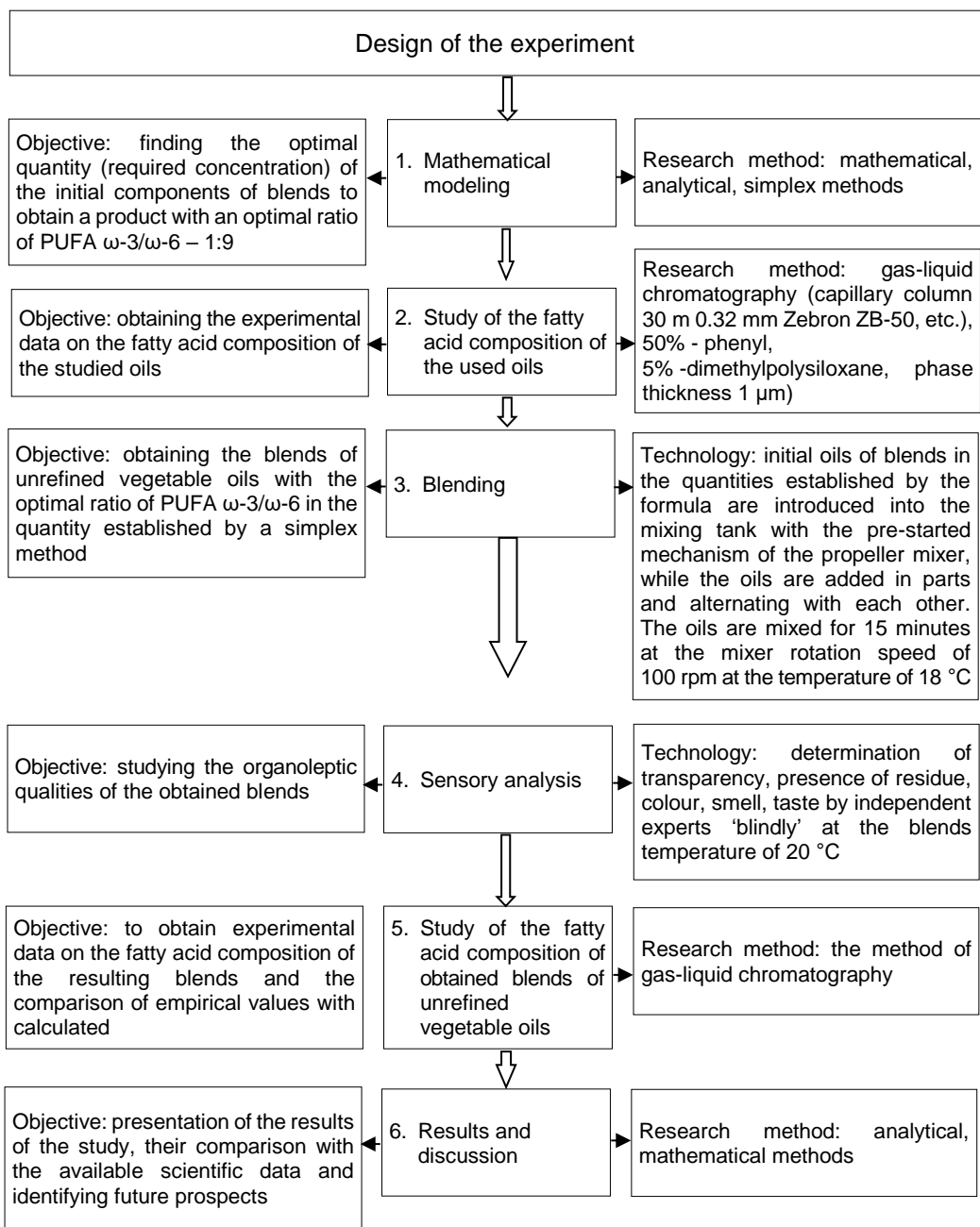


Figure 1. Design of the experiment.

Applying a simplex design of the experiment for determining the effect of fatty acid composition on the functional properties of food compositions, models of blends of unrefined oils were designed (Krasulya et al., 2015). On the basis of the obtained models, formulas of food compositions (Table 3) were proposed, the specified properties of which were confirmed experimentally (Table 4).

Table 3. Formulas, obtained as a result of simplex design

Name of oils in blends	Concentration of oils in blend, %	Design ratio of ω -6: ω -3acids
Safflower + camelina + milkthistle	50 + 13.5 + 36.5	9.94:1
Safflower + camelina + mustard	65 + 10 + 25	10:1
Safflower + milkthistle + mustard	30 + 11 + 59	9.74:1

The obtained quantitative data submit to the normal distribution. Validity of values was determined by Student's t-test, at $P < 0.05$. Statistical processing of the data was carried out according to standard methods using Microsoft Excel software package. The results given in Table 3 prove the compliance of the blends composition to the given ratio ω -6 and ω -3 fatty acids, optimal for daily consumption.

It should be noted that at present the disadvantages of the available fat composite mixtures are the unbalance ω -3/ ω -6 acids, or partial destruction of nutrients under thermal influence in the process of oil manufacturing, high cost of the related initial raw material (various essential oils) for obtaining the finished product, use of oleic safflower oil. A significant disadvantage of the analyzed technologies is the use of refined oils (<https://worldwide.espacenet.com>; <http://www.findpatent.ru>).

The estimated content of vitamin E equivalent (tocopherolequivalent), taking into account the whole group of tocopherol compounds (4 tocopherol and 4 tocotrienol) united by the common term 'vitamin E' was calculated based on the literature data (Skurikhin & Tutelyan, 2002; O'Brien, 2008). To obtain this indicator, the following recalculation factors are used: (α -tocopherol – 1.0; β -tocopherol – 0.4; γ -tocopherol – 0.1; δ -tocopherol – 0.01; α -tocotrienol – 0.3; β -tocotrienol – 0.05; γ - and δ -tocotrienol – 0.01). Information on the study of the estimated degree of the satisfaction of the body's needs in ω -3, ω -6 fatty acids and vitamin E at using the developed blends is presented in Table 5.

One dose (15 mL) of blend supplies the daily need for vitamin E:

- blend No. 1 - by 37.5%;
- blend No. 2 - by 24.9%;
- blend No. 3 - by 18.96%.

For people with low labor activity one dose (15 mL) of blend supplies the daily need for ω -3, ω -6 acids:

- blend No. 1 - by 35% and 70,8%, respectively;
- blend No. 2 - by 30.7% and 61.0%, respectively;
- blend No. 3 - by 22.1% and 43.7%, respectively.

Table 4. Average values of fatty acid composition of the designed blends

Acid name	Fatty acid composition, %		
	Blend No. 1	Blend No. 2	Blend No. 3
C14:0 (myristic)	0.1	0.2	0.1
C16:0 (palmitic)	6.2	7.4	6.9
C16:1 (palmitoleic)	0.2	0.1	0.2
C18:0 (stearic)	3.1	3.7	3.1
C18:1 (oleic)	28.7	18.0	20.5
C18:2 (linoleic)	46.5	56.3	57.2
C18:3 (linolenic)	9.9	8.2	7.7
C20:0 (arachidic)	0.7	1.1	0.6
C20:1 (gondoinic)	3.1	3.4	2.6
C20:2 (eicosadienoic)	0.4	0.4	0.3
C22:0 (behenic)	0.4	0.6	0.2
C22:1 (erucidic)	0.9	0.8	0.4

For people with high labor activity one dose (15 mL) blend supplies the daily need for ω -3, ω -6 acids:

- blend No. 1 - by 24.0% and 47.2%, respectively;
- blend No. 2 - by 20.5% and 40.7%, respectively;
- blend No. 3 - by 14.8% and 28.7%, respectively

Thus, the recommended intake of blend No. 3 for people with high working activity corresponds to two doses (30 mL) per day.

Currently, the study on the experimental determination of vitamin E and various phytochemicals in these blends is being conducted. The developed technology of blending of unrefined vegetable oils does not require technical re-equipment of enterprises, it is simple and convenient. Initial oils of blends in the quantities established by the formula are introduced into the mixing tank with the pre-started mechanism of the propeller mixer, while the oils are added in parts and alternating with each other. The oils are mixed for 15 minutes at the mixer rotation speed of 100 rpm.

The sensory analysis of the developed blends was carried out by practitioners with higher technological education (Fig. 2).

Table 5. Expected degree of supplying the body's needs in ω -3, ω -6 and vitamin E by the developed blends equivalent (tocopherolequivalent)

Content of ω -3, ω -6, vitamin E		% from daily need		
		Blend No. 1	Blend No. 2	Blend No. 3
ω -3, %	in 100 g	158.0	135.7	98.2
	in 1 dose	24.0	20.5	14.8
ω -6, %	in 100 g	314.4	271.5	191.4
	in 1dose	47.2	40.7	28.7
Vitamin E	in 100 g	250.2	166.1	126.4
	in 1 dose	37.5	24.9	19.0

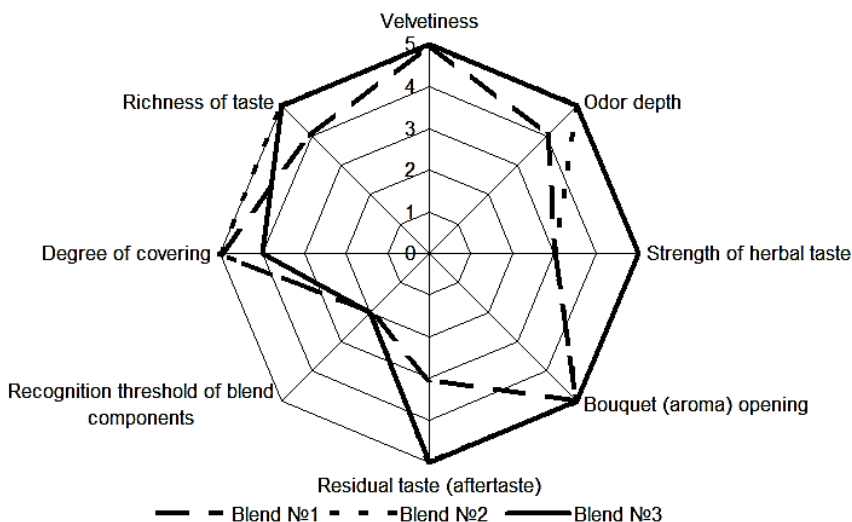


Figure 2. Organoleptic profile of the designed blends.

The descriptive characteristics of the blends are summarized in Table 6. Thus, the range of application of the developed blends is wide and it is rational to use them as a healthy food product both separately and as salad dressings, base for the sauces, mayonnaise and spreads of non-mass production with a short shelf life.

Table 6. Descriptive characteristics of blends

Indicator	Blend No. 1	Blend No. 2	Blend No. 3
Taste	Velvety-chalky, somewhat tasteless with unstable aftertaste	Warm, slightly pronounced herbal, with noble bitterness and moderate aftertaste	Cool, with spicy bitterness and a stable herbal aftertaste
Smell	Moderately cold, moderately intensive flavor of oil crops	Delicate, slightly perceptible herbal aroma	Warm, moderately herbal aroma
Colour	Amber	Intensive, deep amber	Delicate light-amber
Transparency	Transparent, without impurities		

CONCLUSION

Currently, mathematical methods for modeling of food compositions are intensively used in the food industry for the design of food formulas. The choice of the method of mathematical modeling for a particular food system is individual. It is rational to use simplex design in blending vegetable oils. The formulas of blends of regional unrefined vegetable oils from oil crops of the Saratov region with the optimal level and ratio ω -3/ ω -6 acids were designed using this method, the effectiveness of which was confirmed by the results of empirical study. The resulting organoleptic profile of blends confirmed the acceptability of their wide application in food industry. Addition of the developed blends is organically included in the range of a daily diet of a healthy person, as its component and covers a significant part of the daily need of the body in essential substances. The use of this product line in nutrition is designed to contribute to the formation of the society of healthy and active of longevity in general, as well as to the minimizing the deficit of essential factors of nutrition in the child's body, athletes in the popular sports and sports of records.

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Influence of organic and inorganic fertilization on soil properties and water infiltration

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Abstract. Soil aggregate stability (SAS) belongs to the most important parameters determining the quality of soil and fertilizer influence on soil aggregation. We evaluated the relationship between SAS, hydro-physical soil properties and infiltration rates in three long-term field experiments founded in 1956 on different soils. Soil properties under three fertilization regimes—no fertilization, farmyard manure, farmyard manure and mineral fertilization—were evaluated at silty loam Chernozem, silty loam Phaeozem and sandy loam-loam Cambisol. A significant impact of fertilization on SAS was found, even though the differences in SAS were rather low. The lowest SAS was recorded at plots with manure and mineral fertilization (25.1%) compared with plots without fertilization (28.7%) and plots with manure-only fertilization (28.2%). The highest SAS (36.5%) and the highest semi-capillary porosity (SP; 11%) were observed at sandy loam-loam soil. Hydro-physical soil properties were more favourable at fertilized plots (SP 9.6% and bulk density ρ_b 1.31 g cm⁻³) compared with unfertilized ones (SP 8.8% and ρ_b 1.35 g cm⁻³). The lowest SP (8.32%) and the highest ρ_b (1.37 g cm⁻³) were recorded at Phaeozem, which corresponded with the lowest SAS (19.4%). Chernozem had similar soil texture to Phaeozem, but SAS (24.7%), SP (9%) and ρ_b (1.27 g cm⁻³) were more favourable. Despite the low level of statistical significance due to the large variation of infiltration measurements, a higher infiltration rate was recorded at fertilized plots (45 mm hour⁻¹) compared to unfertilized ones (35 mm hour⁻¹).

Key words: soil quality, soil aggregate stability, infiltration, porosity, long-term field trial.

INTRODUCTION

At present, frequency and intensity of climate extremes increase due to ongoing climate change and the soil quality and soil resistance become more significant. At soil quality assessment, soil texture, content of soil organic matter (SOM) and quality of soil structure belong to the key factors determining soil physical properties. Soil structure quality may be expressed by water stability of soil aggregates (SAS), i.e. ability of soil aggregates to resist disintegration by water. SAS is easily determined by laboratory measurements and provides reliable information on soil quality. In some cases, SAS responds better to soil management changes compared to the total SOM content (Stehlíková et al., 2016).

Water stability of soil aggregates refers to complex soil characteristics and is directly related to precipitation infiltration, soil crusting, surface runoff and soil erosion (Barthès & Roose, 2002; Soenne et al., 2016). More stable soil structure leads not only to reduction of erosion, but also to better rainfall utilization, which is important especially in areas with more frequent periods of drought. To improve SAS and soil quality, the importance of crop rotation and fertilization is often mentioned (Naveed et al., 2014; Stehlíková et al., 2016; Suwara et al., 2016). The aim of this paper was to find a relationship between soil aggregates stability and hydro-physical soil properties. For this objective, we investigated soil properties at differently fertilized plots of the oldest multi-site field experiment in the Czech Republic.

MATERIALS AND METHODS

The Crop Rotation Experiment (CRE) was established in 1956 at three locations: Čáslav (GPS: N 49°53.45', E 15°23.77'), Ivanovice na Hané (GPS: N 49°18.84', E 17°5.98') and Lukavec (GPS: N 49°33.45', E 14°58.82'; Tables 1, 2). CRE is a four-field experiment with 12 fertilization treatments, 4 plot replicates and the plot size of 9 x 9 m. Experiment design is described in Kunzová & Hejčman (2009). For the present study, the following treatments of one experimental field were chosen: No. 21–control (No. fertilization), No. 11–manure (40 t ha⁻¹ every four years before maize and potato) and No.14–full fertilization (manure + mineral fertilization in average doses of 66 kg N ha⁻¹, 40 kg P ha⁻¹ and 87 kg K ha⁻¹). The soils were ploughed to the depth of 20–25 cm. Crop residues were removed from plots after the harvest. In the autumn 2014 after the harvest of winter wheat, the manure was applied at the treatments 11 and 14. The crop rotation was as follows: 2011–potato, 2012–spring barley, 2013–clover, 2014–winter wheat, 2015–silage maize, 2016–spring barley, 2017–winter rape, 2018–winter wheat and 2019–potato. Soil samples were collected in four repetitions for each treatment in the following dates: at the end of October 2016 (2 months after winter rape was sown); at the end of October 2017 (1 month after winter wheat was sown); and at the end of April 2018.

Table 1. Characteristics of the researched localities

Locality	Altitude (m a. s. l.)	Annual mean temperature (°C)	Annual precipitation (mm)	Soil type (WRB)	pH (H ₂ O)	Soil texture (USDA)	Parent material
Čáslav	263	8.9	555	Phaeozem	7.05	Silt loam	Loess
Lukavec	610	7.3	683	Cambisol	6.4	Sandy loam–loam	Gneiss
Ivanovice	225	9.2	548	Haplic Chernozem	7.1	Silt loam	Loess

Table 2. Texture (USDA), TOC (total organic carbon) and soil particle density of topsoils

Locality	Clay % < 0.002 mm	Silt % 0.002–0.05 mm	Sand % > 0.05 mm	Soil particle density– ρ_d (g cm ⁻³)	TOC (%)
Čáslav	17.2	68.8	14	2.56	1.323
Ivanovice	18.7	72.3	9	2.53	1.936
Lukavec	9.8	36.3	53.9	2.59	1.378

To assess the soil aggregate stability (SAS), disturbed soil samples to the depth of 7 cm were collected. Samples were gently sieved to obtain the fraction of 1–2 mm. Soil aggregate stability, measured as the proportion of water stable aggregates was assessed by the wet-sieving method of Kandeler (1996), using a laboratory equipment HERZOG (Adolf Herzog GmbH, Vienna, AT) with sieving time of 5 minutes and 3 repetitions per sample.

To determine hydro-physical soil properties, undisturbed soil samples were collected using the steel cylinders with the volume of 100 cm³ to the depth of 7 cm. Three samples were collected for each trial plot, i. e. 12 samples for each treatment were taken. In 2018, the undisturbed samples were collected in July instead of April, after the harvest of winter wheat, as it was necessary to avoid damaging the canopy.

Bulk density (ρ_b), capillary porosity (CP), non-capillary porosity (NP), semi-capillary porosity (SP) and the maximum content of non-gravitational soil water (soil moisture reduced from full saturation after 30 minutes of free drainage– θ_{30} ; this parameter reflects the amount of water held in capillary and semi-capillary pores) were determined according to the methods described in Zbíral et al. (2011) and Pospíšilová et al. (2016). According to the authors, capillary pores have the diameter of < 0.2 μm and retain water for plants. Semi-capillary pores are those of the 0.2–10 μm diameter and are comprised both gravitation pores supporting water infiltration and capillary pores retaining water for plants. Non-capillary pores are characterized by the diameter > 10 μm and they enable the water flow into deeper layers of soil profile.

In summer 2018, measurements of water infiltration were carried out after the harvest. Saturated hydraulic conductivity (K_{fs}) was determined using the method of ponded infiltration (Bagarello et al., 2006) using the cylinders with the diameter 150 mm. In 8 repetitions, the fully fertilized treatment and the unfertilized treatments were evaluated. Cylinders were sunk into the soil to the depth of 10 cm and the soil moisture was recorded using the ML3 Theta Probe sensor in the surrounding soil. Then, the cylinder was in 20 seconds gently filled with 1,000 mL of water using the perforated bowl. The water volume corresponded to precipitation of 56 mm. The time needed to soak the water was recorded. When the water infiltrated completely, soil moisture inside the cylinder was measured. To assess soil texture the parameter α was set to 12. Data were evaluated using the ANOVA and Scheffe's multiple comparison test at $\alpha = 0.05$ determined homogenous groups. Analysis was conducted in STATISTICA 13.3 software (TIBCO Software Inc.).

RESULTS AND DISCUSSION

Soil aggregate stability

Soil aggregate stability (SAS) was predominantly influenced by site characteristics (Fig. 1). In the finer soils of Čáslav and Ivanovice, SAS was significantly lower as compared to the lighter soil of Lukavec. SAS usually increases with the content of fine particles (Le Bissonnais, 1996), but lower SAS values in finer soils were also reported (Stehlíková et al., 2014). Between the experimental sites with finer soils, SAS was slightly higher at Chernozem with higher total organic carbon content (Table 1, 2). Higher SAS at cultivated soils with higher carbon content were reported also by Soinnie et al. (2016).

Fully fertilized treatment showed significantly lower SAS compared to unfertilized ones (Fig. 1). SAS did not significantly differ between plots fertilized with manure and the unfertilized plots. However, the application of manure slightly decreased SAS when compared to unfertilized treatment in Ivanovice and Čáslav, while in Lukavec this effect was not observed. Organic fertilization is usually reported to increase organic carbon (Šimon & Czako, 2014) content and the ratio of macroaggregates (Zhao et al., 2018) and to also have a positive effect on SAS (Kroulík et al., 2010; Badalíková et al., 2015). Šimon et al. (2018) point out the influence of excessive mineral fertilization on mineralization of soil organic carbon. A negative effect of mineral fertilization on SAS is reported e.g. by Stehlíková et al. (2014) and Stehlíková et al. (2016). Brtnický et al. (2017) described a negative influence of increasing mineral fertilization on SAS as well as a reduction in the effect of organic fertilizers on the SAS decrease. On the contrary, Suwara et al. (2016) reported a positive effect of mineral fertilization on higher SAS values at sandy loam Phaeozem.

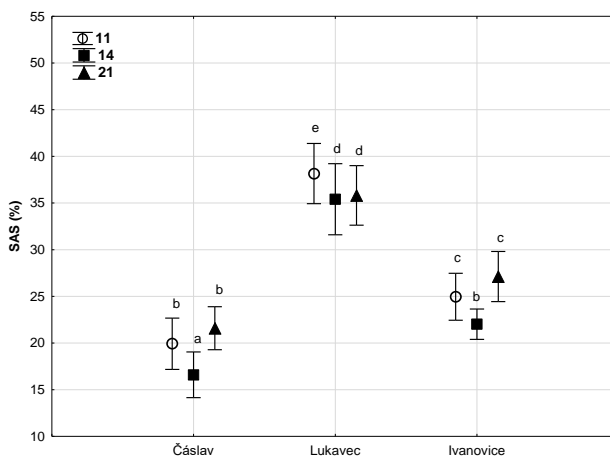


Figure 1. Influence of the treatments on the soil aggregate stability (SAS) at experimental sites (11 – farmyard manure; 14 – mineral + farmyard manure; 21 – no fertilization). Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe’s test. Vertical columns show 0.95 confidence interval.

A significant year-to-year differences in SAS were observed, with the possible effect of seasonal dynamics (Fig. 2). Large year-to-year SAS variability is often reported (Badalíková et al. 2015). Fig. 2 shows significantly higher SAS in 2018 (39.4%), when the samples were collected from wheat plots in spring during the vegetation growth. Significantly lower SAS were then recorded in autumn, when the vegetation is inhibited. The lowest SAS was recorded in autumn 2017, when the samples were collected from winter wheat plots one month after sowing. On the contrary, SAS was higher in autumn 2016, when the samples were collected 2 months after winter rape was sown. The higher SAS in 2016 might be due to a higher rooting intensity of winter rape.

Nevertheless in the event of excessive precipitation or snow melting, soil infiltration and water holding capacity can be exceeded. Finer soils with lower SAS, especially in combination with mineral fertilization, might then be more susceptible to

erosion or degradation. It is for these reasons that bulk density, porosity and rate of infiltration of soil are important.

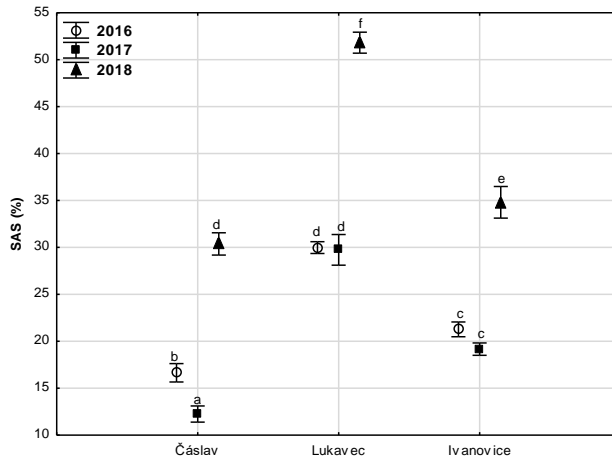


Figure 2. Year to year variability on the soil aggregate stability (SAS) at experimental sites. Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe's test. Vertical columns show 0.95 confidence interval.

Bulk density

Bulk density (ρ_b) was significantly influenced by fertilization. Mean lower ρ_b was recorded at fertilized plots (1.31 g cm^{-3}) compared to 1.35 g cm^{-3} at unfertilized plots (Fig. 3). Lower ρ_b values at fertilized treatments were reported also by Suwara et al. (2016) in sandy loam Phaeozems, by Kroulík et al. (2010) in clay loamy soil and by Šařec & Novák (2017) in silty loam soil. Bulk density varied largely among sampling terms; in 2016 it was significantly lower compared to other samplings terms (Fig. 4).

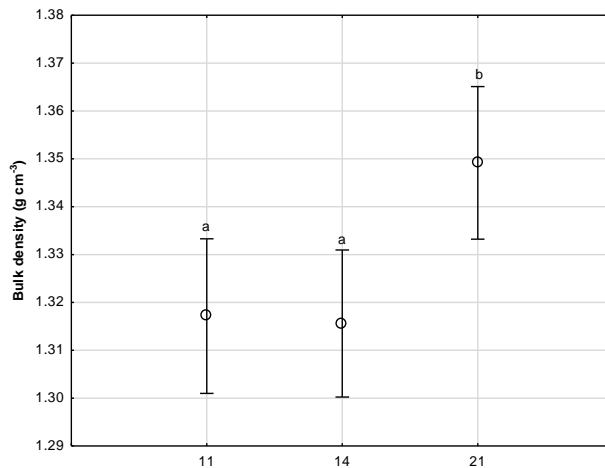


Figure 3. Influence of the treatments (11 – farmyard manure; 14 – mineral + farmyard manure; 21 – no fertilization) on the bulk density of soil. Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe's test. Vertical columns show 0.95 confidence interval.

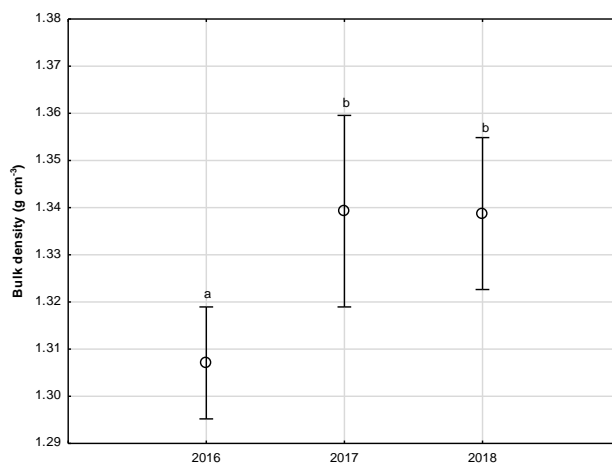


Figure 4. Soil bulk density at three sampling terms. Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe's test. Vertical columns show 0.95 confidence interval.

Higher rooting intensity of winter rape can be possible explanation. No difference in ρ_b was observed between 2017 and 2018. Differences in ρ_b among treatments and years reached 0.05 g cm^{-3} , but difference among sites were two-times higher (Fig. 5). The lowest ρ_b was recorded at Chernozem (Table 1, 2). Samples with similar content of soil carbon and different soil texture showed slightly higher ρ_b in Čáslav when compared to Lukavec. However, the differences were not significant.

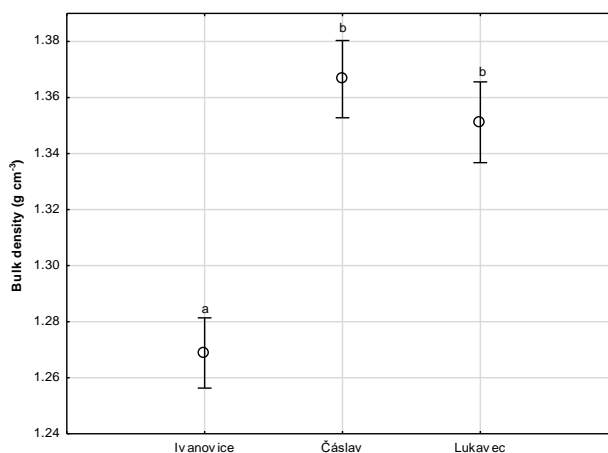


Figure 5. Soil bulk density at experimental sites. Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe's test. Vertical columns show 0.95 confidence interval.

Porosity

Bulk density, SAS and water regime depend on the distribution of soil pores. Fig. 6 compares capillary porosity (CP), semi-capillary porosity (SP) and non-capillary (NP) porosity in three sampling years. Higher rooting intensity in winter rape likely impacted higher NP in 2016. On the contrary, CP was the highest in 2017 in winter wheat. In 2018, CP was lower compared to previous years whereas SP increased. The highest NP was

recorded in Ivanovice and the highest SP was recorded in Lukavec (11%; Fig. 7), which corresponded with the highest SAS. The effect of increased SAS on the amount of micropores refers Regelink et al. (2015). Conversely, the lowest SP values were observed in Čáslav (8.3%), where the lowest SAS as well as the highest differences in SP among treatments were recorded. Significantly higher SP was demonstrated at fertilized treatments (9.6% on average) when compared to unfertilized treatments (8.8%).

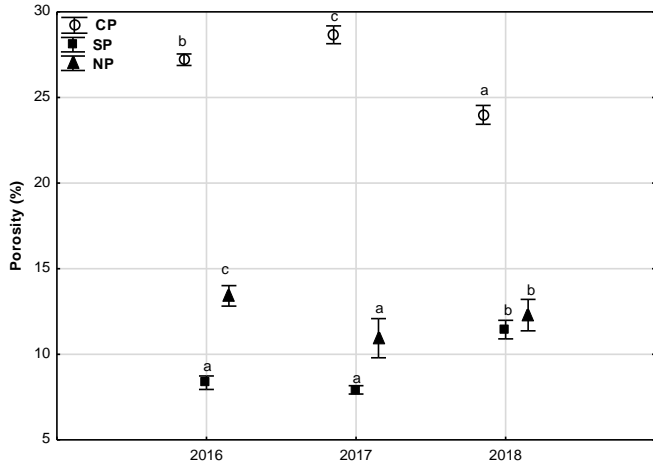


Figure 6. Distribution of porosity between three types of pores (CP – capillary porosity; SP – semi capillary porosity; NP – non – capillary porosity). Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe’s test. Vertical columns show 0.95 confidence interval.

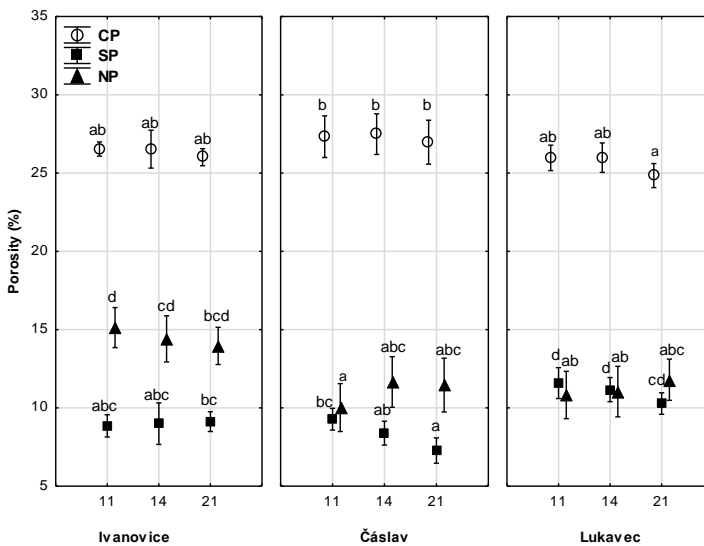


Figure 7. Influence of the treatments (11 – farmyard manure; 14 – mineral + farmyard manure; 21 – no fertilization) at experimental sites on the distribution of porosity between three types of pores (CP – capillary porosity; SP – semi capillary porosity; NP – non – capillary porosity). Different letters indicate significant differences at $\alpha = 0.05$ by Scheffe’s test between sites and treatments within CP, SP, and NP. Vertical columns show 0.95 confidence interval.

Fertilized treatments retained significantly more non-gravitational water compared to unfertilized treatments (Table 3). Significantly more water was held in Lukavec compared to Ivanovice and Čáslav. Higher water retention is related to higher CP and SP ratio at sites and fertilized treatments (Fig. 7). Lower soil moisture at the unfertilized treatment are confirmed by Suwara et al. (2016) in sandy loam Phaeozem and by Badalíková et al. (2015) in clay loamy soil.

Table 3. Non-gravitational soil moisture (θ_{30}) at experimental sites and treatments. The averages marked by the same letter in individual columns did not significantly differ at $\alpha = 0.05$ (Scheffe's test)

Locality	Average (θ_{30}) in %	Standard error
Ivanovice	35.35 ^a	0.17
Čáslav	35.58 ^a	0.30
Lukavec	36.61 ^b	0.23
Treatment		
Not fertilized	34.82 ^a	0.23
Farmyard manure	36.44 ^b	0.23
Farmyard manure + mineral fertilization	36.11 ^b	0.24

Water infiltration

Organic fertilization influences infiltration of water into the soil. Season 2018 was extremely dry from April to September. Drought was probably the main cause of an insignificant effect of fertilization and site on water infiltration rate, measured after harvest of winter wheat in summer 2018. Nevertheless, Fig. 8 shows a lower infiltration rates at the unfertilized treatment (35 mm hour⁻¹) compared to higher rates of fully fertilized treatment (45 mm hour⁻¹). Influence of organic fertilization on higher infiltration rate in sandy loam Cambisol during the years following application were reported by Badalíková & Bartlová (2014) and at clay loamy soils by Kroulík et al. (2010).

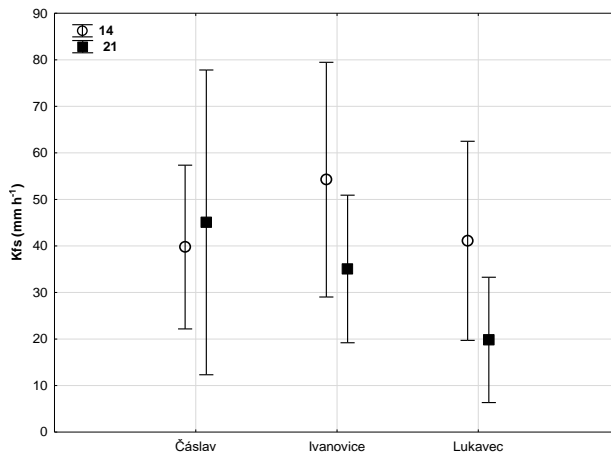


Figure 8. Influence of the treatments (14 – mineral + farmyard manure; 21 – no fertilization) on the saturated hydraulic conductivity (K_{fs}) at three experimental sites. There are not significant differences between treatments ($p > 0.05$). Vertical columns show 0.95 confidence interval, $p > 0.05$.

Despite the expected highest infiltration rate on sandy loam–loam soil in Lukavec, a higher infiltration rate was recorded on silty loam soil in Ivanovice, which was related to the highest non–capillary porosity (14.5%). The significant impact of macropores on higher infiltration rate in clay soils are referenced by Kodešová et al. (2006). The lowest infiltration rate, based on the distribution of pores, was presumed on silty loam soil in Čáslav. Nevertheless, likely due to drought and very low soil moisture at the time of infiltration measurements (8%), the highest infiltration rate was recorded at the unfertilized plots in Čáslav, but rates varied largely. On average, the infiltration rate at the fully fertilized treatment in Čáslav was comparable to the unfertilized treatment.

CONCLUSIONS

Higher SAS and higher semi–capillary porosity leads to higher water retention; this can be concluded for the trial in sandy loam–loam Cambisol, but not for two trials in silty loam soils. Significantly lower SAS were recorded at fully fertilized plots of fine texture soils. However the differences were small. Despite slightly lower SAS, fertilized plots showed significantly higher semi–capillary porosity and content of non–gravitational water. The lowest SAS and semi–capillary porosity and the highest soil bulk density were observed at Phaeozem. Semi–capillary and capillary pores determine maximum content of non–gravitational soil water. On the other hand, semi–capillary and non–capillary pores are related to bulk density and infiltration rate. We found that fertilization promotes higher infiltration rates. The results show that even small differences in SAS, porosity and soil bulk density were reflected by increased infiltration rates at fertilized treatments. Organic fertilization had a positive impact on SAS, semi–capillary porosity and soil bulk density. Significant year–to–year differences in soil properties were also shown, therefore the influence of seasonal climate can not be excluded. However, the differences in infiltration rates not always corresponded with the change of SAS. Within finer soils especially, higher infiltration rates were probably impacted by macropores and soil cracks caused by period of drought prior the measurements.

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The effect of sowing date on cover crop biomass and nitrogen accumulation

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Abstract. Cover crops are important tools for reducing nitrogen (N) leaching from the soil and improving the nutrition of cash crops. In northern regions with short autumns it is important to maximise the growing season of cover crops to achieve sufficient biomass and N accumulation. The objective of the study was to evaluate the biomass and N accumulation of cover crops at different sowing dates in August.

Field experiment at Estonian Crop Research Institute was conducted in 2017 and 2018 with white mustard (*Sinapis alba* L.), phacelia (*Phacelia tanacetifolia* Benth), buckwheat (*Fagopyrum esculentum* Moench), berseem clover (*Trifolium alexandrinum* L.), field pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.). Cover crops were sown on August 3, 8, 14 and 18 in 2017 and August 3, 8, 13, 17 and 23 in 2018.

The two year experiment showed that biomass and N accumulation of cover crops were reduced with delayed sowings, but the reduction mainly depended on cover crop species.

White mustard, field pea and faba bean accumulated significantly higher amount of biomass and N than phacelia, buckwheat and berseem clover at all sowing dates in both years. Because of a rapid decrease in biomass, the optimum sowing time for phacelia and buckwheat should not be later than middle of August. In both year berseem clover produced the modest amount of biomass and therefore more suited as spring sown cover crop in Estonian conditions.

Key words: biomass, cover crops, nitrogen accumulation, sowing date.

INTRODUCTION

Cover crops can reduce N losses from the soil and improve the nutrition of cash crops (Thorup-Kristensen et al., 2003; Arlauskienė & Maikštėnienė, 2008; Sapkota et al., 2012; Munkholm & Hansen, 2012). They provide many other valuable ecosystem services such as to build soil organic matter, and to support beneficial soil organisms. They can also inhibit weeds, pests, and diseases, as well as improve soil and water quality (Snapp et al., 2005; Kaspar & Singer, 2011).

The commonly used cover crop species were selected in the study. White mustard (*Sinapis alba* L.), phacelia (*Phacelia tanacetifolia* Benth.) and buckwheat (*Fagopyrum esculentum* Moench) were chosen because of their rapid emergence and growth in the fall, and their effectiveness of taking up residual nutrients from the soil (Talgre et al., 2011; Björkman et al., 2013; Brust et al., 2014). Legumes field pea (*Pisum sativum* L.),

faba bean (*Vicia faba* L.) and berseem clover (*Trifolium alexandrinum* L.) were selected because of biological nitrogen fixation ability (Talgre et al., 2011; Clark, 2012).

The nitrogen accumulation of cover crop is largely dependent on the amount of biomass they can produce by the termination time. Early planting is major factor that influences cover crop biomass yield (Etemadi et al., 2018). However, the unfavourable weather conditions often cause later harvest of cash crop and therefore delay establishment of cover crops.

Previous research has shown that the cover crop biomass and nitrogen accumulation decreased with delayed sowing. Studies in northeast United States showed that two weeks of planting delay caused 55–62% reduction of faba bean biomass (Etemadi et al., 2018). Zaniewicz-Bajkowska et al. (2013) reported about 45% biomass reduction of phacelia, amaranth, sunflower, seradella and faba bean after four weeks planting delay in Poland.

In Estonia white mustard, phacelia, faba bean and field pea have shown to be great biomass producers in the beginning of August (Talgre et al., 2011). However, the studies with delayed sowing of cover crops have not been previously investigated. Therefore the experiment was conducted to evaluate the biomass and nitrogen accumulation of these species and new potential species (buckwheat and berseem clover) at different sowing dates.

MATERIALS AND METHODS

Field experiments were established in 2017 and 2018 at Estonian Crop Research Institute (58° 44' 59.41" N, 26°24' 54.02" E) at a climate zone with an average annual temperature of 5.3 °C and precipitation of 670 mm (Estonian weather service). The soil type in the experimental area was Cambic Phaeozem (Loamic) soil (IUSS 2015). The soil characteristics were as follows: pH_{KCl} 6.9, P 104 mg kg⁻¹, K 195 mg kg⁻¹, Ca 3,700 mg kg⁻¹, Mg 510 mg kg⁻¹, C_{org} 2.1% and N_{tot} 0.16%. The following cover crop species were used in the study (seeding rate in brackets): white mustard cultivar (cv.) 'Braco' (18 kg ha⁻¹), phacelia cv. 'Stala' (12 kg ha⁻¹), buckwheat cv. 'Aiwa' (70 kg ha⁻¹), berseem clover cv. 'Akenaton' (15 kg ha⁻¹), field pea cv. 'Kirke' (280 kg ha⁻¹) and faba bean cv. 'Kontu' (280 kg ha⁻¹). Cover crops were sown on August 3, 8, 14 and 18 in 2017 and August 3, 8, 13, 17 and 23 in 2018, after harvest of winter wheat (*Triticum aestivum* L.). The soil was disc harrowed before cover crop sowing. Plots with an area of 4 x 6 m were arranged in a randomized complete block design with four replications. The above and below ground biomass samples were collected from four randomly placed squares of 0.25 m² in each plot at the end of vegetation period (20th and 23th of October in 2017 and 2018, respectively), before ploughing the cover crops into the soil. Above ground biomass was cut on the ground level. Roots were taken with a shovel to a depth of 25 cm and washed from soil on a sieve with a mesh size 0.5 mm.

The samples were oven-dried at 65 °C to a constant weight for dry matter (DM) determination and milled for elemental analysis. Plant total N concentrations were determined in Soil Science and Agrochemistry laboratory at Estonian University of Life Sciences by the Dumas Combustion method on a varioMAX CNS elemental analyser ('Elementar', Germany).

Precipitation and air temperature were measured daily at a meteorological station located near the field trial site. In 2017 the average air temperature was moderately higher than the long-term average in August by 0.6 °C, and in September by 1.2 °C. Rainfall in August (83 mm) was similar to the long-term average (89 mm), but it was considerably higher in September (86 mm) and October compared to the long term average (66 mm) (Table 1).

The weather conditions in 2018 autumn were not favourable for cover crop establishment. Average air temperature in July was 20.3 °C, which is 3.4 °C higher than the long term average. The soil conditions were very dry as the amount of precipitation was only 15 mm. The average air temperature in August was 2.4 °C higher than the long term average. The rainfall started from 4th of August, but the amount of precipitation in August was still 22 mm lower than the long term average. The average temperature in September was even 4.1 °C higher than the long term average. Rainfall (72 mm) was similar to the long-term average (66 mm), but occurred mostly at the end of the month (Table 1). The first night frost occurred at 29th of September in both years. The plant vegetation period ended at 20th and 23th of October in 2017 and 2018, respectively.

Statistical analyses were carried out by statistical package Agrobase 20TM. To test the differences of biomass and nitrogen (N) accumulation of cover crops, the 3-factor ANOVA (cover crop, sowing date, year and their interaction) was used. In case of significant factors Fisher’s LSD test was performed.

Table 1. Average air temperature and precipitation per ten-day period and monthly sums of effective air temperatures > +5 °C (ETS) during the experimental period

Long-term average	Air temperature per 10 day period			Long-term average 1922–2017) (per month)	Rainfall			Long-term average 1922–2017) (per month)	ETS	Long-term average 1922–2017) (per month)
	1	2	3		1	2	3			
	2017									
July	13.8	14.8	16.1	16.8	8	45	4	79	308	365
August	17.3	17.5	13.1	15.3	22	35	26	89	337	320
September	12.4	12.5	10.5	10.6	14	71	1	66	206	177
October	7.4	7.1	0.3	5.3	54	38	15	66	53	60
2018										
July	15.6	21.7	23.2	16.8	10	5	0.5	79	474	365
August	21.1	17.3	15.6	15.3	31	29	16	89	401	320
September	17.2	14.1	9.5	10.6	1	26	45	66	260	177
October	7.9	9.3	2.7	5.3	33	1	43	66	86	60

RESULTS AND DISCUSSION

The variation in biomass and nitrogen accumulation was significantly influenced by cover crop species, sowing date, years and their interaction (Table 2). Biomass accumulation was mainly influenced by cover crop species (53%) and sowing date (31%) in both years; for nitrogen it was 67 and 21%, respectively.

Table 2. Analyses of variance for cover crop biomass and nitrogen accumulation depending on cover crop species, sowing date, year and their interaction

Characteristic	Source of variation	df	SS	MS	F	p
Biomass	Year	1	2,358,755	2,358,755	75	0.000
	Cover crop	5	118,228,073	23,645,615	754	0.000
	Sowing date	3	69,540,674	23,180,225	739	0.000
	Crop x Year	5	4,399,229	879,846	28	0.000
	Crop x sowing date	15	13,866,705	924,447	29	0.000
	Sowing date x year	3	4,220,966	1,406,989	45	0.000
	Crop x year x sowing date	15	3,397,583	226,506	7	0.000
Nitrogen	Year	1	1,669	1,669	53	0.000
	Crop	5	154,195	30,839	980	0.000
	Sowing date	3	47,936	15,979	508	0.000
	Crop x Year	5	3,565	713	23	0.000
	Crop x sowing date	15	12,086	806	26	0.000
	Sowing date x year	3	4,836	1,612	51	0.000
	Crop x year x sowing date	15	2,598	173	6	0.000

Biomass and N accumulation of cover crops in 2017

In 2017, at the earliest sowing date (Aug 3), white mustard produced significantly highest DM yield (4,980 kg ha⁻¹) (Fig. 1). Similar amount of biomass (3,500–5,500 kg ha⁻¹) has been reported by Björkman et al. (2015), who concluded that mustard performed best in cool and moist conditions by producing higher biomass in autumn compared to spring planting. Earlier studies in Estonia reported white mustard’s biomass of 2,500 kg ha⁻¹ (Toom et al., 2017) and 3,000 kg ha⁻¹ (Talgre et al., 2011). N accumulation by cover crops depends on the total amount of biomass produced and the percentage of N in the plant tissue. Due to its large biomass, white mustard accumulated significantly higher amount of N (128 kg ha⁻¹) (Fig. 2). Faba bean had significantly higher biomass (3,050 kg ha⁻¹) than field pea (2,600 kg ha⁻¹), however their N accumulation (100 kg ha⁻¹) did not differ significantly. Similar biomass (1,570–1,870 kg ha⁻¹) and N (30–41 kg ha⁻¹) accumulation were recorded for phacelia, buckwheat and berseem clover.

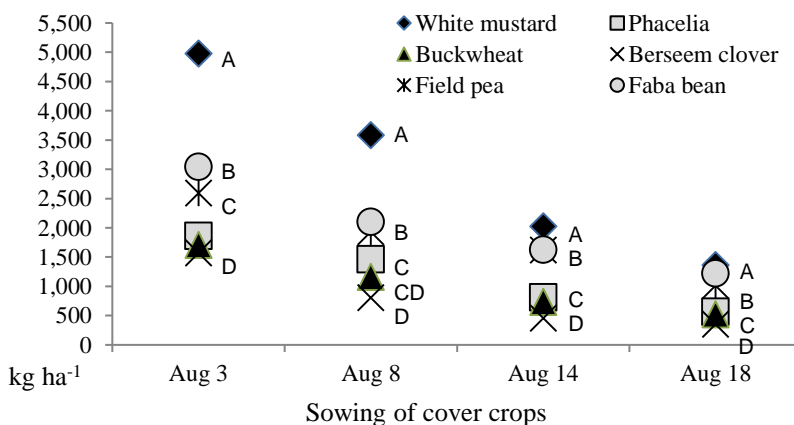


Figure 1. The biomass (above and below ground) production of cover crops (dry matter kg ha⁻¹) depending on date of sowing in 2017. Statistically significant differences ($p < 0.05$; ANOVA, Fisher LSD test) within the sowing date are marked with different letters.

At the sowing dates of Aug 8 and 14, white mustard's biomass decreased 28 and 59%, respectively, from the first sowing date. It produced significantly higher amount of biomass (3,590 and 2,030 kg ha⁻¹, respectively) of all species. At the same sowing dates, there was no significant difference between the biomass of field pea and faba bean (1,940–2,100 and 1,620 kg ha⁻¹, respectively).

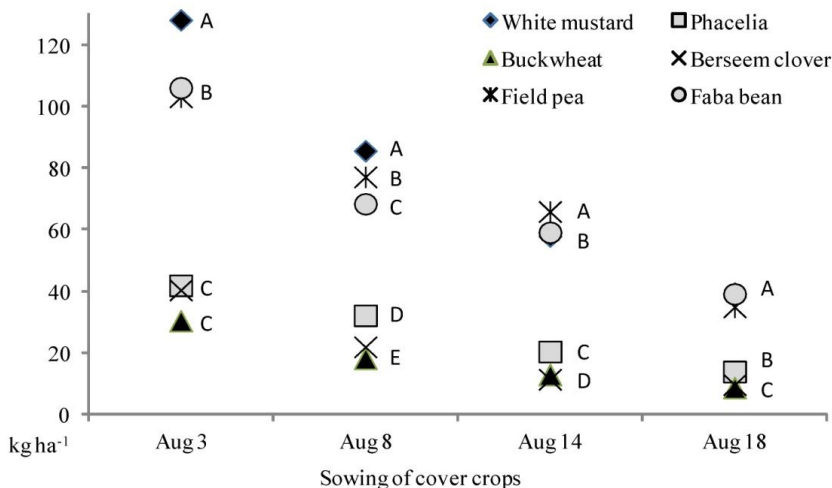


Figure 2. The nitrogen accumulation of cover crops (N kg ha⁻¹) depending on date of sowing in 2017. Statistically significant differences ($p < 0.05$; ANOVA, Fisher LSD test) within the sowing date are marked with different letters.

By the last sowing date (Aug 18), white mustard (73% reduction from the first sowing date) and faba bean (60% reduction) produced significantly higher biomass (1,220–1,370 kg ha⁻¹), followed by field pea (1,000 kg ha⁻¹). All these three species produced significantly higher amount of N (35–40 kg ha⁻¹) compared to other tested cover crops.

At second planting date (Aug 8), phacelia's and buckwheat's biomass decreased 22 and 32%. At later sowings Aug 14 and 18, it was reduced sharply (57 and 70%, respectively), producing biomass of 520–570 kg ha⁻¹ by last sowing date. One of the reason of Buckwheat's low yield was the damage by frosts that occurred at the end of September. In other studies, buckwheat has shown rapid growth in the beginning of the growing season, but in some years, lower yield is explained by sensitivity to the irregular rainfall and low ground temperatures that end its growth (Handlířová et al., 2017). Conversely, phacelia has been recommended as one of the least sensitive cover crop to delayed sowing (Zaniewicz-Bajkowska et al., 2013) and unfavourable temperature and rainfall conditions (Handlířová et al., 2017). Berseem clover produced significantly lowest biomass at Aug 8, 14 and 18 sowings. At the second sowing date it was reduced already 49%. By the last sowing date, the biomass decreased 77%, resulting in yield of 360 kg ha⁻¹ and the nitrogen accumulation of only 10 kg ha⁻¹.

Biomass and N accumulation of cover crops in 2018

In 2018, because of the dry soil conditions, cover crops sown at first (Aug 3) and second (Aug 8) planting dates had no differences in biomass as well as in N accumulation. As in the previous year, white mustard produced significantly higher (3,790–3,810 kg ha⁻¹) biomass compared to other species (Fig. 3). The lower biomass yield compared to 2017 was possibly associated with warm temperatures that caused earlier flowering and reduced root activity and nutrient uptake (Talgre et al., 2011). Legumes faba bean and field pea were not affected by drought and produced similar biomass as in the previous year (Fig. 4). Faba bean had significantly higher yield (3,230–3,340 kg ha⁻¹) compared to field pea (2,660–2,690 kg ha⁻¹), however their N accumulation value did not differ significantly and was precisely the same as in the previous year (100 kg ha⁻¹). Studies have shown that faba bean sown at early August produced 100 kg ha⁻¹ N (Talgre et al., 2011; Zaniewicz-Bajkowska et al., 2013) and in favourable conditions even 192 kg ha⁻¹ N (Etemadi et al., 2018). Our results showed that Phacelia produced 2230 kg ha⁻¹ of biomass and accumulated 46 kg ha⁻¹ N, which were higher than in the previous year. Zaniewicz-Bajkowska et al. (2013) reported much higher biomass (5,500 kg ha⁻¹ DM) and N accumulation (90 kg ha⁻¹) of phacelia in Poland, sown on 4th of August. Buckwheat's biomass was practically the same as in 2017 (1,650 kg ha⁻¹), with N accumulation of 27 kg ha⁻¹. Significantly lower yield (720 kg ha⁻¹) and N accumulation (18 kg ha⁻¹) compared to other species was found in berseem clover. Clark et al. (2012) concluded that poor seedling emergence and reduced growth of berseem clover are caused by dry and warm conditions before sowing.

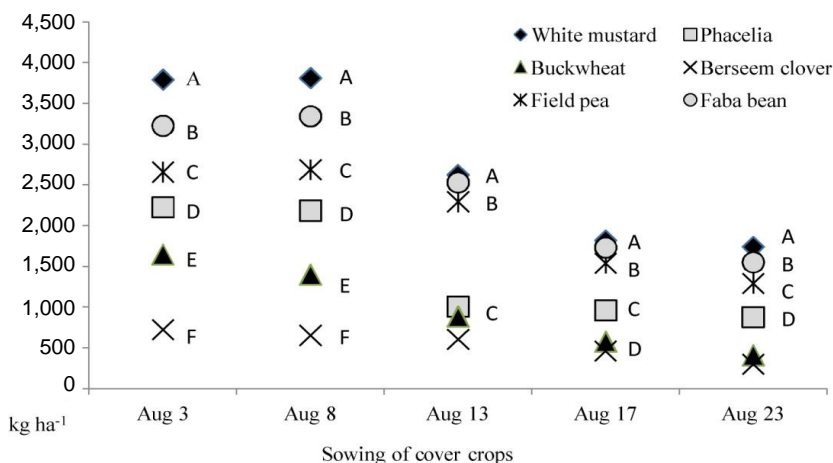


Figure 3. The biomass (above and below ground) production of cover crops (dry matter kg ha⁻¹) depending on date of sowing in 2018. Statistically significant differences ($p < 0.05$; ANOVA, Fisher LSD test) within the sowing date are marked with different letters.

At sowing dates Aug 13–23, white mustard (2,620–1,740 kg ha⁻¹), faba bean (2,530–1,550 kg ha⁻¹) and field pea (2,290–1,290 kg ha⁻¹) were the best biomass producers and N accumulators (90–50 kg ha⁻¹). Because of the smaller decrease at later sowings compared to the previous year, it can be concluded that these species were not negatively affected by low rainfall and they preferred warmer temperatures. The yield

of phacelia and buckwheat was reduced sharply from Aug 13, whilst it stayed nearly the same at sowing dates of Aug 17 and 23 (960 and 880 kg ha⁻¹). As in the previous year, buckwheat was damaged by night frosts, and by last sowing date its biomass yield was only 410 kg ha⁻¹, which was not significantly different from berseem clover (300 kg ha⁻¹). Both crops accumulated only 7–8 kg ha⁻¹ N. Buckwheat and phacelia are valued because of their weed suppression ability and disease resistance (Brust et al., 2014; Björkman et al., 2013). Earlier studies have also demonstrated their ability to solubilize and take up phosphorus that is otherwise unavailable to crops and release it to subsequent crops (Eichler-Löbermann et al., 2009; Teboh & Franzen et al., 2011). Therefore these species could be used in a mixture with other species that produce higher biomass.

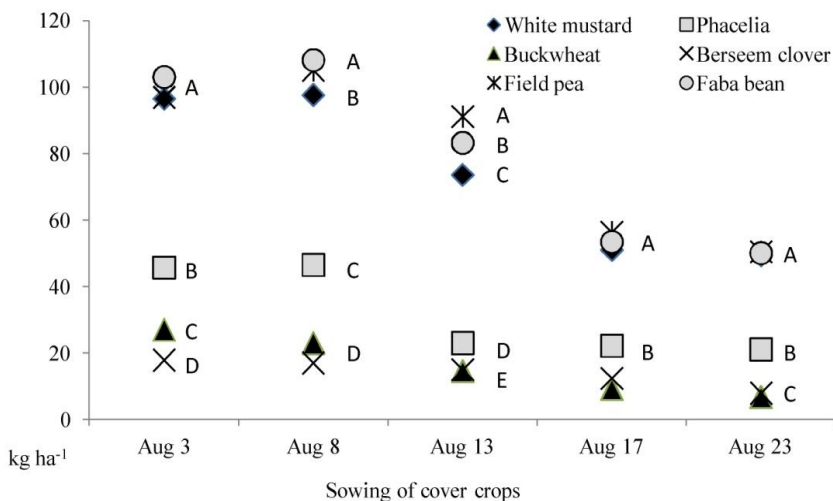


Figure 4. The nitrogen accumulation of cover crops (N kg ha⁻¹) depending on date of sowing in 2018. Statistically significant differences ($p < 0.05$; ANOVA, Fisher LSD test) within the sowing date are marked with different letters.

Although the poor emergence and growth of berseem clover was caused by unfavourable weather conditions, it also showed rapid biomass reduction in the previous year when sown after the first week of August. It can be concluded that Estonian weather conditions in the fall are not favourable for berseem clover. However, when sown in spring, berseem clover produces high biomass and can be used as a green manure crop before winter cereals (Tamm et al., 2016). However, when sowing of berseem clover is performed early August and weather conditions are not as extremely dry as in 2018, it might be a valuable addition to other cover crop species used here in Estonia. This is in accordance with Ghaffarzadeh (1997) who found that berseem clover is suitable for mixing cropping to increase total DM yields and subsequent crop yield.

CONCLUSIONS

The two year experiment showed that biomass and N accumulation of cover crops were reduced with delayed sowings, but the reduction mainly depended on cover crop species.

In 2017 with moderate temperatures and high rainfall conditions, cover crop biomass was reduced with all delayed sowings (Aug 8–18). In 2018, because of lack of soil moisture before sowing, cover crops sown at Aug 3 and 8 produced the same amount of biomass, but at later sowings (Aug 13–23) the biomass was reduced.

White mustard, field pea and faba bean accumulated significantly higher amount of biomass and N than phacelia, buckwheat and berseem clover at all sowing dates in both years. Although their biomass also decreased with delayed sowing dates, they accumulated adequate biomass and N in latest sowings. Phacelia's biomass decreased rapidly in both years, therefore the optimum sowing time should be until the middle of August. Buckwheat and berseem clover were the most susceptible to unfavourable weather conditions and their biomass decreased quickly in both years and can be recommended for sowing at the beginning of August

ACKNOWLEDGEMENTS. The research was supported by Estonian Ministry of Rural Affairs' project 'Varieties suitable for organic cultivation in Estonia' (10.1-2/430 p.4; PA1-RUP-026) and by 'Designing of an agrotechnical system including evaluation of suitable catch crop species, their seed mixtures and their cultivation methods' (T170143PKTM).

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Agrobiological evaluation of *Allium ampeloprasum* L. variety samples in comparison with *Allium sativum* L. cultivars

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Abstract. The purpose of investigation is to study the biological and morphological features, to establish the economic and valuable characteristics of the introduced and local forms of *Allium ampeloprasum* L. in comparison with *Allium sativum* L. and to study the effect of removal of the scape in the yield. The studies used field, statistical, settlement and analytical methods. The weight of the bulb without removing the inflorescence shoot of the cultivar Sofiiivskyi was 28.4–53.3 g smaller than the variety samples No. 2 and No. 3 respectively. With removing the scape the difference increased by 60.5–68.6 g. The yield of No. 2 variety sample *Allium ampeloprasum* L. without removing the scape was lower than the standard by 1.7 t ha⁻¹ while the variety sample No. 3 exceeded the standard by 1.1 t ha⁻¹. With the removal of inflorescence shoot the yields of variety samples No. 2 and No. 3 exceeded the cultivar Sofiiivskyi by 1.6 and 2.2 t ha⁻¹. It has been established according to the researches that introduced forms of *Allium ampeloprasum* L. have high indicators of economic and valuable characteristics, but they are limited in the first years of cultivation, by the period of adaptation to the new soil and climatic conditions.

Key words: bulb, cultivar, garlic, variety sample, yield.

INTRODUCTION

In the context of global climate changes and the deterioration of soil and climatic conditions, the lack of stable and high yielding varieties of winter garlic, an issue becomes relevant in selection of local forms (varieties) that are characterized by high adaptive capacity and stable yields.

The cultivars of garlic in Ukraine are not very diverse, two types: hardneck and softneck, as well as a less common species both in Ukraine and in the world of *Allium ampeloprasum* L. We believe that the investigation of biology and growing technology of cultivation for the introduction of *Allium ampeloprasum* L. will contribute to Ukraine's conditions will promote the expansion of the variety of vegetable plants.

Allium ampeloprasum L. – an onion garlic, an Egyptian garlic or onion, a Spanish garlic or just a garlic-onion, an Elephant garlic, as soon as this plant is not called but most often – rocambole (Danin, 2004; Ludilov & Ivanova, 2009). Today they grow two different forms of species origin of rocambole: actually rocambole – varieties derived from *Allium scorodoprasum* var. *Babingtoni* (Ron, 1992; Stace, 2010; Dimpoulos et al., 2013) and the elephant garlic varieties derived from *Allium ampeloprasum* var. *ampeloprasum* (Sulistiorini & Van der Meer, 1993; Brewster, 1994; Flora of North America; Christopher et al., 2013;).

Since ancient times to the present the peoples of Africa and Asia use *Allium ampeloprasum* L. as an anti-helminthic, diuretic, anti-hypertensive agent (Haciseferogullari, 2005; Guarrera & Savo, 2013) and for improving digestion (Triano et al., 1998). The shredded bulbs are used to treat the initial stages of cough, sore throat and mucous membranes. The fresh juice is taken as an antispasmodic (Malafaia et al., 2015). Despite the considerable medical and economic potential of this genus, the researchers, as a rule, are concentrated to cultivated species *A. cepa* L., *A. fistulosum* L., *A. sativum* L. (Ben Arfa et al., 2015).

Allium ampeloprasum L. includes not only Elephant garlic but also cultivated forms of the leeks (Hanelt et al., 1992). It creates the inflorescence shoot with a small amount of the non-viable seeds or without them. The center of origin is North Africa and Southwest Asia (Mc Collum, 1987). Within this group of species the divergence was aggravated by different climates and technology of culture in different regions (Astely et al., 1982). According to the analysis of chloroplastic DNA (Havey, 1991; Mes et al., 1997), a close genetic similarity between *Allium ampeloprasum* L. and *Allium sativum* L. was confirmed.

The aim of the research was to study the biological and morphological peculiarities, to establish economically valuable features and correlations with the productivity of the introduced and local forms of *Allium ampeloprasum* L. in order to subsequently create an Elephant garlic based on the local form. The comparison of *Allium ampeloprasum* L. variety samples with *Allium sativum* L. cultivars due to differences in biological characteristics, yield and nutritional value such as *Allium ampeloprasum* L. has a higher nutritional value and it is characterized by less sharpness that it can be consumed by people who do not use ordinary garlic for some reason. Elephant garlic also has higher yield and higher product cost which is economically important.

MATERIALS AND METHODS

The research of the collection was carried out in 2017–2018 at the experimental field of the Department of Vegetable Growing of Uman National University of Horticulture in accordance with generally accepted methods (Bondarenko & Yakovenko, 2001; Volkoday, 2016), excepting the schemes of planting of *Allium ampeloprasum* L (Figliuolo et al., 2001). The soil of the experimental field is black, puddle, heavy loam with a well developed humus horizon (about 2.9% of humus) in the deep of 40–45 cm.

The total area: for the experiment 400 m², for plot 100 m²; for sampling – 10 m². The plots were arranged in a systematic order with a four replication. The predecessor – early vegetables. Planting was carried out by the scheme of 45×6 cm for *Allium sativum* L. and 45×10 cm for *Allium ampeloprasum* L. at the end of the first decade of October. The location of the plots was systemic. During the investigation characters

including the length and width of the leaf, the area of the leaf blade and the total leaf area per plant on the 30th, 60th, 90th days after planting (DAP) were determined. The height and diameter of the scape and the pseudostem – before harvesting using the trammel. The No. of leaves (per plant, pcs) was determined by the method of calculation, the area of the leaf blade by a calculated (linear) method, using the parameters of the length and width of the leaf by the formula 1:

$$S_n = 0.67 \times ab \quad (1)$$

where S_n – the area of one leaf, cm²; a – the largest leaf width, cm; b – leaf length, cm; 0.67 is the coefficient that reflects the configuration of the leaf.

The cold resistance were judged by the counting of alive plants after spring regrowth. The coefficient of adaptability (CA) of the winter garlic was determined by the method of A. N. Podolskyh (Podolskykh, 2004), as the ratio of the number of plants that formed the commercial bulb to the total number of planted once, and the closer the value to 1, the more adaptive is the variety. The material of the research was two breeding varieties of the species *Allium sativum* L.: Sofiiivskiy (St.), Prometei, and two introduced forms of *Allium ampeloprasum* L. – variety samples No. 2 and No. 3.

Also we studied the effect of removing scape on increasing the yield of *Allium sativum* L. and *Allium ampeloprasum* L.

Proteins, fats, carbohydrates and ash content were determined using standard methods described in the procedures of the American Organization of Analytical Chemists (International Organization of International, AOAC International) (Horwitz & Latimer, 2005). Crude fat was determined using a Soxhlet apparatus with petroleum ether, according to the AOAC 920.85 methodology (Horwitz & Latimer, 2016). The content of ash was determined by burning at 600 °C to constant mass in accordance with procedures AOAS 923.03 (Horwitz & Latimer, 2016). Energy was calculated by the formula 2:

$$\text{Energy (kcal)} = 4 \times (\text{protein}) + 4 \times (\text{carbohydrate}) + 9 \times (\text{g fat}) \quad (2)$$

Free sugar was determined using HPLC, coupled with a refractive index detector using the internal standard methodology (IS, mesostiosis) (Guimarães et al., 2013).

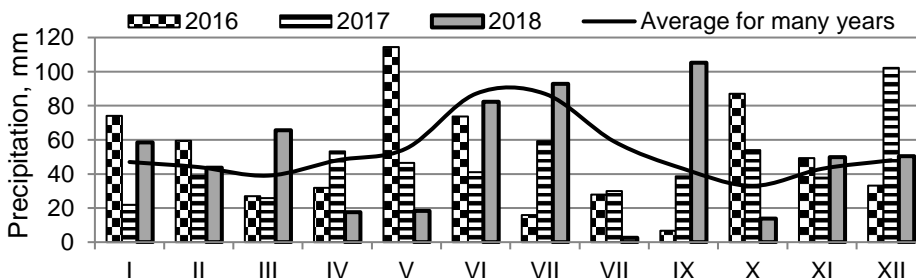


Figure 1. The amount of precipitation in 2016–2018.

For the food and chemical composition, three samples were analyzed in each genotype, and all analyzes were performed in three replicates. The results were expressed as averages and standard deviation (SD). The chemical composition and antioxidant activity were analyzed using a one-way dispersion analysis, followed by the

Tvyxi's Honesty Difference (TQ) test with $\alpha = 0.05$ using statistical analysis program (SAS) v. 9.1.3. All results are expressed as g per 100 g f.v.

According to Uman meteorological station the hydrometeorological conditions of 2017 were characterized by a slightly lower amount of precipitation relative to the average perennial indicators, but the uniformity of their fall during the period of the garlic vegetation was observed. The amount of precipitation for this period in 2018 was more relative to 2017, which is close to medium-long-term data, but the main number of them fell at the beginning and at the end of the vegetation which testifies to their lack of a phase of intensive growth and development of the plant, but it did not have a significant effect of precipitation, so the investigation was carried out under the drip irrigation.

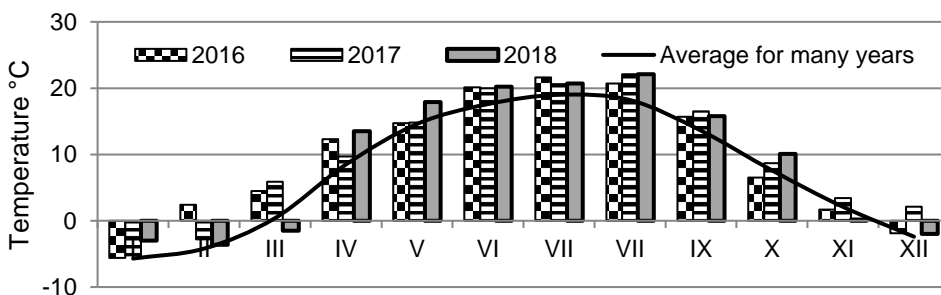


Figure 2. Average air temperature °C in 2016–2018.

The temperature of air 2016–2017 from the date of planting to the emergence of the sprouts was somewhat lower but close to the perennial which positively influenced the development of germination of the garlic plants in autumn-winter period. The temperature indexes of 2017–2018 from the date of planting to the restoration of the spring vegetation were atypically warm and it resulted the emergence of the sprouts of garlic during the autumn-winter period (Novak, 2017; Novak & Novak, 2018).

The data were statistically analyzed using Analysis of variance Microsoft Office Excel.

RESULTS AND DISCUSSION

Garlic, as a vegetatively propagated plant, is plastic and it reacts sharply to the changes in growing conditions, which may result to partial or complete freezing of crops. In the conditions of the Forest-Steppe of Ukraine for planting in the optimal period the garlic cloves are well rooted and no freezing is observed. According to the data the percentage of overwintering plants of *Allium ampeloprasum* L. in the experiment is within the range of 98.0–100% (Table 1), which indicates their high winter resistance, and plants *Allium sativum* L. – 100%. The lower level of

Table 1. Overwintering and adaptability of the studied garlic genotypes (average for 2017–2018)

Cultivar/ sample	Origin	Winter hardiness, %	Coefficient of adaptability
Sofiivskiyi St.	Ukraine	100	0.95
Prometei		100	0.97
No. 2	Greece	98.0	0.45
No. 3	Ukraine	100	0.49

winter resistance of variety sample No. 2 is due to its origin from Greece where the climate is milder, but the indicator of wintering is excellent for such significant change in climatic conditions.

Cold resistance and adaptability of the studied garlic genotypes

The variety samples No. 2 and 3 showed the low adaptability (CA) because in the first year of cultivation (2017) they did not form the commercial bulbs, all plants of both variety samples formed massive single-clove bulbs while in the second year of cultivation (2018) all plants formed the commercial bulb, indicating a sharp negative reaction to changing conditions of cultivation.

The cultivars of winter garlic Sofiiivskiyi and Prometei have high adaptability – 0.95–0.97 according to the cultivar, due to the fact that these cultivars did not change the place and the conditions of cultivation.

Morphometric indices of studied garlic genotypes

An important varietal sign is this leaf width, because this indicator is less variable among others (Skorina et al., 2014). According to this feature garlic is divided into three groups: I (up to 2.5 cm) – with the narrow leaves; II (2.6–2.9 cm) – with the leaves of medium width; III (≥ 3 cm) – with the wide leaves. The biometric measurements for 60th DAP showed that the first group includes: the standard-cultivar Sofiiivskiyi with a leaf width of 2.3 cm, to the second group – the cultivar Prometei – 2.5 cm, the variety samples No. 2 and No. 3 belong to the group with wide sheets – 4.5 and 4.9 cm in accordance with the specimen and exceed the standard by 95.7 and 113.0% (2.2 and 2.6 cm) (Table 2). The length of the leaf of the experimental variety samples of *Allium ampeloprasum* L. was slightly less than the cultivars of winter garlic. Thus, during the vegetation on the 30th, 60th, 90th DAP the difference between No. 2 and the standard decreased from 9.9 cm (68.8%) on 30th DAP to 5.6 cm (15.0%) on the 90th DAP. The variety sample No. 3 formed a shorter leaf 30th DAP than the standard by 7.6 cm (45.5%), 90th DAP the difference decreased to 2.6 cm (6.5%). The number of leaves of the variety samples No. 2 and No. 3 30th DAP was insignificantly lower than the standard by 0.3 pcs per plant, 60th DAP the number of leaves of No. 2 and No. 3 increased to 7.7 and 8.9 pcs per plant, where the given index was greater than the standard by 0.3 and 1.5 cm. On 90th DAP the variety sample No. 2 had 8.9 leaves per plant which is more than the standard by 1.1 pcs per plant. No. 3 was higher by this indicator than the cultivar-standard Sofiiivskiyi by 2.2 pcs per plant.

Table 2. Morphometric indices of the studied garlic genotypes during the vegetation on the 30th, 60th 90th days after planting (2017–2018)

Cultivar/sample	Leaf width, cm			Leaf length, cm			Number of leaves, pcs.		
	days after planting (DAP)								
	30	60	90	30	60	90	30	60	90
Sofiiivskiyi St.	1.5	2.3	2.5	24.3	41.9	42.8	4.5	7.4	6.5
Prometei	1.8	2.5	2.7	26.2	43.8	44.3	5.7	8.7	7.1
No. 2	2.5	4.5	3.5	14.4	33.4	37.2	5.7	8.7	7.1
No. 3	3.0	4.9	4.2	16.7	36.7	40.2	4.2	7.7	8.2
LSD _{0.05}	0.16	0.15	0.22	1.27	2.64	2.19	0.17	0.37	0.52

Area of the leaf and leaf area index of studied garlic genotypes

The area of the leaf blade of the variety samples No. 2 and No. 3 in the initial stages of growth (30th DAP) (Table 3) was less than the standard by 7.8 and 3.3 cm², on the 60th DAP of *Allium ampeloprasum* L. variety samples exceeded the standard by 31.4% and 65.0% accordingly, 90th DAP of the standard exceeded was 12.5 and 39.5 cm², the same trend was maintained in the analysis of the total leaf area per plant. So, it is possible to assume that these variety samples are more soon-ripe than the usual garlic.

Table 3. Area of the leaf of the studied garlic genotypes during the vegetation on the 30th, 60th 90th days after planting (2017–2018)

Cultivar/sample	Area of the leaf, cm ²			Area of the leaves per plant, cm ²			Leaf area index (LAI)		
	days after planting (DAP)								
	30	60	90	30	60	90	30	60	90
Sofiivskyi St.	23.2	64.9	77.4	60.0	290.0	300.0	0.22	1.07	1.11
Prometei	25.2	72.5	89.8	110.0	380.0	390.0	0.41	1.40	1.44
No. 2	25.2	72.5	89.8	110.0	380.0	390.0	0.24	0.84	0.86
No. 3	15.4	85.3	87.1	60.0	450.0	420.0	0.13	0.99	0.93
LSD _{0.05}	0.99	5.51	5.23	0.06	0.29	0.22	–	–	–

Leaf area index of *Allium ampeloprasum* L. variety samples was smaller than the winter garlic cultivars according to the scheme of planting the number of plants per unit of the area is smaller. Thus, during the vegetation of plants, Prometei cultivar had a higher leaf area index than the standard by 86.4; 30.8; 29.7% on the 30th, 60th, 90th DAP, at the same time, the variety sample No. 2 exceeded the standard by 9.1% on the 30th DAP and it was lower by 21.5 and 22.5% on the 60th and 90th DAP and the variety sample No. 3 was less one than standard by 40,9; 7.5; 16.2%.

Plant height and morphometric indices of bulbs and bulbils of studied garlic genotypes

The biometric measurements have been shown that the introduced forms of *Allium ampeloprasum* L. differ significantly from each other. The variety sample No. 2 had a height of 58.7 cm which is 8.4 cm (14.3%) lower than the standard, while the variety sample No. 3 predominated the cultivar Sofiivskyi by 1.2 cm but it was smaller by 1.1 cm of the cultivar Prometei (Table 4). The variety samples No. 2 and No. 3 had the scape higher than the standard by 11.6–17.3 cm and 18.0–23.7 cm of the cultivar Prometei. By the diameter of the pseudostem, both variety samples of the species *Allium ampeloprasum* L. the cultivars of winter garlic Sofiivskyi and Prometei dominated significantly. Thus, variety samples No. 2 and No. 3 had a diameter of the pseudostem of 13.0–15.1 mm, which is higher than the standard by 4.4–6.5 mm or 51.2–75.6%. A similar dynamics is observed in the diameter of the scape, but the variety sample No. 2 had a slightly higher figure than No. 3. The diameter of the scape of the variety samples No. 2 and 3 was bigger by 4.0–3.9 mm, accordingly, and by 2.5–2.4 mm of the cultivar Prometei.

Allium ampeloprasum L. does not form the aerial bulbs (bulblet), but on the bottom and under the sheath the bulbs from 3–5 to 10 pcs. of large size bulb which have a very dense outer covering and internal transparent parchment scales.

According to the number of bulbils per stem or capsule the cultivars of winter garlic are very much dominated the variety samples of *Allium ampeloprasum* L. whereas the weight of little bulblet of one plant of the variety samples No. 2 and No. 3 is less than the standard by 61.9% and 51.1%, by 119.0% and 104.0% of the cultivar Prometei according to the sample (Table 4). The variety samples No. 2 and No. 3 have a mass of 1,000 pcs of the scape. The scape which is higher than the standard by 670.8 g and 831.1 g whereas the difference between these variety samples and the garlic cultivar Prometei is more than doubled.

It is evident of the obtained data that the number of cloves of the variety samples No. 2 and No. 3 is significantly less than the winter garlic cultivar Sofiiivskyi and is more similar to the cultivar Prometei. So, the variety samples No. 2 and No. 3 have in their structure 7.1 and 5.1 large cloves according to the variety sample which is less than the standard by 2.5 and 4.5 pcs. (Table 4).

Table 4. Morphometric indices of the studied garlic genotypes (2017–2018)

Cultivar/sample	PH,	ISH,	PSD,	ISD,	NoB,	IM,	W _{1,000}	NoC,	NoS,
	cm	cm	mm	mm					
Before harvesting									
Sofiiivskyi St.	67.1	105.9	8.6	4.9	166.8	6.8	40.7	9.6	4.1
Prometei	69.4	99.5	10.1	6.4	78.3	9.2	120.2	5.8	6.0
No. 2	58.7	117.5	13.0	8.9	5.1	4.2	711.5	7.1	5.1
No. 3	68.3	123.2	15.1	8.8	5.2	4.5	871.8	5.1	4.7
LSD _{0.05}	4.23	7.21	0.44	0.37	4.67	0.38	35.05	0.31	0.27
CV (%)	7.4	9.7	24.9	26.9	120.3	37.7	95.6	28.7	16.0

PH – plant height; ISH – scape height; PSD – pseudostem diameter; ISD – scape diameter; NoB – No. of bulbils (bulblet) per stem or capsule, pcs; IM – inflorescence mass; W_{1,000} – weight 1,000 pcs., bulbils (bulblet); NoC – No of cloves, pcs; NoS – Number of sheath on bulb, pcs.

The presence of a large number of covering scales affects the length of storage period of the commercial garlic and reduces the damage by pests and diseases. *Allium ampeloprasum* L variety samples have almost the identical numbers of common sheath and insignificantly dominate the standard cultivar of winter garlic Sofiiivskyi, but their covering scales are very thin, white or almost transparent and have a violation of their integrity even before harvesting and during logistics operations, but this phenomenon does not make a significant influence because *Allium ampeloprasum* L. have a very thick and dense covering scales of the clove from cream to light brown color. The varieties of winter garlic do not possess such characteristics of the covering scales therefore it is important for them to preserve their integrity.

Both variety samples of *Allium ampeloprasum* L. are very similar with each other, so the bulblet of variety samples Nos. 2 and 3 are apparently similar to nut, but the variety sample No. 2 has the bulblet aligned in size, while the bulblet of the variety sample No. 3 differ significantly, the diameter of which may be from 4.0 to 20.0 mm. The bulbils' sheath are creamy and very dense, which requires pre-scarification to be planted. Under the covering scales is a thin parchment scoop similar to a film of white or almost transparent color.

Weight of bulbs and yield of studied garlic genotypes

The average weight of bulb for two years without removing the scape of the cultivar Sofiivskiyi was 35.8 g, which was 28.4 and 53.3 g less than the variety samples No. 2 and No. 3 respectively (Table 5). With the removal of the scape the difference increased to 60.5–68.6 g.

The yield (Table 6) was differed considerably over the years due to the fact that in the first year of cultivating *Allium ampeloprasum* variety samples formed only one-clove bulbs, this phenomenon can be explained by changing of the climatic conditions of cultivation and passing of the adaptation period, as the planting material of both years was the same fraction, and the storage (temperature and humidity) before planting were also the same.

In 2017 the productivity of removal of the scape in both cultivars of garlic grew by 3.4 t ha⁻¹, the yield of the Sofiivskiyi cultivar grew by 27.9%, the Prometei cultivar – by 25.6%. This difference between the No. 2 and No. 3 variety samples could not be detected since one-clove bulbs were formed this year. In 2018 the increase of the yield of garlic cultivars of Sofiivskiyi and Prometei for removal of the scape by 24.5% (2.5 t ha⁻¹) and 27.4% (3.2 t ha⁻¹). Increasing the yield of *Allium ampeloprasum* variety samples were almost the same – 35.0% (4.1 t ha⁻¹) and 35.5% (4.3 t ha⁻¹).

Correlation coefficient of studied garlic genotypes

Bulb yield was found to be significantly and positively correlated with plant height, number of leaves per plant, bulb weight, number of cloves per bulb. This indicated that these attributes were more influencing the bulb yield in garlic and therefore, were important for bringing improvement in bulb yield.

Correlation analysis revealed that *A. sativum* and *A. ampeloprasum* had different levels of correlation between yield and morphometric characteristics. So, *A. sativum* depends on a greater extent on the weight of the bulb ($r = 0.77$), and in turn it depends on the leaf width, plant height and number of leaves (Table 7).

A. ampeloprasum had a greater relation between yield and weight of the bulb ($r = 0.90$), and the weight of the bulb considerably depended on leaf width and their number and height of the plant (Table 8).

Table 5. Weight of bulb (g) of the studied garlic genotypes

Cultivar/ sample	2017		2018		Average	
	RIS	WR	RIS	WR	RIS	WR
Sofiivskiyi St.	43.0	48.2	28.5	36.5	35.8	42.4
Prometei	44.8	54.9	32.7	40.2	38.8	47.6
No. 2	49.1	–	79.2	103.0	64.2	103.0
No. 3	92.6	–	85.5	111.1	89.1	111.1
LSD _{0.05}	3.94	2.41	2.96	3.86	–	–

RIS – without removing the scape; WR – with removing the scape.

Table 6. Yield (t ha⁻¹) of the studied garlic genotypes

Cultivar/ sample	2017		2018		Average	
	RIS	WR	RIS	WR	RIS	WR
Sofiivskiyi St.	12.2	15.6	10.2	12.7	11.2	14.2
Prometei	13.3	16.7	11.7	14.9	12.5	15.8
No. 2	7.3	–	11.7	15.8	9.5	15.8
No. 3	12.4	–	12.1	16.4	12.3	16.4
LSD _{0.05}	0.53	0.76	1.03	1.11	–	–

RIS – without removing the scape; WR – with removing the scape.

Obtained data can be used in perspective by breeders to select the initial forms of garlic classically and by clonal selection.

Table 7. Correlation coefficient of the studied characteristic in *Allium sativum* L. cultivars

Characters	1	2	3	4	5	6	7
1	1						
2	0.18	1					
3	0.29	-0.21	1				
4	0.43	0.05	0.40	1			
5	-0.15	-0.14	0.37	0.58	1		
6	0.42	-0.35	0.30	0.38	0.08	1	
7	-0.09	0.21	-0.39	-0.52	-0.43	-0.03	1
8	0.41	-0.39	0.41	0.03	-0.19	0.77	0.14

Table 8. Correlation coefficient of the studied characteristic in *Allium ampeloprasum* L. variety samples

Characters	1	2	3	4	5	6	7
1	1						
2	-0.42	1					
3	0.64	0.06	1				
4	0.45	0.27	0.53	1			
5	-0.07	0.48	0.26	0.52	1		
6	0.71	-0.12	0.68	0.52	0.11	1	
7	-0.33	-0.31	-0.49	-0.52	-0.32	-0.24	1
8	0.68	-0.40	0.56	0.23	-0.23	0.90	-0.08

1 – leaf width; 2 – leaf length; 3 – number of leaves; 4 – plant height; 5 – scape height; 6 – weight of bulb; 7 – No. of cloves; 8 – yield.

Nutritional value of studied garlic genotypes

According to the results of nutritional value (Table 9), Elephant garlic samples had the better indices than the garlic cultivars for the content of ash and carbohydrates and it influences the caloric content.

Table 9. Nutritional value of the studied garlic genotypes

Cultivar/sample	Ash	Proteins	Fat	Carbohydrates	Energy
	g per 100 g f.w.				
Sofiivskyi St.	1.19	6.3	0.20	20.9	110,60
Prometei	1.62	6.2	0.31	26.7	134,39
No. 2	1.82	6.1	0.12	31.7	152,28
No. 3	1.75	4.5	0.15	35.1	159,75
LSD _{0.05}	0.07	0.34	0.01	1.55	8.84

The content of ash in sample No. 2 is greater than in the garlic cultivars of Sofiivskyi and Prometei by 0.63 and 0.20 g per 100 g f.w., sample No. 3 by 0.56 and 0.13 g per 100 g f.w.

The content of protein in sample No. 2 is lower than Sofiivskyi and Prometei cultivars by 0.2 and 0.1 g per 100 g f.w., sample No. 3 had a lower protein content by 1.8 and 1.7 g per 100 g f.w.

The content of fat in the garlic cultivars of Sofiiivskiyi and Prometei was greater than in the sample No. 2 on 0.08 and 0.19 g per 100 g f.w., in sample No. 3 on 0.05 and 0.16 g per 100 g f.w.

The carbohydrates content of No. 2 and No. 3 is greater in Sofiiivskiyi cultivar by 51.7–67.9%, and by calorie content - 27.6–37.3%.

The caloric value of 100 grams of samples of Elephant garlic was higher than Sofievsky cultivar by 37.7 and 44.4%, and by 13.3 and 18.9% for Prometei cultivar.

The total content of sugar samples of *Allium ampeloprasum* was between cultivar Sofiiivskiyi at 31.2 and 43.5%, cultivar Prometei at 29.6 and 41.7 which explains its lesser taste (Table 10).

Table 10. Free sugars of the studied garlic genotypes (g per 100 g f.w.)

Cultivar/ sample	Free sugars (g per 100 g f.w)			Total Sugars
	Fructose	Glucose	Sucrose	
Sofiiivskiyi St.	0.15	0.11	2.11	2.37
Prometei	0.10	tr	2.30	2.40
No. 2	0.27	tr	2.84	3.11
No. 3	0.15	tr	3.25	3.40
LSD _{0.05}	0.01	–	0.12	0.18

tr – traces.

CONCLUSION

It has been established according to the researches that introduced forms of *Allium ampeloprasum* L. have high indicators of economic and valuable characteristics, but they are limited in the first years of cultivation, by the period of adaptation to the new soil and climatic conditions, which creates some difficulties in their cultivation and reproduction, so as in the first year of cultivation, the coefficient of reproduction with cloves may be zero, but, in general, these variety samples are more productive than cultivars of the winter garlic.

The positive effect of the removal of the scape on increasing the yield of both species was established, where the increase of the garlic cultivars yield was varied within the range of 24.5–27.9% depending on the cultivars, while the increment of the yield in variety samples of Elephant garlic reached to 35.0–35.5%.

The correlation analysis showed that the representatives of both closely related species have significant interdependence between yield, bulb weight, width and number of leaves, but they vary according to the strength of these bonds.

The analysis of nutritional value showed that *Allium ampeloprasum* variety samples had a higher content of ash and carbohydrates, which depended more on calorie content, while garlic cultivars had significantly higher protein content relative for No. 2 and higher fat content relatively to both *Allium ampeloprasum* variety samples.

Therefore, according to the performed analyzes, it can be concluded that *Allium ampeloprasum* L. samples have better chemical composition and higher nutritional value.

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Torrefaction – the process for biofuels production by using different biomasses

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Abstract. Torrefaction process is a mild pyrolysis, where biomass material is converted into solid fuel with higher heating value. The results of torrefaction at different temperatures in a range from 220 to 400 °C for three varied materials, oak wood, mixed wood and dehydrated, granulated sewage sludge are presented. The torrefaction process started with warm up stage, which took place for 30 minutes, after that sample was torrefied for 2 hours at constant temperature. The process continued with cool down stage. The energy demands were covered by electric power, while the flue gasses were not integrated in the process. The influence of the operating temperatures are analysed in order to determine optimal operation parameters to get the torrefied biomass with highest calorific value. Furthermore, the optimal operation time according to the largest increase in calorific value for each material is evaluated. The results of calorific value, mass drop and chemical compositions such as elemental analyses are also presented. Results show that heating values increase with raising temperature for both wood samples. The heating values for sewage sludge increases to approximately 320 °C, after that temperature are unchangeable. Torrefied oak wood samples were more fragile at higher temperatures in comparison to raw or torrefied oak wood samples at lower temperatures. At torrefied sewage sludge samples the changes in fragility are not detected due to pre-prepared granulates of sludge.

Key words: solid fuel, torrefaction, oak and mixed wood, sewage sludge, biomass, energetic evaluation.

INTRODUCTION

Biomass is one of the more important sources to produce energy and synthetic fuels, especially in Slovenia being one of the more forested countries in Europe with over 50% of its area covered by forests. Even though biomass is more expensive than coal, the carbon-trading laws are good motivation for greater usage of biomass. Tenacity of raw biomass is especially challenging, which prevents efficient pulverisation of biomass to use it in higher temperature gasifiers or in boilers of thermal power plants and heating plants. The torrefaction process (mild pyrolysis) is coming to the fore as a possible thermochemical conversion route that enhances the biomass properties obtaining ecologically acceptable energy source, which has similar properties as coal (Trop et al., 2014; Correia et al., 2017). Torrefied biomass is hydrophobic, resistant to biodegradation and is suitable for storage. Furthermore, the homogeneity and heating value of torrefied biomass is greater than that of wood. An important advantage of torrefied biomass is

also its reduced tenacity. The grind ability of the product is higher and easier milling and application in industrial equipment is achieved (Iroba et al., 2017; L. Wang et al., 2017a).

Pyrolysis of wood is used mainly for the energetic exploitation, as the product can replace the fossil fuels (Van der Stelt et al., 2011). Pyrolysis is a thermal decomposition of organic materials at the inert conditions or at a limited inflow of air. This process leads to a release of volatile substances and the formation of product. Furthermore, waste can be converted to products with high heating value by using the pyrolysis process. It is difficult to achieve an atmosphere totally devoid of oxygen; therefore, oxygen is present in small concentration within every pyrolysis system, causing minor oxidation. The process takes place at a controlled concentration of oxygen, consequently careful reaction control is necessary with options for rapid cooling and heating (Yue et al., 2017).

MATERIALS AND METHODS

The comparison between three materials was performed to evaluate the influence of temperature on heating value of the torrefied biomass and to determine optimal operation time according to energy demands.

The materials were oak wood, dehydrated sewage sludge from waste water treatment plant and mixed wood. The calorific value and chemical composition for all raw materials are given in Table 1.

The ash content was determined according to the standard SIST EN ISO 18122: 2016, analytical humidity according to the standard SIST EN ISO 18134-3: 2015, heating value according to the test method of EN 14918: 2010. The total carbon, hydrogen and nitrogen content were determined according to the standard SIST EN ISO 16948: 2015. The sulphur content was determined using the test method ASTM D4239-14e2 by incineration in a tube.

The materials were processed in Bosio electric resistance furnace with nominal power of 2.7 kW. The container was filled with the sample and covered with ceramic lid that the inert atmosphere conditions were reached and air inflow was limited. Ceramic lid was placed in the way that the combustion gasses could discharge. All samples were treated in three parallels.

Table 1. Properties of raw samples

Parameter	Oak wood	Sewage sludge	Mixed wood
GVC/LHV [kJ kg ⁻¹]	19,074/17,793	15,520/14,421	19,722/18,405
Analytical moisture [%]	10.45	8.5	8.78
Nitrogen [%]	0.34	5.87	0.22
Volatiles [%]	79.12	61.14	78.54
Carbon [%]	48.53	36.59	49.6
Ash [%]	3.24	32.58	1.05
Hydrogen [%]	5.89	5.09	6.05
Sulphur [%]	0.02	0,8	0.02

The temperature influence

The process started with warm up stage, which took place for 30 minutes, after that sample was torrefied for 2 hours at constant temperature. The process continued with cool down stage for 30 minutes when the temperature of the furnace reached 50 °C. At

the end the sample was cool down to the room temperature. The energy demands were covered by electric power, while the flue gasses were not integrated in the process.

The experiments were done at 220 °C, 240 °C, 260 °C, 280 °C, 300 °C, 320 °C, 340 °C and 400 °C, according to previous research (Medic et al., 2012; Nanou et al., 2015; Barta-Rajnai et al., 2017; Białowiec et al., 2017; Wang et al., 2017b). The analyses of heating value were performed for each sample.

Optimal operation time

The torrefaction process was performed as it is described in previous sub-section. The materials were treated at 260 °C and for different time periods (0.5 h, 1 h, 1.5 h and 2 h) as it is presented on Fig. 1.

The invested energy was evaluated according to Eq. 1 and 2. The electricity (Eq. 1) was evaluated from furnace nominal power. The invested energy (Eq. 2) was than calculated per sample mass.

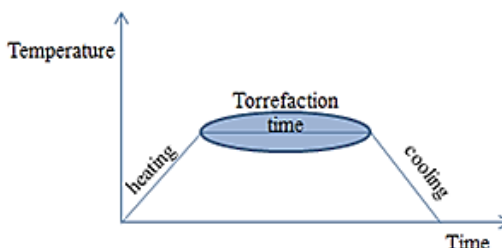


Figure 1. Schematic presentation of the process operation.

$$E_{electricity} = P_n \cdot t \quad (1)$$

where $E_{electricity}$ – electricity (kWh); P_n furnace nominal power (kW), t – time (h).

$$E_{invested} = \frac{E_{electricity} \cdot 3,600}{m_v} \quad (2)$$

where $E_{invested}$ – invested energy (kWh); m_v – mass of the sample (kg).

RESULTS AND DISCUSSION

The samples of oak wood, sewage sludge and mixed wood were processed at different condition. The sewage sludge particles were the same size, because they were previously dehydrated and granulated, while the wood particles were mixed. Optimal torrefaction temperature was determined at the beginning and in the next step optimal operation time was experimentally specified for each material.

Temperature

The comparison of higher heating values (GVC) and low heating values (LHV) for torrefied oak wood, sewage sludge and mixed wood at different temperatures are given on Fig. 2 and Fig. 3.

Fig. 2 presents the values of GVC and LHV for each sample, while on Fig. 3 the differences between torrefied and raw material are presented.

The heating values increase with raising temperature for both wood samples. The heating values for sewage sludge increases to approximately 320 °C, after that temperature are unchangeable or are lower than for raw sample.

Torrefied oak wood samples were more fragile at higher temperatures in comparison to raw or torrefied oak wood samples at lower temperatures. At torrefied sewage sludge samples the changes in fragility could not be detected due to pre-prepared granulates of sludge.

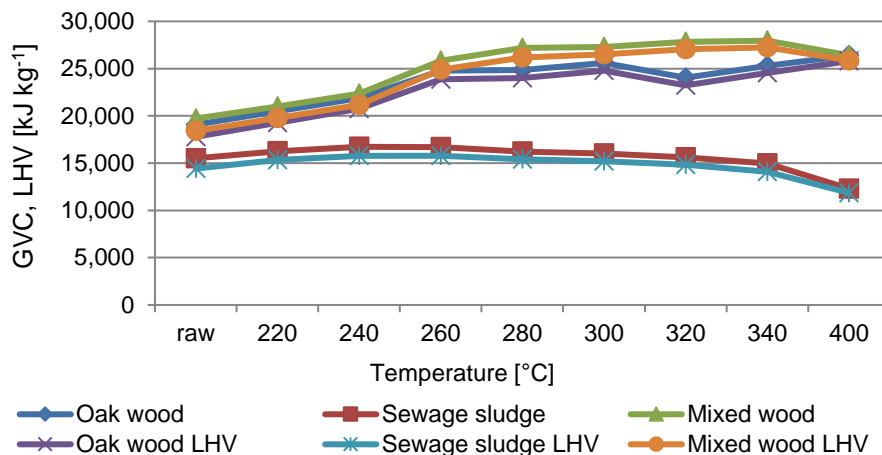


Figure 2. The GVC and LHV for torrefied materials depending on temperature.

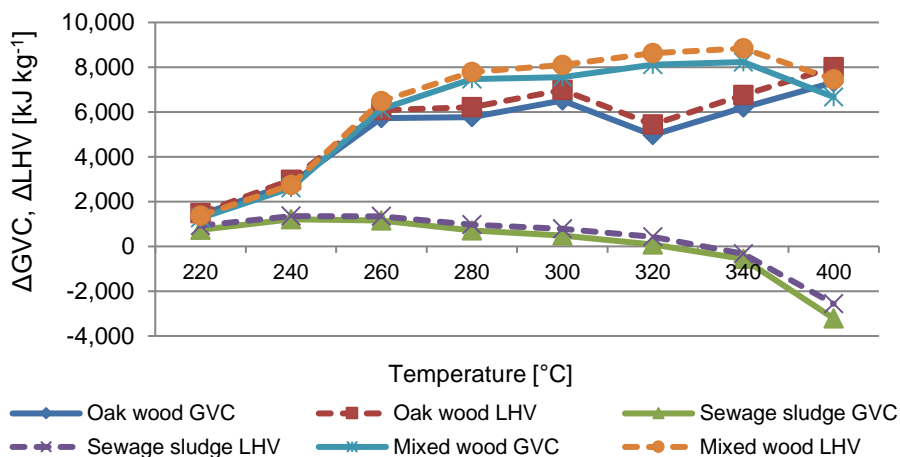


Figure 3. The difference in GVC and LHV depending on temperature.

Operation time

The experiments at different operation time of the torrefaction process were proceed at the constant temperature of 260 $^{\circ}\text{C}$ according to the results from previous sub-section. The temperature was chosen, according to the largest increase of GVC and according to the literature (Barta-Rajnai et al., 2017; Białowiec et al., 2017; Medic et al., 2012; Nanou et al., 2015; Wang et al., 2017a). The elemental analyses of torrefied samples at 260 $^{\circ}\text{C}$ are presented in Table 2.

Samples were torrefied for 0.5 h, 1 h, 1.5 h and 2 h at constant conditions and according to literature (Medic et al., 2012; Li et al., 2015; Nanou et al., 2015; Strandberg et al., 2015; Chen et al., 2016).

Fig. 4 presents the mass drop for all samples depending on operation time.

The LHV and GVC are increasing with time for oak wood and mixed wood (Fig. 5), while the GVC and LHV for sewage sludge is almost the same for different operation time.

Table 2. Properties of torrefied samples at 260 °C

Parameter	Oak wood	Sewage sludge	Mixed wood
Analytical moisture [%]	1.59	0.61	4.72
Nitrogen [%]	0.42	6.26	0.32
Volatiles [%]	47.94	50.75	46.85
Carbon [%]	65.01	39.9	66.66
Ash [%]	5.03	39.61	1.7
Hydrogen [%]	4.24	4.27	4.45
Sulphur [%]	0.01	0.79	0.03

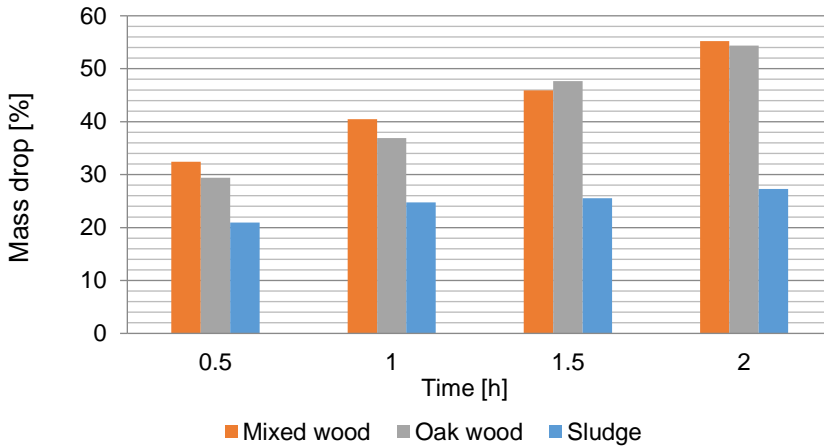


Figure 4. Mass drop for torrefied materials depending on operation time.

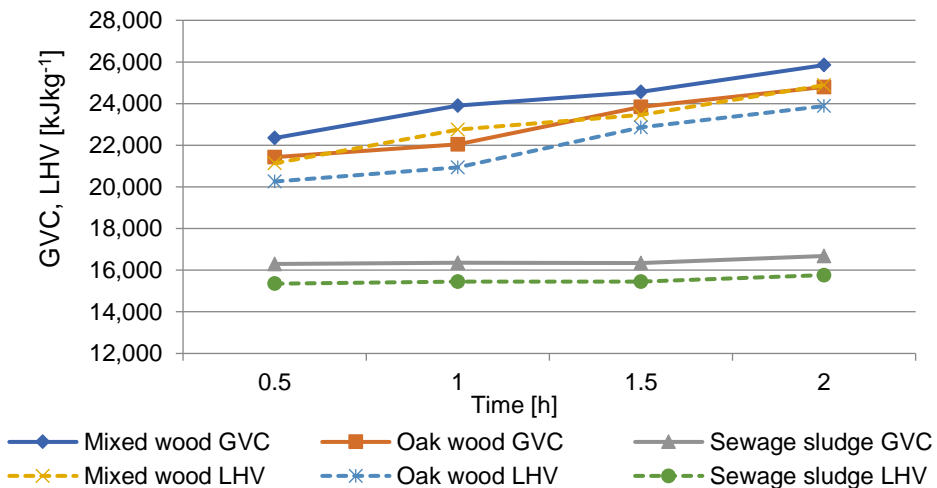


Figure 5. The GVC and LHV for torrefied materials depending on operation time.

The LHV and GVC are increasing with time for oak wood and mixed wood (Fig. 5), while the GVC and LHV for sewage sludge is almost the same for different operation time.

Fig. 6 presents the difference in calorific value between torrefied material and raw material. Also, the invested energy (Fig. 7.) is included, which was evaluated from furnace energy demands, the material mass and operation time according to equation 1 and 2.

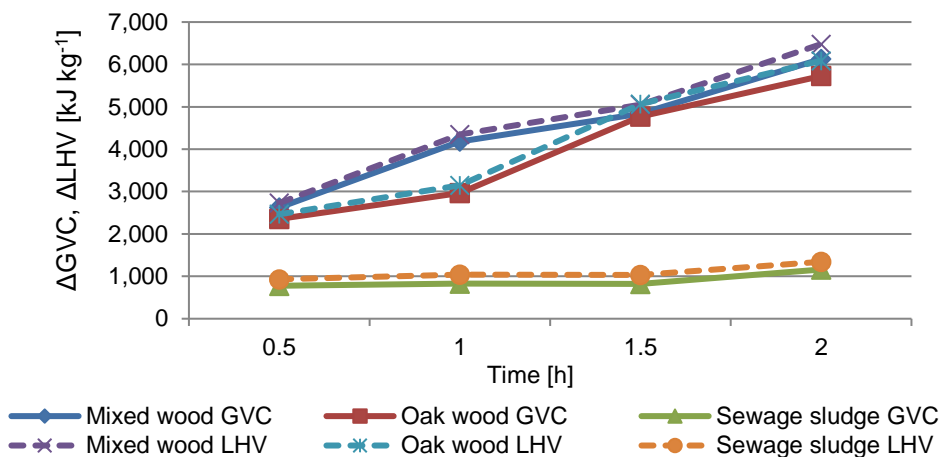


Figure 6. The difference in calorific value between torrefied material and raw material depending on operation time.

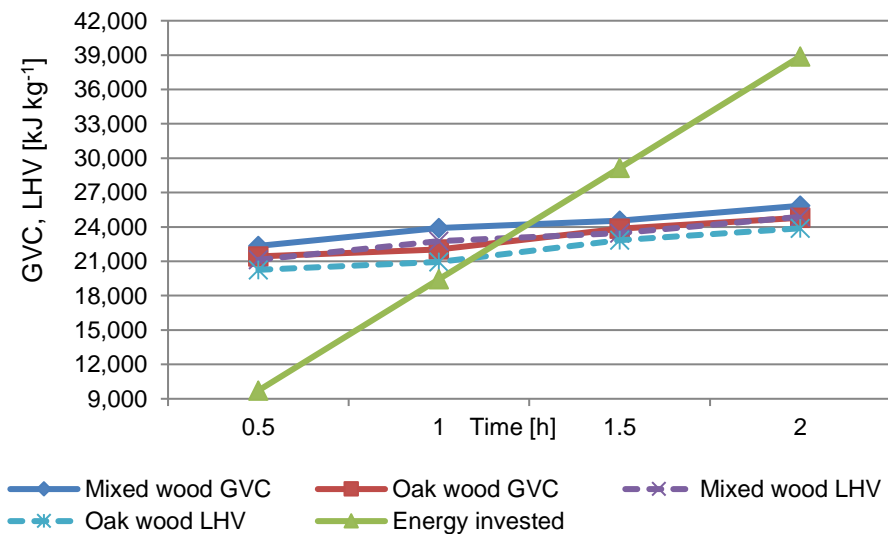


Figure 7. The invested energy, LHV and GVC for mixed and oak wood depending on operation time.

The results on Fig. 6 and Fig.7 show that the optimal operation time in case of oak and mixed wood is around 1.2 h, because till that time the solid fuel with higher heating value is gained. The operation time could be longer if the flue gases would be integrated for energetic exploitation.

CONCLUSIONS

The torrefaction of different biomasses was researched and optimal conditions were experimentally determined. Oak wood, dehydrated sewage sludge and mixed wood were processed at different temperatures, but for the same time (2 h) according to torrefaction conditions. The heating value of all materials increases with the temperature. According to the experimental results it was found out that for this material optimal operation temperature is at around 260 °C, where the higher increase of heating values is achieved. Similar results are presented in various literatures (Li et al., 2015; Strandberg et al., 2015; Chen et al., 2016).

The further research was purposed to determine the optimal operation time of the torrefaction process at previously determined optimal temperature of 260 °C. The results show that the torrefaction is favourable for both kinds of wood and it should take place for around 1.2 h, because there is the higher increase of heating values in comparison with invested energy. On the other hand, the results show that from invested energy point of view the sewage sludge torrefaction is not justified in case, if the flue gasses are not integrated in the process.

In a future work, the integration of flue gases in the process will be done and its influence will be evaluated. Also TGA analyses will be done.

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Enrichment of meat products with dietary fibers: a review

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Abstract. In recent years, much attention has been paid to the development of food products with properties that can promote well-being and prevent disease. Tested strategies to improve the functional value of meat products through the introduction of dietary fiber are reviewed here. Based on the literature reviewed, the addition of dietary fiber in meat products contributes to the fabrication of products which enhance physiological functions. Furthermore, fibers can be used to improve the quality and yield of meat products.

Key words: dietary fiber, meat product, health, functional value.

INTRODUCTION

The meat industry is one of the most important in the world. One of the directions, for the development of the meat industry, is the production of healthy foods characterized by a lower fat content. This development also includes the enhancement of meat products to achieve a higher content in minerals, vitamins, antioxidants or dietary fibers (Cofrades et al., 2008; Zhang et al., 2010; Bis-Souza et al., 2018).

Dietary fibers are recognized to have an important physiological role in the human body. For this reason, dietary fibers are considered before other nutrients to ensure a healthy nutrition. The lack of fibers in the diet is often associated with gastrointestinal diseases, including constipation, colon cancer; increased risks of cardiovascular diseases, including hypercholesterolemia, stroke, and ischemic heart; metabolic diseases, including obesity and diabetes (Jimenez-Colmenero et al., 2001; Fuller et al., 2016).

The fat content significantly affects organoleptic characteristics of meat products. This is because fat has binding properties and contribute to juiciness, tenderness and taste (Mallika et al., 2009). For example, in sausages, it was noted that a low fat content reduces the taste and texture qualities of foods (Choi et al., 2011). Various dietary fibers have, therefore, been used to improve the texture of meat products with a low fat content

(Pintado et al., 2016). The addition of dietary fibers was shown to improve texture properties by Schmiele et al. (2015) and Bis-Souza et al. (2018). Replacing fats with dietary fibers contributes to lowering the calorie content of meat products (Hu et al., 2015).

The recent increased consumer interest in healthy and low fat nutrition, opens the door to the development of a large market for fiber-rich foods. The purpose of this study is to review the actual information on dietary fibers application in the formulations of fortified meat products as well as analysis of their effects on technological properties of meat.

SPECIFICATION OF DIETARY FIBERS

Dietary fibers are generally known as the indigestible portion of food. In fact, the term ‘dietary fiber’ was first used in an article by Hipsley (1953). Trowell et al. (1985) defined dietary fiber as ‘the sum of polysaccharides and lignin not digested by the human gastrointestinal tract’. Later, Jimenez-Colmenero et al. (2001) defined dietary fibers as carbohydrate food components which are not hydrolyzed by the endogenous enzymes in the small intestine.

It is also known that diverse types of fibers have different properties within the gastro-intestinal track such that it is challenging to come up with a definition that encompasses all aspects. Zielinski & Rozema (2013) reviewed the definitions of ‘dietary fiber’, used by the Institute of Medicine, AACC International and Codex Alimentarius Commission and noted one commonality in these definitions: each mentioned positive physiological effects. This reflected that, in the international scientific community, the importance of dietary fibers in human nutrition was increasingly recognized.

Although the definition of dietary fibers is still elusive, Fuller et al. (2016) point to dietary fibers’ modes of action being related to chemical compounds defined by structure, or functional properties, and/or a combination of both structural and functional properties. The various classifications methodologies used to describe dietary fibers reflect such a complexity.

Different characteristics can be used as a basis for the classification of dietary fibers. The type of raw material, the structure of polymers, the amounts and proportions of raw materials and of co-agents, the ability to dissolve in water and water binding capacity can be used to classify dietary fiber. Other characteristics such as the ion exchange, sorption or physiological properties have also been used to classify dietary fibers. The most common classification strategies are described below.

USE OF DIETARY FIBERS IN MEAT PRODUCTS TECHNOLOGIES

Dietary fibers come from grains, vegetables, fruits, and enriched preparations of food products. Traditional sources of fibers and β -glucan in the diet are cereals, such as wheat, oats and barley (Sze et al., 2017). Fruits, vegetables, legumes, soy, psyllium husk and oat bran are good sources of SDF while whole grains are good sources of IDF (Fernandez-Gines et al., 2005). Preparations of cellulose are generally produced from wheat, rice, soybeans, citrus and bamboo. Pectin is extracted from citrus peels and apple pomace. Alginates are anionic polysaccharides isolated from marine algae (e.g. brown algae) or some bacteria. All of these are used in the preparation of meat products.

Oat products are of particular interest for many reasons. To start with, they tend to cost less than meat. In addition, they contain both SDF and IDF (Gramza-Michałowska et al., 2018). Oat fibers also have good water-absorption capacity and, as a result, they are often used in the production of emulsion-type products such as sausages, pâtés, as well as in minced meat products (like burgers). Finally, they enhance the flavor and texture of some meat products, like minced meat and pork sausages (García et al., 2002). It follows that oat flour (Serdaroglu, 2006), oat bran (Talukder & Sharma, 2010) and different preparations of oat fibers are used for the enrichment of an array of meat products.

Fruits are also an important source of dietary fiber. This source can also be cost effective because fruit fibers can be obtained as byproducts of the production of juices and other plant products. These fibers can be used alone or in combination with cereal fibers, in the formulation of meat products. Dietary fibers present in the skin of the fruit are considered functional ingredients in the formulation of meat products due to their water-holding capacity and low cooking loss (García et al., 2002). In this category, watermelon rind powder (WRP) is a rich source of dietary fibers and bioactive compounds that could be used in the development of functional foods. The high total dietary fiber content in the WRP indicates promises for food product enrichment applications to produce high-fiber, value-added, healthy foods (Naknaen et al., 2016).

Other interesting sources of dietary fibers are also chitin and chitosan. The chitin (polymer of β -(1,4)-linked 2-acetamido-2-deoxy-D-glucopyranose) is found mainly in the animal kingdom, it is structurally similar to cellulose (Kardas et al., 2012). Chitin is available from the shells of marine crustaceans such as crabs and shrimps; shells and skeletons of mollusks, krill, and insects as well as the cell walls of fungi (Kurita, 2006; Kardas et al., 2012; Cheung, 2013). Chitosan is a linear polysaccharide obtained by extensive, although not total, deacetylation of chitin. Chitin and chitosan are mainly composed of the random association of two kinds of β -1,4 linked structural units: 2-amino-2-deoxy-D-glucose and N-acetyl-2-amino-2-deoxy-D-glucose. Chitin and chitosan impose stability on food emulsions. The addition of chitin and chitosan to diets as a source of fibers has been deemed completely safe.

Dietary fibers are also found in the rhizomes of plants. Their high content in the lotus rhizome, chory root, Jerusalem artichoke is well known (Tomaschunas et al., 2013; Afoakwah et al., 2015; Ham et al., 2017).

Functional and technological effects of dietary fibers used in meat products

Dietary fibers have helpful functional properties which can enhance the quality and sensory characteristics of meat products. These functional properties depend on the types of dietary fiber incorporated to products. The most important functional properties of dietary fibers used in meat products are water-holding and fat-binding capacity, viscosity, gel-forming ability and emulsification properties (Kim & Paik, 2012). The water-holding capacity of dietary fibers is dependent on the structure, the chemical composition (Chau, 2003) and the relative proportions of different types of fibers. The addition of fibers to meat products can cause the following technological effects:

- 1) increase the moisture-retaining capacity of minced products,
- 2) improve the stability of emulsions,
- 3) substitute fat, reduce fat content,
- 4) increase the yield of the product,
- 5) improve the texture of meat products,

- 6) retain the shape of the product after heat treatment and
- 7) Stabilizes fats and proteins, which leads to increased storage stability.

When formulating meat products, the type of dietary fiber, chemical organization of the additive, and consumer properties of the enriched meat product must be taken into account. Different types of additions associated functional properties and concomitant technological effects are summarized in Table 1.

Applications of different additives in the technology of meat products

Most scientific analytical reviews of the applications of dietary fibers are based on the source of dietary fibers (cereals, vegetables, fruit, etc.). However, such a classification does not take into account how the dietary fiber additives were derived. Additives processing influences chemical composition. Thus, the impact of these additives on the properties of the product can also be different. For example, oat flour, oat bran and a purified preparation of dietary fiber from oat can present significant differences in the concentration of dietary fiber and other nutrients. Accordingly, the dosage of these additives, as well as how the additives were derived can influence the properties of the product. Consequently, the applications of different additives in the technology of meat products were reviewed for the three groups of sources of derived dietary fibers listed below:

- 1) raw natural materials (vegetables, fruits, grains, flour, etc.),
- 2) secondary products of food processing (soy okara, bran, etc.), and
- 3) preparations of dietary fibers (wheat fiber ‘Vitacel’ and ‘Jelucel’; soy fiber ‘Protocol’, citrus fiber ‘Citri-Fi’, potato fiber ‘Potex’ and ‘Lyckery’, etc.).

Applications for natural raw materials

Flours are included in this category. Flours are obtained from various natural raw materials. The effect of cereal and legume flour (wheat, barley, oat, rye, rice, corn, soy, chickpea and yellow lentil flour) on the physical, chemical, and sensory properties of beef patties has been determined. Cereal and legume flour increased yield, moisture, and fat retention and limits diameter reduction values. Oat flour increased moisture retention, texture, flavor and overall acceptability values of the cooked beef patties. Enriching minced meat with oat flour was found to increase fat and moisture retention but significantly change the cooking properties of patties. It, however, did not negatively affect organoleptic properties. This addition has also economic repercussions as it increases the profitability when selling burgers (Serdaroglu, 2006). Among the legume flours, chickpea flour had higher performance on the sensorial properties of beef patties (Kurt & Kili n ekker, 2012). The addition of chia flour as chicken skin substitute (15%) allowed to produce chicken nuggets with ‘high fiber content’ (Barrosa et al., 2018). Others have noted that the inclusion of 10% chia flour and olive oil in sausages allowed to increase the total amount of dietary fiber (98% IDF) (Pintado et al., 2016).

Vegetable fibers can be an attractive option to enrich certain meat products. These dietary fibers can be used as fat substitute and they tend to be used as additives to enhance the texture (juiciness, and tenderness) of certain types of meat products. Despite the fact that vegetables and fruits are a good source of dietary fiber, using them in raw form for enrichment of meat products is inefficient. This is because they contain a large amount of moisture, which can adversely affect the functional and technological parameters. In this case, the quantity of introduced dietary fiber has to be kept to a very small proportion. Alternatively, a larger portion of dried fruit and vegetables can be used.

Table 1. Dietary fibers and their technological properties in meat products

Fiber sources	Dietary fiber components	Functional properties	Technological effect in the meat product	Reference
Cereal grains and bran: wheat, ray, rice. By-products: hulls, husk.	Cellulose, hemicellulose	Water-holding and fat-holding capacity	Regulate the moisture content in the meat products and crystallization during freezing increase cooking yield and firmness; substitute fat in chicken meatballs and beef patties	Talukder & Sharma, 2010; Gibis et al. 2015; Hu & Yu, 2015
Fruits and vegetables. By-products: seeds of berries and fruits; apple, pear, tomato pomace; citrus peel	Cellulose, hemicellulose, pectin	Water-holding capacity, viscosity, gel-forming ability due to the high content of polyphenols, it exhibits antioxidant properties	Modify moisture, texture and color brightness of meat products. Improve emulsion stability and cooking yield, increase shelf-life, prevent lipid oxidation of chicken product	Turksoy & Ozkaya, 2011; Cava et al., 2012
Oats, barley grains, bran and flour	Beta-glucan, cellulose, hemicellulose	Viscosity, Water-holding and Fat-holding capacity	Decrease cooking losses and reduce fat content improve the flavor, texture, and palatability of beef and pork patties, sausages, and meatballs	Yilmaz & Daglioglu, 2003; Serdaroglu, 2006; Choi et al., 2011; Schmiele et al., 2015
Legumes: soybeans, beans, peas flour	Resistant starch, cellulose, hemicellulose	Water-holding capacity, Gel-forming ability	Reduce fat content, increase cooking yield and protein content. Improve emulsion stability, minimize the production cost of bologna sausage and chicken nuggets	Serdaroglu, 2006; Pietrasik & Janz, 2010
Chicory root, Jerusalem artichoke	Inulin, fructooligosaccharides, cellulose, hemicellulose	Water-holding capacity, gel-forming ability	Substitute fat in low-fat meat products. Promote the development of acceptable color as well as textural and sensory properties of meat products	Sun et al., 2010; Tomaschunas et al., 2013; Afoakwah et al., 2015

Table 1 (continued)

Gum guar, gum arabic, algae, agar, alginates	Gums, carageenans, alginates	Viscosity, water-holding capacity, fat-binding capacity	Replace some portion of fat in meat products. Provide stability of emulsion, and gel texture improve cooking yield, texture and water retention in the sausages and frankfurters improve cooking characteristics and decrease mass transfer and diameter reduction, provide high yield, improves the thickness of products extend the shelf-life of minced pork patties	Beriain et al., 2011; Moroney et al., 2013
Fungi and shellfish, shells of marine crustaceans	Chitin/chitosan	Gel-forming ability, high viscosity, water-holding capacity.	Stabilize the structure of meat products that leads to minimized shrinkage and improved product density improve stability of the meat emulsion	Cheung, 2013

Aloe vera is one of the interesting sources of natural dietary fiber that can be used in the technology of meat products. The results of studies on beef burgers with added Aloe vera, as well as goat meat nuggets with fresh Aloe vera gel, shown that it could be used to improve the quality of meat products. The authors noted 2.5% of Aloe vera, lead to an improved cooking yield of goat meat nuggets (Soltanizadeh & Ghiasi-Esfahani, 2015; Rajkumar et al., 2016).

The interesting source of dietary fiber is jabuticaba skin flour, obtained from jabuticaba fruit planting in Brazil. Alves et al. (2017) determined that the restructured hams with addition of 0.5% jabuticaba skin flour had virtually no changes in the physicochemical properties, except for the increase of fiber, phenolic compounds and hardness and reduced brightness.

Applications for secondary food processing additives

Bran is an example of secondary processing additive. As a source of dietary fibers in chicken patties, Talukder & Sharma (2010) found that oat bran contains more SDF than wheat bran. They also found that IDF were higher in wheat bran. The addition of bran resulted in an increase in water holding capacity and emulsion stability, as well as a significant increase in yield. The authors recommended the introduction of 10% oat and 15% wheat bran in chicken patties (Talukder & Sharma, 2010). In the studies of Choi et al. (2015), fat was partially replaced with rice bran fiber in sausages. The authors have measured the chemical, textural and sensory properties of these lower fat sausages. It was determined that the addition of rice bran fibers (2%) improved the taste while not significantly affecting textural attributes and it allowed to reduce the fat content by 12 to 30%. In Yilmaz studies (2004) rye bran was used in various amounts (between 5 and 20%) to replace fat in meatballs. The product saw an increase in its nutritional value and a decrease in its total content of trans-fatty acids. Thus, the authors concluded that rye bran can be used as a source of dietary fiber in meat products.

The processing of large quantities of vegetables and fruits generally produces by-products that are rich in dietary fibers. This wide spread and low cost raw material can be used as dietary fibers by the industry (Seo & Kyung, 2015). By-products of citrus, such as lemon albedo and orange fiber powder was added in various concentrations to cooked and dried sausages (Fernández-López et al., 2004). The authors determined that the addition of lemon albedo in an amount of 2.5% to 7.5% did not worsen the organoleptic properties of sausages.

Okara is a by-product of the soy and tofu industries (Turhan et al., 2009). It is used in the production of beef patties in raw (Turhan et al., 2007) and in dried form (Turhan et al., 2009). The authors recommend adding no more than 7.5% of raw okara and not more than 22.5% of dry okara (Turhan et al., 2007; Turhan et al., 2009) in beef patties.

Applications of preparations of dietary fiber additives

Oat, rice and rye fibers are used by the food industry to enrich certain types of meat products. These types of dietary fibers tend to provide good water absorption capacity, they can enhance taste and they can offer an economical advantage as they are less costly than meat.

High absorption or bleached oats fibers have been added to determine their effects on quality characteristics of light bologna and fat-free frankfurters (Steenblock et al., 2001). The results showed that the addition of both types of oat fibers produces higher

yields but, in the sausages, oat bran fibers produced a harder product (high shear stress) and contributed to the production of sausages containing less humidity. High absorption and bleached oats fibers appear to produce different textures depending on the product in which they are used (Steenblock et al., 2001).

Jongaroontaprangsee et al. (2007), and Nilnakara et al. (2009) have obtained fibers from the outer leaves of cabbage and Seo & Kyung (2015) produced dietary fibers from Chinese cabbage waste. From these studies, it was determined that the powder from cabbage outer leaves possessed high water-holding and swelling capacities, indicating potential for use in many food applications (Jongaroontaprangsee et al., 2007). In addition, Seo & Kyung (2015) noted that dietary fibers derived from Chinese cabbage waste have probiotic, hypoglycemic and hypolipidemic effects.

Potato, cactus pear and pineapple fibers were also tested to improve meat products. The addition of dietary fiber from dry potato pulp, Potex and enzymatically extracted non-starch polysaccharides in pâtés allowed not only to reduce the content of animal fat, but also to improve the texture of the product. Diaz-Vela et al. (2017) used cactus pear and pineapple fibers as a source of dietary fibers in boiled sausages.

Soy fibers are used in meat products to increase yields because of their high water- and fat-holding capacities. Soy fibers are used to improve the structure of the sausage (Cofrades et al., 2008). In minced meat, the introduction of a soy fiber 'Protocol' allows to condense the structure of the finished product by creating a 'three-dimensional skeleton'. This is particularly useful when low grade raw materials are used.

Inulin is a SDF which can be used as a fat substitute in meat products. For example, Mendoza et al. (2001) conducted a study using dry fermented sausages with a fat content close to 50 and 25% of the original amount by adding 7.5 and 12.5% of inulin, respectively. The results showed that the dry fermented sausages obtained had technological properties similar to conventional sausages, while they had a softer texture and tenderness, springiness and adhesiveness. The low-calorie dry-fermented sausages are currently available on the market and they contain approximately 10% inulin (Mendoza et al., 2001).

Pea fibers (obtained from the inner cell wall of yellow peas) contain about 48% fat, 44% starch, and 7% protein. This additive was added to beef patties, in a dry form. The aim was to lower fat by 10% to 14%. The recipe led to an improvement of the tenderness and increased the yield with no detriment on juiciness and flavor (Anderson & Berry, 2000).

Lopez-Marcos et al (2015) obtained dietary fibers from different agro-industrial co-products. These included lemon dietary fibers, grapefruit dietary fibers, pomegranate dietary fiber, lemon albedo dietary fiber, and tiger nut fiber. Studies conducted using the obtained products showed that the emulsifying capacity and emulsion stability was generally high. On that basis, the authors suggest the possibility of using such types of dietary fibers in products that require emulsifiers and have a long shelf life (as they require long-term stability). The authors determined that lemon dietary fibers and grapefruit dietary fibers samples, with the highest soluble dietary fiber content, showed higher water holding capacity values than tiger nut fiber samples (with the lowest soluble dietary fiber content). In addition, the highest fat / oil binding values were obtained for lemon dietary fibers and lemon albedo dietary fiber samples, suggesting that the higher the ratio of the soluble dietary fiber / insoluble dietary fiber, the higher the fat / oil binding capacity. The authors also indicated that these fibers have the potential to reduce the adsorption of cholesterol (Lopez-Marcos et al., 2015).

Garcia and others (2002) conducted studies on reduced-fat dry-fermented sausages prepared with the addition of 1.5 and 3% cereal (wheat and oat) and fruit (peach, apple, and orange) fibers (García et al., 2002). The authors noted that the best sensory characteristics were obtained in sausages with the introduction of 1.5% fiber, especially orange fibers. In this case, it was found that a higher amount of fibers worsened the texture (Fernandez-Gines et al., 2005).

The sections above provided examples of how materials containing fibers can affect physical properties of meat products. Data reported in the literature on the effect of dietary fibers in various meat products with an indication of the established optimal dosages are summarized in Table 2.

Using combinations of dietary fibers in the formulation of meat products

Recently, a number of studies have been conducted on the usage of combinations of various types of dietary fiber in meat products. For example, Petridis et al. (2014) studied the cumulative synergistic effects of citrus fiber, rice bran and collagen on the texture and selected sensory characteristics of frankfurter-type sausages. An emulsion from pork skin has been used as a source of collagen. The most acceptable formulations were with 13% of collagen addition, 1.5% of citrus fiber and 0.5% of rice bran or 13% of collagen addition with 2% of citrus fiber. The authors mentioned that, for both formulations low fattiness and brittleness were achieved while a moderate elasticity was obtained. Using both formulations, sausages were adequately hard and cohesive and differed solely in color intensity (moderate vs light reddish) (Petridis et al., 2014).

Kılınçeker & Kurt (2018) studied the effects of inulin, carrot, and cellulose fiber additions on chicken meatballs. The authors noted that the use of fibers in meatballs can boost product quality and improve color properties (L, a, b values). Cellulose and carrot fibers augmented the yield and moisture absorption values while preventing diameter reduction of the fried samples.

Combinations of different types of fibers additives as part of meat formulations are currently being studied. It follows that we will be seeing more of these types of products in the market place.

Various non-traditional dietary fibers are used in other food products. Their high functional and technological potential show great promise for their integration in meat product formulations.

CONCLUSION

Although existing definitions of dietary fibers have common concepts and elements, no consensus has been reached to date. This may be due to the diversity of dietary fibers, the multiplicity of forms in which they may be available and, therefore, the complexity of possible physical and chemical interactions that may be taking place within the gastro-intestinal tract. The modern classification systems for dietary fibers reflect this. It is wide and diverse and can be based, for example, on origin, structure, solubility or physiological effect. Numerous positive physiological effects of the use of dietary fibers have been documented. These include curative and preventive effects for diseases or conditions such as obesity, certain types of cancers, cardiovascular diseases, diabetes, and constipation. Research on various types of dietary fibers continues to contribute new data on the health benefit of dietary fibers.

Table 2. Effects of dosages of dietary fibers on the properties of various meat products

Groups	Source of fibers	Meat product	% Recommended	Determined effect	References
Natural product	Barley flour	Sausages	3.9 to 6.9*	Increase water absorption index and viscosities, reduce fat content	Choi et al., 2011
	Chia flour	Chicken nuggets	10	Decrease the moisture, saturated fatty acids and monounsaturated fatty acids contents; lower the acceptability of the meat product	Barrosa et al., 2018
		Sausages	10	Increase the total amount of dietary fiber	Pintado et al., 2016
	Oat flour	Beef patties	4.0	Increase the juiciness scores, no effect on other sensory properties, improve the cooking characteristics	Serdaroglu, 2006
			5.0	Increase moisture retention, odor, texture, flavor and overall acceptability values	Kurt & Kili�n�ç�eker, 2012
	Legume flour	Beef patties	5.0	Increase yield, moisture, and fat retention and decrease diameter reduction values	
	Aloe vera gel	Goat meat nuggets	up to 2.5	Decrease pH value and protein content; reduce the lipid oxidation and microbial growth during storage	Rajkumar et al., 2016
Jabuticaba skin flour	Restructured hams	0.5	Higher contents of phenolic compounds; greater weight loss; a darker shade; texture profile with smaller parameters of stiffness, cohesiveness, adhesiveness, flexibility and chewiness	Alves et al., 2017	
Secondary food processing	Rye bran	Meatballs	20	Reduce total trans fatty acid, increase ratio of total unsaturated fatty acids to total saturated fatty acids, reduce weight losses, improve nutritional value, health benefits and color	Yilmaz, 2004
	Chickpea hull flour	Chicken nuggets	5	Decrease in total cholesterol and glycolipid content, reduce sensory scores	Verma et al., 2012
	Rice bran	Frankfurters	2	Reduce the moisture, ash, carbohydrate, energy value, cooking loss, and total expressible fluid, improve flavor and overall acceptability	Choi et al., 2015
	Wheat bran	Chicken meat patties	15	Increase the water holding capacity and emulsion stability; increase in cooking yield, firmness, reduction in sensory attributes, moisture, protein, fat and cholesterol content.	Talukder & Sharma, 2010
	Oat bran		10		
	Okara powder	Beef patties	up to 7.5	Reduce the cholesterol content, increased the energy values; improve whc, cook loss and shrinkage, increase the pH, lightness and yellowness values	Turhan et al., 2009

Table 2 (continued)

Preparations of dietary fibers	Oat fiber	Light bologna, frankfurters	3	Increase yields and hardness and contribute to lighter red color	Steenblock et al., 2001
	Fruit (orange) fiber	Dry fermented sausages	1.5	Reduce energy value; reduce fat without loss of sensory quality	García et al., 2002
	Inulin	Emulsion type sausages	6	Reduce fat, energy content and color measurement; sensory evaluation comparable to the traditional product	Berizi et al., 2017
		Fermented sausages	10	Reduce calorie, improve softness, tenderness, springiness, and adhesiveness	Mendoza et al., 2001
	Bacterial cellulose (Nata)	Chinese-style meatball	10	Acceptable textural and sensory qualities, decrease in cohesiveness value	Lin & Lin, 2004
	Soy fiber	Bologna sausages	2.5	Improve fat and water binding properties, reduce fat, decrease textural properties and increase weight loss	Cofrades et al., 2008
	Guar gum, xanthan gum, gum Arabic	Fried beef patties	1.5	Effect on yield and diameter reduction; increase the moisture retention; gum arabic increase lightness and yellowness values	Kilincceker & Yilmaz, 2016
	Microcrystal-line cellulose	Beef patties	2.0	Decrease moisture loss; improve the texture in the sensory evaluation had more juiciness than the control and had a fat-like mouthfeel	Gibis et al., 2015
Combined	Citrus fiber	Frankfurter sausages	1.5	Positive effect on the acceptability of the samples. Adequately hard and cohesive, differing solely in the intensity of color (moderately and low reddish)	Petridis et al., 2014
	rice bran		0.5		
	pork skin		13.0	Improve color properties (lightness, yellowness values), improve the technological properties	Kılınççeker & Kurt, 2018
	Inulin,	Chicken meatballs	3		
	carrot fibres		9		
cellulose fibres		6 or 9			

*for different fraction.

In recent years, more and more sources of dietary fibers have been discovered and tested. In this review, the information relevant to meat products was gathered. Traditional sources of dietary fibers added to meat products include cereals, vegetables and fruits. Non-traditional sources of dietary fibers include fungi, and secondary products of animal processing. Fibers can be used in raw and processed forms. Formulations now comprise combinations of dietary fibers.

All classes of dietary fibers can be used in the fabrication of healthy meat products (usually with lower fat content). In most cases, formulations can be adjusted such that additional sensory and functional-technological indicators (moisture binding, emulsifying ability, palatability, color) benefits can be offered. In some cases, the taste can also be improved.

Consumers recognize that their health can be improved through diet and this review showed that, as results, there is a trend for an increasing use of products containing dietary fibers amongst consumers. In fact, at the moment, the use of dietary fiber in the production of meat products is becoming widespread. It follows that manufacturers are looking to improve the functional and technological properties of their products. Meat manufacturers are also interested in increasing the yield of their products. With both consumers and manufacturers engaged, the use of dietary fiber will no doubt contribute to improving the health of the population. It will also contribute to the enhancement of functional properties of many meat products while promoting the usage of available raw materials from other food industry sectors.

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Danielyan, S.G. & Nabaldiyan, K.M. 1971. The causal agents of meloids in bees. *Veterinariya* **8**, 64–65 (in Russian).

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