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VIGOUR OF MAIZE SEEDS PRODUCED AT THE MAIZE RESEARCH INSTITUTE "ZEMUN POLJE"

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Abstract: Maize is one of the economically most important crops. Its immense significance arises from its various uses, high yielding and the production scope. In order to establish a lucrative production, first-rate seeds are required. Therefore, the aim of this study was to test the vigour of maize seeds conventionally and organically produced. The seeds of the maize variety Rumenka were used and tested at the Maize Research Institute "Zemun Polje" in its Seed Testing Laboratory. According to the results, it can be concluded that although there was a minimal difference in the percentages of the first count of germinated seeds between the two types of seeds, the total germination was equal (98%). The results of the germination test performed after accelerated seed ageing indicate that total seed germination was higher in organic than in conventional seeds (39% vs. 33%). The electrical conductivity of the leachate per gram of weight of conventional maize seeds averaged 4.90 µS/cmg and 3.66 µS/cmg for organic maize seeds. According to stated values, it can be concluded that maize seeds of both production types are characterised by high viability and are suitable for earlier sowing under unfavourable environmental conditions. The results show that the radicle emergence was uniform amounting to 84% in conventional seeds and to 85% in organic seeds.

Key words: *Zea mays* L., germination, accelerated ageing, electrical conductivity.

Introduction

Maize (*Zea mays* L.), a field crop of universal significance, is primarily intended for human and animal nutrition, as well as a raw material for the processing industry (Pandurović, 2014). Furthermore, maize belongs to the family of grasses (*Poaceae*) and is an annual, monoecious, cross-pollinated crop that differs from other members of this family by its morphological traits. The maize

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kernel is actually a one-seeded fruit/grain and contains on average about 8–9% protein, 5% oil, 1.5% cellulose and 70% starch (Vančetović et al., 2012). In addition to its high energy value, the maize kernel has a relatively low nutritional value, because it is poor in digestible proteins and some other important nutritional substances. Breeders worldwide and in our country have been working very intensively to develop different forms of maize with specific production and quality grain traits in order to develop new genotypes such as red-seeded maize hybrids. The grains of the recently developed genotypes have a higher nutritional value for organisms of humans and domestic animals (Glamočlija et al., 2016). Maize is a very important part of every state economy, as it can be the basis for significant financial resources as an export item, which means that maize is of strategic importance for every state. The Republic of Serbia is an important producer of cereals at the regional level and in the European market. In recent years, maize has been grown on an area of around 1,200,000 ha (about 35% of arable land in Serbia), with an average yield of about 4.4 t/ha. Globally, the average maize yield increased by almost 70% during the period between 1980 and 2020. This increase was the result of a permanent progress in the breeding process and the development of increasingly high-yielding hybrids, as well as improvements in cultivation technology and the development of the new agricultural machines, mineral fertilisers and pesticides (Starčević and Latković, 2006). Organic farming positively affects natural biodiversity as it excludes the application of pesticides and GMOs. Contrary to conventional farming, organic farming favours preventive rather than corrective measures and integral plant protection technology (Bekavac, 2012). In addition, organic food production, especially in rural areas, utilises indigenous species and local varieties. Organic maize production in Serbia still takes place on small areas in comparison with conventional maize production. GMO seeds are not used in the organic maize production. Furthermore, the application of mineral fertilisers and the use of synthetic plant protection products are not allowed in this type of production. Therefore, the final product is a healthier food with a higher nutritional value compared to classical/conventional farming (Golijan and Marković, 2018).

The objective of the present study was to evaluate the vigour of conventional and organic maize seeds by comparing parameters of germination, accelerated ageing, electrical conductivity and the rate of radicle emergence and to determine possible differences between conventionally and organically produced seeds.

Material and Methods

The seeds of the red-seeded maize variety Rumenka, conventionally and organically produced in the 2014 production year, were tested. The field trial was set up in the location of Zemun Polje in 2014. Two plants were sown per hill and

per row. The distance between the hills was 40 cm, while the inter-row distance was 75 cm. The sowing density was 66,700 plants ha-1. The elementary plot size amounted to 300 m^2 (0.03 ha) (6 m^2 x 50 rows). The harvest was performed in October and yielded 246 kg (fresh cobs). The following traits were tested and determined in the laboratory of the Maize Research Institute "Zemun Polje" during 2016: standard germination, electrical conductivity, rate of radicle emergence and the accelerated seed ageing test was performed.

1. The **seed germination test** was carried out in the standard germination chamber. The seed testing paper was used for the germination test. The test was done on four replicates of 100 maize seeds. Selected samples were grown on the moist paper. They were covered with filter paper, rolled up and placed into the chamber with the altering temperature of 20/30°C. The first count was made four days after the seeds had been placed into the chamber. The standard germination test for maize seeds lasted seven days (ISTA, 2016).

2. **The accelerated seed ageing test** was conducted in the accelerated ageing chamber. First, the working sample was drawn. Two hundred seeds of each genotype were placed in the accelerated ageing boxes. The seeds were placed on a mesh screen and suspended over 40 ml of distilled water. The seeds should not come into contact with water. Then, each box was covered with the lid and placed in the accelerated ageing chamber. The duration of the test period was 72 h from the moment when the maximum humidity was reached and when the temperature in the chamber reached 43°C (ISTA, 2016).

3. **The electrical conductivity test of seeds** was done using a conductivity meter that directly reads the values with the direct or the alternating current using a submersible cell. From the fraction of pure seeds with a moisture content ranging from 10% to 14%, two replicates of 50 maize seeds each were randomly counted and the weight was measured. Erlenmeyer flasks (eight for the seed samples and two as controls) were used for this test. They were first washed with distilled water, then filled with 250 ml of distilled water with a conductivity below 5 μS/cm and then covered. The prepared flasks were stored at a temperature of 20°C for 24 h. The reading had to be taken within 15 minutes of taking the flasks in order not to distort results. Prior to the use, the conductivity metre was calibrated using two standard solutions. When measuring the electrical conductivity, the electrical conductivity of the control Erlenmeyer flasks was read first and then that of the samples themselves. The Erlenmeyer flasks with the samples were slightly shaken and the cell was immersed. The reading was completed when stable conductivity values were obtained (ISTA, 2016).

The seed membrane integrity is indirectly determined by the electrical conductivity test. This test is used to assess the seed vigour, because it detects the deterioration process of the seed already in its early phase. The basic water conductivity was measured. Then, the conductivity per gram of the seed weight

was calculated for each replicate. The average of two replicates was a test result for a certain seed lot. The conductivity of each replicate was estimated by the use of the following formula:

$$\text{conductivity}\left(\frac{\mu S}{cm \times g}\right) = \frac{\text{conductivity value}\left(\frac{\mu S}{cm}\right) - \text{basic value}}{\text{replication weight (g)}}$$

4. **Radicle test.** A working sample of 8 x 25 seeds was drawn and placed on the wet testing paper at 20°C. The number of seeds from which radicles developed was determined after 66 hours. Based on this number, their proportion was calculated (ISTA, 2016).

The obtained data were processed and graphically displayed by using the Microsoft Excel 2010 programme.

Results and Discussion

Seed germination

Figure 1 shows the first count of the tested maize seeds produced by conventional and organic methods, which was done four days after sowing for the standard germination tests. The results show that the first count of germinated maize seeds produced conventionally amounted to 88%, 93%, 90% and 82% over the replications, which means that the average first count was 88%. In the case of organically produced maize seeds, these percentages amounted to 90%, 91%, 91% and 91% over the replications, meaning an average of 91%. According to these data, higher percentages of the first count were read in organically produced maize seeds. Golijan (2020) read the first count of organically and conventionally produced seeds after sowing for the standard germination test and found that organically produced seeds had a higher first count than conventional seeds (70.75% vs. 34.25%).

The final result of seed germination of conventionally and organically produced maize is shown in Figure 2.

Figure 2 shows that the total germination of conventionally produced maize seeds amounted to 97%, 100%, 98% and 98% over the replications, or 98% on average. The corresponding values for organically produced maize seeds were 96, 96%, 100% and 100%, i.e., 98% on average. Ilbi et al. (2009) tested the germination of maize seeds using the standard germination test, but also observed plant emergence in the field under conditions that were certainly far from the ideal laboratory conditions. Eight samples containing 50 seeds each were tested under the laboratory conditions of the standard germination test. There were no

statistically significant differences in seven of these eight samples. The germination of these samples was above 90%. Contrary to these results, plant emergence in the field was inferior. Four samples expressed a high percentage of plant emergence (90–96%), i.e., a high degree of viability, while the viability of the remaining samples was lower (emergence – 62–75%). Golijan Pantović et al. (2022) observed that the germination determined by the standard germination test was twice as high for organically produced maize seeds (88.25%) as for conventionally produced maize seeds (43.25%).

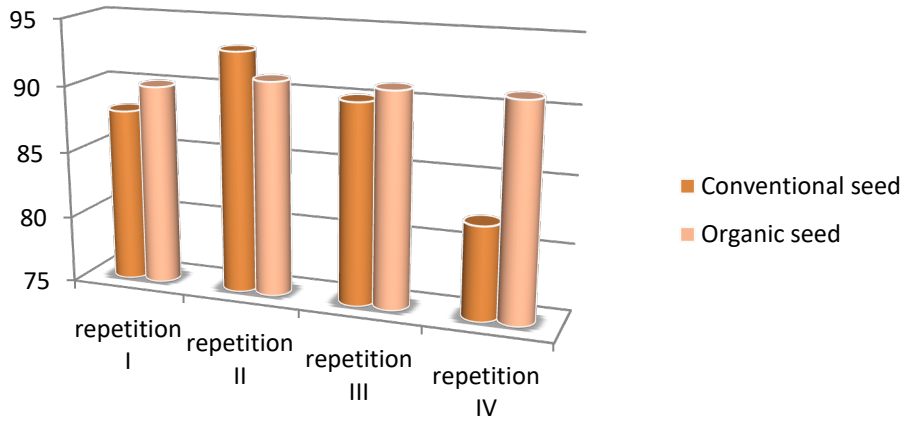

Figure 1. The first count of maize seeds produced conventionally and organically.

Standard germination tests are indicators of seed quality that can be used to predict plant emergence in the field when soil conditions are close to ideal (Durrant and Gummerson, 1990). However, the conditions under which seeds are tested are usually far removed from those prevailing in the field. The emergence of plants in the fields depends on the seed vigour (Milošević et al., 2010). In the majority of countries, maize is sown early in the spring when soil temperatures are low. At low temperatures, the seeds swell, but very often do not germinate and are invaded by pathogens (fungi). For these reasons, germination tests conducted according to the international rules under optimal conditions of temperature and humidity do not provide reliable predictions of plant emergence in the field. Under these circumstances, it is necessary to conduct tests under sub-optimal conditions and it is recommended to apply the cold germination test to estimate the maize seed vigour more accurately (Radić and Milošević, 2004).

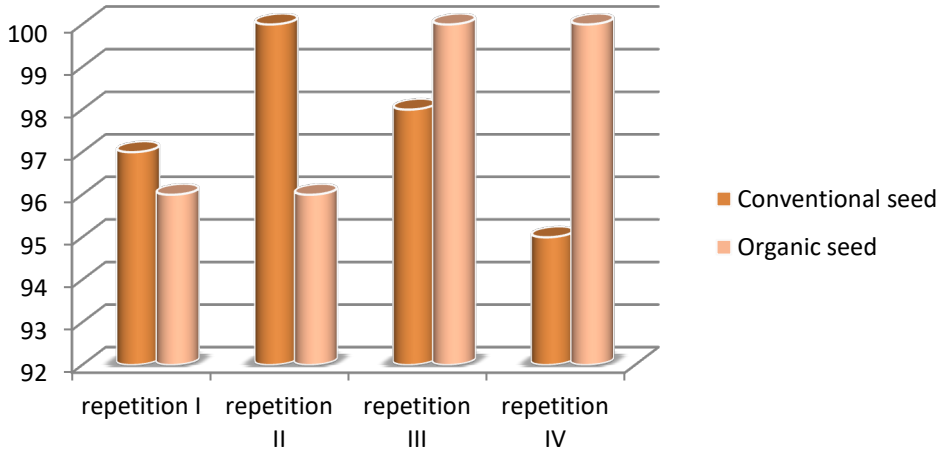

Figure 2. Germination of conventional and organic maize seeds.

Seed accelerated ageing

After accelerated ageing, the seeds were sown for germination under identical conditions as in the standard germination test. Figure 3 shows the first count of the observed maize seeds after accelerated ageing. Four days after sowing the seeds, the first count of the germination test was made.

Figure 3 shows that the first count of conventional maize seeds after accelerated ageing reached 14% and 16% (15% on average) over the replications. The corresponding values for the organic maize seeds amounted to 23% and 24% (24% on average). The results show that the first count of seeds after the accelerated ageing treatment was lower (below 25%) than for seeds not exposed to this treatment, and that organically produced seeds had a higher first count (24%) after the accelerated ageing treatment than the seeds of the conventionally produced crop (15%).

The total germination of maize seeds after the accelerated ageing treatment is shown in Figure 4.

Figure 4 shows that the total germination of conventional maize seeds after accelerated ageing amounted to 33% and 34% over replications (33% on average), while the values of organic maize seeds were somewhat higher and amounted to 38% and 40% (39% on average). The data collected show that the germination percentage after the accelerated ageing treatment was low (below 40%) for both conventionally and organically produced seeds, but that the organically produced maize seeds showed a slightly higher germination rate. The values of seed germination determined after accelerated ageing were significantly lower than the

values determined in the standard germination test. Therefore, these values indicate the lower viability of the seeds and the fact that seeds sown in the field trials, where conditions are far from perfect laboratory conditions would almost certainly show low levels of germination. According to Golijan Pantović et al. (2022), who applied the accelerated ageing test to organic maize seed, there was an increase in the first count (78.5%) in comparison to the standard laboratory test (70.75%). High relative humidity and air temperature during the test reduced seed germination (from 88.25% to 84.25%), increased the percentage of non-germinated seeds and decreased the percentage of off-type seedlings. However, none of the stated differences were statistically significant. The seed germination (84.25%) of organic maize seeds obtained in the test conducted under stress conditions was lower than the percentage obtained in the standard laboratory test (88.25%). On the other hand, the germination of conventionally produced seeds was higher in the accelerated ageing test (46.25%). Nevertheless, the percentage of seed germination was below 80% in both cases, which was considered low vigourous seed lots. Milivojević (2016) tested seed quality of maize inbred lines of various maturity groups and genetic backgrounds and estimated that the average values of total seed germination amounted to 87.87% and 63.07% for seeds produced in 2014 and 2010, respectively, using an accelerated ageing test. Kavitha et al. (2017) found that accelerated seed ageing resulted in lower germination, shoot and root length, dry matter production and vigour index.

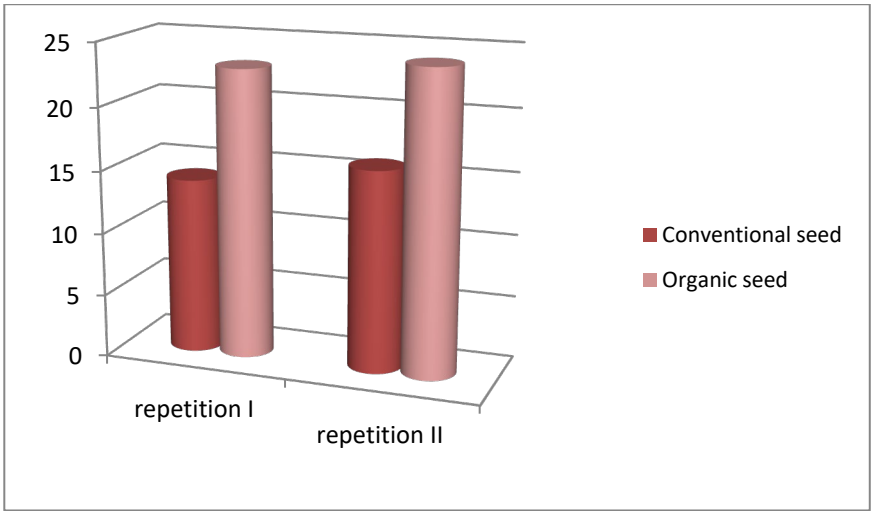

Figure 3. First count of conventional and organic maize seeds after accelerated ageing.

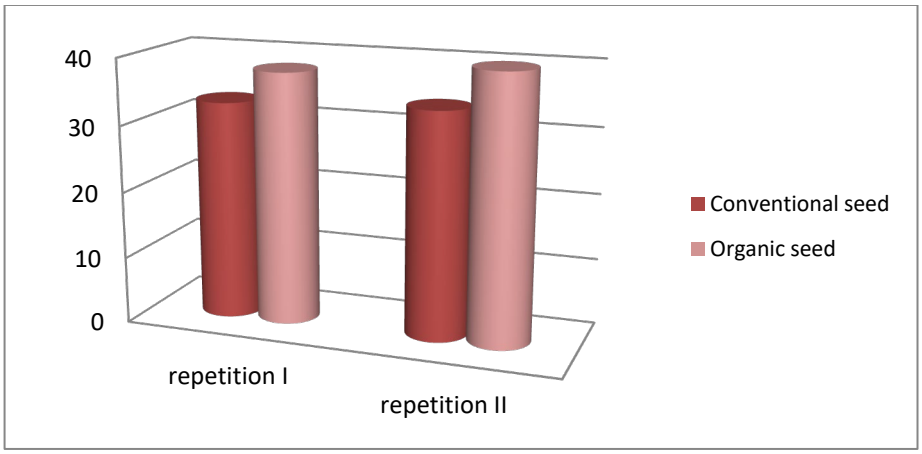

Figure 4. Seed germination of conventional and organic maize seeds after accelerated ageing.

Hussein et al. (2012) conducted the accelerated seed ageing test and found that the following properties of seedlings were reduced: dry and fresh weights, shoot and root lengths, vigour index, and germination speed index. The authors assumed that this was a consequence of the reduction in biochemical activities in seeds, because seed ageing adversely affected enzymes liable for converting reserve nutrients in the embryo into a form that could be taken up by the cell, thus impairing the formation of normal seedlings (Iqbal et al., 2002). According to Kapoor et al. (2010), the reduction in the length of shoots and roots as well as the seedling vigour index could be ascribed to breaking down DNA into smaller fragments during seed ageing, resulting in disrupted transcription that might cause the deficient or damaged synthesis of enzymes necessary for the initial stages of germination.

Woltz and TeKrony (2001) observed maize seed viability, i.e., maize seed germination after two different ageing treatments: at a temperature of 45°C for 72 h and at a temperature of 41°C for 96 h. The results indicated that maize seeds subjected to accelerated ageing at a temperature of 41°C for 96 h had a higher germination rate.

Electrical conductivity of seeds

Measuring the electrical conductivity of seed leachates provides an assessment of seed viability based on the release of electrolytes from plant tissues. If the electrolyte release is strong, that is, if the conductivity of the leachate is high, the seed is considered poorly viable, but if the electrolyte release is weak (low conductivity) seeds are considered more viable (ISTA, 1995).

The conductivity of the leachate per gram of seed weight was estimated based on the conductivity values obtained in this study, the initial seed weight and the baseline values using the formula given above and the results are shown in Figure 5.

Figure 5 shows that the leachate conductivity per gram of weight of conventional maize seeds averaged 4.90 µS/cmg. On the other hand, the conductivity of the leachate per gram of weight of organic maize seeds amounted on average to 3.66 µS/cmg. According to these values, it can be concluded that the seeds were highly viable. Sivritepe et al. (2015) obtained almost identical results: at temperatures of 20°C, 25°C and 30°C, electrical conductivity ranged from 1.7 to 5 µS cm^{-1} g^{-1}, 2.1 to 6 µS cm^{-1} g^{-1}, and from 2.2 to 7.3 µS cm^{-1} g^{-1}, respectively.

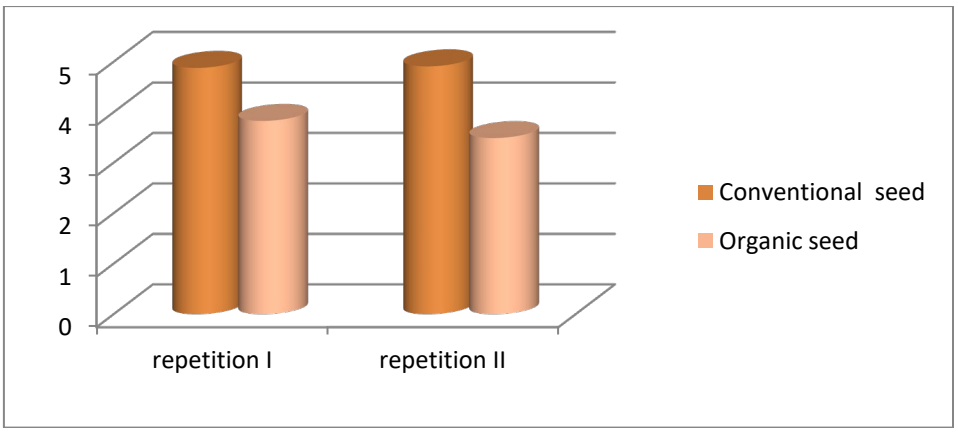

Figure 5. Conductivity of the leachate per gram of maize seed weight.

According to Hussein et al. (2012), seed germination decreases when the leakage of solutes is intensified. Lipid peroxidation is intensely related to the electrical conductivity of seeds. It could be said that the impacts of accelerated ageing and disruptions of the seed membrane affect the increase in peroxidation products and electrical conductivity (Al-Maskri et al., 2002). The loss of the quality of the complete membrane can result in reduced germination capacity, lower vigour and finally lower viability (Khan et al., 2003). Al-Maskri et al. (2002) showed in their study that the electrical conductivity of carrot seeds was higher with the enhanced ageing time. The electrical conductivity test of a seed lot shows the condition of the seed membrane. This test of single seeds indicated that the damage to the seed membrane was lower when the ageing time of the seeds was shorter. Khan et al. (2003) assumed that the delay in growth and germination in aged seeds probably depended on the membranes.

Radicle test

The radicle emergence tests were performed with conventional and organic maize seeds in eight replications with 25 seeds each. Figure 6 shows the results of radicle emergence of conventional and organic maize seeds. Based on the data in Figure 6, it can be observed that the radicle emergence of conventional maize seeds amounted to 19, 19, 22, 24, 23, 22, 21 and 18 out of 25 seeds in the replications, which corresponds to an average of 84% of germinated seeds. The corresponding values for organically produced maize seeds were 23, 21, 24, 22, 19, 21, 19 and 22 out of 25 seeds, corresponding to an average of 85% of germinated seeds. The results show that the percentage of radicle emergence of conventionally and organically produced seeds was quite uniform, that is, almost identical. Previous studies have not determined whether maize radicle emergence is reduced by soil moisture that is sufficient for seed imbibition, but not for radicle emergence. Slower germination, which is an indicator of early physiological ageing of seeds, is the main cause of reduced seed viability.

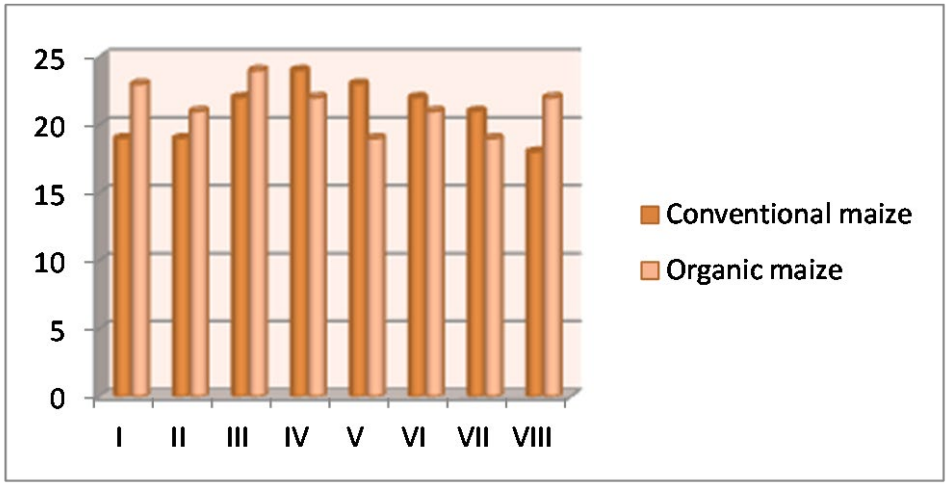

Figure 6. Radicle emergence in conventionally and organically produced maize.

Analysts from several laboratories have developed the radicle emergence test for maize seeds (Matthews et al., 2011a, b). The seed germination rate is accurately reflected in a single assessment of emerged radicles at the beginning of germination and this assessment is closely related to other parameters of the germination rate. The higher the number of radicles during the evaluation is, the higher the viability of the seeds, and vice versa, a lower number indicates a lower

viability of the seed. A similar study was conducted by Helms et al. (1997). These authors showed that radicle emergence in maize was >85%, then the percentage of emerged radicles in sunflower increased from 59% under conditions of lower soil moisture to 90% under conditions of high initial soil moisture, while this percentage was very low in soya bean (22%).

Luo et al. (2015) conducted the study to evaluate the number of radicles at 20°C and 13°C for 84 hours and 150 hours, respectively. They then compared the results obtained with the results of the following tests conducted on seven sweet maize seed lots: complex stressing vigour test, cold test, germination energy, germination percentage, germination index, vigour index and mean germination time. The number of radicles at 20°C after 84 hours and at 13°C after 150 hours was significantly related to six estimations of vigour and germination percentages. The attained results imply that single numbers of radicles may be useful in seed vigour testing of sweet maize.

Conclusion

Based on the test results of standard germination, germination after accelerated ageing, the conductivity of the leachate per gram of weight of conventionally and organically produced maize seeds, as well as radicle emergence, the following can be concluded:

- The total germination of the tested seeds either conventionally or organically produced was equal (98%);
- After accelerated ageing, organically produced seeds had a higher first count (24%) than conventionally produced seeds (15%);
- The germination of organically produced seeds after accelerated ageing was higher (39%) than the germination of conventionally produced seeds (33%);
- The test results on the electrical conductivity of leachates show that seed viability was high for both conventionally and organically produced seeds; these seeds were equally resistant to unfavourable conditions at the time of sowing and emergence of the crop;
- The radicle test points out to the uniform germination rate for both conventionally and organically produced seeds;
- The results of the tests presented do not indicate significant differences between the seeds of conventionally and organically produced crops.

Acknowledgements

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ŽIVOTNA SPOSOBNOST SEMENA KUKURUZA PROIZVEDENOG U INSTITUTU ZA KUKURUZ „ZEMUN POLJE”

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R e z i m e

Po svom privrednom značaju kukuruz je jedna od najvažnijih ratarskih biljaka. Veliki značaj kukuruza proizilazi iz raznovrsne upotrebe, velike rodnosti i obima proizvodnje. Kako je za uspešnu proizvodnju neophodno kvalitetno seme, cilj ovog rada bio je da se ispita životna sposobnost semena kukuruza poreklom iz dva različita načina proizvodnje – organskog i konvencionalnog. Korišćeno je seme kukuruza sorte rumenka (konvencionalno i organski proizvedeno), a ispitano je u Laboratoriji za ispitivanje semena Instituta za kukuruz „Zemun polje”. Prema dobijenim rezultatima može se zaključiti da je nakon minimalnih razlika u procentu energije klijanja konvencionalnog i organskog kukuruza, ukupna klijavost bila identična (98%). Rezultati ispitivanja klijavosti nakon ubrzanog starenja semena ukazuju da je ukupna klijavost kukuruza bila viša kod organskog semena (39%) u odnosu na konvencionalno seme (33%). Provodljivost ekstrakta po gramu mase semena konvencionalnog kukuruza u proseku daje 4,90 µS/cmg, dok kod semena organskog kukuruza provodljivost iznosi 3,66 µS/cmg. Iz navedenih vrednosti može se zaključiti da ispitivano seme konvencionalnog i organskog kukuruza poseduje visoku životnu sposobnost i pogodno je za raniju setvu pri nepovoljnim uslovima sredine. Prema dobijenim rezultatima, pojava primarnog korena je bila ujednačena i iznosi 84% kod konvencionalnog semena i 85% kod organskog semena.

Ključne reči: *Zea mays* L., klijanje, ubrzano starenje, električna provodljivost.

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EFFECT OF MYCORRHIZAL INOCULATION AND PHOSPHORUS FERTILIZER ON MAIZE ROOT INFECTIVITY IN THREE SOIL SERIES

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Abstract: An experiment was carried out to examine the influence of mycorrhizal inoculation and phosphorus fertilizer on maize root infectivity in three soil series at the Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. The treatments included three soil series (Gambari, Itagunmodi and Iwo series), three levels of phosphorus fertilizer (0, 30 and 60 kg P_2O_5/ha) and three levels of mycorrhizal inoculation (0, 10 and 20 g per 15 kg soil). The experimental design used was a 3×3×3 factorial trial in a completely randomized design with three replications. Root infectivity was evaluated by the grid line intersect method. After harvesting, the phosphorus (P) uptake was determined by multiplying the P concentration in the plant by the total dry weight. The data were analyzed using analysis of variance and the significant means of the treatment were compared using the Duncan's multiple range test at the 5% significance level. The results revealed that P uptake was influenced significantly ($p < 0.05$) by the soil series. The order of decrease in P uptake across the soil series was as follows: Itagunmodi (0.54 mg/kg) < Iwo (0.90 mg/kg) < Gambari (2.52 mg/kg). Root infectivity significantly enhanced the uptake of P. The highest root infectivity and the highest P uptake were achieved at 20 g inoculation. Mycorrhizal infection levels were reduced by moderate to high rates of soluble phosphorus fertilizer. Thus, increasing levels of phosphate fertilizer stimulate maize root growth but significantly reduce root infection levels.

Key words: mycorrhizal inoculation, soil series, root infectivity, phosphorus uptake.

Introduction

Arbuscular mycorrhizal fungi (AMF) create a key functional group of soil organisms as a result of their effect on soil properties (Dal Cortivo et al., 2018). The phylum Glomeromycota comprises the AMF, which also belongs to the class

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Glomeromycetes (Brundrett and Tedersoo, 2018); and easily forms symbiotic associations with most terrestrial plants. Bushy, brush-like hyphae known as arbuscules are formed in the root cell of the host plant, enabling the exchange of carbohydrates and the absorption of water and nutrients between the host and fungi (Gutjahr and Parniske, 2013). However, the relationship is significant for the fungi because the plants serve as their only source of energy. The importance of maize cannot be overestimated, as it serves both as food for humans and animals and as an industrial raw material for the production of corn starch, beer, etc.

Phosphorus (P) is one of the three crucial nutrients required for plant growth. However, it is not readily available to plants, thus limiting biomass production in ecosystems (Rodrigues et al., 2021). Phosphorus as a principal component of deoxyribonucleic acid (DNA), and a genetic inheritance of ribonucleic acid (RNA) is essential for protein synthesis in both plants and animals. Plants require a significant amount of phosphorus in the early stages of growth for optimum crop production (Zhu and Whelan, 2018), and this element is highly mobile within the plant. If the plant does not contain enough phosphorus, the older leaves turn purple and premature senescence occurs, signifying phosphorus deficiency. However, the availability of phosphorus is also influenced by the type of soil. However, the quantity of available phosphorus and mycorrhizal inoculation are independent of root infectivity.

Maize (*Zea mays* L.) is an essential cereal crop worldwide (Ashraf et al., 2016). It is limited by many factors such as soil fertility, imbalanced nutrition, disturbed soil properties, cultivars, and weed infestation. Maize has some advantages compared to other cereals: high production, easy processing, easy digestibility and lower price. The high net energy content, fiber content and high protein level of maize make it an important food for humans and feed for livestock (Oladejo and Adetunji, 2012).

Different soil series have been used for maize cultivation in southwestern Nigeria, but little or no attention has been paid to the influence of the different soil series on mycorrhizal infectivity and phosphorus availability. Therefore, this study is crucial to examine the phosphorus level at which mycorrhizal infectivity is maximized and to determine the most suitable quantity of mycorrhizal propagules and soil series that significantly enhance mycorrhizal root infectivity in maize.

Material and Methods

Description of the sample locations and soil sample collection

Three soil series (Itagunmodi, Iwo and Gambari) were used for the study. The top soil samples were collected using shovel at the 0–15-cm depth from three study areas. These were the areas near Ilesha (Itagunmodi series), Ede – Iwo Area (Iwo series) and a series break of slope before the valley bottom location within the

Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Teaching and Research Farm (Gambari series). The soil types and potential land use patterns were described by Charles et al. (2004).

Experimental design

The experiment was carried out in pots at the Teaching and Research Farm, LAUTECH Ogbomoso. The experiment was a 3×3×3 factorial trial comprising: 3 types of soil series (Gambari, Itagunmodi and Iwo series); 3 levels of phosphorus application: 0 kg P_2O_5/ha, 30 kg P_2O_5/ha and 60 kg P_2O_5/ha and 3 levels of mycorrhizal application (*Glomus mossae*), 0 g/15 kg soil, 10 kg/15 kg soil and 20 g/15 kg soil. The trial was arranged in a completely randomized design (CRD) with three replications.

Field preparation and planting

The pots were placed at a distance of 0.75 m from each other. Three seeds of the maize variety SUWAN -1- SR-Y were sown in the middle of the pots and later, one week after sowing, thinned out to one plant per pot. Before sowing, three levels of the mycorrhizal *Glomus mossae* strain ([0 g/15 kg soil], [10 g/15 kg soil] and [20 g/15 kg soil]) were applied at a depth of 3 cm in the center of the pots. Phosphorus in the form of single superphosphate was applied at a rate of 0 kg P_2O_5/ha (0 g/15 kg soil), 30 kg P_2O_5/ha (1.3 g/15 kg soil) and 60 kg P_2O_5/ha (2.5 g/15 kg soil). Weeding was carried out manually three weeks after sowing (3 WAS) and six weeks after sowing (6 WAS).

Laboratory analyses of the soil samples

The particle size of each soil sample was determined by air-drying, crushing and sieving the soil samples through 2-mm and 0.5-mm meshes. Thereafter, pH (H_2O), total nitrogen (N), organic carbon, available phosphorus (P), iron (Fe), copper (Cu), zinc (Zn), the exchangeable cations (Ca, Na, Mg and K) and exchangeable acidity were determined using standard methods. Soil pH was determined in a 1:1 soil-water suspension using a digital pH meter (Bentop pH meter, pH-B200E/PH-B200EM by Infitek), while particle size distribution was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962) using sodium hexametaphosphate as a dispersant. The exchangeable cations (Ca, Mg, K and Na), available phosphorus, Cu, Zn, Fe and Mn were also determined by extracting the soil with the Mehlich-3 extractant in a ratio of 1:10 soil/extractant. Potassium and Na were determined in the extract using the flame photometry method while Ca, Mg, Mn, Zn, Fe and Cu were determined by the atomic absorption spectrophotometer (AAS, 325-1100Nm, India). The available

phosphorus was determined colorimetrically by the ascorbic acid method. The exchangeable acidity (H^+, Al^{3+}) was determined by titrating 0.01N HCl with the 1N KCl extract of the soils using the phenolphthalein indicator (Kumar et al., 2014). Total nitrogen was determined from the concentrated sulphuric acid digestion using the Adapted Technicon Salicylate/Sodium Nitroprusside colorimetric method. Organic carbon was analyzed by the colorimetric complete oxidation method using a spectrophotometer (UV-Vis spectrophotometer, Hach company, Danahar) (Heanes, 1984).

Botanical data collection and plant analyses

At the twelfth week after sowing (12 WAS), cobs, roots and shoots were harvested. After harvesting, all shoots and roots were oven-dried at a temperature of 80^0C to a constant weight for five days. These were later used for the determination of dry weight and total biomass production. The plant samples were milled in a Wiley mill to pass through a 1-mm sieve and the plant samples were analyzed for phosphorus according to the plant analysis procedure outlined in Selected Methods for Soil and Plant Analysis Manual of International Institute of Tropical Agriculture, Ibadan (IITA, 1979). A portion (4.0 ml) of nitric/perchloric acid mixture (3:1 ratio) was added into 0.2 g of each sample in a 25-ml conical flask and left overnight. The content was heated until white fumes were formed, then 1.0 ml of hydrochloric acid/distilled water mixture (1:1 ratio) was added. It was heated further for 30 minutes before the heat was removed. Distilled water was added to the digestion and shaken before cooling to room temperature to avoid the formation of insoluble perchlorate compounds. The digestion was washed into a 50-ml volumetric flask. It was then made up to the mark with distilled water. Total phosphorus was determined by the colorimetric vanadomolybdate method on a spectrophotometer. The nutrient accumulated in the plant parts was calculated as follows: nutrient uptake = % nutrient concentration X sample dry weight, according to Ombo (1994) and Gungunla (1999).

The determination of mycorrhizal root infectivity was carried out by the grid line intersect method. After harvesting, root samples were cut into 1-cm length and stored in 50% ethanol. The root samples were later carefully rinsed with slow running tap water to remove the ethanol (before the commencement of the root staining procedure). The root particles were put in sample bottles with 10% KOH overnight. The root particles were placed in a water bath and steamed at 80°C for 30 minutes (Philip and Hayman, 1970). They were then poured into a sieve, rinsed under running water and returned to the sample bottles. They were then bleached in alkaline H_2O_2 for 10 minutes, rinsed with water and soaked in 1% HCl acid for 10 minutes. Trypan blue staining solution was used for the roots, containing 0.05% trypan blue; 10 ml of 50% glycerol was added to each bottle, shaken well and left for 24 hours for proper staining. The stained root particles were poured inside Petri

dishes with glycerol to prevent desiccation. The degree of mycorrhizal infectivity was assessed by spreading 25 root pieces per slide and observing them under a dissecting microscope at low magnification. The total number of roots and the infected roots intersecting the grids were counted using the grid line intersect method (Vierheilig et al., 2005). The percentage mycorrhizal root infectivity was calculated by the ratio between the number of intersects with infection and the total number of intersects multiplied by 100 (Fagbola et al., 2001).

$$\% \text{ root infectivity } = \frac{EP}{N \times Mp} \times \frac{100}{1}$$

where,
EP = estimated population,
N = number of lines per slide,
Mp = maximum root particles per slide.

Statistical analysis

The experimental design was a 3×3×3 factorial trial in a completely randomized design with three replications. The data were subjected to analysis of variance. Comparison of the various treatment means was done using the Duncan's multiple range test at the 5% level of significance.

Results and Discussion

Table 1 shows the physico-chemical properties of the soil series used. The Itagunmodi series had the lowest pH value and available phosphorus, while the Gambari series had the highest pH value and available phosphorus.

Table 1. Physical and chemical properties of the soil samples of the three studied soil series.

Properties	Gambari series	Itagunmodi series	Iwo series
Soil pH	5.00	3.60	4.40
Available P (mg/kg)	2.52	0.54	0.90
Fe (mg/kg)	63.30	42.50	69.80
Total N (g/kg)	1.00	1.50	0.70
Organic C (g/kg)	12.20	13.60	7.60
Ex. Ca (Cmol/kg)	0.67	0.21	0.05
Ex. Mg (Cmol/kg)	0.48	0.50	0.27
Ex. K (Cmol/kg)	0.17	0.26	0.09
Ex. Na (Cmol/kg)	0.01	0.06	0.02
Base saturation (%)	88.67	76.30	57.44
Sand (g/kg)	853.00	563.00	753.00
Silt (g/kg)	117.00	227.00	157.00
Clay (g/kg)	30.00	210.00	90.00

The Itagunmodi series had the lowest Fe and sand value, while the Iwo series had the highest value of Fe and the Gambari series had the highest sand value. The Itagunmodi series also had the highest value of silt and clay, while the Gambari series had the lowest value of silt and clay. The Iwo series had the lowest values of exchangable Ca, exchangeble Mg, exchangeable K, exchangeable Na and base saturation.

Phosphorus uptake

Table 2 shows that the soil series had a significant effect on phosphorus uptake. The Itagunmodi series had the highest mean phosphorus uptake (4.27 g/plant), followed by the Iwo series (3.44 g/plant), while the Gambari series had the lowest phosphorus uptake (2.28 g/plant). Mycorrhizal inoculation at 20 g/15 kg soil had a significant effect on phosphorus uptake, which was significantly ($p<0.05$) higher (4.58 g/plant) than 2.61–3.01 g/plant observed at lower doses of mycorrhizal inoculation. The combination of the Itagunmodi series with 20 g of mycorrhizal dose resulted in the highest phosphorus uptake (Figure 1).

Table 2. The effect of mycorrhizal inoculation, soil series and P fertilizer on phosphorus uptake (g/plant).

Factor	Treatments	Phosphorus uptake (g/plant)
P fertilizer		
	0gP_2O_5/15 kg	3.51a
	1.3gP_2O_5/15 kg	2.88a
	2.5gP_2O_5/15 kg	3.81a
Mycorrhiza		
	0g/15 kg soil	2.61b
	10g/15 kg soil	3.01b
	20g/15 kg soil	4.58a
Soil series		
	Gambari	2.28b
	Itagunmodi	4.27a
	Iwo	3.44ab

For each factor, means followed by the same alphabet in the column are not significantly different by the Duncan's multiple range test at the 5% level of probability.

Root infectivity

Table 3 shows that mycorrhizal root infectivity had a significant effect on maize root. The highest root infectivity (36%) was recorded at 20 g inoculum/15 kg soil and the lowest root infectivity (11.9%) was recorded at 0 g inoculum/15 kg soil.

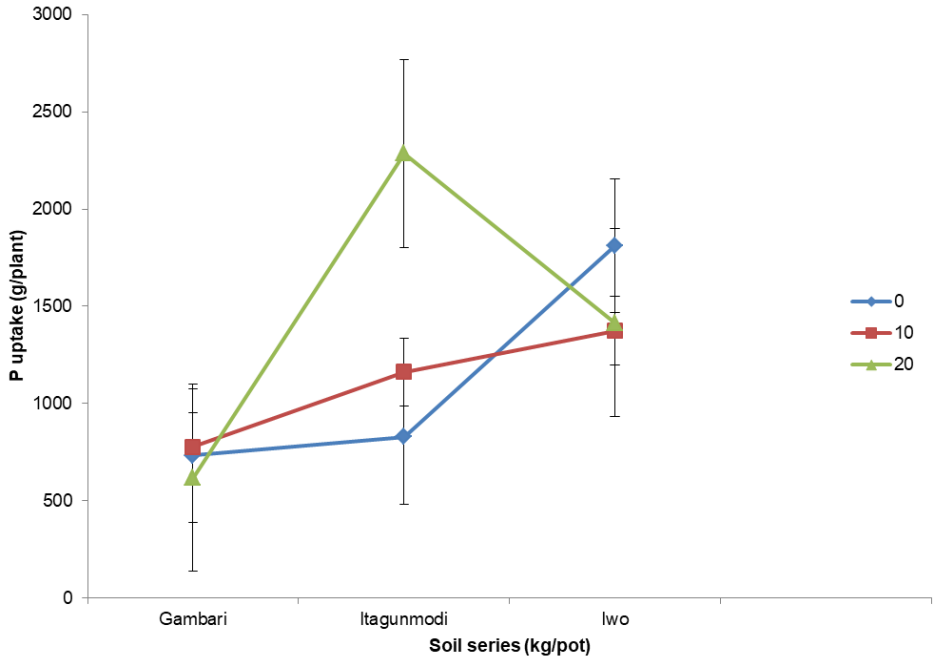

Figure 1. The interactive effect of P fertilizer, mycorrhizal inoculation and soil series on P uptake (g/plant) in maize.

Table 3. The effect of mycorrhizal inoculation, soil series and P fertilizer on maize root infectivity.

Factor	Treatments	Root infectivity (%)
P fertilizer		
	0gP_2O_5/15 kg	25.1a
	1.3P_2O_5/15 kg	26.7a
	2.5P_2O_5/15 kg	28.0a
Mycorrhiza		
	0g/15 kg soil	11.9c
	10g/15 kg soil	31.4b
	20g/15 kg soil	36.0a
Soil series		
	Gambari	27.4a
	Itagunmodi	25.3a
	Iwo	27.4a

For each factor, means followed by the same alphabet in each column are not significantly different by the Duncan's multiple range test at the 5% level.

The results showed no response of root infectivity to P fertilizer application, irrespective of the levels while mycorrhizal inoculation responded to root infectivity and the effect was significant with increasing quantity of inoculum. The result agrees with Cheng et al. (2013). Arbuscular mycorrhizal fungi increased the root length in all root diameter classes, although the proportion of medium and coarse roots was increased to a higher degree than that of fine roots. The interactions are dependent on plant-fungus compatibility, as some arbuscular mycorrhizal fungus-plant combinations are more beneficial than others (Kandhasamy et al., 2020). In this study, the Itagunmodi series gave the highest phosphorus uptake with 20 g mycorrhiza per 15 kg soil.

Phosphorus uptake was the highest in the crops on the Itagunmodi series. This was due to the low amount of available P and Fe from Table 1 compared to the other two soil series. Kowalska et al. (2015) have also reported that soil P plays the most significant role in regulating plant mycorrhizal infectivity. High P application was also found to have negative effects on root infectivity (Cheng et al., 2013). However, low fertilizer application optimizes the plant mycorrhizal infectivity (Rana et al., 2020). According to Ma et al. (2019), fertilizer application has been shown to have negative effects on AMF infectivity traits and thus affects plant responses to AM fungi.

The results therefore show that root infectivity responded to mycorrhizal inoculation but not to P fertilizer application.

Conclusion

This paper summarizes the influence of mycorrhizal inoculation and phosphorus fertilizer on root infectivity in three soil series. Root infectivity was only recorded with mycorrhizal inoculation and no significant infectivity was observed with fertilizer application. However, root infectivity was significantly enhanced with 20 g inoculum, irrespective of the soil series. The study therefore showed that high phosphorus levels had a negative influence on AMF infectivity of crops. However, phosphorus uptake was significantly enhanced by root infectivity. Therefore, excessive P application to maize would have negative impacts on maize growth. Apart from this observation, high P levels can also lead to incremental costs of production, which should be discouraged to promote farmer profitability. The results show that mycorrhizal inoculation significantly improved root infectivity and P uptake. Therefore, it is recommended to promote cropping systems that maintain mycorrhiza in the root zones of maize.

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UTICAJ MIKORIZNE INOKULACIJE I FOSFORNOG ĐUBRIVA NA INFEKTIVNOST KORENA KUKURUZA NA TRI ISPITIVANA PODRUČJA

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R e z i m e

Izveden je eksperiment kako bi se ispitalo uticaj mikorizne inokulacije i fosfornog đubriva na infektivnost korena kukuruza u tri ispitivana područja na Tehnološkom univerzitetu Ladoke Akintola (engl. *Ladoke Akintola University of Technology* – LAUTECH), Ogbomoso, Nigerija. Tretmani su obuhvatali tri lokacije (Gambari, Itagunmodi i Iwo), tri nivoa fosfornog đubriva (0, 30 i 60 kg P_2O_5/ha) i tri nivoa inokulacije mikorizom (0, 10 i 20 g na 15 kg zemljišta). Eksperiment je bio dizajniran kao trofaktorski ogled (3×3×3) u potpuno slučajnom dizajnu sa tri ponavljanja. Infektivnost korena procenjivana je metodom preseka linija mreže. Nakon žetve, usvajanje fosfora (P) određivano je množenjem koncentracije P u biljci sa ukupnom suvom masom. Podaci su analizirani primenom analize varijanse, a značajnost srednjih vrednosti tretmana upoređena je Dankanovim testom višestrukog opsega na nivou značajnosti od 5%. Rezultati su pokazali da je lokacija zemljišta značajno ($p < 0,05$) uticala na usvajanje fosfora (P). Redosled smanjenja usvajanja fosfora po lokacijama zemljišta bio je sledeći: Itagunmodi (0,54 mg/kg) < Iwo (0,90 mg/kg) < Gambari (2,52 mg/kg). Infektivnost korena značajno je povećala usvajanje fosfora. Najveća infektivnost korena i najveće usvajanje fosfora postignuti su pri inokulaciji od 20 g. Stepeni mikorizne infekcije smanjeni su pri umerenim do visokim dozama rastvorljivog fosfornog đubriva. Dakle, povećanje nivoa fosfornog đubriva podstiče rast korena kukuruza, ali značajno smanjuje nivo infekcije korena.

Ključne reči: mikorizna inokulacija, lokacije zemljišta, infektivnost korena, usvajanje fosfora.

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EFFECTS OF STORAGE TEMPERATURE ON THE QUALITY AND QUANTITY OF DNA EXTRACTED FROM MAIZE LEAVES

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Abstract: This study was carried out to evaluate the effect of temperature during storage of maize leaves and extracted DNA on its quality and quantity in order to be efficiently amplified using PCR. Leaves were collected from the four-week-old plants and divided into three groups of 20 samples. The first group of leaves was processed immediately, while the other two were stored at -20°C or -80°C for 30 days. The DNA extracted from the fresh leaves was divided into three portions with the first being amplified immediately and the other two were stored at -20°C or -80°C for 30 days. The DNA quality and quantity were examined using a biospectrometer, after which the samples were diluted for the PCR assay. The quality of all DNA samples was at an acceptable level with their average $OD_{260/280}$ ratio in the range from 1.85 to 1.87. The concentration of the DNA extracted immediately from fresh leaf tissue was not statistically different from the stored samples. Both the quality and quantity of DNA in all samples were sufficient for successful PCR amplification with two *opaque2*-specific molecular markers. Phi057 amplified a ~170bp fragment in QPM and ~160bp in non-QPM, while umc1066 amplified a ~150bp fragment in QPM and ~160-170bp in non-QPM. Our results suggest that appropriate storage conditions do not affect the DNA quality and quantity. This could be useful in marker-assisted selection of target genes, when a large number of samples must be processed prior to pollination, allowing breeders to discard plants lacking the desired alleles and reduce the size of the breeding population.

Key words: DNA quality, DNA quantity, extraction, maize, storage temperature.

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Introduction

Marker-assisted selection (MAS) has become an efficient method for crop improvement programs due to its precise and accelerated approach (Vivekananda et al., 2018). According to Eathington et al. (2007), MAS methodologies have increased the mean performance of progeny as compared to the conventional breeding methodologies. As a method used worldwide to rapidly incorporate valuable traits into new cultivars, it is used in breeding to select progenies with the desired genes. Once the DNA has been extracted from the plants, genetic markers are used to tag and track the genetic variation in the DNA samples, which can be identified by polymerase chain reaction (PCR) and electrophoresis (Begna, 2020).

The extraction of DNA with good quality and high yield is a limiting factor in the genetic analysis of plants (Abdel-Latif and Osman, 2017). The quality and quantity of the DNA extracted from cereals are often affected by the presence of polysaccharides, proteins, and DNA polymerase inhibitors, rendering the sample non-amplifiable (Sarwat et al., 2006). Although many DNA extraction protocols have been developed for plants (Dellaporta et al., 1983; Saghai-Maroof et al., 1984; Doyle and Doyle, 1987), some of them include time-consuming steps, laborious works, or the use of liquid nitrogen, which can be hard to procure. Furthermore, some DNA isolation methods from plant tissues produce either small amounts or DNA of inconsistent quality (Abdel-Latif and Osman, 2017). On the other hand, there are methods of DNA extraction that can be performed within a short period of time in a laboratory with basic facilities, especially suitable for foreground selection in the MAS program (Dorokhov and Klocke, 1997; Vivekananda et al., 2018). The quality and quantity of the extracted DNA can be assessed spectrophotometrically. The A_{260}/A_{280} absorption ratio provides an estimation of DNA purity by considering contaminants that absorb UV light, such as proteins (Vahdani et al., 2024). Besides the acceptable DNA purity, the sufficient quantity of DNA for successful PCR amplification must also be considered.

Researchers and scientists from the Maize Research Institute Zemun Polje have integrated conventional and molecular breeding programs aimed at converting standard maize inbred lines to quality protein maize (QPM) genotypes for growing in temperate regions (Kostadinovic et al., 2016; Ignjatovic Micic et al., 2020). Marker-assisted breeding involves a large number of individual plants that need to be analyzed prior to pollination. Therefore, in addition to a rapid and reliable DNA extraction protocol, adequate storage conditions are important due to its large-scale requirements. The aim of our study was to evaluate the effect of storage temperature on the quality and quantity of DNA extracted from maize leaves for use in marker-assisted selection to improve maize quality.

Material and Methods

Leaves were collected from the four-week-old plants that are parental lines in the marker-assisted conversion of maize inbred lines to quality protein maize (QPM) adapted to temperate climate. The collected maize leaves were divided into three groups of 20 samples. The first group was processed immediately, while the other two were stored at -20°C or -80°C for 30 days. The genomic DNA was extracted following the modified protocol of Dorokhov and Klocke (1997). The DNA extracted from fresh leaves was divided into three portions with the first being processed immediately and the other two stored for 30 days at -20°C or -80°C. The DNA quantity and quality were examined using a biospectrometer (BioSpetrometer kinetic, Eppendorf, Germany). The DNA concentration was measured directly in µg/mL, while the DNA quality was measured as DNA/RNA ratio ($A_{260/280}$). The t-test (Microsoft Excel) was used to compare the mean values among the treatments at the probability level $P<0.05$.

All samples were diluted to a working concentration of 20 ng/µl for the application in polymerase chain reaction (PCR). Two SSR markers specific for the *opaque2* gene (phi057 and umc1066) were employed in the PCR assay to evaluate the efficiency of DNA amplification (Table1). The polymerase chain reaction was carried out in a reaction volume of 20 µl containing the following: DreamTaq™ Green PCR Master Mix (Thermo Scientific, USA), 0.25 µM primers and 20 ng of DNA. Amplifications were performed in the Biometra TProfessional Standard 96 thermocycler (Biometra, Germany) with the following program: an initial denaturation at 94ºC /2min, followed by 40 cycles each of denaturation at 94ºC/1min, annealing at 60ºC/2min and extension at 72ºC/2min, with final extension at 72ºC/10min. The amplified fragments were resolved by electrophoresis on 8% polyacrylamide gel (Mini Protean Tetra-Cell, Bio-Rad, USA).

Table 1. The set of *opaque2* gene-specific markers used for PCR.

Primer		Sequence (5'-3')	Fragment size
phi057	F	CTCATCAGTGCCGTCGTCCAT	160–170 bp
	R	CAGTCGCAAGAAACCGTTGCC	
umc1066	F	ATGGAGCACGTCATCTCAATGG	150–160 bp
	R	AGCAGCAGCAACGTCTATGACACT	

After staining with ethidium bromide, they were visualized under UV transilluminator and documented in the gel documentation system BioDocAnalyze (Biometra, Germany). The approximate size range of the amplification products for each SSR locus was determined based on the positions of the bands relative to the 50 bp molecular weight ladder.

Results and Discussion

Extraction and purification of DNA represent one of the basic steps in molecular biology and therefore the preparation of high-quality DNA from various sources, such as fresh and frozen tissue, is the most important first step (Samoo et al., 2017). Although fresh tissue is the best source for high molecular weight DNA extraction, in some cases fresh tissue cannot be obtained or only previously collected and stored samples are available (Alrokayan, 2000). Furthermore, some methods and large-scale experiments require DNA extraction of a large number of samples in a short period of time, so the storage conditions are of great importance.

In this research, the concentration and purity of DNA extracts from fresh maize leaves were compared to extracts and leaves stored at -20°C or -80°C for 30 days. The quality and purity of the DNA is presented as $A_{260/280}$ ratio. A high A_{260}/A_{280} value indicates RNA contamination, while a low A_{260}/A_{280} ratio indicates DNA contamination with proteins (Meyer, 2003). With an average $OD_{260/280}$ ratio in the range from 1.85 to 1.87, the quality of all DNA samples was at an acceptable level with values between 1.8 and 2.0 (Gryson, 2010). No significant differences were found for A_{260}/A_{280} values between the DNA extracted from fresh leaf tissue immediately after sampling and stored samples. Similarly, there were no significant differences between the concentration of the DNA extracted from fresh leaf tissue immediately after sampling and the samples that were stored. However, concentration of DNA was numerically the highest in the samples extracted from the leaves stored at -20°C. The concentration and purity of DNA extracted from maize leaves are presented in Table 2.

Table 2. The concentration and purity of DNA extracted from maize leaves.

	Fresh	Fresh/-20°C	Fresh/-80°C	-20°C	-80°C
DNA concentration (μg/mL)	523.84^{ns}	536.28^{ns}	538.64^{ns}	544.57^{ns}	534.08^{ns}
DNA purity ($A_{260/280}$)	1.87^{ns}	1.87^{ns}	1.85^{ns}	1.85^{ns}	1.87^{ns}

Both the quantity and quality of DNA in all samples were sufficient for successful PCR amplification with two *opaque2*-specific molecular markers. The phi057 amplified a ~170bp fragment in QPM and ~160bp in non-QPM, while umc1066 amplified a ~150bp fragment in QPM and ~160-170bp in non-QPM. The amplification with the SSR marker umc1066 is given in Figure 1. The dominant homozygotes (lanes 1, 3 and 12) were clearly distinguished from the recessive homozygotes (lanes 7, 8 and 10) and the heterozygous individuals (lanes 2, 4, 5, 6, 9 and 11).

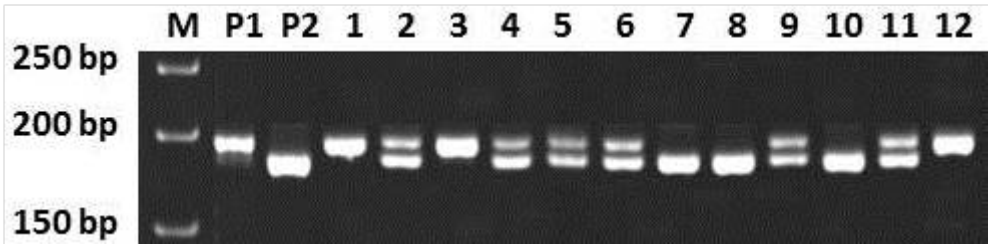

Figure 1. Amplification with the *opaque2*-specific marker umc1066. M: 50 bp DNA ladder, P1: standard line, P2: QPM line, 1–12: analyzed individual plants.

Based on these results, it can be concluded that the DNA was intact and the banding patterns showed no obvious difference or any form of DNA degradation. This is an indication that the extracted DNA from all samples is of good quality and suitable for PCR analysis (Adetumbi et al., 2013). This could be useful in marker-assisted selection of target genes, when a large number of samples must be processed prior to pollination, allowing breeders to discard plants without alleles of interest and reduce the size of the breeding population. The time elapsed between sampling and DNA extraction depends principally on the experiment and storage conditions. If tissue preservation conditions and sampling are appropriate, the storage time will not be a factor at least for short storage periods (Samoo et al., 2017).

Conclusion

This study provides useful information for marker-assisted breeding, where a large number of plants need to be processed and therefore a large number of DNA extracts need to be handled. Our results indicate that adequate storage of leaves/extracted DNA does not have a negative effect on DNA yield in terms of quality, quantity, and integrity, at least for short storage periods. The samples extracted from stored leaves as well as the stored extracts, exhibited not only a sufficient DNA amount, but also ensured their purity for PCR applications.

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UTICAJ TEMPERATURE SKLADIŠTENJA NA KVALITET I KOLIČINU DNK IZOLOVANE IZ LISTA KUKURUZA

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R e z i m e

Cilj ovog istraživanja je bio ispitivanje efekta temperature skladištenja listova kukuruza i iz njih izolovane DNK na njen kvalitet i količinu radi efikasnog umnožavanja pomoću lančane reakcije polimeraze. Listovi su uzeti sa biljaka starih četiri nedelje koji su podeljeni u tri grupe od po 20 uzoraka. Prva grupa listova je analizirana odmah, dok su druge dve čuvane 30 dana na -20°C i -80°C. DNK izolovana iz svežih listova je podeljena u tri grupe, od kojih je prva analizirana odmah, a druge dve su skladištene 30 dana na -20°C i -80°C. Kvalitet i količina DNK određene su na biospektrofotometru, nakon čega su uzorci razblaženi za lančanu reakciju polimeraze. Kvalitet svih uzoraka DNK bio je na zadovoljavajućem nivou sa prosečnim vrednostima odnosa $OD_{260/280}$ od 1,85 do 1,87. Koncentracija DNK koja je izolovana odmah iz svežih listova nije se statistički razlikovala od uzoraka koji su čuvani 30 dana. I kvalitet i količina DNK u svim uzorcima bili su dovoljni za uspešno umnožavanje pomoću lančane reakcije polimeraze sa dva molekularna markera specifična za gen *opaque2*. Pomoću markera phi057 umnožen je fragment od ~170bp kod linije visokog kvaliteta proteina (VKP) i ~160bp kod linije koja nije VKP, dok je pomoću markera umc1066 dobijen fragment od ~150bp kod VKP i ~160-170bp kod ne-VKP. Rezultati su potvrdili da odgovarajući uslovi čuvanja ne utiču ni na kvalitet ni na količinu DNK. Ovo može biti korisno u selekciji pomoću molekularnih markera kada veliki broj uzoraka mora biti obrađen pre polinacije, što omogućuje selekcionerima da odbace biljke bez željenih alela i smanjuje obim populacije koju treba testirati.

Ključne reči: kvalitet DNK, količina DNK, ekstrakcija, temperatura skladištenja.

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POTATO MINITUBER PRODUCTION BY AEROPONICS: EFFECTS OF GENOTYPE AND PLANT ORIGIN

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Abstract: Aeroponics is a modern farming technology for soilless potato cultivation that enables the efficient production of high-quality pre-basic seed potatoes (minitubers). In this system, the roots and underground stems (stolons) of the potato plants grow within closed modules, suspended in a fine mist of a nutrient-rich solution that continuously recirculates. This setup enables the formation of numerous minitubers with a length greater than 10 mm during the growing period. Our study aimed to evaluate the impact of the genotype and origin of the planting material on minituber production in an aeroponic facility in Guča, Serbia. Three potato cultivars were analyzed: Cleopatra, Kennebec, and Désirée, using two types of planting material: acclimated microplants and plants derived from the previous season's minitubers. The plants were cultivated aeroponically from late May to December 2019, with a planting density of 24 plants per square meter and harvest intervals of approximately 14 days. The Désirée cultivar produced the highest average number of minitubers per plant (19.89), followed by Kennebec (15.71) and Cleopatra (11.05). The average weight of minitubers was significantly greater in plants grown from last season's minitubers compared to plants grown *in vitro*. The Kennebec plants originating from minitubers achieved the highest yield of 10.27 kg per square meter. Additionally, the plants originating from minitubers consistently produced tubers throughout the entire cultivation period in the aeroponic growing system.

Key words: soilless production, potato cultivars, pre-basic seed potato.

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Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most significant food crops, with commercial production based on vegetative propagation by seed tubers. In Serbia, the average potato yield in 2022 was 21.1 t ha-1 (Statistical Office of the Republic of Serbia, 2023), while in the developed countries of Europe and North America it can reach 40–50 t ha-1 (FAO, 2023). Low yields are due to various limitations, with the condition of the planting material being a key factor (Broćić et al., 2021). High-quality, healthy seed material derived from potatoes grown from minitubers (pre-basic seed potatoes) is essential for achieving high yields. Minituber production is a step between in vitro propagation of disease-free plant material (microplants, microtubers) under sterile conditions and seed production in the open field (Struik, 2007). Therefore, the minitubers serve as the initial material for field propagation, leading to the production of basic seeds and other seed categories (da Silva Filho et al., 2020).

Traditional seed potato production systems are based on the production of minitubers, grown on substrate in controlled environments, such as greenhouses. This process involves the acclimation and growth of plant material previously propagated in vitro. A low reproductive rate, usually 2–5 tubers per cultivated microplant, is considered the main limitation of substrate-based potato minituber production (Struik, 2007; Otazú, 2010; Broćić et al., 2021). To address this limitation, a common practice is the production of the final seed tubers (certified following the legislation of the respective country) after three to five generations of propagation in open field conditions (Struik and Wiersema 1999; Broćić et al., 2022). However, pathogens such as fungi, bacteria, and particularly viruses can easily be transmitted from one vegetative generation to the next, which increases the risk of infection with each subsequent reproduction cycle (Naik and Buckseth, 2018). To overcome these challenges in conventional seed production, soilless seed potato production technologies such as hydroponics, semi-hydroponics, and aeroponics have been developed over the last 20 years (Ritter et al., 2001; Mateus-Rodriguez et al., 2013). The world's largest potato producers are moving from conventional to high-tech seed production systems to improve seed quality and increase minituber production rates (Buckseth et al., 2022). In addition, researchers are making constant efforts to further develop innovative technologies for the rapid multiplication of minitubers.

Aeroponics is an advanced soilless potato growing technology that allows the production of a substantial quantity of healthy minitubers. The advantages of this technology, promoted by the International Potato Center (CIP), are high multiplication rates (up to 1:45), high production efficiency per area (>900 minitubers per m^2), as well as water, chemicals and energy savings (Mateus-Rodriguez et al., 2013). Aeroponics can potentially reduce the number of generations of seed multiplication in the field, with lower costs and maintain high phytosanitary quality (Nichols, 2005). In aeroponics, the leaves of the plants are exposed to the open air and light source of the greenhouse, while

the roots and stolons grow suspended in a fine mist of nutrient solution inside the closed modules. The front part of the modules is hinged and can be opened for harvesting minitubers of the desired size (Otazú, 2010; Lakhiar et al., 2018; Andrade-Piedra et al., 2019). The full access to available oxygen and carbon dioxide in the modules stimulates the growth of roots and stolons, leading to increased plant growth rates and productivity. Consequently, a significant number of tubers larger than 10 mm can develop on the stolons during the growth period. The sequential collection of tubers is conducted at intervals of 7 to 15 days (Farran and Mingo-Castel 2006). The minitubers are harvested when they reach the desired size, usually > 3 g. The harvested minitubers are left to harden for about two weeks before being placed in refrigerators at 2–4°C to be utilized for planting in the next crop season. Multiplication rates in aeroponic systems are significantly higher than those obtained in conventional systems and can range from 36 to over 100 minitubers per plant depending on the cultivar (Ritter et al., 2001; Farran and Mingo-Castel, 2006; Tierno et al., 2014; Broćić et al. 2021).

In addition to acclimated microplants and microtubers obtained from in vitro culture, the starting plant material for aeroponic potato production can also include minitubers from aeroponic cultivation of the previous seasons (Broćić et al., 2021, 2022), shoots or rooted stem cuttings delivered from microplants after acclimatizations (Muthoni et al., 2017), and rooted sprouts previously separated from seed tubers (da Silva Filho et al., 2020). Previous research has shown that the aeroponic technology is potentially efficient for specific potato cultivars and that there is variability between cultivars in terms of production in an aeroponic system under uniform conditions (Mateus-Rodriguez et al., 2012; Chang et al., 2011).

Cleopatra, Kennebec, and Désirée are popular and frequently cultivated potato cultivar in Serbia. This study aimed to evaluate the dynamics of minituber production and the aeroponic performance (the number of minitubers per plant, minituber mass, and yield per square meter) of these three cultivars when two types of planting material, acclimated microplants and plants originating from the previous season's minitubers, were used. Our results provide valuable information for selecting cultivars/genotypes and the type of planting material that enables a high production level of pre-basic seed potatoes in aeroponics.

Material and Methods

Experimental setup and plant material

The experiment was conducted from late May to December 2019 in an aeroponic facility in Guča (Serbia). A completely randomized block design with three replications for each combination of cultivar and plant origin was used for this study, with each replication comprising ten plants. The study involved three potato cultivars: Cleopatra (early maturing), Kennebec and Désirée (late maturing). Two types of planting material were used in the experiment: acclimated

microplants and plants derived from the minitubers harvested in the previous season. The potato microplants were *in vitro*-grown in environmentally-controlled conditions, (21 ºC, photoperiod 16 h, illumination 90 μmol m^{-2} s^{-1}, relative humidity 70%) and were transferred to nutritive medium every 30 days using single-node cuttings according to Momčilović et al. (2014). The potato microplants were planted in the substrate consisting of sand and perlite (4:1) and subsequently acclimated in the pest-free greenhouse for 25 days prior to their transfer to the aeroponic system (Figure 1A). Sprouted minitubers (obtained during the preceding season of aeroponic cultivation) were sown 10 days earlier in the same substrate, and 35 days later, the developed plants of similar size and physiological age compared to the acclimated microplants were transferred to aeroponics.

Figure 1. Potato production in an aeroponic facility. (A) Acclimation of potato microplants in the greenhouse. (B) Plants positioned in plant holders before transfer to the aeroponic module. (C) Aeroponic facility for potato cultivation. (D) Roots of plants, stolons and minitubers in the aeroponic module. Minitubers collected from aeroponically grown plants of (E) cv. Cleopatra, (F) cv. Désirée, and (G) cv. Kennebec.

The roots of the plants were rinsed with water, the stems were inserted into the holders, and the holders were placed in the module (Figure 1B). In the aeroponic system, the subterranean plant organs (roots and stolons) of the potato plants are

suspended in the air and supplied with water and nutrients via a nutrient solution dispersed as fine fog particles (30–100 microns). Concurrently, the foliage develops above the module under controlled greenhouse conditions. The planting density within the aeroponic module was established at 24 plants per m^2 (Figure 1C). The minitubers were collected approximately every 14 days (from July to December), and the number of minitubers (tuber length ≥ 2 cm) per plant, along with the mass of the minitubers, were measured at the end of each harvest interval. In addition, the productivity parameters of the cultivars (minitubers per plant, minituber mass and yield per m^2) were measured at the end of the growing season.

Temperature conditions

During the experiment, temperature measurements were made in the greenhouse (in the area of the plant leaves) and in the aeroponic module, where the plant roots and stolons were located. The average daily temperatures are presented in Table 1.

Table 1. Daily average temperatures in the greenhouse and aeroponic module during the experimental period in 2019.

Month	Greenhouse	Aeroponic module
	Temperature (°C)	
June	27.8	23.4
July	25.1	21.9
August	27.3	22.6
September	23.2	19.3
October	20.0	17.0
November	15.7	13.4
Average	23.2	19.6

Statistical analysis

Statistical analysis was performed using STATISTICA 12 (StatSoft, Inc. 1984-2014, Tulsa, OK, USA). The mean minituber mass or number of tubers per plant was correlated with harvest time. A regression analysis was performed and the Pearson's correlation coefficient was calculated. The data regarding the total number of minitubers per plant, the mass of minitubers and yield were analyzed using a two-factor analysis of variance (ANOVA) with plant origin and genotype (cultivar) as the categorical predictors. To compare the means, the Tukey's multiple comparison test was used at the significance level of $p < 0.05$.

Results and Discussion

Cultivation of potato plants in aeroponics

The research study involved plants from three commercial potato cultivars (Cleopatra, Désirée, and Kennebec), obtained from two types of planting materials: microplants and minitubers. The microplants were acclimated in the greenhouse for 25 days prior to transfer into the aeroponic system, while the plants developed from sprouted minitubers were transferred to the aeroponic modules 35 days after tuber-sowing. These plants differed in some morphological and anatomical traits before and after relocation to aeroponics. Plants developed from minitubers possessed a vigorous primary shoot, greener foliage and a larger root system compared to those originating from the *in vitro* culture, which produced multiple shoots.

The temperature, as a significant factor for the initiation, development and enlargement of potato tubers, was recorded in both the aeroponic system and the greenhouse throughout the duration of the experiment (Table 1). In the aeroponic module, the daily average temperatures ranged from 22.6 ºC in the initial stage of the growing period to 16.6 ºC in the second stage of the growing period, which is close to the optimal temperature range for potato tuberization (14–22°C). The temperatures in the insect-free greenhouse were higher, namely 26.7°C in the initial phase of the growing period and 19.7°C in the second phase, but adequate for foliage growth due to the different temperature requirements.

Dynamics of minituber production in aeroponics

The dynamics of minituber production varied among the three genotypes studied. For plants originating from minitubers, tuberization in the Cleopatra, Kennebec, and Désirée cultivars began 30 days after planting in the aeroponic system, and tuber formation occurred consistently throughout the entire cultivation period (Figure 2A). However, Kennebec and Désirée exhibited more vigorous tuberization, producing a significantly higher number of minitubers compared to Cleopatra. The highest numbers of minitubers were recorded in Kennebec and Cleopatra plants at the seventh harvest and in Désirée at the final, tenth harvest. Overall, a greater number of minitubers was collected from Cleopatra plants during the mid-point of the cultivation period, whereas Kennebec and Désirée produced more minitubers in the latter half of the growing season (Figure 2A). Both Kennebec and Désirée are classified as medium-late to late cultivars, with a vegetative cycle of 120–135 days, which was extended by 35 days due to aeroponic cultivation.

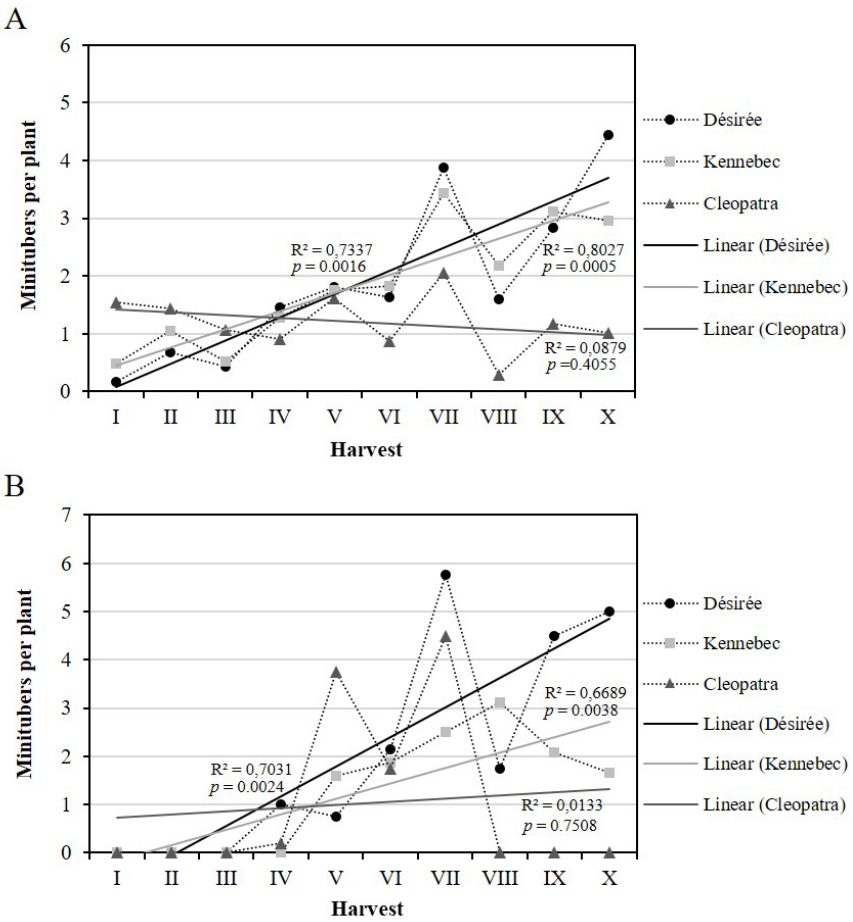

Figure 2. The dynamics of minituber formation for three potato cultivars grown in the aeroponic system**.** (A) Plants of minituber origin. (B) Plants of *in vitro* origin.

For plants of *in vitro* origin, the first minitubers from the investigated cultivars were harvested at the end of August (harvest IV). The Cleopatra cultivar was the first to complete its vegetative cycle at the end of October, with the final number of minitubers collected at the seventh harvest (Figure 2B). This cultivar is characterized as early-maturing, with a vegetation period of 85–100 days in open field production in Serbia; in particular, this developmental trait appears unaffected by aeroponic cultivation. In the Kennebec plants of *in vitro* origin (Figure 2B), the highest number of minitubers was collected during the middle of the growing period (harvests V–VIII), while Désirée exhibited maximum values for the number

of minitubers per plant in the second half of the cultivation period (harvests VI-X). Additionally, Désirée produced a significantly larger number of minitubers compared to the other two cultivars.

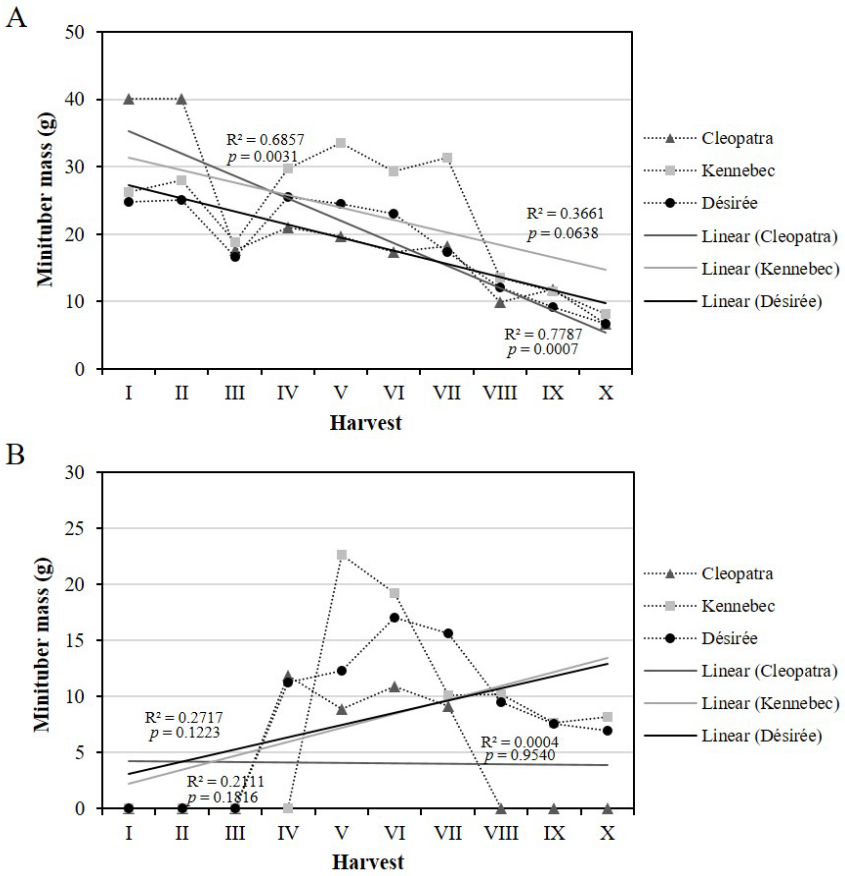

Figure 3. The dynamics of minituber mass per harvest for three potato cultivars grown in the aeroponic system. (A) Plants of minituber origin. (B) Plants of *in vitro* origin.

The heaviest tubers formed by Cleopatra plants of minituber origin (Figure 3A) were recorded early in the cultivation period (harvests I and II), while the mass of minitubers decreased in later harvests during the second half of the growing period. These findings align with those of Broćić et al. (2019a). In contrast, for Cleopatra plants of *in vitro* origin, only four harvests were conducted during the middle of the cultivation period (Figure 3B). The highest masses of minitubers for both Kennebec and Désirée, produced by plants of both origins (Figure 3A and B),

were recorded in the middle of the cultivation period (harvests V–VII). According to Broćić et al. (2022), the decrease in minituber mass during the final harvests is likely due to rapid maturation caused by low environmental temperatures, a phenomenon also noted in our experiment (Table 1, Figure 3).

Effects of cultivar and plant origin on minituber production

The results of the two-way ANOVA showed the significant effect of both factors, genotype (cultivar) and plant origin, on minituber production in an aeroponic system (Table 2).

Table 2. Results of the two-way ANOVA for production of potato minitubers in an aeroponic system.

Parameter	Factor	df	SS	MS	F	*p*	Sig.
Number of minitubers per plant	Genotype	2	231.977	115.989	50.269	1.47×10^{-6}	***
	Plant origin	1	15.290	15.290	6.627	2.44×10^{-2}	*
	Genotype x plant origin	2	45.408	22.704	9.840	2.95×10^{-3}	**
Minituber mass	Genotype	2	46.570	23.285	12.540	1.15×10^{-3}	**
	Plant origin	1	319.286	319.286	171.949	1.79×10^{-8}	***
	Genotype x plant origin	2	6.803	3.401	1.832	2.02×10^{-1}	-
Yield per m^2	Genotype	2	40.8498	20.4249	38.789	5.78×10^{-6}	***
	Plant origin	1	67.6076	67.6076	128.395	9.14×10^{-8}	***
	Genotype x plant origin	2	12.6105	6.3052	11.974	1.38×10^{-3}	**

Note: SS, MS, df, and F are test parameters; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The investigated factors significantly affected the total number of minitubers per potato plant. A significant two-way interaction between genotype and plant origin indicated that the effect of plant origin varied among cultivars (Table 2). The highest number of minitubers per plant (20.90) was recorded in Désirée plants of *in vitro* origin, while the lowest number (10.22) was observed in the Cleopatra cultivar of the same origin (Figure 4). Similar findings for the Désirée and Cleopatra cultivars were reported by Broćić et al. (2018). The Désirée and Kennebec plants, derived from minitubers, produced a significantly larger number of minitubers compared to the Cleopatra plants. A post-hoc analysis revealed that plant origin did not significantly affect the number of minitubers formed in Cleopatra and Désirée, while it resulted in significant changes in Kennebec (Figure 4). Specifically, Kennebec plants originating from minitubers formed a significantly higher number of minitubers than those of *in vitro* origin. The results of the present study are not in accordance with some previous

findings (Muthoni et al., 2017). Muthoni et al. (2017) investigated how different planting materials (microplants, stem cuttings, and minitubers) impact the productivity of two potato cultivars, Asante and Tigoni, in an aeroponic minituber production system. The authors reported that potato plants derived from *in vitro* sources produced a significantly higher quantity of minitubers compared to plants derived from minitubers, 1.64- and 1.84-fold higher in the first and the second growing season, respectively. However, the results were not shown separately for each cultivar, Asante and Trigoni.

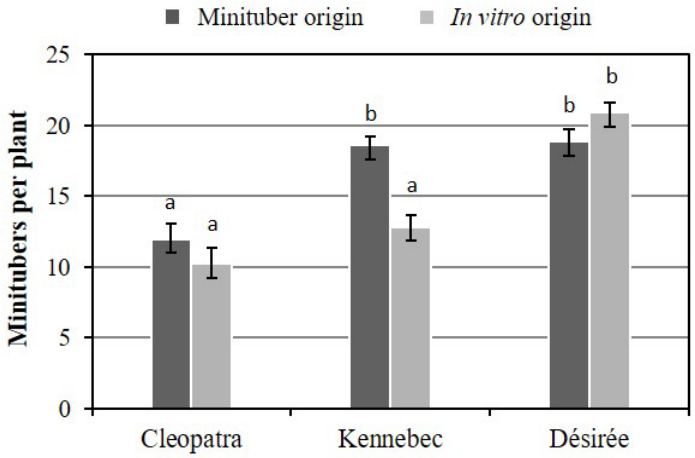

Figure 4. Effects of cultivar and plant origin on the formation of minitubers in an aeroponic system.

The mass of minitubers was also affected by the genotype and plant origin (Table 2). Plants of *in vitro* origin produced tubers of similar mass across all three investigated cultivars, while Kennebec plants from minituber origin yielded significantly heavier tubers than both Désirée and Cleopatra (Figure 5). Furthermore, the average mass of minitubers for all cultivars was significantly higher in plants originating from minitubers compared to those of *in vitro* origin (Figure 5), which is consistent with the findings of Broćić et al. (2023). In agreement with our previous research from the 2018 season (Broćić et al., 2019a, 2019b), Kennebec plants of minituber origin had the highest average mass of minitubers at 23.01 g, while Cleopatra and Désirée plants of minituber origin achieved a mass of 18.46 g. These minituber masses are notably higher than those reported by other researchers (Farran and Mingo-Castel, 2006; Struik 2007; Rykaczewska 2016) and in our previous studies (Broćić et al., 2018, 2019a, 2019b).

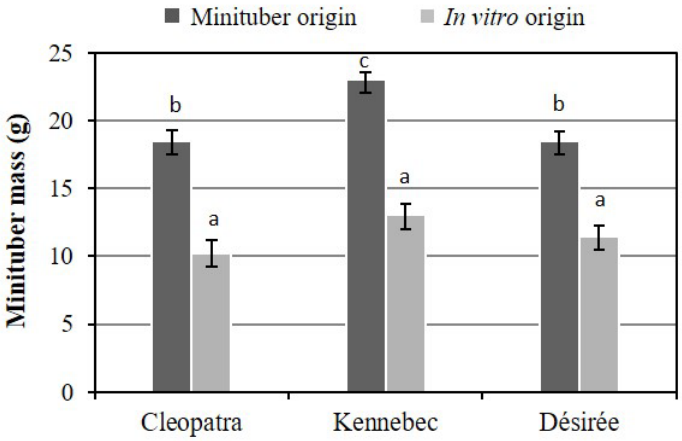

Figure 5. Effects of cultivar and plant origin on the minituber mass in an aeroponic system.

The yield was influenced by the factors of genotype and plant origin. A significant two-way interaction between genotype and plant origin revealed that the impact of plant origin varied among the cultivars (Table 2). Specifically, there was no significant difference in yield between the plants of minituber origin of the Désirée and Kennebec cultivars, while the Cleopatra plants of minituber origin produced a notably lower average yield compared to the other cultivars (Figure 6).

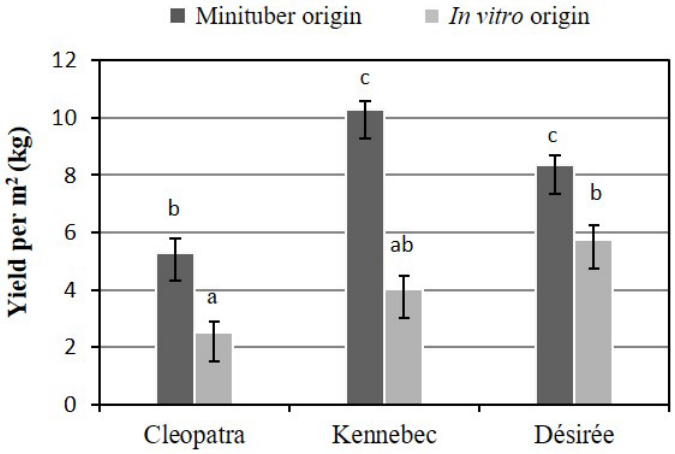

Figure 6. Effects of cultivar and plant origin on the minituber yield in an aeroponic system.

The Kennebec, Désirée, and Cleopatra plants of minituber origin produced higher yields per square meter compared to those from *in vitro* origin. The highest yield was observed in the Kennebec plants from minitubers, with 10.27 kg/m², followed by Désirée with 8.35 kg/m² and Cleopatra with 5.30 kg/m², both from the same origin (Figure 6).

Conclusion

The findings of our study indicate different performance of three potato cultivars in aeroponic cultivation, as well as a significant impact of the origin of the plant material on minituber production. The cultivars Kennebec and Desiree are classified as medium-late to late varieties with a growing season of 120–135 days that was extended by aeroponic cultivation to approximately 150 days. However, the life-cycle length of the Cleopatra cultivar depended on the plant origin. This cultivar is characterized as an early ripening plant with a vegetation period of 85–100 days in open-field cultivation in Serbia, and this period was prolonged only for plants derived from minitubers during aeroponic cultivation. Kennebec and Désirée were more productive than the Cleopatra cultivar during aeroponic cultivation in the 2019 season. Désirée and Kennebec plants derived from minitubers formed a significantly higher number of minitubers than Cleopatra plants, while Désirée plants of *in vitro* origin formed a significantly larger number of tubers compared to the other two cultivars of the same origin. Furthermore, the average mass and yield of minitubers of all the cultivars were significantly higher in plants grown from minitubers harvested in the previous season than in plants of *in vitro* origin.

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AEROPONSKA PROIZVODNJA MINI KRTOLA KROMPIRA: UTICAJ GENOTIPA I POREKLA SADNOG MATERIJALA

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R e z i m e

Aeroponika je savremena poljoprivredna tehnologija gajenja krompira bez zemljišta, koja omogućava efikasnu proizvodnju visokokvalitetnog predosnovnog semenskog krompira (mini krtola). U ovom sistemu, koreni i podzemna stabla (stolone) biljaka krompira rastu unutar zatvorenih modula, suspendovani u finoj magli rastvora bogatog hranljivim materijama koji neprekidno cirkuliše. Ova postavka omogućava formiranje brojnih mini krtola dužine veće od 10 mm tokom perioda rasta. Naša studija je imala za cilj da proceni efekat genotipa i porekla sadnog materijala na proizvodnju mini krtola u aeroponskom objektu u Guči, Srbija. Analizirane su tri sorte krompira: kleopatra, kenebek i dezire, upotrebom dve vrste sadnog materijala: aklimatizovanih mikrobiljaka i biljaka dobijenih od mini krtola iz prethodne sezone. Biljke su uzgajane aeroponski od kraja maja do decembra 2019. godine, sa gustinom sadnje od 24 biljke po kvadratnom metru i intervalima žetve od približno 14 dana. Sorta dezire produkovala je najveći broj mini krtola po biljci (19,89), zatim kenebek (15,71) i kleopatra (11,05). Prosečna težina mini krtola bila je značajno veća kod biljaka gajenih od prošlosezonskih mini krtola u odnosu na biljke *in vitro* porekla. Najveći prinos od 10,27 kg po kvadratnom metru, ostvarile su biljke sorte kenebek poreklom od mini krtola. Pored toga, biljke poreklom od mini krtola su postojano proizvodile krtole tokom čitavog perioda gajenja u aeroponskom sistemu.

Ključne reči: uzgoj bez zemljišta, sorte krompira, predosnovni semenski krompir.

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POSSIBILITY OF USING THE BOTANICAL INSECTICIDE AZADIRACHTIN AND SYNTHETIC AND SEMI-SYNTHETIC INSECTICIDES TO CONTROL *HELICOVERPA ARMIGERA* IN SWEET PEPPER

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Abstract: During 2019 and 2020, field experiments were performed on sweet pepper crops to determine the efficacy of chemical insecticides (lambda-cyhalothrin, flubendiamide), semi-synthetic (emamectin benzoate), and biological pesticide (azadirachtin) in controlling cotton bollworm (*Helicoverpa armigera*). The experiments were performed in a randomized complete block design with four replications according to the standard EPPO method at the site of Veliko Gradište (Serbia). Flubendiamide was applied at a rate of 50 g/ha, lambda-cyhalothrin at a rate of 7.5 g/ha, emamectin benzoate at a rate of 375 g/ha, and azadirachtin at a rate of 0.75 g/ha. The intensity of the 2nd generation cotton bollworm infestation on sweet pepper at this locality was higher during 2020 compared to 2019. After performing two treatments for the 2nd generation, flubendiamide showed the highest efficacy, ranging from 92.42% (3 days after treatment – DAT, 2020) to 95.56% (9DAT, 2019). Lambda-cyhalothrin had a satisfactory efficacy in the range of 81.93% (9DAT, 2020) to 90.63% (3DAT, 2019), and emamectin benzoate showed similar efficacy from 80.72% (9DAT, 2020) to 90.63% (3DAT, 2019). Azadirachtin could gain a significant place as a botanical insecticide in integrated pest management programs for sweet pepper protection from *H. armigera*. However, it statistically showed a significantly lower efficacy than other insecticides (77.27%: 3DAT, 2020 to 86.67%: 9DAT, 2019).

Key words: effects, insecticides, botanical insecticide, cotton bollworm, *Capsicum annuum*.

Introduction

The cotton bollworm (*Helicoverpa armigera* Hübner, Lepidoptera: Noctuidae) is a polyphagous pest (Fathipour and Sedaratian, 2013) with high fecundity, a wide range of host plants, and a high potential for developing insecticide resistance. The larvae cause damage to peppers by burrowing into fruits and feeding on their

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internal contents. Fruits damaged in this way are susceptible to attack by pathogens that cause wilting and rot (*Fusarium* spp., *Alternaria* spp., *Erwinia carotovora*). As such, they are not suitable for human consumption (Sekulić et al., 2004). The producers must pay special attention to cotton bollworm monitoring and control as it can potentially cause significant damage. Various non-chemical (agrotechnical, mechanical, biological) and chemical measures are used to control *H. armigera*. Of all the available measures, the application of insecticides is the most common, especially in the conditions of growing sweet peppers in the field. However, for insecticides to give good results, treatments must be timely, i.e., they should be performed when earlier larval stages are present and before they penetrate the fruits.

Various insecticides are available worldwide for the control of *H. armigera*. These include non-selective pyrethroids and newer, highly selective diamides, semi-synthetic insecticides, and some compounds of natural origin. Natural products have been increasingly used to control various pests of cultivated plants. Bioinsecticides have some favorable characteristics such as a biological basis and an excellent toxicological and ecotoxicological profile. Azadirachtin belongs to the biochemical bioinsecticides and is one of the limonoids extracted from the Indian neem tree, *Azadirachta indica*. Structurally, azadirachtin is similar to the insect hormone ecdysone and labeled as an ecdysone blocker. The antifeeding effect occurs because of the action of azadirachtin on the taste receptors and the paralysis of the oral apparatus, which results in the termination of feeding (Mordue et al., 2010). Azadirachtin is widely used in the world for the protection of various cultivated plants because of its biological origin, its favorable toxicological and ecotoxicological properties, different modes of action, low potential for pest resistance development, and promising effectiveness in controlling pests from distant insect orders (Lepidoptera, Coleoptera, Hemiptera, and Homoptera). Flubendiamide and emamectin benzoate, semi-synthetic bioinsecticide, are highly selective insecticides with exceptional biological activity in controlling Lepidoptera larvae, especially *H. armigera* (Ameta and Bunker, 2007). In addition, their selectivity for beneficial arthropods makes them suitable for inclusion in integrated pest management (IPM) programs. This contrasts with pyrethroids, which damage beneficial arthropods, and where resistance problems develop. *H. armigera* is a species whose populations rapidly develop resistance to insecticides. This pest has developed resistance to many synthesized chemicals from the group of pyrethroids, organophosphates, carbamates, etc. The resistance of the cotton bollworm to pyrethroids was found in southern France, where the resistance factor for deltamethrin was 32-fold (Buès et al., 2005). High resistance to cypermethrin was also found in southern India, where the resistance factor for this compound was 48-fold, while resistance to chlorpyrifos was low to moderate (Chaturved, 2007). The ecotoxicological and toxicological consequences of resistance manifest through environmental pollution due to the increase in insecticide application rates

and the increased number of treatments during the growing season. These consequences are critical reasons for the mandatory introduction of natural products in IPM plant protection programs, where bioinsecticides and semi-synthetic products are crucial.

This study aimed to determine the efficacy of insecticides of chemical, semi-synthetic and biological origin in the control of cotton bollworm (*H. armigera*) on sweet pepper in field conditions to justify their use.

Material and Methods

The field trials were performed according to the standardized and partially adapted EPPO method PP 1/295 (1) (EPPO Standards, 2016) to test insecticide efficacy in controlling *H. armigera* on vegetables and ornamentals (EPPO).

The experiments were carried out during 2019 and 2020 in the sweet pepper crops at the locality of Veliko Gradište (GPS: N 44° 44.477204; E 21° 24.177048) in a randomized complete block design with four replications. The size of the experimental plot was 25 m^2. The "Solo" backpack sprayer was used for the treatments. The insecticide preparations were applied with a water consumption of 500 l/ha. The effects of the following insecticides were investigated: flubendiamide, emamectin benzoate, lambda-cyhalothrin, and azadirachtin (Table 1).

Table 1. Insecticides examined in the trials.

Insecticide	Trade names of the insecticides (the content of a.i.)	Amount of insecticide preparation	Amount of insecticide (g/ha)
Flubendiamide	FLUBENDIAMIDE SC (200 g a.i./l)	0.25 l/ha	50
Emamectin benzoate	AFFIRM 095 SG (250 g a.i./l)	1.50 kg/ha	375
Lambda-cyhalothrin	LAMDEX 5 CS (50 g a.i./l)	0.15 l/ha	7.5
Azadirachtin	NIMBECIDINE EC (0.3 g a.i./l)	2.5 l/ha	0.75
Control (untreated)	-	-	-

Two treatments of the sweet pepper crop were performed to control the second generation of cotton bollworm during each year of examination. The first treatment was established based on monitoring the flight of the butterflies with light traps and visual inspection of the fruit to determine the presence of pest eggs. The first treatments were performed after the confirmed presence of laid eggs, and the first hatched larvae before their penetration into the fruits. The treatments were carried out in the evening to ensure optimal temperature conditions for the insecticides to take effect and to avoid direct sunlight. The usual agrotechnical and plant

protection measures were implemented since the crop was established. The first generation of *H. armigera* was regularly controlled. The evaluation of the trial results was performed in two periods: three days after the second treatment (3DAT) and nine days after the second treatment (9DAT) (Table 2).

Table 2. Dates of insecticide treatments and result evaluations.

Locality	Veliko Gradište, Serbia	
Year	2019	2020
Date of the first treatment	August 6	August 12
Date of the second treatment	August 15	August 21
The first evaluation	August 18 (3DAT*)	August 24 (3DAT)
The second evaluation	August 24 (9DAT)	August 30 (9DAT)

*DAT: days after the second treatment.

The evaluation was conducted by examining 100 randomly selected fruits in each experimental plot and determining the number of fruits damaged by *H. armigera* larvae.

The standard EPPO method PP 1/152 (4) (EPPO Standards, 2012) was used for the statistical processing of the test results. The average damage of fruits (Ms) by treatments and the standard deviation (Sd) were determined, as well as the comparison of means, i.e., the significance of the differences between the treatments (Student's t-test). The analysis of variance was processed in the Microsoft Excel computer program. The percentages of fruit damage in treatment replications (x) were previously transformed using statistics: $\sqrt{x + 0{,}5}$.

Immediately before each treatment, it was found that there were no damaged fruits, only a certain number of eggs laid. Therefore, the efficacy of the insecticides was calculated using the Abbott's formula based on the damage observed in the post-treatment assessments.

Results and Discussion

The test results are shown in Tables 3 and 4.

At the locality of Veliko Gradište, the intensity of infestation of the second generation of the cotton bollworm on sweet pepper was higher during 2020 compared to 2019, so that the average fruit damage in the untreated plot was 8% (3DAT) and 11.25% (9DAT) (Table 3), while in 2020, it was 16.5% (3DAT) and 20.75% (9DAT) (Table 4).

In our experiments, lambda-cyhalothrin (7.5 g/ha) had a satisfactory efficacy in the control of *H. armigera* on sweet pepper at the locality of Veliko Gradište,

but the efficacy was better at lower levels of this pest. In the first assessment after the second treatment (3DAT), the efficacy of this compound was 90.63% (2019) and 86.36% (2020). In the later assessment (9DAT), there was a particular decline in the degree of efficacy, and it amounted to 88.89% (2019) and 81.93% (2020). The field trials conducted during 2012 and 2013 confirmed that the efficacy of lambda-cyhalothrin ranged between 88.03% and 90.89% in controlling the cotton bollworm on chickpea (Yogeeswarudu and Venkata Krishna, 2014).

Table 3. The efficacy of the insecticides applied in the control of *H. armigera* on sweet pepper (Veliko Gradište, 2019).

Treatments	Average damage of fruits (Ms ± Sd)*	
	Efficacy %	
	3DAT	9DAT
Lambda-cyhalothrin (7.5 g/ha)	$0.75^{a**} \pm 0.96$	$1.25^{a} \pm 0.96$
	90.63	***88.89***
Flubendiamide (50 g/ha)	$0.5^{a} \pm 0.58$	$0.5^{b} \pm 0.58$
	93.75	***95.56***
Emamectin benzoate (375 g/ha)	$0.75^{a} \pm 0.96$	$2.0^{c} \pm 0.82$
	90.63	***82.22***
Azadirachtin (0.75 g/ha)	$1.25^{b} \pm 0.96$	$1.5b^{a} \pm 0.58$
	84.38	***86.67***
Control (untreated plot)	$8.0^{c} \pm 1.83$	$11.25^{d} \pm 3.59$
$LSD_{0.05}$	0.1182	0.1033
$LSD_{0.01}$	0.1957	0.1709

*Data are expressed as average damage of fruits (Ms) ± standard deviation (Sd) of four replications of each insecticide treatment; **Mean values followed by the same superscript letter (s) within the same column are insignificantly different ($P \leq 0.05$; $P \leq 0.01$) according to the Student's *t-test.*

Of all the tested insecticides, flubendiamide (50 g/ha) showed the highest efficacy during both years. In the evaluations at 3DAT and 9DAT, flubendiamide showed good efficacy, and it amounted to 93.75% and 95.56% during 2019. The excellent efficacy of this insecticide was also recorded in 2020, namely 3DAT: 92.42% and 9DAT: 93.98%.

During 2019 and 2020, emamectin benzoate (375 g/ha) showed a statistically significantly lower efficacy than flubendiamide in our experiments, while it also had a weaker prolonged effect at 9DAT. It had a satisfactory initial efficacy (3DAT) of 90.63% (2019) and 87.88% (2020). The weaker efficacy of emamectin benzoate was achieved at 9DAT, and it amounted to 82.22% (2019) and 80.72% (2020).

According to the results of the field trials conducted during 2005 and 2006 in the state of Tamil Nadu (India), the efficacy of flubendiamide (Flubendiamide 480 SC, 125 ml/ha) in controlling *H. armigera* on tomatoes ranged from 86.24% to

99.51%, while emamectin benzoate had a lower efficacy (64.14–79.76%) (Kubendran et al., 2008). Ameta and Kumar (2008) reported the excellent efficacy of flubendiamide in controlling the cotton bollworm on the chili pepper. Murugaraj et al. (2006) emphasized a high efficacy of 91.46% for emamectin benzoate in the control of *H. armigera* on tomatoes.

Table 4. The efficacy of the insecticides applied in the control of *H. armigera* on sweet pepper (Veliko Gradište, 2020).

Treatments	Average damage of fruits (Ms ± Sd)	
	Efficacy %	
	3DAT	9DAT
Lambda-cyhalothrin (7.5 g/ha)	2.25^{a} ± 0,96 ***86.36***	3.75^{a} ± 1.50 ***81.93***
Flubendiamide (50 g/ha)	1.25^{b} ± 0.50 ***92.42***	1.25^{b} ± 1.26 ***93.98***
Emamectin benzoate (375 g/ha)	2.0^{c} ± 0.82 ***87.88***	4.0^{c} ± 0.82 ***80.72***
Azadirachtin (0.75 g/ha)	3.75^{d} ± 0.50 ***77.27***	4.25^{d} ± 0.96 ***79.52***
Control (untreated plot)	16.5^{e} ± 4.20	20.75^{e} ± 3.30
$LSD_{0.05}$	0.0393	0.0288
$LSD_{0.01}$	0.0651	0.0477

In our experiments during 2019 and 2020, azadirachtin (0.75 g/ha) showed a statistically significantly lower efficacy compared to the other tested insecticides ($P \leq 0.05$; $P \leq 0.01$), with a more pronounced and prolonged effect at 9DAT. However, although its efficacy was satisfactory or even weak in the conditions of less persistent attacks of *H. armigera*, it offers certain advantages over the synthetic insecticides due to its biological origin. Azadirachtin showed an efficiency of 84.38% (2019) and 77.27% (2020) at the 3DAT assessments. In the later assessment at 9DAT, there was an increase in the degree of efficacy, and it amounted to 86.67% (2019) and 79.52% (2020). In trials conducted by other authors during 2013 and 2014, the efficacy of azadirachtin A (Nimbecidine, 0.4%) in the control of *H. armigera* on tomatoes was 83.33% (Kumar et al., 2016). Yankova and Todorova (2011) found that the efficacy of azadirachtin (NeemAzal T/S 0.3%) was 77.12% in controlling the cotton bollworm on peppers. Gayi et al. (2016) discussed the excellent efficacy of azadirachtin and bifenthrin in *H. armigera* larvae control on cotton. Good efficacy of azadirachtin and emamectin benzoate in the control of *H. armigera* on tomatoes was also found by Shah et al. (2013). An efficacy of 87.37% in the control of *Tuta absoluta* was achieved by applying azadirachtin in tomatoes (Sammour et al., 2018).

Conclusion

All tested insecticides could be found within the regular IPM programs to protect sweet pepper from the cotton bollworm, depending on the intensity of the infestation by this pest. Flubendiamide as the most effective tested insecticide with a short withdrawal period (3 days) would be a suitable solution in conditions of intensive activity of *H. armigera*, especially the second generation, but also later generations that occur when the harvest has already begun. Lambda-cyhalothrin exhibited poorer efficacy in intense cotton bollworm attack conditions, so it could be positioned in IPM programs when the pest population density is lower, and this is mostly the typical case during the development of the first generation. The semi-synthetic insecticide emamectin benzoate and the bioinsecticide azadirachtin have shown lower efficacy in severe infestation of *H. armigera*. However, they should also be included in IPM programs due to their favourable ecotoxicological and toxicological properties. They could be recommended for sweet pepper protection when this pest is present in lower population densities. In addition, due to the short withdrawal period, biological insecticides should be used in case of pest activity at the beginning of ripening and between fruit harvests.

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MOGUĆNOST PRIMENE BIOINSEKTICIDA AZADIRAHTINA I NEKIH SINTETIČKIH I POLUSINTETIČKIH INSEKTICIDA U SUZBIJANJU *HELICOVERPA ARMIGERA* NA PAPRICI

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R e z i m e

Tokom 2019. i 2020. godine vršeni su poljski ogledi na usevima paprike u cilju utvrđivanja efikasnosti sintetičkih insekticida (lambda-cihalotrin, flubendiamid), polusintetičkih (emamektin benzoat) i bioinsekticida (azadirahtin) u suzbijanju pamukove sovice (*Helicoverpa armigera*). Ogledi su izvedeni prema tipu potpunog slučajnog blok sistema u četiri ponavljanja prema standardnoj metodi EPPO na lokalitetu Veliko Gradište (Srbija). Flubendiamid je primenjen u količini od 50 g/ha, lambda-cihalotrin u 7,5 g/ha, emamektin benzoat u 375 g/ha, a azadirahtin u količini od 0,75 g/ha. Intenzitet infestacije larvama druge generacije pamukove sovice na paprici na ovom lokalitetu bio je veći tokom 2020. godine u odnosu na 2019. godinu. Nakon obavljena dva tretiranja za suzbijanje druge generacije, flubendiamid je pokazao najveću efikasnost, u rasponu od 92,42% (3 dana posle tretiranja – DPT, 2020) do 95,6% (9DPT, 2019). Lambda-cihalotrin je imao zadovoljavajuću efikasnost u rasponu od 81,93% (9DPT, 2020) do 90,63% (3DPT, 2019), a emamektin benzoat je pokazao sličnu efikasnost od 80,72% (9DPT, 2020) do 90,63% (3DPT, 2019). Azadirahtin bi kao bioinsekticid mogao da zauzme značajno mesto u integralnim programima zaštite paprike od *H. armigera*. Međutim, statistički je pokazao značajno nižu efikasnost od drugih insekticida (77,27%: 3DPT, 2020, do 86,67%: 9DPT, 2019).

Ključne reči: efekti, insekticidi, bioinsekticid, pamukova sovica, *Capsicum annuum.*

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RAMAN SPECTROSCOPY IN THE CHARACTERIZATION OF AUTOCHTHONOUS SWEET CHERRY (*PRUNUS AVIUM* L.) CULTIVARS FROM THE BALKAN REGION

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Abstract: The quality assessment and evaluation of fruits and vegetables are crucial in their postprocessing, shelf life, and price. Most of the techniques applied to evaluate fruit and vegetable quality are invasive. However, there is a growing interest in non-invasive techniques for assessing fruit quality, which are gaining traction due to their application and operation mechanism. The present study demonstrates, for the first time, the applicability of the Raman spectroscopy for spectral signature assessment of sweet cherry (*Prunus avium* L.) cultivars ('Đuti', 'Canetova', 'Ohridska crna', and 'Dolga Šiška'). Combined with principal component analysis (PCA), Raman spectroscopy was used in assessing nutritionally similar samples, such as the studied sweet cherry cultivars. Sugars (glucose, sucrose, and fructose), anthocyanins, phenolic acids, and flavonoids, quantified by comparison to reference standards using high-performance liquid chromatography, exhibited Raman bands (at 337, 399, 455, 538, 617, 1327, and 1600 cm^{-1}, respectively) of varying intensities, indicating differences among cultivars. Compared to the other cultivars, the 'Ohridska crna' cultivar had the highest nutritional and health-promoting compounds. A correlation was found between the Raman bands and the sugar and phenolic content obtained by chemical analysis. The results indicate the applicability of chemometric modeling associated with Raman spectroscopy for rapid sweet cherry authentication.

Key words: sweet cherry, vibrational modes, multivariate analysis, HPLC analysis, anthocyanins, phenolic compounds, carbohydrates.

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Introduction

The sweet cherry tree (*Prunus avium* L.) is one of the most valuable species of stone fruit trees. Sweet cherries, typically consumed fresh and unprocessed, are considered early-season fruits (Usenik et al., 2008). Their regular consumption is associated with a balanced diet and an array of health benefits owing to the abundant presence of bioactive and nutraceutical compounds in this fruit (Faienza et al., 2020). The chemical composition of cherries is contingent on several factors, most notably the specific cultivar. Additionally, factors such as maturation age, agricultural practices, and environmental conditions can significantly influence the chemical profile of cherries. Carbohydrates, sugars (e.g., fructose, glucose, sorbitol), constitute the primary chemical compounds in cherries. Sweet cherries are esteemed as excellent sources of dietary phenolic compounds, encompassing phenolic acids (p-coumaric and chlorogenic acids) and flavonoids (anthocyanins, flavan-3-ols, and flavonols), as well as valuable sources of vitamins, particularly vitamin C, and minerals such as calcium, magnesium, and potassium (Schmitz-Eiberger and Blanke, 2012; Ferretti et al., 2010; Yıgıt et al., 2009).

Epidemiological research has revealed a link between the intake of fruits and vegetables abundant in bioactive compounds or phytochemicals and a decreased risk of developing degenerative diseases caused by oxidative stress, such as cancer (Kang et al., 2003), atherosclerosis, diabetes mellitus, and cardiovascular disease (Faienza et al., 2020). Moreover, scientific investigations suggest that the consumption of sweet cherries may have beneficial effects, including easing arthritis-related pain and inflammation (Jacob et al., 2003; Seeram et al., 2001) and protecting against neurodegenerative disorders (Filaferro et al., 2022). Kent et al. (2016) provided evidence that the consumption of sweet cherries, whether as fruit or juice, can lead to a significant decrease in both systolic and diastolic blood pressure and heart rate, especially among individuals with hypertension.

Up to this point, the majority of research endeavors have employed high-performance liquid chromatography (and/or spectrophotometric methods) as their primary method for pinpointing and measuring bioactive substances within sweet cherries (Ballistreri et al., 2013; Clodoveo et al., 2023). Nonetheless, conventional approaches to evaluate fruit quality primarily revolve around sensory assessment and chemical analysis, which are prone to external influences, lack precision, have limited detection speed, and demand significant resources. Consequently, researchers across the globe have been actively exploring and advancing novel, non-invasive detection technologies, with Raman spectroscopy being a prominent example (Xu et al., 2020).

The development of a rapid, highly accurate, non-destructive, and cost-effective fruit quality testing technology is of paramount practical importance. In addition to its inherent advantages of high efficiency and non-destructiveness,

Raman spectroscopy (RS) is unaffected by water and can be applied to aqueous solutions (Neng et al., 2020). Its strong penetration capability makes it suitable for assessing the internal quality of fruits and vegetables. Thus, Raman spectroscopy plays a crucial role in studying food chemical composition and quality control (Seidler-Lozykowska et al., 2010; Petersen et al., 2021; Nakajima et al., 2023; Xu et al., 2023). This type of spectroscopy typically provides a special structural fingerprint that is used to identify different molecules. In general, Raman spectra can provide some minor changes that allow the isolation of specific local variations of certain phytochemicals.

Raman spectroscopy, combined with chemometric data analysis, proves to be a formidable method for detecting chemical structures, even from complex matrices (Xu et al., 2020). The application of Raman spectroscopy coupled with chemometrics represents a step forward in sweet cherry fruit authentication approaches. To the best of our knowledge, this is the first study of the nutritional composition of cherry fruits using Raman spectroscopy.

The present work aims to make a comparative study of the four autochthonous sweet cherry cultivars originating from Serbia and North Macedonia. Raman spectroscopy coupled with chemometrics was applied to evaluate the differences in the nutritional profile, phenolic composition, and health qualities of cherries. In addition, high-performance liquid chromatography was also employed to quantify the individual sugars and phenolic compounds of the sweet cherry cultivars to gain a deeper understanding of the outcomes acquired through the non-invasive Raman technique. Anthocyanins, phenolic acids, and flavonoids, which are primarily responsible for the health-promoting effect of these fruits, were quantified.

Material and Methods

Five autochthonous sweet cherry cultivars originating from Serbia and North Macedonia were harvested manually in the period from May to July 2022 and used for the studies: ‘Đuti’ (May 20, Belgrade, 1), ‘Canetova’ (June 2, Čačak, 3), ‘Ohridska crna’ (June 24, Ohrid, 4), and ‘Dolga Šiška’ (June 26, Ohrid, 5) (Figure 1). The harvested fruits were transported to the laboratory, where the samples were adequately stored before further analyses.

Chemicals used as reference standards for HPLC analysis: cyanidin-3-*O* rutinoside, cyanidin-3-*O* glucoside, p-coumaric acid, glucose, fructose and sorbitol were supplied from Sigma-Aldrich (Germany), while chlorogenic acid and rutin analytical standards were purchased from Acros Organics (US). Methanol was purchased from J.T.Baker (Netherlands).

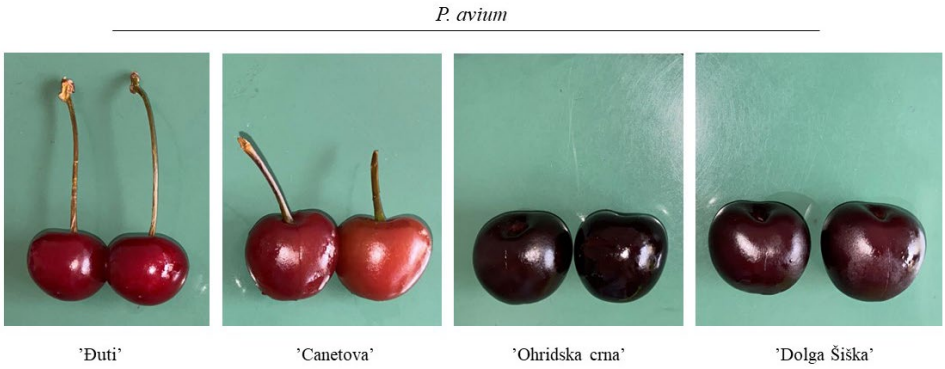

Figure 1. Sweet cherry (*Prunus avium* L.) cultivars used in the present study.

Fruit material preparation

Fresh fruits delivered to the laboratory were pitted and stored at -80°C until further analysis. For the extraction of anthocyanins and phenolic compounds, the fruits were first freeze-dried using a Beta 2-8 LD freeze-dryer (Martin Christ, Osterode, Germany) at -60°C for 48h and under a pressure of 0.012 mbar. The freeze-dried fruits were then ground in a lab mill, and the extraction was carried out following the procedure of Średnicka-Tober et al. (2019) with slight modifications. Two grams of freeze-dried fruit sample were mixed with 6 mL of methanol and 2 mL of 1M HCL in a plastic test tube. The samples were vortexed for 15 min at 30°C in a shaker and then centrifuged for 5 min at 7,000 rpm. The supernatant was filtered and cooled at 5°C. Obtained extracts were used for analyses. For the content of individual sugars, a mashed (3 g) fruit sample was dissolved with 30 mL of distilled water for 30 min at room temperature. The extracted sample was centrifuged at 7,000 rpm for 5 min (IKA centrifuge, USA). The supernatant was filtered and the obtained extracts were transferred into vials and used for analyses.

Raman spectroscopy of different fruit cherry extracts

Raman spectroscopy of fruits of *Prunus avium* L. cultivars was focused on the direct measurement of storage parenchyma cells. A Raman spectrometer system (Horiba Jobin Yvon, France) equipped with the Olympus BX 41 microscope was used. During the spectral recording, the 785 nm laser was focused onto the sample using the 50 LWD objective (Olympus, Tokyo, Japan). The spectrometer is equipped with 600 lines/mm grating, in the range from 200 to 1800 cm^{-1} in the extended mode. The measurement was conducted with a 5s integration time, with 5 spectral accumulations. The spectral resolution was approximately 3 cm^{-1} and the calibration was verified using the 520.47 cm^{-1} line of silicon. Ten spectra were

recorded per sample, making 150 spectra in total. The assignment of the bands was carried out using the literature data. The spectra were preprocessed using the Spectragryph software, version 1.2.14. (Menges, 2021), while the PC analysis was performed using the PAST software (Hammer et al., 2001). The principal components are composed of scores and loadings. When using PCA, it is possible to visualize the data while reducing the data size, allowing segregation between classes. Particularly, the scores and loadings reveal the differences between the samples.

Determination of sugars using HPLC

Samples were analyzed using a Dionex Ultimate 3000, Thermo Scientific (Waltham, MA, USA) HPLC system. The analysis was performed using deionized water as the mobile phase with an elution rate of 0.6 mL/min, on a carbohydrate column (Hi-Plex Ca^{2+},300 mm x 7.7 mm, 8 mm) incubated at 80°C. The product was detected using the RI detector (RefractoMax 520, ERC GmbH, Riemerling, Germany) preheated at 40°C. All data acquisition and processing were done using the Chromeleon 7.2 software.

Determination of anthocyanin and phenolic compounds using HPLC

The HPLC system (Dionex Ultimate 3000 Thermo Scientific, Waltham, USA) and a reverse phase column (XBridgeTM C18, 100 mm × 3 mm, particle size 3.5 μm) were used for the quantitative analysis of the samples. Solvent (A) H_2O: HCOOH = 100:0.1 % and solvent (B) MeOH were used as mobile phases. Elution was conducted in the following way: 0–5 min isocratic 0% B, 5–20 min gradient from 0 to 10% B, then 20–40 min isocratic 10% B, 40–60 min gradient from 10 to 20% B, 60–70 min isocratic 20% B, 70–95 min gradient from 20 to 50% B, 95–105 min isocratic 50% B, then 105–105.1 min gradient from 50 to 0% B and 105.1–110 min isocratic 0% B. The flow rate was 0.5 mL/min and the column was thermostated at 30°C. The injection volumes of the samples ranged from 5 to 30 μL. The products were detected by a UV detector at 310 and 520 nm. The standard curves for the analyzed compounds (cyanidin-3-O rutinoside, cyanidin-3-O glucoside, p-coumaric acid, chlorogenic acid, rutin) were constructed using different concentrations of standards and the obtained slopes were used for the calculations.

Statistical analysis

In the present study, the statistical analysis of the data obtained by HPLC was performed using analysis of variance (one-way ANOVA) followed by the Duncan's *post hoc* test within the statistical software, STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$, n=3.

Results and Discussion

Qualitative and quantitative analysis of sweet cherries using Raman and HPLC

Sweet cherry fruit analysis was performed by Raman microspectroscopy, and high-performance liquid chromatography, and the averages related to *Prunus avium* L. cultivars: 'Đuti', 'Canetova', 'Ohridska crna', and 'Dolga Šiška' are shown in Figure 2, Tables 1 and 2, respectively. The characteristic vibrational bands and the corresponding preliminary identification in the Raman spectra are listed in Table 3.

The Raman spectra of the extracted fruit samples show an increase or decrease in the intensity of the bands and the appearance of new bands (Figure 2) depending on the cultivar. An increase in the intensity of the bands in the carbohydrate region ranged from 200 to 500 cm^{-1}, especially at 455 cm^{-1}. The appearance and increase in the intensity of the band at 1327 cm^{-1} probably indicate an increase in the phenol concentration or anthocyanins in the fruit samples (Edwards et al., 1997; Zaffino et al., 2015; Farber et al., 2020), especially in 'Ohridska crna', while this band was not clearly observed in the 'Đuti' cultivar. A correlation was found between the mentioned Raman bands and the sugar and phenolic contents obtained by HPLC quantitative analysis (Table 2).

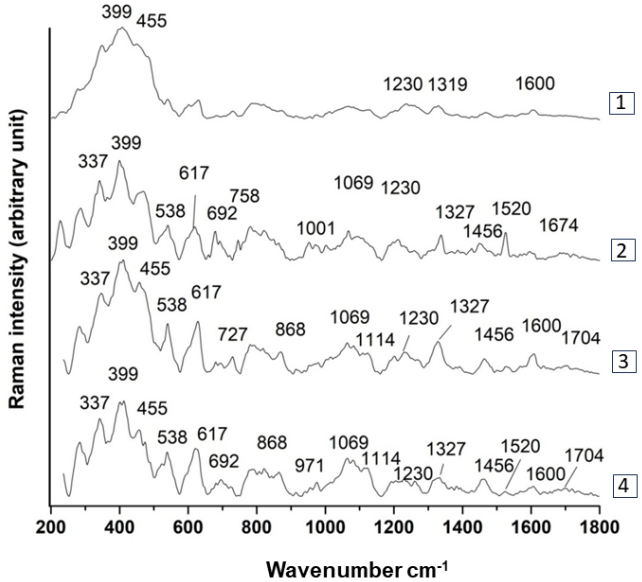

Figure 2. Averages of normalized Raman spectra of four *Prunus avium* cultivars ('Đuti' – 1, 'Canetova' – 2, 'Ohridska crna' – 3, 'Dolga Šiška' – 4) extracted fruit samples, recorded in the spectral range from 200 to 1800 cm^{-1}.

More precisely, significant bands of higher intensity were observed in the Raman spectra (Figure 2) at 337, 399, and 455 cm^{-1}, which are associated with the essential components of the fruits and assigned to the glucosidic ring vibrations, fructose, cellulose, and pectic acid (Boyaci et al., 2015; da Silva et al., 2008; Camerlingo et al., 2017; Zeise et al., 2018). These bands can be assigned to the C-C-C and C-O-C bending vibrations, indicating the presence of carbohydrates as major components of the storage parenchyma cells of cherries as the main tissue of the pericarp. The fruit samples were also analyzed for the content of individual sugars (glucose, fructose, and sorbitol) using HPLC analysis. Generally, glucose was found to have the highest content, ranging from 46.44 to 80.93 g/100g FW (fresh weight). The content of fructose varied from 39.93 g/100g FW ('Canetova') to 64.03 g/100g FW ('Ohridska crna'), and the sorbitol concentration was in the range 7.53–31.56 g/100g FW. The highest sum of sugars was found in 'Ohridska crna' and 'Dolga Šiška' from North Macedonia (Table 1). The results obtained in our work confirm the results of Usenik et al. (2008).

Table 1. Mean sugar content in g/100g FW ± standard deviation of the different sweet cherry cultivars.

	Glucose	Fructose	Sorbitol
'Đuti'	47.59±1.96^{a}	41.85±3.17^{a}	7.53±0.42^{a}
'Canetova'	46.44±0.73^{a}	39.93±1.55^{a}	8.17±0.37^{b}
'Ohridska crna'	80.93±3.44^{b}	64.03±3.05^{b}	31.56±1.23^{c}
'Dolga Šiška'	74.73±2.12^{c}	62.31±2.98^{b}	24.75±0.93^{d}

Different letters indicate significantly different values at $p<0.05$.

The medium intensity band positioned at 1327 cm^{-1} could indicate significant differences between 'Ohridska crna' and other cultivars, and this band could be associated with phenylpropanoids, anthocyanins, or cellulose (Zaffino et al., 2015; Farber et al., 2020). Furthermore, medium-intensity bands at 538 and 617 cm^{-1} are associated with polygalacturonic (pectic) acid and cyanidins, respectively (Edwards et al., 1997; Boyaci et al., 2015; Camerlingo et al., 2017). According to these bands, the only difference between the samples was observed in the band at 617 cm^{-1} associated with anthocyanidin content (Zaffino et al., 2015); for the 'Đuti' cultivar (lower intensity band) and the 'Ohridska crna' cultivar (higher intensity band) (Figure 2).

Two different anthocyanins and three phenolic compounds have been identified and quantified after applying the extraction process and the chromatographic method described previously. Variations in the anthocyanin concentrations were found between the cultivars. The most abundant anthocyanin was cyanidin 3-*O*-rutinoside (ranging between 4.175 and 289.275 mg/100g FW), followed by cyanidin 3-*O*-glucoside (0.453-49.625 mg/100g FW) in all cultivars.

As can be observed in Table 2, the sweet cherry cultivar showing the highest level of anthocyanin belonged to 'Ohridska crna'.

Very weak bands have been identified in the range from 650 to 1300 cm^{-1} and from 1440 to 1800 cm^{-1}, tentatively attributed to polygalacturonase (pectic acid), pectin, and the lower amounts of carotenes and phenols (Synytsya et al., 2003; Agarwal, 2006; da Silva et al., 2008; Boyaci et al., 2015; Kang et al., 2016; Farber et al., 2020). A band at ~1600 cm^{-1} could indicate the C=O ring vibration of phenylpropanoids, flavonoids, and chlorogenic acid (Maiti et al., 2013; Zaffino et al., 2015; Krysa et al., 2022), and this band was the most intense in 'Ohridska crna' (Figure 2). Three phenolic compounds were detected and identified in sweet cherry cultivars by comparison with reference standards: p-coumaric acid (phenolic acid), chlorogenic acid (phenolic acid) and rutin (flavonoid). 'Ohridska crna' showed the highest levels of phenolic compounds (26.26 mg/100g FW as the sum of the three identified compounds), while the 'Đuti' cultivar showed the lowest amount of phenolics (3.93 mg/100g FW as the sum of the three identified compounds). In all cultivars, rutin was the most abundant, except in 'Canetova', with the highest amount of chlorogenic acid. The results obtained for phenolic compounds are similar to those reported by González-Gómez et al. (2010) and higher than those reported by Usenik et al. (2008), probably because of the differences in geographical origin of the cultivars (Balkans, Spain, and Slovenia, respectively). The results of this work show a correlation between the Raman band intensities and the concentrations of the studied compounds.

Table 2. Anthocyanin and phenolic content of the different sweet cherry cultivars in mg/100g FW ± standard deviation.

	Cyanidin-3-*O* Rutinoside	Cyanidin-3-*O* Glucoside	p-coumaric acid	Chlorogenic acid	Rutin
'Đuti'	4.17 ± 0.03^{a}	0.45 ± 0.01^{a}	0.95 ± 0.08^{a}	0.66 ± 0.01^{a}	2.32 ± 0.09^{a}
'Canetova'	18.44 ± 0.92^{b}	4.48 ± 0.03^{b}	n.d.	3.34 ± 0.10^{b}	1.91 ± 0.04^{b}
'Ohridska crna'	289.27 ± 7.99^{c}	49.63 ± 2.22^{c}	7.07 ± 0.21^{b}	4.46 ± 0.12^{c}	14.73 ± 0.76^{c}
'Dolga Šiška'	35.14 ± 1.35^{d}	1.68 ± 0.02^{d}	1.31 ± 0.04^{c}	1.82 ± 0.02^{d}	3.38 ± 0.15^{d}

n.d. not detected, different letters indicate significantly different values at $p<0.05$.

Principal component analysis (PCA)

PCA (Fig. 3A) was applied to the data obtained from the Raman spectra in the 200–1800 cm^{-1} range to obtain the criteria for distinguishing the cherry cultivar samples. The first PCA model for the cherry samples resulted in two principal components that explained 65.77% of the total data variance. The first principal component (PC1) explained 48.30% of the total data variance, while the second (PC2) accounted for 17.47%. The mutual projections of the factor scores and their loadings for the first two PCs are shown in Figure 3.

The loading plot of the PC1 (Figure 3B) shows the positive loadings responsible for the separation between the 'Canetova' and 'Dolga Šiška' cultivars from the other cultivars. Examination of the PC1 shows many medium-positive contributions at 538, 677, and 617 cm^{-1}, which could be attributed to pectic acid and anthocyanins (Boyaci et al., 2015; Zaffino et al., 2015). In the higher range of differentiation, the bands of influence were at 1065, 1108, 1450, 1526, and 1336, 1593 cm^{-1}, which are likely from carbohydrates, especially cellulose, methyl and acetyl ester groups in pectin and phenylpropanoids, chlorogenic acid or flavonoids, respectively (da Silva et al., 2008; Pompeu et al. 2018; Farber et al., 2020).

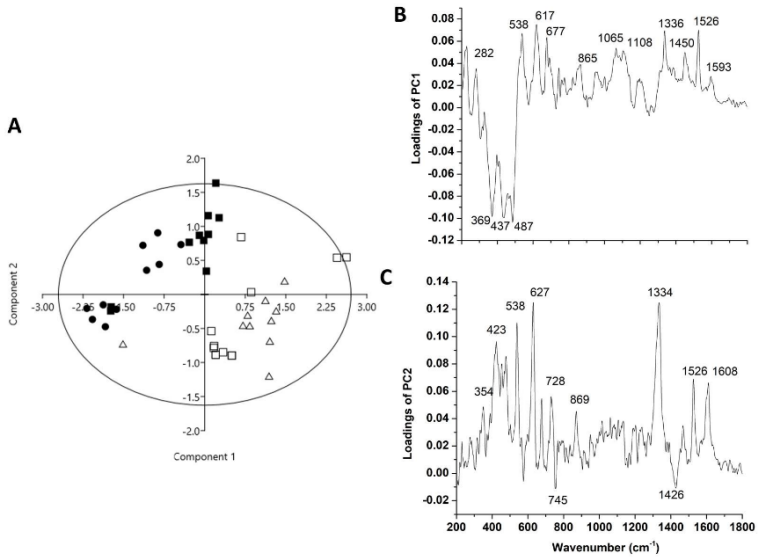

Figure 3. PCA applied to the Raman spectra data of extracted cherry samples: (A) score plot, (B, C) loading plots (symbols on the fruit samples: 'Đuti'– cycle, 'Canetova' – open square, 'Ohridska crna' – closed square, 'Dolga Šiška' – open triangle).

According to the PC2, the key differentiation between the 'Ohridska crna' cultivar and all other cultivars is related to the higher intensity of positive loadings at 423, 538, 627 cm^{-1} involving the C-C-O bending vibration of α-glucose and pectic acid (Boyaci et al., 2015) and the highest intensity of positive loading at 1334 cm^{-1} (Figure 3C), which could be Ziska related to CH_2 or $=C(CH_3)_2$ bending vibrations of plant fibers or flavonoids (Edwards et al., 1997; Maiti et al., 2013; Pompeu et al., 2018). Furthermore, these cultivars differed to a higher extent by loadings at 1526 and 1608 cm^{-1} (Figure 3C), and these differences might be related to phenolic compounds, e.g. chlorogenic acid (da Silva et al., 2008; Maiti et al.,

2013; Farber et al., 2020; Pompeu et al. 2018). In the lower extent of the separation, the negative loadings on PC2 at 245 and 1426 cm^{-1} probably indicate the glucosidic ring vibration (Synytsya et al., 2003; da Silva et al., 2008), or 1426 cm^{-1} could indicate chlorogenic acid (Eravuchira et al., 2012).

From a practical standpoint, the combination of Raman spectroscopy with principal component analysis (PCA) holds promise for distinguishing between the same fruit cultivars. The selectivity of the method is crucial for further investigation related to the quality of sweet cherry fruits. The Raman technique offers a more efficient and selective approach to the investigation of nutritionally similar samples than HPLC. The evolution of various Raman spectroscopy techniques has expanded its utility in identifying raw materials, pushing the boundaries of fruit investigation. Our further investigation will be focused on the application of the Raman method to the non-processed (fresh) cherry fruit, as there is no interference from water molecules compared to other methods.

Table 3. Assignment of vibrational bands observed in the spectra collected from the extracted cherry fruit samples.

Extract samples	Literature data	Vibrational mode	Chemical moiety	Reference
108		δ(C-C-C)	Glucosidic ring	da Silva et al., 2008
308		δ(C-C-C)	Glucosidic ring	da Silva et al., 2008
337	344, 348	δ(C-C-C), δ(C-O-C)	Glucosidic ring, fructose	da Silva et al., 2008, Camerlingo et al., 2017, Maiti et al., 2013
399	400	δ(C2-C1-O1) bending	α-Glucose	Boyaci et al., 2015
410	415 (pure compound)	δ(C2-C1-O1) bending	α-Glucose	Boyaci et al., 2015
455	441 449	C–O–C Phenyl ring	Polygalacturonic (pectic) acid Chlorogenic acid	Synytsya at al., 2003, Maiti et al., 2013
-	518-527	C-O-C, C-C-O	Glucosidic ring, cellulose	Edwards et al., 1997, da Silva et al., 2008, Camerlingo et al., 2017, Nekvapil et al., 2018
538	537		Polygalacturonic (pectic) acid	Boyaci et al., 2015
617	614		cyanidin	Zaffino et al., 2015
692, 677	686 677	Low frequency vibrations of pyranoid ring, Phenolic group	Polygalacturonic (pectic) acid, Chlorogenic acid	Boyaci et al., 2015, Eravuchira et al., 2012
758	747	γ(C–O-H) of COOH	Pectin	Synytsya et al., 2003, Eravuchira et al., 2012; Boyaci et al., 2015, Farber et al., 2020

Continuation Table 3.

Extract samples	Literature data	Vibrational mode	Chemical moiety	Reference
850	849–853	(C6–C5–O5–C1–O1)	Pectin	Farber et al., 2020
868, 865	870	CH and CH_2, C-C	Furanose	Boyaci et al., 2015, Camerlingo et al., 2017, Nekvapil et al., 2018
971	974	$\rho(CH_2)$, ν(C-O-H)	Glucosidic link stretch	da Silva et al., 2008, Eravuchira et al., 2012
1001	1000-1008	ν(C-C), CH_3	Carotene	Schulz et al., 2005, Schulz and Baranska, 2007, da Silva et al., 2008, Boyaci et al., 2015, Farber et al., 2020
1065	1056	υ(C-O-C), υ(C-C)	Carotenoids, carbohydrates	Edwards et al., 1997, Schulz et al., 2005, Wiercigroch et al., 2017, Farber et al., 2020
1108	1107-1122	ν(C-O-C) ν (C-O-C)	Cellulose	Baranski et al., 2005, da Silva et al., 2008, Farber et al., 2020,
1230		δ(C-C-H)	Carotenoids, xylan	Yu et al., 2007, Flores et al., 2008
1319, 1327	1325-1341	$\delta(CH_2)$ bending	Aliphatics, cellulose, Polygalacturonic (pectic) acid phenylpropanoids Anthocyanins	Edwards et al., 1997, Boyaci et al., 2015, Farber et al., 2020, Zaffino et al., 2015
1334-1337	1337-1340	ν(C-O-C) CH_2 $=C(CH_3)_2$ phenyl group	Cellulose phenylpropanoids, chlorogenic acid flavonoids	Zeise et al., 2018, Edwards et al., 1997, Pompeu et al. 2018, Eravuchira et al., 2012, Maiti et al., 2013
1426	1440-1444	$\delta(CH_2)$ phenyl group	Lipids and glucosidic signal chlorogenic acid	Da Silva et al., 2008, Eravuchira et al., 2012
1456 1450	1456 1444	$\delta(CH_2) + \delta(CH_3)$, δ(COH) Phenyl ring	Methyl and acetyl ester groups in pectins, fructose chlorogenic acid	Boyaci et al., 2015, Maiti et al., 2013
1520, 1526	1525 1518	-C=C- ν(C=C) benzopyrilium phenyl ring stretch	Carotenoids chlorogenic acid anthocyanins	Farber et al., 2020, Maiti et al., 2013 ,Zaffino et al., 2015
1600, 1593	1590-1608	ν(C=C), υ(C-C) ring + δ(CH) carbonyl group	Lignin, henylpropanoids chlorogenic acid anthocyanins rutin	Agarwal, 2006, da Silva et al., 2008; Eravuchira et al., 2012; Kang et al., 2016; Farber et al., 2020; Maiti et al., 2013; Zaffino et al., 2015; Krysa et al., 2022
1674	1680	COOH conjugated C=C and C=O modes	Carboxylic acids coniferyl alcohol and conifer aldehyde, chlorogenic acid	Farber et al., 2020; Zhu et al. 2018; Eravuchira et al., 2012

Conclusion

Although phenolic composition and sugar contents are significant factors in determining the potential of cherries, no rapid analytical method has been established so far for the authentication of these fruits. In this work, Raman spectroscopy allows the selective observation of nutritional and bioactive compounds from fruit samples by presenting a successfully performed authentication of sweet cherries. According to RS, the cherry samples are rich in carbohydrates, glucose, fructose, cellulose, and pectic acid, which are stored in the parenchyma tissue of the pericarp. Phenols, anthocyanins, and flavonoids are also present in lower concentrations. According to the PCA, 'Ohridska crna' differs from the other cultivars mainly in glucose, pectic acid, flavonoids, and phenolic compounds. The differences observed among samples through Raman and chemometric analysis correlate well with the results obtained using reference standards, indicating the reliability of the method. The potential demonstrated by Raman spectroscopy in this study can be extended to the analysis of fresh fruits, offering even greater efficiency. The capability of Raman spectroscopy to identify raw materials without interference from water molecules makes it particularly advantageous in this regard.

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PRIMENA RAMANOVE SPEKTROSKOPIJE ZA KARAKTERIZACIJU AUTOHTONIH SORTI TREŠNJE (*PRUNUS AVIUM* L.) POREKLOM SA BALKANA

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R e z i m e

Procena kvaliteta voća i povrća je od ključne važnosti za njihovu dalju obradu, predviđanje roka trajanja i formiranje cene. Većina tehnika koje se primenjuju za analizu kvaliteta ovih namirnica su invazivne. Međutim, sve veće interesovanje se javlja za neinvazivnim tehnikama za ocenjivanje kvaliteta voća, koje dobijaju na značaju zbog svoje jednostavnije primene i mehanizma rada. Ova studija po prvi put demonstrira primenljivost Ramanove spektroskopije za merenje spektralnih karakteristika različitih sorti trešnje (*Prunus avium* L.) (‘đuti’, ‘canetova’, ‘ohridska crna’ i ‘dolga šiška’). U kombinaciji sa analizom glavnih komponenti (engl. *principal component analysis* – PCA), Ramanova spektroskopija je korišćena za procenu uzoraka nutritivno sličnog sastava, kao što su proučavane sorte trešnje. Šećeri (glukoza, saharoza i fruktoza), antocijanini, fenolne kiseline i flavonoidi, kvantifikovani poređenjem sa referentnim standardima, korišćenjem tečne hromatografije visokih performansi (engl. *high-performance liquid chromatography* – HPLC), pokazali su Ramanove pikove (na 337, 399, 455, 538, 617, 1327 odnosno 1600 cm^{-1}) različitih intenziteta, što ukazuje na razlike između sorti. Sorta ‘ohridska crna’, u poređenju sa drugim sortama, sadrži najveću količinu nutritivnih i bioaktivnih jedinjenja. Nađena je korelacija između Ramanovih pikova i rezultata sadržaja šećera i fenola dobijenih hemijskom analizom. Ostvareni rezultati su ukazali na primenljivost hemometrijskog modelovanja povezanog sa Ramanovom spektroskopijom za brzu autentifikaciju trešnje.

Ključne reči: trešnja, vibracioni režimi, multivarijantna analiza, HPLC analiza, antocijanini, fenolna jedinjenja, ugljeni hidrati.

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VALUE OF PERMANENT CROPS IN THE GROSS VALUE ADDED IN AGRICULTURE

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Abstract: The aim of the research is to analyze the impact of the value of the production of permanent crops (fruit and viticulture sector) on the realized gross value added (GVA) in agriculture in Serbia and the EU from 2012 to 2022. The results were obtained by applying a multiple regression model where the dependent variable is the GVA in agriculture, and the independent variables represent the production values of the fruit and viticulture sector (in EUR mln). The coefficients of the model were tested using the t-test, and the model was verified using the F-test at a significance level of 0.05. The value of the standardized beta coefficient shows that the fruit-growing sector had a greater influence on the realized GVA of agriculture in Serbia and the EU (0.532 vs. 0.852), the t-values for Serbia belonged to the critical area in both sectors, while the t-values for the viticulture sector in the EU did not belong to the critical area. The F-test values show that the fitted model was significant at the 0.05 level for both observation areas. An analysis of the presence of multicollinearity in the independent variables was also conducted, and the results showed that there was a weak multicollinearity originating from the value of viticulture production.

Key words: gross domestic product (GDP), gross value added (GVA), intermediate consumption, agricultural output, permanent crops.

Introduction

The economic categories GDP and GVA are very important because they measure the economic strength of the state and the economic sector. We have explained the difference between these economic categories as follows: GVA represents the value added for the improvement of certain product indicators, while GDP expresses the total amount of products produced in a country (Sahu and Gartia, 2022).

Agricultural production is a very important activity for Serbia and the Serbian economy (Užar and Radojević, 2019; Grujić et al., 2022). It also plays a significant role in total exports and employment (Nikolić et al., 2017; FAO, 2020a; Volk et al.,

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2019; Grujić et al., 2021). Its specificity is contained in the fact that it achieves a high gross value added (GVA), which results in a high participation in the GDP structure. Agricultural production in Serbia generates a high value for agricultural output, which includes the value of so-called intermediary consumption. Specifically, the value of agricultural output includes the values of taxes, subsidies, and intermediary consumption in the total agricultural output (Užar and Radojević, 2019; Popescu et al., 2021a). Serbia achieves a constant increase in the value of agricultural production and GVA, even though agricultural production is characterized by different levels of regional development. According to Brankov and Matkovski (2022), agriculture contributes considerably to the development of GVA, which is higher in Western Balkan countries than in the EU. A group of authors (Grujić et al., 2022) applied the multiple linear regression method to determine the impact of different areas of agricultural production on the GVA of agriculture in Serbia, finding that crop production, especially grain production, significantly contributes to the creation of the GVA of agriculture. The aforementioned research also showed the significant participation of the fruit-growing and viticultural sectors in the creation of GVA agriculture, while Dašić et al. (2022) believe that fruits and grapes from Serbia are recognizable on the markets of other countries, achieve a high price and are more competitive compared to products from other countries.

If we look at the EU level, we conclude that the GVA of agriculture in the EU records a significant share in the total GDP of the EU (Popescu et al., 2021a; Popescu et al., 2021b). Furthermore, agricultural production in the EU (as a whole) has a significant impact on the global agricultural market (Popescu et al., 2021a; Pawlak et al., 2021), despite the fact that member countries have varying levels of economic growth (Baer-Nawrocka, 2016). The EU should provide enough food for its population and market, but also create surpluses for export to the markets of non-EU countries (Megyesiova, 2021). The production process should also ensure quality-controlled food, which is implemented under the umbrella of the Common Agricultural Policy (CAP) (FAO, 2020b). The CAP reforms aim to make agricultural production more market-oriented (Giannakis and Bruggeman, 2015). In Romania, the participation of GVA of agriculture in the creation of GDP is weak. In 2022, it was about 4.6% (Ionitescu, 2023). Lithuania records a constant increase in agricultural output and GVA in agriculture (Kriščiukaitienė and Baležentis, 2011).

Agriculture is also an important economic sector in other non-EU countries of the world. Teshome and Lupi (2018) point out that agriculture in Ethiopia has great importance for the country's economy, especially in terms of employment. In Nigeria, the importance of agriculture is reflected in the reduction of poverty and the increase in income, as the share of agricultural GVA in total GDP is steadily increasing. Matthew and Mordecai (2016) have applied the multiple linear regression method to determine whether per capita

income in agriculture in Nigeria is more influenced by the value of agricultural output or public agricultural expenditure and found that both predictors have a significant impact. About 70% of the population lives in the rural areas of Bangladesh, where the agricultural sector accounts for 14.23% of GDP (2015), which shows that agriculture is the main source of income (Dey, 2022). According to the same source, it was determined that the analyzed predictors (rice, jute, wheat, potato, and sugarcane), as areas of plant production, described 97.4% of the total variations of the dependent variable (value added in agriculture) in the set model. During 2022, the share of GVA in agriculture, forestry and fisheries (abbreviated as AFF) and rural population in the total population decreased in Bangladesh (11.2% versus 60%) (FAOSTAT, 2024). The success of the economy in India is based predominantly on the results achieved in the agricultural sector. In 2014, women outnumbered men (60:40) in this sector (Reddy and Dutta, 2018). In this country, the state provides significant subsidies for agricultural inputs (pesticides, seeds, fertilizers). Therefore, these authors used multiple regressions to analyze the impact of certain agricultural inputs on the GVA of agriculture (in %). They have found that seeds and pesticides have a significant impact, while the influence of fertilizers has no significant impact on the GVA of agriculture.

Since the study covered a number of the previously specified economic categories, mathematical equations were used in the following sections to illustrate them. Užar and Radojević (2019) set up the equation as follows (Equation 1):

$$GDP = PV + T - Sb - IC \quad (1)$$

where *PV – production value, T – taxes, Sb – subsidies, IC – intermediate consumption.* The initial formula can be further used to calculate other economic categories relevant to this research (Equations 2 and 3).

$$GVA = PV + IC \quad (2)$$

$$GDP = GVA + T - Sb \quad (3)$$

We can further use the mathematical equations set in this way to calculate the value of intermediate consumption, and its form was presented by Albu et al. (2020). With this formula, we obtain the value of intermediate consumption in a simple way (Equation 4).

$$IC = AOV - GVA \quad (4)$$

where *AOV – agricultural output value.*

Equation 4 was used to set up a mathematical form that can be used to easily calculate the value of final consumption or the so-called direct GVA (Albu et al., 2020) and is shown in Equation (5).

$$AOV - IC = direct\ GVA\ (final\ consumption) \quad (5)$$

The theoretical frameworks of the exhibited components of the national accounts, articulated by mathematical equations, were examined in the subsequent

phase of the research, while the results were given in tabular or graphical format. Their modifications were accompanied by sufficient annotations.

Table 1 shows the average annual rate of change (abbreviated as AARC) of GDP and GVA (at current prices) in Serbia and the EU from 2012 to 2022. The values are expressed in percentages.

Table 1. AARC of GDP and GVA in AFF in Serbia and the EU, 2012–2022 (in %).

Territory	AARC of GDP	AARC of GVA
Serbia	7.1	7.3
EU	3.4	3.6

Source: Calculation of the authors based on EUROSTAT and SORS databases.

According to the data shown in Table 1, we can see that from 2012 to 2022 Serbia's average annual GDP increased by 7.1%, and GVA in AFF by 7.3%. In the territory of the EU, a positive AARC was also recorded for the observed indicators, but the value was twice as low. The fact that the AARC of GVA in AFF in Serbia is twice as large can be explained by the fact that in Serbia the agricultural sector is more important for the development of the economy than in the case of EU countries, as well as that the agricultural sector is more represented in Serbia compared to the area of the EU member states.

Table 2 shows the structure of the participation of GVA of AFF in total GDP according to the observed areas.

Table 2. The share of GVA of AFF activity in GDP in Serbia and the EU, 2012–2022 (in %).

Variables	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Average*
Share of GVA in AFF in total GDP *in Serbia*	7.5	7.9	7.7	6.7	6.8	6.0	6.3	6.0	6.3	6.3	6.5	6.7
Percentage of GVA in AFF in total GDP *in the EU*	1.7	1.7	1.7	1.6	1.6	1.7	1.6	1.6	1.6	1.6	1.7	1.6

Source: EUROSTAT and SORS. *Note: Calculation of the authors based on EUROSTAT and SORS databases.

When analyzing the participation of GVA in the AFF in total GDP, we notice that Serbia also recorded almost four times higher average annual participation in this respect compared to the EU (Table 2). Megyesiova (2021) states that the lower contribution of GVA in agriculture is characteristic of developed countries, and vice versa, in less developed countries, the participation of GVA in agriculture is higher in the total GDP of the country.

Table 3 provides a comparative overview of the realized values of agricultural production and GVA in agriculture for 2012 and 2022 for Serbia and the EU. We observe that in Serbia the value of agricultural production increased on average by 4.9% per year, reaching a 61.3 higher value in 2022 compared to 2012. When we look at the EU, we see that the AARC agricultural output and the rate of change recorded lower growth compared to Serbia. More precisely, the calculated AARC of agricultural output value in the EU showed a growth of 3.3% per year on average and a positive rate of change of 38.6% in 2022 compared to 2012, and from 2010 to 2015 (Zsarnóczai and Zéman, 2019) the output value of agriculture in the EU increased by 8.6%.

Table 3. Agricultural output value and GVA in Serbia and the EU, 2012 and 2022.

Territory	Agricultural output value				GVA in agriculture				Average GVA in agriculture , in EUR mln (2012–2022)	Share of GVA in agriculture in agricultural output value, in %*	
	2012, in EUR mln	2022, in EUR mln	Rate of change, in %	AARC, in %	2012, in EUR mln	2022, in EUR mln	Rate of change, in %	AARC, in %		2012	2022
Serbia	4,443.5	7,165.8	161.3	4.9	1,930.1	3,903.5	202.2	7.3	2,646.916	43.4	54.5
EU	369,514.6	511,975.5	138.6	3.3	152,030.5	215,599.1	141.8	3.6	169,974.639	41.1	42.1

Source: EUROSTAT and SORS. *Note: Calculation of the authors based on EUROSTAT and SORS databases.

If we look at the realized values of GVA in agriculture, we see that Serbia doubled the realized GVA in 2022 compared to 2012 (+102.2%). Both Serbia and the EU recorded positive changes in the realized value of GVA in agriculture, and the increase in this value in 2022 compared to 2012, expressed by the rate of change, was 41.8%.

As we can see in Table 3 and according to Equations 4 and 5, GVA in agriculture represents only a part of the total realized agricultural value. Accordingly, we can say that in the EU, the share of the value of the intermediate consumption was higher (around 60%) than in Serbia (45–55%). We also concluded that intermediate consumption in Serbia in 2022 was 29.8% higher than in 2012, while in the EU it was 36.3% higher.

Popescu et al. (2021a) analyzed individual EU countries and found that in 2020, compared to 2011, the largest increase in agricultural output value was recorded in France (18.3%), Germany (13.8%) and Italy (13%) and the largest GVA growth was achieved by Latvia (128.38%).

By further analysis of the ratio of realized values of GVA and intermediate consumption, we found that in Serbia, one euro spent on intermediate consumption increased from 0.8 euro (2012) to 1.2 euro (2022) of GVA for agriculture. In the EU, one euro spent on intermediate consumption produced about 0.7 euro of agricultural GVA and did not change significantly in the period 2012–2022.

After providing an overview of the total values of agricultural production, we now proceed to show the structure of these values. We know that the overall value of agricultural output is made up of the realized values of crop and livestock production, as well as agricultural services, and their respective shares are shown in Table 4.

Table 4. Share of the value of each agricultural sector in the total value of the production of agricultural goods and services (average for the period 2012–2022, in %).

Territory	Crop production	Animal production	Agricultural services
Serbia	68.4	29.1	2.5
EU	55.2	39.8	5.0

Source: Calculation of the authors based on EUROSTAT and SORS databases.

As can be seen in Table 4, plant production had a dominant share in generating the value of total agricultural production, both in Serbia and in the EU. However, agricultural production involvement was greater in Serbia than in the EU, although animal production participation was higher in the EU.

Since we have found that the value of plant production dominates the total value of agricultural production, we have decided to investigate the structure of the value of plant production by area and type of production (Figure 1).

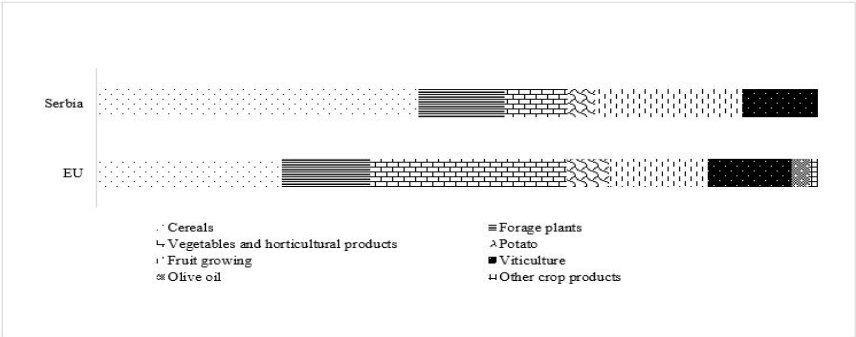

Figure 1. Structure of the value of vegetables and horticultural products in Serbia and the EU (average for the period 2012–2022, in %).

Figure 1 shows that the value of grain production had the largest average share in the realized value of plant production in Serbia (40%). In second place was the value of the production of the fruit sector (with 18.4%), followed by industrial and fodder plants, and the value of the production of the viticulture sector (with a 9.2% share) was in the fifth place.

In the EU, products from the group of vegetables and horticultural products accounted on average for the largest share of the value of plant production (24.7%), followed by grain, then by the fruit sector with 12.4%, and the value of the production of fodder plants, and in the fifth place was the value of the production of the viticulture sector (10.6%).

If we were to add up the share of the production value of the fruit and viticulture sector, which is the share of the value of the production of permanent plantations in the total value of agricultural production, we would arrive at a sum of 27.6% for Serbia and 23% for the EU. In other words, in Serbia and the EU, a quarter of the total realized value of agricultural production came from permanent plantings.

Table 5 presents three influential areas of crop production with their average values from 2012 to 2022, the rate of change (2022/2012) and the AARC for the period 2012–2022 in Serbia and the EU. The following areas were singled out: arable, fruit-growing and viticultural production. In our case, the value of crop production consisted of the value of the production of grain, industrial and fodder plants, vegetables and potatoes.

As seen in Table 5, agricultural production had the greatest average value of production in Serbia and the EU. The rates of change in the value of production reveal that permanent plants developed faster year after year. In Serbia, the increase in the production value of the viticulture sector by 216.4% and the fruit sector by 128.9% in 2022 compared to 2012 stood out. In the EU, the fruit sector recorded a growth rate of 46.7%, while the viticulture sector achieved a growth rate of 45.1%.

Table 5. Average value, rate of change and AARC for three areas of crop production in Serbia and the EU (2012–2022).

	Serbia		
	Average, in EUR mln	Rate of change (2022/2012), in %	AARC, in %
Crop farming	2,738.4	153.4	4.4
Fruit growing	663.9	228.9	8.6
Viticulture	334.4	316.4	12.2
	EU		
	Average, in EUR mln	Rate of change (2022/2012), in %	AARC, in %
Crop farming	161,705.5	140.9	3.5
Fruit growing	27,123.5	146.7	3.9
Viticulture	22,983.6	145.1	3.8

Source: Calculation of the authors.

Looking at the AARC, it can be seen that the value of agricultural production grew the slowest in both Serbia and the EU. Table 5 also shows that the value of Serbian fruit-growing sector production increased at an average yearly rate of 8.6% (4.4% in agriculture) and 12.2% in viticulture. In the EU area, there were no significant fluctuations in the AARC of certain crop production sectors (3.9% in fruit production and 3.8% in viticulture production).

Previous research has revealed that grain production accounts for a considerable portion of the total agricultural output value, but does not have a significant AARC, as is the case in the fruit and viticulture sectors. As a result, permanent plantations play a significant role in our research.

Material and Methods

The structure of the participation of each sector in the formation of the overall value of agricultural production revealed a considerable engagement in plant agricultural output, with permanent plantings also noted. Although agricultural production showed high average values, it cannot be said that it recorded high rates of change and AARC. Therefore, the research is focused on the analysis of the impact of production values originating from permanent plantings on the GVA of agriculture in Serbia and the EU in the period 2012–2022. The results were obtained using the multiple linear regression method. Permanent plantations included the sectors of fruit growing (i.e., the value of the fruit produced) and viticulture (i.e., the value of the wine produced).

At the beginning of the study, the results of the descriptive statistics of the value structure of plant production in Serbia and the EU were presented (coefficients of correlation and determination, standard error of regression, AARC, structures of indicators, etc.). The Durbin-Watson test *(d-test)* was used to determine the possible presence of autocorrelation between the variables in the set regression model (Akter, 2014). The results of this test lead to the conclusion that the predictors are significant for the set regression model.

The values of the coefficients of the *d-test* range from 0 to 4. If the obtained value is in the interval *from 0 to 2*, we consider that there is a positive first-order autocorrelation; if it is *from 2 to 4*, then we conclude that there is a negative first-order autocorrelation between the variables, but *if the value of this coefficient is 2* then we state that there is no autocorrelation between the variables. It is best when the d-test coefficient values are in the interval from 1.50 to 2.50 (Investopedia, 2024). The Durbin-Watson test (Chen, 2016) is calculated according to the following formula in Equation (6)

$$\mathrm{d} = \frac{\sum_{i=2}^{n}(\hat{\varepsilon}_i - \hat{\varepsilon}_{i-1})^2}{\sum_{i=1}^{n}\hat{\varepsilon}_i^2} \tag{6}$$

where n – sample size, i – number of elements ($i = 1, 2, \ldots, n$), $\hat{\varepsilon}_i$ – prediction error.

This was followed by an analysis of the impact of the realized value of fruit and viticulture production on the GVA of agriculture in Serbia and the EU in order to see which group of permanent plantations contributed more and had a greater influence on the realized value of the GVA of agriculture. In order to examine the influence of the independent variables on the dependent variable, the authors used a multiple linear regression model, and the SPSS software package was used for the analysis. The fitted multiple regression model is shown in Equations (7) and (8). The meaning of the individual variables can be found in Table 6.

$$\beta_1 L_1 + \beta_2 N_1 + \beta_0 = C_1 \quad (7)$$

$$\beta_1 L_2 + \beta_2 N_2 + \beta_0 = C_2 \quad (8)$$

Table 6. Explanation of the variables.

Variables	Description	Unit of measure	Source	Type of variable
C_1	GVA in agriculture in Serbia	Current prices, in RSD* mln	SORS, Statistical yearbook	Dependent
C_2	GVA in agriculture in the EU	Production value at producer price, in EUR mln	EUROSTAT	Dependent
L_1	The production value of the fruit-growing sector in Serbia	Producer prices of the current year, in RSD* mln	SORS, Statistical yearbook	Independent
L_2	The production value of the fruit-growing sector in the EU	Production value at producer price, in EUR mln	EUROSTAT	Independent
N_1	Production value of the viticulture sector in Serbia	Producer prices of the current year, in RSD* mln	SORS, Statistical yearbook	Independent
N_2	Production value of the viticulture sector in the EU	Production value at producer price, in EUR mln	EUROSTAT	Independent
β_1, β_2, and β_0	Model coefficients	-	SPSS program report	Regression parameters

Source: Author's view. * Given that the data for Serbia were expressed in local currency (RSD), it was necessary to convert them into EUR to ensure data comparability. Converting values from RSD to EUR was carried out using the average annual exchange rate available on the website of the National Bank of Serbia (abbreviated as NBS). The average annual mean exchange rate of RSD against foreign currencies represents the arithmetic mean of the mean exchange rates calculated during working days.

The research included the testing of β coefficients using the t-test and the set model using the F-test. The defined null and alternative hypotheses are presented in tabular form (Table 7).

Table 7. Hypotheses of the research.

*Testing of the β coefficients – **t-test***	
The null hypothesis H_0: coefficient β_i=0 means that the observed coefficient is not statistically significant.	*An alternative hypothesis Ha:* coefficient $\beta_i \neq 0$ means that the observed coefficient is statistically significant.
*Testing of the established model – **F-test***	
The null hypothesis H_0: The model is not statistically significant at the 0.05 significance level.	*An alternative hypothesis Ha:* The model is statistically significant at the 0.05 significance level.

Source: Author's view.

First, a matrix form of the centralized data values was formed by Equation (9).

$$Y^* = X^* \beta^* \varepsilon^* \quad (9)$$

where Y^* – vector of the dependent variable (GVA in Serbia and the EU), X – matrix of independent variables (realized value of production of the fruit-growing and viticulture sector in Serbia and the EU), β – the vector of coefficients, ε – error of the model. The estimation of the unknown coefficients β_i was performed, where i takes the values 1 and 2 (Equation 10), as well as the rating of the coefficient β_0 (Equation 11).

$$\beta^* = (X^{*'} X^*)^{-1} X^{*'} Y^* \quad (10)$$

$$b_0 = \bar{Y}_n - b_1 \bar{x}_{1n} - \cdots - b_k \bar{x}_{kn} \quad (11)$$

where $\bar{Y}_n, \bar{x}_{1n}, \ldots, \bar{x}_{kn}$ are the arithmetic means of the corresponding data.

The indicators of the possible presence of multicollinearity in the observed variables were also analyzed in the research. The verification of the appearance of multicollinearity was carried out by evaluating the values obtained for the tolerance level, the VIF coefficient and the Eigenvalues. Mathematical formulas for their calculation were presented by Adeboye et al. (2014).

According to the set regression model, the downloaded report from the SPSS program showed the predictive values of the indicators of descriptive statistics, including the limits at which the values should move.

In addition to the above-mentioned methods of descriptive statistics and multiple linear regression, the methods of induction and deduction were used during the research when drawing appropriate conclusions. The methods of analysis and synthesis were also used in the interpretation of statistical data. All results, whether presented tabularly or graphically, are accompanied by appropriate comments by the author.

The research is based on the analysis of literature that includes the results of previous studies on the same or a similar topic by domestic and foreign authors.

The statistical analysis of the secondary data was carried out using the SPSS software package.

Results and Discussion

Since the research results reported in the introductory section have demonstrated that crop production dominates the value structure of overall agricultural output, the research will be continued using inputs from this type of production in Serbia and the EU. To support this, Table 8 presents the basic results of the descriptive data for various crop producing areas.

Table 8. Descriptive statistics of plant production in Serbia and the EU from 2012 to 2022 (in EUR mln).

Serbia									
	Cereals	Industrial crops	Forage plants	Vegetables and horticultural products	Potato	Fruit growing	Viticulture	Olive oil	Other crop products
Average	1,431.6	537.0	375.3	270.9	123.6	663.9	334.4	0.0	4.6
Min	937.6	401.7	145.4	220.7	96.3	476.7	167.3	0.0	3.9
Max	1,921.1	757.3	1,914.7	329.6	207.1	1,091.4	529.3	0.0	5.2
Std. dev.	290.8	117.2	513.5	34.7	33.5	192.7	108.6	0.0	0.3
Cv, in %	20.3	21.8	136.8	12.8	27.1	29.0	32.5	0.0	7.5
EU									
	Cereals	Industrial crops	Forage plants	Vegetables and horticultural products	Potato	Fruit growing	Viticulture	Olive oil	Other crop products
Average	51,374.2	20,725.0	24,001.5	53,933.9	11,670.9	27,123.5	22,983.6	4,964.1	2,181.4
Min	40,077.6	17,801.8	22,157.0	46,735.9	9,022.5	21,892.0	18,484.4	3,255.7	1,894.4
Max	80,187.5	30,685.3	26,189.5	65,747.3	15,390.0	32,117.5	27,392.1	6,699.0	2,563.1
Std. dev.	11,505.7	3,766.0	1,268.4	6,093.0	2,042.2	3,306.1	2,443.6	1,006.3	224.1
Cv, in %	22.4	18.2	5.3	11.3	17.5	12.2	10.6	20.3	10.3

Source: Calculation of the authors.

Table 8 shows that Serbia had the largest percentage deviation from the average value in the production of fodder plants, which reached 136.8%. There were no major fluctuations in the other crops. As for the EU, there were no significant deviations from the arithmetic mean and the values were around the average values.

Table 9 shows the statistical indicators of the set regression model for Serbia and the EU. More precisely, the variability of the assumed model of the dependent variable Y from the independent variable X is presented.

Table 9. Statistical indicators of the set model.

Model	R	R square	Adjusted R square	Std. error of the estimate	Durbin-Watson (d-test)	Type of autocorrelation	Sig. F change
Serbia	.950	.902	.877	182.1481	2.622	Negative	.000
EU	.840	.706	.632	11,397.4244	1.970	Positive	.007

Source: Calculation of the authors. Output from the SPSS program.

The correlation coefficient shows us that there was a strong positive relationship between the predictors, which was greater in Serbia. The coefficient of determination shows that 90.2% of variations in the GVA of Serbian agriculture can be explained by the influence of the fruit and viticulture sector, and 70.6% of the variations in the EU. The corrected coefficient of determination shows that 87.7% for Serbia, or 63.2% of the variability of agricultural VAT for the EU, depended on the value of production created by the fruit and viticulture sector. With these results, we have shown that permanent plantings occupy a significant place in the GVA structure of agriculture in Serbia and the EU because they describe more than half of the changes under whose influence the GVA structure changes. The values of the *d-test* were in the optimal intervals. Finally, we can conclude that the set model was significant (sig. value), whereby the significance for Serbia was higher than for the EU.

According to the report of the SPSS program, we obtained the scores of the coefficients β_1, β_2, and β_0, as well as indicators of the possible presence of multicollinearity between the predictors (Table 10).

Table 10. Results of the estimated regression model.

	Model	Unstandardized coefficients		Standardized coefficients	t	Sig.	Collinearity statistics	
		B	Std. error	Beta			Tolerance	VIF
Serbia	(constant)	940.211	208.343		4.513	.002		
	Fruit growing	1.437	.495	.532	2.902	.020	.364	2.746
	Viticulture	2.250	.879	.469	2.560	.034	.364	2.746
EU	(constant)	41,382.844	34554.679		1.198	.265		
	Fruit growing	4.847	1.685	.852	2.876	.021	.419	2.389
	Viticulture	-.125	2.280	-.016	-.055	.958	.419	2.389

Source: Calculation of the authors. Output from the SPSS program.

According to the results shown in Table 10, the set regression model was given a new form, which is shown in Equations 12 and 13.

$$1.437 * L_1 + 2.250 * N_1 + 940.211 = C_1 \quad (12)$$

$$4.847 * L_2 - 0.125 * N_2 + 41{,}382.844 = C_2 \quad (13)$$

Table 10 shows that, in Serbia and the EU, the fruit-growing sector had a greater influence on the realized GVA of agriculture than the viticulture sector. This conclusion was drawn based on the observed higher value of the standardized beta coefficient in the fruit-growing sector than in the viticulture sector. The obtained results for the unstandardized beta coefficients, depending on the area of observation, can be interpreted as follows: "If the value of fruit production increases by EUR 1 mln, then the GVA value of agriculture in Serbia increases by EUR 1,437 mln, and in the EU by EUR 4,847 mln". Therefore, if we were to increase the GVA value of agriculture, then the production value of the fruit-growing sector would also have to increase.

Furthermore, the null hypothesis H_0 was tested, coefficient $\beta_i = 0$ (the observed coefficient was not statistically significant), against the alternative hypothesis H_1, coefficient $\beta_i \neq 0$ (coefficient was statistically significant), with the index i assuming the values 1 and 2. Testing was performed using the t-test, and the realized values of the test statistic for the regression coefficients are given in Table 11. The t-statistic was distributed according to the Student's-distribution, provided that the null hypothesis was true, with 8 degrees of freedom, and its theoretical value amounted to $t(0.05; 8) = 2{,}306$.

If we look at the level of Serbia, we find that the values of the coefficients β_1 and β_2 belonged to the critical area, therefore we accept the corresponding alternative hypotheses for the observed coefficients. (t_{β_1} = 2.902 and t_{β_2} = 2.560) at a significance level of 0.05 (Table 11).

Table 11. Realized test statistic values for the regression coefficients in the set regression model for Serbia and the EU.

Serbia			
Coefficients	Estimate	t-value	*p*
(Intercept)	940.211	4.513	0.002
β_1	1.437	2.902	0.020
β_2	2.250	2.560	0.034
EU			
(Intercept)	41382.844	1.198	0.265
β_1	4.847	2.876	0.021
β_2	-0.125	-0.055	0.958

Source: Calculation of the authors. Output from the SPSS program.

If we look at the EU level, we can see that the realized t-value for the coefficient β_1 p belonged to the critical area. Thus, we accept the appropriate alternative hypothesis for the observed coefficient (t_{β_1} = 2.876) at a significance level of 0.05. Therefore, the t-value for the coefficient β_2 was not in the critical

area, was not significant at the 0.05 significance level, because $t_{\beta_2} = -0.055$ and we accept a null hypothesis.

Using the F-test, the entire model was tested (H_0: the model was not statistically significant; H_1: the model was statistically significant), and the obtained results show that the model was statistically significant, at a significance level of 0.05, which can be seen in Table 12.

Table 12. The F-statistic values at the significance level of 0.05.

Territory	*F*	F (0.05,2,8)	*p*
Serbia	36.813	4.459	0.000
EU	9.600	4.459	0.007

Source: Calculation of the author. Output from the SPSS program.

According to the results shown in Table 12, we can conclude that the regression model set for the level of Serbia was more significant than that for the EU, because p = 0.000 < p = 0.007.

The rest of the paper analyzes the indicators of the possible presence of multicollinearity in the variables. The basis for the conclusions is presented in Tables 10 and 13.

Table 13. Results of the multicollinearity check of the variables.

Serbia						
Model	Dimension	Eigenvalue	Condition index	Variance of proportions		
				(constant)	Fruit growing	Viticulture
1	1	2.935	1.000	.01	.00	.00
	2	.049	7.726	.93	.05	.17
	3	.016	13.675	.07	.95	.83
EU						
Model	Dimension	Eigenvalue	Condition index	Variance of proportions		
				(constant)	Fruit growing	Viticulture
1	1	2.990	1.000	.00	.00	.00
	2	.007	20.526	.89	.24	.04
	3	.003	33.442	.11	.76	.96

Source: Calculation of the author. Output from the SPSS program.

The values in the *Tolerance* column (0.364 for Serbia and 0.419 for the EU) and *VIF* (2.746 for Serbia and 2.389 for the EU) shown in Table 10 indicate a weak presence of multicollinearity. The set regression model was valid, so we conclude that fruit and wine production were only weakly collinear. In Table 13, the

Eigenvalues show the degree of closeness between the variables. When the Eigenvalues are close to zero, then the condition index achieves a very high value. For example, a high value of the β_2 coefficient, which represents the viticulture sector, influenced the presence of multicollinearity in the set model (13.675 for Serbia and 33.442 for the EU).

Finally, the results of the SPSS program provided the potential predictive values of the observed variables, primarily the parameters of descriptive statistics. The results are tabulated in Table 14.

Table 14. Prediction values of the set model.

	Serbia			
	Minimum	Maximum	Mean	Std. deviation
Predicted value	2,001.789	3,699.854	2,646.927	494.2450
	EU			
	Minimum	Maximum	Mean	Std. deviation
Predicted value	145,180.531	193,701.109	169,974.639	15,792.4216

Source: Calculation of the authors. Output from the SPSS program.

Based on the given data for Serbia, the statistical program SPSS showed the expected predicted mean value of GVA of EUR 2,646,927 mln, which was EUR 0.011 mln higher than the mean value of the analyzed period.

When it comes to the EU, the expected mean value of GVA would be unchanged at EUR 169,974 mln compared to the average of the observed period.

Conclusion

According to the findings, Serbia had a higher average yearly proportion of GVA in agriculture in total GDP (6.7%) than the EU (1.6%) for the study period (2012–2022), whereas the EU had a higher share of intermediate consumption.

In Serbia and in the EU, plant production contributed significantly more than livestock production to the creation of GVA for agriculture. Within crop production, the value of production under permanent plantings stood out, principally due to greater AARCs than for cereal production and crop farming.

The *d-test* values show that there was an autocorrelation between the predictors. Using the multiple regression method, the authors found that GVA in agriculture in Serbia and in the EU had a greater influence on the production value of the fruit-growing sector (sig. = 0.020 and sig. = 0.021, respectively), and the set model was statistically significant at the significance level of 0.05. The test of the analyzed predictors shows that they were significant for Serbia, while for the EU the value of viticulture production did not fall within the critical area ($t_{\beta_2} =$

-0.055). In the set model, a weak multicollinearity between the fruit-growing and viticulture sectors was observed, and the multicollinearity originated from the viticulture sector.

The results of this research reveal that permanent crops, which have a greater AARC output value than grain, have contributed considerably to the development of GVA in agriculture in Serbia and the EU between 2012 and 2022.

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VREDNOST STALNIH ZASADA U BRUTO DODATOJ VREDNOSTI POLJOPRIVREDE

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R e z i m e

Cilj istraživanja je analiza uticaja vrednosti proizvodnje stalnih zasada (voćarskog i vinogradarskog sektora) na ostvarenu bruto dodatu vrednost (skr. BDV) u poljoprivredi Srbije i EU od 2012. do 2022. Rezultati su dobijeni primenom modela višestruke regresije gde je zavisna promenljiva BDV u poljoprivredi, a nezavisne promenljive predstavljaju vrednosti proizvodnje voćarskog i vinogradarskog sektora (u mil. EUR). Pomoću t-testa testirani su koeficijenti modela, a primenom F-testa izvršena je provera postavljenog modela na nivou značajnosti od 0,05. Vrednost standardizovanog beta koeficijenta pokazala je da veći uticaj na ostvarenu BDV poljoprivrede u Srbiji i EU ima sektor voćarstva (0,532 prema 0,852), t-vrednosti za Srbiju pripadaju kritičnoj oblasti u oba sektora, dok u EU t-vrednosti za vinogradarski sektor ne pripadaju kritičnoj oblasti. Vrednosti F-testa pokazale su da je postavljeni model značajan na nivou značajnosti od 0,05 za oba područja posmatranja. Sprovedena je i analiza na prisustvo multikolinearnosti kod nezavisnih varijabli, a rezultati su pokazali da postoji slaba multikolinearnost koja potiče od vrednosti vinogradarske proizvodnje.

Ključne reči: bruto domaći proizvod (BDP), bruto dodata vrednost (BDV), međupotrošnja, proizvodnja poljoprivrednih dobara i usluga, stalni zasadi.

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The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

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The examples of listing references are the following:

Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon, France.

Thesis
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Summary
The summary in Serbian is given at the end of the paper and should comprise 200 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

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Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

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All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

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Abbreviations and units
Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature
The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae
All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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Journal of Agricultural Sciences

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Originalan naučni rad – Rad koji sadrži prethodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.
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Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

Rezultati i diskusija

U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

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Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

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Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

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