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INFLUENCE OF DIFFERENT NUTRIENT SOURCES AND GENOTYPES ON THE CHEMICAL QUALITY AND YIELD OF LETTUCE

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ABSTRACT

The aim of this study was to examine the effect of different fertilizers on the yield and antioxidant capacity of two lettuce genotypes “Santoro RZ” and “Kiribati RZ”.

Lettuce genotypes are fertilized with organic fertilizer (Slavol) and organic-inorganic NPK fertilizer (Fitofert hemisuper plus) during the vegetation. The analyzed parameters were root length and head weight of lettuce, total phenols, and flavonoids, as well as antioxidant capacity. Lettuce genotypes “Santoro RZ” and “Kiribati RZ” fertilized with organic fertilization showed the highest content of total phenols (358.13 ± 1.30 mg RU/100 g of fresh sample), the total content of flavonoids (114.22 ± 0.3 mg RE/100 g of fresh sample) and antioxidant capacity (neutralization of DPPH radicals 58.72 ± 1.88%).

The results revealed that the yield and antioxidant capacity of lettuce can be improved by using organic fertilizers.

Keywords: *lettuce, genotype, fertilizer, total phenols, antioxidant capacity*.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a very important and widespread vegetable plant. The largest producers of lettuce in Europe are Spain, Italy, and Germany (FAO, http:// www.fao.org/faostat/en/#data/QC). Lettuce is consumed as a fresh vegetable, mostly as a salad or as a minimally processed vegetable. It is important in the human diet because of its content of vitamins, minerals, and antioxidants. The advantage of lettuce, eaten raw, is that it maintains more nutrients than thermally processed food [1].

The biological characteristics of lettuce and its specific growth and development are the basis for establishing the optimal method of cultivation. Fertilization of lettuce with various organic, mineral, and microbiological fertilizers is performed to achieve higher yields. Different fertilization systems can affect the high and good yield of vegetables in greenhouse production [2,3]. Organic fertilizers are of primary importance in plant growth and development. This fertilizer has all the necessary macro and microelements, and during mineralization, they improve the physical and chemical properties of the soil [4]. Various types of organic fertilizers should be applied in combination with mineral fertilizers, to achieve the best yields [5,6].

Manipulation of mineral fertilizers is much easier because nutrients are introduced into the soil in a directly accessible form. Overdressing of mineral fertilizers can cause various problems, such as soil contamination and underground water after harvesting crops [7]. Mineral fertilizers can have a detrimental effect on plant quality, declining dry matter content, increasing soil acidity, degeneration of physical characteristics, and increased erosion, and instability of soil aggregates [8,9].

Biostimulators are popular in sustainable agriculture because they activate several physiological processes that improve food efficiency, stimulate plant growth, and reduce fertilizer consumption [10]. The use of biofertilizers has ecological and economic justifications. These fertilizers occupy a special place in organic, sustainable agricultural production. Biostimulators help the plant to resist the effects of biotic and abiotic stresses, increasing the quality and yield of the plant [11].

The aim of our study was to determine the effect of different fertilization methods on the yield, nutritional value, and antioxidant capacity of lettuce.

MATERIAL AND METHOD OF WORK

Plant material

Two genotypes of lettuce “Santoro RZ” and “Kiribati RZ” were used during the study. The experiment was set up at the Lukavica site, in the area of the city of East Sarajevo (43.8269 0N 18.3832 0E) from spring 2021. in greenhouse conditions.

Experimental design

The test plants were planted by block method in three replicates with the size of the experimental plot $2m^2$ (1 x 2 m). Lettuce was grown by planting seed 530 x 310 x 60 mm. One month of seedlings with well-formed 4–5 true leaves was transplanted at a distance of 30x20cm. Dripping irrigation was used during the experiment.

Fertilizers

We applied different types of fertilization in the experiment. One part of the plants were fertilized with a biostimulator-Slavol. Slavol is a preparation that contains nitrogen-fixing bacteria and phosphorus mineralizer, growth stimulators that produce auxins (indole-3 acetic acid) in the fermentation process in the range from 0.01 to 0.1 mg/L. It was used in the amount of 200 mL/10 L of water.The other part of the plants was fertilized with organic-inorganic NPK fertilizer-Fitofert hemisuper plus. In addition to organically active components, this fertilizer contains humic and fulvic acid, carbohydrates, betaine, and lignosulfonates. It is used in the amount of 100 mL/10 L of water.A third of the experimental plants were not treated with fertilizer. These were control plants.

Fertilization was performed in the following stages of lettuce development: rooting phase, rosette leaf formation phase, and head formation phase.

Harvesting and handling after harvest

Lettuce samples were harvested at the stage of technological value. After harvest, the fresh weight of the rosette (head) (FW) was measured using a digital scale to two decimal places and the results are shown in grams (g) as the mean value for three replicates.

Applied methods:

Determination of total phenols and flavonoids

The plant material (50 g) was extracted with 75% ethanol, and the extraction with the same plant material was repeated three times with five times the volume of solvent relative to the weight of the crushing plant, for 24 h. The resulting extracts were filtered, combined, and concentrated in a vacuum.The content of total phenolic compounds was determined by the Folin-Ciocalte spectrophotometric method [12] and was expressed as gallic acid equivalent (mg GAE/100 g fresh sample).The total amount of flavonoids was determined by the method with $AlCl_3$, described by [13], and the results are presented as equivalently rutin (mg RE/100 g fresh sample). All results are expressed as the mean of the three measurements of the analyzed samples (± standard deviation).

Determination of antioxidation activity

DPPH „scavenger" activity was determined by the method which spectrophotometrically monitored the reaction between a stable DPPH radical (1,1-diphenyl-2 picrylhydrazyl radical) and a sample whose antioxidant activity was examined [14]. A modified method was used to determine the antioxidant activity by the ABTS test (Total Equivalent Capacity assay). Antioxidant activity is expressed in % inhibition.

Statistical analysis

All samples were analyzed in triplicates. The results are expressed as mean values ± standard deviation (SD). Differences were considered significant if *P*-values were under 0.05. Statistical analysis of experimental data was performed using the Statistica (Tukey HSD Test) (StatSoft Inc. "STATISTICA Data Analysis Software System, (Version 10).

PCA

Principal component analysis (PCA) is a statistical technique applied to a set of variables when the researcher is interested in which variables of the set form coherent subsets that are relatively independent of each other [15].

RESULTS AND DISCUSSIONS

Root length and rosette weight were measured from growth parameters, and the results are given in Figure 1.

The highest root length was recorded in the genotype "Kiribati RZ" treated with the fertilizer Slavol (11.21 cm), while the lowest value was recorded in the genotype „Santoro RZ" in the control variant (8.44 cm). Use of biostimulators actively helps the development of the root system [16,17].

The weight of the rosette varied depending on the genotype and the type of fertilizer. The highest weight was in the genotype „Kiribati RZ" treated with Slavol fertilizer (249.48 g), followed by the genotype „Santoro RZ" treated with the same fertilizer (222.99 g). In their research, a group of authors concluded that the use of biostimulators stimulates the growth of the aboveground part of the plant by an average of 29% compared to the control variant [4,18,19].

Chemical characterization of lettuce leaves

Total phenolic content and antioxidant activity

In the extracts of lettuce, the content of total phenols, flavonoids, and antioxidant capacities was determined, the results are shown in Figure 2. All samples contain total phenols and flavonoids. The content of total phenols and flavonoids depends on the genotype and the type of fertilizer. The highest concentration of total phenols was measured in genotype "Kiribati RZ" fertilized with Slavol (358.13 ± 1.30 mg RE/ 100 g fresh sample).

The lowest concentration was in the genotype "Kiribati RZ" which treated with Fitofert hemisuper plus (from 115.35 ± 1.64[a] mg GAE / 100 g fresh sample). All samples treated with fertilizer showed higher phenol content compared to the control sample. Application of fertilizers affects the yield, physiological characteristics, and content of phenolic compounds in wheat grain *(Triticum aestivum L. ssp. aestivum)* [20,21,22].

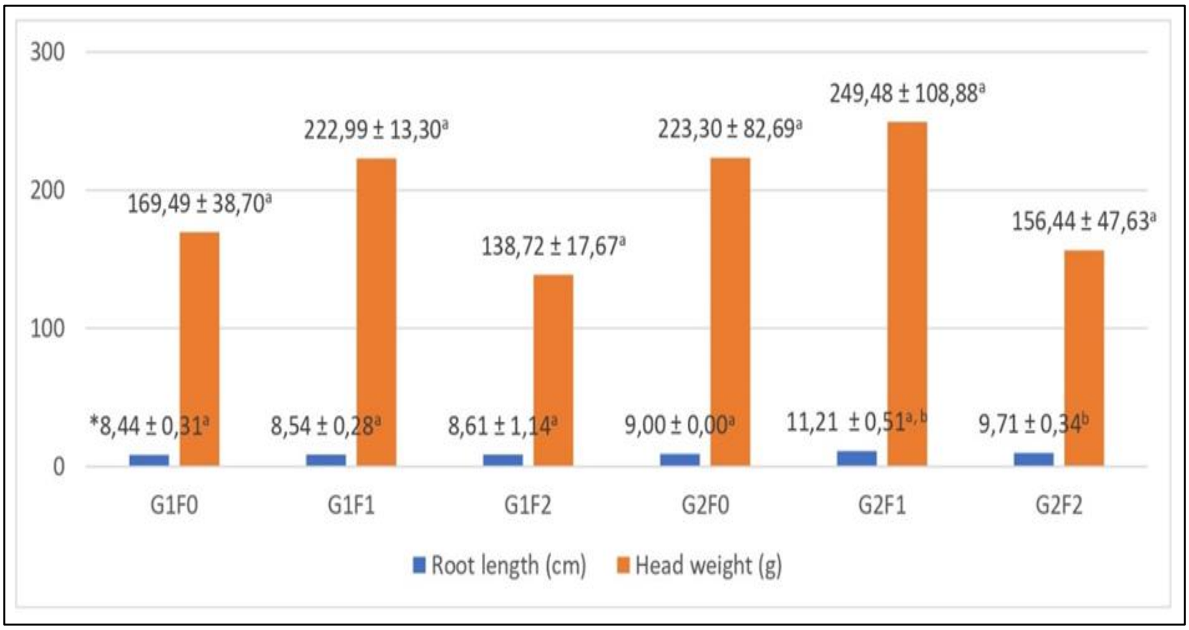

Figure 1. Effect of different fertilization methods on root length and head weight in lettuce

G – genotypes (G_1 – Santoro RZ, G_2 – Kiribati RZ); F – Fertilizers (F_0 – control, F_1 – Slavol, F_2 – Fitofert hemisuper plus)
* = Mean value ± standard deviation (n = 3)
$^{a\text{-}b}$ Different letters in superscript indicate the statistically significant difference between values, at a significance level of p<0.05 (based on Tukey's HSD test)

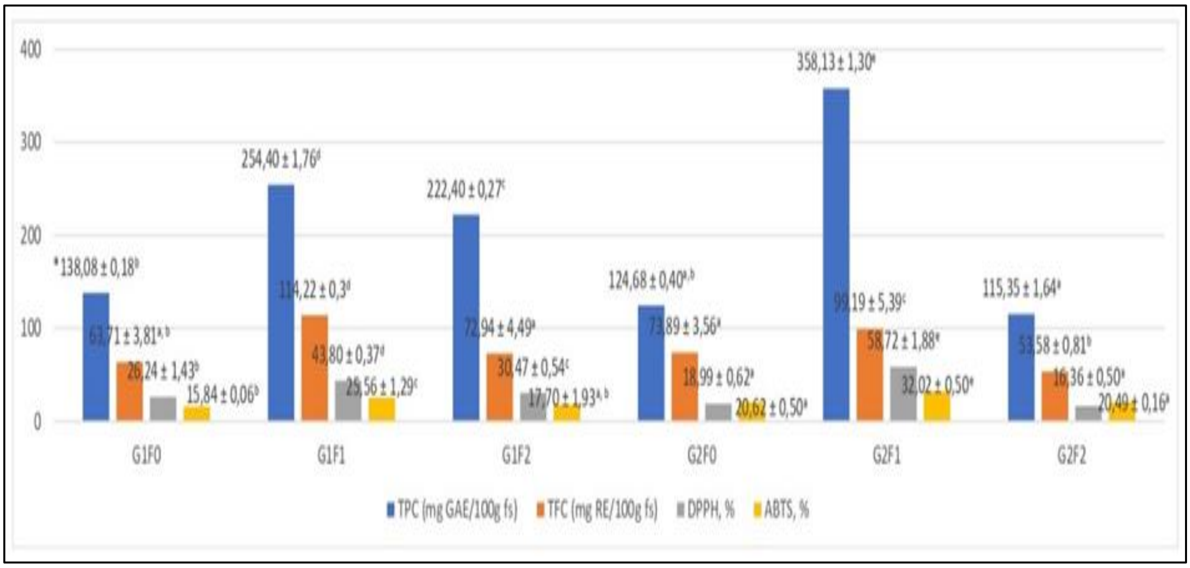

Figure 2**.** Total phenols, flavonoids and antioxidant capacity of lettuce leaves

G – genotypes (G_1 – Santoro RZ, G_2 – Kiribati RZ); F – Fertilizers (F_0 – control,F_1 – Slavol,F_2 – Fitofert hemisuper plus)
* = Mean value ± standard deviation (n = 3)
$^{a\text{-}b}$ Different letters in superscript indicate the statistically significant difference between values, at a significance level of *p*<0.05 (based on Tukey's HSD test)

The flavonoid content is dominated by genotype „Santoro RZ" treated with Slavol fertilizer (114.22 ± 0.3^{d} mg RE/100 g fresh sample). The lowest content of flavonoids (53.58 ± 0.81^{b} mg RE/100 g of fresh sample) was in genotype "Kiribati RZ" treated with Fitofert hemisuper plus fertilizer. It can be concluded that the type of fertilizer affects the phenolic and flavonoid content [2,6].

In our study, values for total phenolic content and flavonoids were higher for plants treated with organic fertilizers. The effect of organic fertilizers, fish pellets and manure mixtures affect the phenol content, and the overall fruit quality of tomato varieties [23]. Also the influence of fertilizers during the growth and development of the plant, shows an increased content of phenols and flavonoids compared to the control soil [24].

The antioxidant activity of lettuce was determined by DPHH and ABTS tests. All samples showed antioxidant capacity. The percentage of neutralization of DPHH radicals ranges from 58.72 ± 1.88 e% for genotype "Kiribati RZ", treated with Slavol fertilizer, to 16.36 ± 0.50% for "Kiribati RZ" sample treated with Fitofert hemisuper plus fertilizer (Figure 2).

The examined samples also show a high degree of $ABTS^{+}$ „scavenging" activity. Both methods confirmed that fertilizer-treated lettuce samples had a much higher antioxidant potential compared to the control sample. The results are in agreement with previous research that different ways of fertilizing vegetables adding biostimulants, significantly affect the antioxidant capacity [25, 26].

A similar observation for an increase in total phenols content and antioxidant capacity was reported after treatment with organic fertilizers in peppers ,cucumbers, watermelons and melon fruits [27].

Thus, the results revealed that the quality and nutritional value of lettuce leaves could be improved by applying organic fertilization for the production of foods for human healthy nutrition.

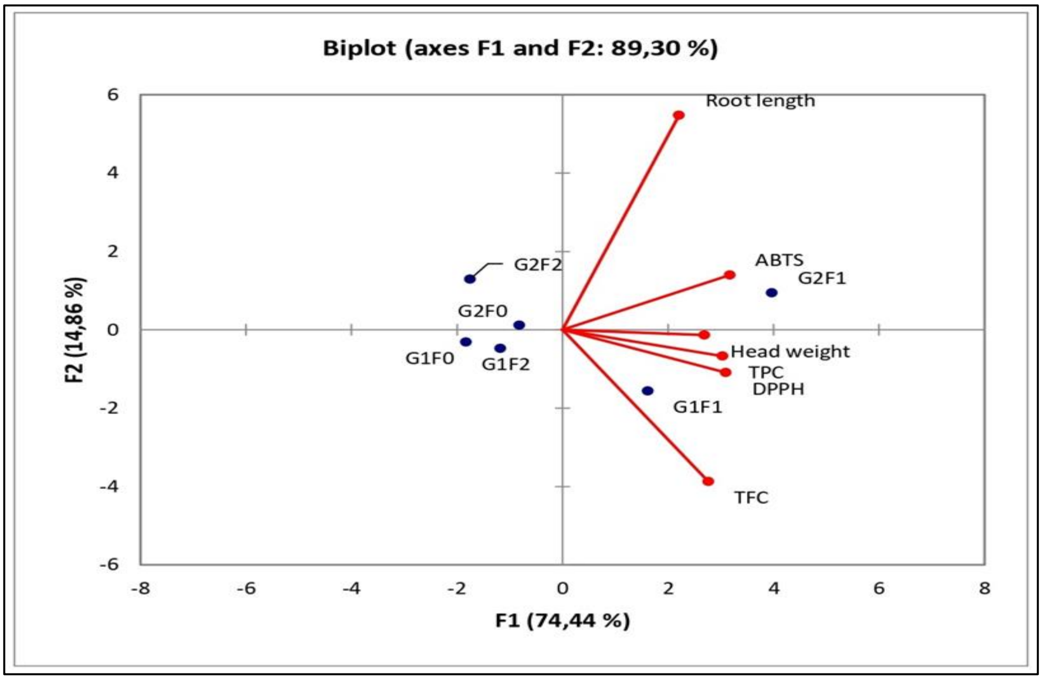

Figure 3. PCA of independent variables and responses of root length/rosette weight and bioactive components

The results of the PCA are shown in Figure 3. A scatter plot was created with the first two principal components from PCA of the data matrix, with the first principal component at the x-axis and the second at the y-axis, to visualize trends in the displayed data and demonstrate the discriminating effectiveness of the descriptors used. The contribution of the variables (%) showed that ABTS (20.69%), DPPH (19.94 %), TPC (18.95%) and head weight (14.87%) most participated in F_1, and root length (61.63%), and TFC (30.92%) in F_2.

The position of the samples in Figure 3 was primarily influenced by the type of fertilizer than the genotype of the values of the first principal component. Samples G_2F_1 and G_1F_1 were characterized by higher values of all analyzed parameters (oriented on the positive side of the x-axis by the positive value of the F_1 component), compared to other samples G_1F_0, G_2F_0, G_1F_2, and G_2F_2 oriented on the negative side of the x-axis (by the negative value of the F_1 component). Therefore, sample G_2F_1 was characterized by the high values of the following parameters: root length and ABTS, and sample G_1F_1 was characterized by the high values of the following parameters: head weight, DPPH, TPC, and TFC.

The high concentrations of flavonoids contribute to the higher antioxidant activity of lettuce as well. Various studies have shown cohesion between total phenol content and antioxidant activity of fruits, plants, and vegetables.

A comparative study, of antioxidant properties, and phenolic profile [28] shows the cohesion of antioxidant properties and phenolic profile of the most consumption of berry species. Different investigations have shown a relationship between the total phenolic contents and the antioxidative activity of fruits, plants and vegetables [29,30].

CONCLUSION

The application of biostimulants gave statistically significant effects on the components of the growth and development of lettuce. It strongly influenced the development of the root system and aboveground mass.

The application of biostimulants had the greatest impact on the accumulation of secondary metabolites of phenol and total flavonoids compared to mineral fertilization. These results also support the concept of organic fertilization of lettuce genotypes “Santoro RZ” and “Kiribati RZ” that have strongly increased the antioxidant potential of vegetables.

The application of biostimulants can be an important strategy to increase the quality and yield of lettuce.

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REFERENCES

[1] Aćamović-Đoković, G., Pavlović, R., Mladenović, J., Đurić, M. (2011). Vitamin C content of different types of lettuce varieties. Acta Agriculturae Serbica,vol.16, (32): 83–89.

[2] Bogdanović, D., Ilin, Ž., Čabilovski, R., Marijanušić, K., Adamović, B. (2014).Effect of direct and residual fertilization with organic and mineral fertilizers on tomato yield. Chronicle of Scientific Papers, Faculty of Agriculture Novi Sad,38(1): 59–68. https://doi.org/10.5937/lnrpfns1401059B

[3] Adejero, S.A., Arije, D. N., Adegaye, A. C. (2019). Residual effects of neem Azadirachta indica A. Juss.*(Sapindales: Meliaceae)* seed-based fertilizer and NPK on the performance of Basella alba L.*(Caryophyllales: Basellaceae)* plant. Brazilian Journal of Biological Sciences, 6(12): 141–148. http://dx.doi.org/10.21472/bjbs.061213

[4] Chatterjee, R., Bandhopadhyay, S., Jana, J. C. (2014). Organic amendments influencing growth, head yield and nitrogen use efficiency in cabbage *(Brassica Oleracea var. Capitata L.)*.American International Journal of Research In Formal, Applied & Natural Sciences 5(1): 90–95. http://www.iasir.net/

[5] Fatimah Obaid Saeed Ali Kalbani, Mohammed, A. Salem., Abdul, J. Cheruth,, Shyam, S. Kurup., A, Senthilkumar (2016). Effect of some organic fertilizers on growth, yield and quality of tomato (Solanum lycopersicum). International Letters of Natural Sciences Submitted: ISSN: 2300–9675, 53: 1–9. http://dx.doi.org/10.18052/www.scipress.com/ILNS.53.1

[6] Palia, M., Saravanan, S., Prasad, V.M., Upadhyay, R.G., Kasera, S.(2021). Effect of Different Levels of Organic and Inorganic Fertilizers on Growth, Yield and Quality of Brinjal (*Solanum melongena* L.). Agricultural Science Digest 41 (special issue), 203–206. https://arccjournals.com/journal/agricultural-science-digest/D-5157

[7] Gordon, W. B., Whitney, D. A., Raney, R. J. (1993). Nitrogen Management in Furrow Irrigated, Ridge-Tilled Corn. Journal of production agriculture, 6(2): 213–217. https://doi.org/10.2134/jpa1993.0213

[8] Hammad, H. M., Khaliq, A., Abbas, F., Farhad, W., Fahad, S., Aslam, M., Bakhat, H. F. (2020). Comparative effects of organic and inorganic fertilizers on soil organic carbon and wheat productivity under arid region. Communications in Soil Science and Plant Analysis, 51(10): 1406–1422.

[9] Adeniji, A.A. (2020). Effects of Incorporated Green Manure and Inorganic Fertilizer on Amaranth *(Amaranthus Caudatus.* L.) Vegetable. International Journal of Trend in Scientific Research and Development (IJTSRD),4(6): 1414–1419. http://eprints.federalpolyilaro.edu.ng/id/eprint/1466

[10] Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., Brown, P.H. (2017). Biostimulants in plant science a global perspective.Frontiersin in Plant Science 7, article 2049, 1–32. https://doi.org/10.3389/fpls.2016.02049

[11] Banaker, S. N., Kumar, M. P. (2020). Foliar application of red seaweed *(Kappaphycus alvarezii)* bioformulations increased the levels of chlorophyll content in rice. Journal of Pharmacognosy and Phytochemistry, vol.9, Issue 1, 408–410.

[12] Singleton, V. L., Orthofer, R., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

[13] Brighete, I.M.C., Dias, M., Verdi, L.G., Pizzalatti, M.G. (2007). Antioxidant activity and total phenolic content of some Brazilian species. Pharmaceutical Biology 45:156–161. https://doi.org/10.1080/13880200601113131

[14] Kumarasamy, Y., Byres, M.,Cox, P.J., Jasapars, M., Nahar, L., Sarker, S.D. (2007): Screening seed of some Scottish plants for free-radical scavenging activity. Phytotherapy Research 21: 615–621. http://dx.doi.org/10.1002/ptr.2129

[15] Rajčić, K. (2015). Comparison of principal component and factor analysis, Master's thesis, University of Zagreb, Faculty of Science.

[16] Zeljković, S., Parađiković, N., Babić, T., Đurić, G., Oljača, R., Vinković, T., Tkalec, M. (2010). Influence of biostimulators on the growth and development of *(Salvia splendens L.)* seedling roots. Journal of Agricultural Sciences, 55: 29–36. http://www.doiserbia.nb.rs/img/doi/1450-8109/2010/1450-81091001029Z

[17] Ergović, L. (2019). Influence of biological preparation on salad seedling adaptation, Doctoral dissertation, Josip Juraj Strossmayer University of Osijek. Faculty of Agrobiotechical Sciences Osijek.Department for agroecology. https://urn.nsk.hr/urn:nbn:hr:151:965440

[18] Cvijanović, G., Petrović, G. B., Marinković, J., Cvijanović, V., Đurić, N., Roljević Nikolić, S. (2017). Microbiological activity of land and productivity of different genotypes of wheat in a sustainable system of production.Knowledge–International Journal,20(5): 2351–2356.

[19] Zeljković, S., Parađiković, N., Tkalec Kojić, M., Mladenović, E. (2021). Effect of biostimulant application on development of pansy *(Viola tricolor var. hortensis dc.)* seedlings. Journal of Central European Agriculture, 22(3): 596–601. https://doi.org/10.5513/JCEA01/22.3.3191

[20] Stumpf, B., Yan, F., Honermeier, B. (2019). Influence of nitrogen fertilization on yield and phenolic compounds in wheat grains *(Triticum aestivum L. ssp. aestivum*). Journal of Plant Nutrition and Soil Science, 182(1): 111–118. http://dx.doi.org/10.1002/jpln.201800342

[21] Khan, M. A., Basir, A., Saeed, B. (2020). Biochar Improves Phenological and Physiological Attributes of Wheat in Soil Amended with Organic and Inorganic Nitrogen Sources. Sarhad Journal of Agriculture, 36(4): 1214–1226. http://dx.doi.org/10.17582/journal.sja/2020/36.4.1214.1226

[22] Tian, W., Wilson, T. L., Chen, G., Guttieri, M. J., Nelson, N. O., Fritz, A., Li, Y. (2021). Effects of environment, nitrogen, and sulfur on total phenolic content and phenolic acid composition of winter wheat grain. Foods10 (11): 2857. https://doi.org/10.3390/foods10112857

[23] Bileva, T., Petkova, N., Babrikov, T.(2020). Influence of Organic Fertilization on Nutritional Characteristics and Antioxidant Capacity of Melon Fruits. Bulletin of University of Agricultural Science and Veterinary Medicine Cluj-Napoca. Food Science and Technology 77(2): 17–25.http://dx.doi.org/10.15835/buasvmcn-fst:2020.0013

[24] Malenčić, Đ., Kiprovski, B., Rajković, M., Miladinović, J., Kljakić, S., Šućur, J. (2019). Changes in polyphenol content in soybeans *(Glycine max L.)* and tatula (*Datura stramonium* L.) after treatment with herbicides and Delfan Plus.Annals of Agronomy,43(1): 26–37. http://fiver.ifvcns.rs/handle/123456789/2187

[25] Chu, Y., Sun, J., Wu, X., Liu, R. (2002). Antioxidant and antiproliferative activities of common vegetables. Journal of Agricultural and Food Chemistry, 50(23): 6910–9616. https://doi.org/10.1021/jf020665f

[26] Emeghara, U., Olukotun, O., Olagunju, O. E., Akanni-John, R., Oni, B.O., Ganiyu, L., Rasheed, F. M. (2020). Effect of Different Organic Manures on the Growth and Yield of Water melon *(Citrullus lanatus)*.Asian Soil Research Journal, 37–43. https://doi.org/10.9734/asrj/2020/v4i230091

[27] Babrikov, T., Bileva, T., Tzvetkov, P., Petkova, N., Ivanov, I., Denev, P. (2016). Influence of biofertilizers and biopesticides on the growth, development and production quality of some vegetable crops, Agricultural University – Plovdiv, Scientific Works, LX (2), 61–70. https://mc04.manuscriptcentral.com/agriculture

[28] Okatan, V. (2020). Antioxidant properties and phenolic profile of the most widely appreciated cultivated berry species: A comparative study. Folia Horticulturae, 32(1): 79–85. https://doi.org/10.2478/fhort-2020-0008

[29] Gao, Y., Guo, X., Liu, Y., Zhang, M., Zhang, R., Abbasi, A. M., You, L., Liu, R.H. (2018). Comparative assessment of phytochemical profile, antioxidant capacity and anti-proliferative activity in different varieties of brown rice (*Oryza sativa* L.).Food Science and Technology 96:19–25. https://doi.org/10.1016/j.lwt.2018.05.002

[30] Fitriana, A. S., Royani, S. (2020). Identifying Antioxidant Activities of Guava Fruit Using DPPH Method. Proceedings of the 1st International Conference on Community Health (ICCH 2019) https://doi.org/10.2991/ahsr.k.200204.027