11th INTERNATIONAL SCIENTIFIC CONFERENCE RURAL DEVELOPMENT 2023
BIOECONOMY FOR THE GREEN DEAL
26-28th September, 2023, Vytautas Magnus University Agriculture Academy, Lithuania

VYTAUTAS MAGNUS UNIVERSITY AGRICULTURE ACADEMY

Proceedings of the 11th International Scientific Conference Rural Development 2023

Edited by assoc. prof. dr. Judita Černiauskienė

ISSN 1822-3230 (Print)
ISSN 2345-0916 (Online)

Article DOI: https://doi.org/10.15544/RD.2023.029

THE APPLICATION OF BIOLOGICAL ORIGIN PRODUCTS IN AGRICULTURAL PLANT GROWING TECHNOLOGIES

Sonata KAZLAUSKAITE, Department of Agroecosystems and soil science, Faculty of Agronomy, Vytautas Magnus University, address: K. Donelaičio str. 58, LT-44248 Kaunas, Lithuania, sonata.kazlauskaite@.vdu.lt *(corresponding author)*
Elena SURVILIENE, Department of Landscape Architecture and Recreation, Kaunas Forestry and Engineering University of Applied Science, address: Liepų g. 1, Girionys, LT 53101 Kaunas distr., Lithuania, esurviliene@yahoo.com
Kimete LLUGA, Department of Biology, Faculty of Natural Sciences, University of Prishtina, Prishtina, Str. "George Bush", No.31, 10 000 Prishtinë, Republic of Kosov, kimete.lluga@uni-pr.edu
Povilas MULERCIKAS, Department of Agroecosystems and soil science, Faculty of Agronomy, Vytautas Magnus University, address: K. Donelaičio str. 58, LT-44248 Kaunas, Lithuania, povilas.mulercikas@.vdu.lt

Due to implementing the requirements of the green course, the use of both mineral fertilizers and synthetic plant protection products should be drastically reduced. Therefore, products of biological origin (bioproducts) are gaining more and more importance in crop production and especially in horticulture. The effect of a product based on bacteria *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus vallismortis* on the growth and development of two varieties of fava bean 'Bobas' and 'Tiffany' was investigated. The product improved plant development and productivity. It is claimed that the product reduces the stress of lack of moisture on plants, and thus, increases resistance to diseases. The purpose of the research was to determine the effect of the used bacterial preparation on the immunity and productivity indicators of two different varieties of fava beans.

Keywords: *Bioproducts; Bacillus licheniformis; Bacillus subtilis, Bacillus vallismortis.*

INTRODUCTION

Currently green course encourages the choice of sustainable farming methods, the use of new technologies in order to preserve resources for both current and future generations. Over the past few decades, technological advances in the production of chemicals have led to a revolution in agriculture. Chemicals that are used for crop fertilization, for pest and disease control ensure a successful farming system, abundant and wholesome harvests, but in some cases, it has a significant impact on the environment and public health. On the other hand, the long-term application of various chemicals in agriculture leads to a decrease in the resistance of plants to diseases and pests, as well as to soil degradation. An innovative solution to avoid these problems is the use of biological preparations (Vasilevski, 2003; Hossain et al., 2017). The application of biological preparations, compared to the use of traditional chemicals, gives a lower yield, but does less harm to the environment and significantly improves the quality of the production, since there are no pesticide residues in the products, and the nutritional value remains the same, and sometimes even higher (Reganold et al., 2016). Many biological preparations act as stimulators of the physiological functions of plants and microorganisms. The composition of these preparations usually includes essential oils, various microorganisms, plant extracts, seaweed extracts, etc. After spraying such preparations on the soil, the decomposition process of the residues is intensified, thus the soil is enriched with organic matter, from which it is easier for the plants to absorb the necessary nutrients. Some species of bacteria have many beneficial properties, such as promoting plant growth by secreting various plant growth hormones and mobilizing many important inorganic macro and microelements, and are therefore often referred to as biofertilizers (Dasgupta et al., 2021). Some biological preparations contain nitrogen-fixing bacteria and biologically active substances. Bacteria take nitrogen from the environment, consume part of it themselves, and the other part remains the soil in a form available to plants. Such products can compensate for the missing amount of mineral nitrogen in the soil and facilitate its assimilation by plants. There are also biological preparations that contain bacteria that allow for easier dissolution and mineralization of phosphates, thus increasing their effectiveness. Many nutrients and micronelements important to plants are found in soil in a form unavailable to plants and must therefore be fixed or mobilized by rhizobacteria (Hayat et al., 2010; Muras et al., 2021). Also, these bacteria promote the establishment of beneficial bacteria and thus improve the colonization of local symbiotic rhizobacteria (Elkoca et al., 2007). Due to these bacteria, conditions are created that improve resistance to diseases and pests, as well as increase the resistance of plants to lack of moisture (Sukkasem et al., 2018). In addition,

rhizospheric activity of bacteria confers a strong antagonistic potential against a wide range of plant pathogens by releasing various antimicrobial secondary metabolites and hydrolytic enzymes (Kaur et al., 2016; Ratul et al., 2018). Preparations of biological origin are an ecological alternative to the application of synthetic pesticides. *Bacillus licheniformis* inhibits the reproduction of some microorganisms, thus these bacteria increase plant disease resistance (Tendulkar et al., 2017; Sukkasem et al., 2018). *Bacillus subtilis* bacteria, often are included in the composition of biological preparations, are used as plant growth stimulants and for biocontrol of various plant pathogens (Wang et al., 2018). These bacteria are able to form spores that are resistant to drought, temperature, and lack of nutrients, which makes them perfectly suitable for use to ensure disease control in the crop and improve its yield. The beneficial properties of *B. subtilis* have already been used in the composition of many currently used biological preparations (Blake et al., 2020). *Bacillus vallismortis* is characterized by the ability to inhibit the growth and development of pathogenic fungi (Zhao et al., 2010). These bacteria inhibit a wide range of pathogenic fungi, reducing the spread of fungal diseases by 70 to 85 percent, and timely application of these bacteria can prevent the onset of diseases (Falardeau et al., 2013).

The purpose of the conducted field experiment was to determine the effect of the applied bacterial preparation on the immunity and productivity of fava beans of two different varieties.

RESEARCH METHODS

The experiment was carried out in 2021 at Vytautas Magnus University Botanical Garden. The effect of a biological preparation containing spores of three species of bacteria - *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus vallismortis* - on fava beans varieties 'Bobas' and 'Tiffany' was studied. The experiment was carried out in three replicates. It was performed with three variants: control (K), fava bean seeds were treated with bioproduct (800 g t^{-1}) (SA), fava bean seeds were treated with bioproduct (800 g t^{-1}) + plants sprayed with bioproduct (750 g ha^{-1}) at growing period BBCH 40-49 (SA+A). The size of each site was 1.05 m^2.

According to the FAO, the soil cover is classified as Cambisols. The average productivity score is 49. The soil is slightly acidic (pH 5.6-6.0) and suitable for growing agricultural crops. The depth of the carbonate layer is 70-120 cm.

The accounting of fava bean diseases was carried out in the BBCH 50-59 period following the methodology according to Gaurilčikienė and Šurkus (2002).

Fava beans are moisture-loving agricultural plants. A sufficient amount of moisture from germination to flowering periods is extremely important for these plants. However, fava beans are not heat demanding. The optimum temperature for them is 15-20°C. Seeds of fava bean start to germinate at 3-4°C, although it takes a long time to germinate in cold soil (Šiuliauskas, 2015). According to the data of the Kaunas Meteorological Station, the year 2021 was not favourable for the growth and development of fava beans due to the low amount of precipitation, although the temperature was optimal for them throughout the growing season (Figure 1).

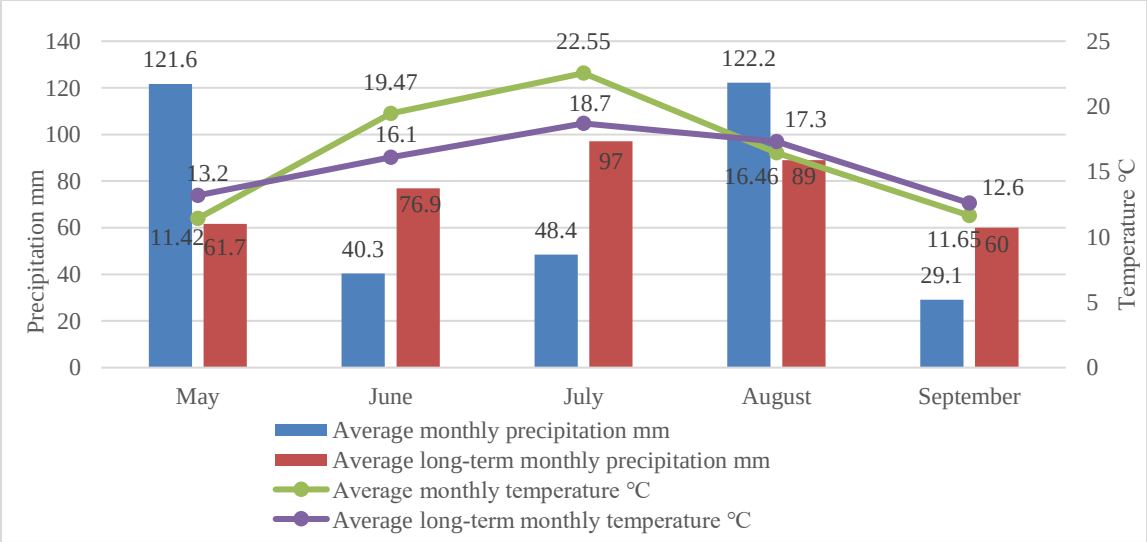

Figure 1. Meteorological data during the growing season of fava beans in 2021.

Plant biometric indicators were evaluated in accordance with the methodology Žemės ūkio augalų veislių ūkinio vertingumo tyrimų metodikos (2002) in each site: plant height (was measured before harvesting), root length, the average number of root nodules on the roots of the plant, and the average number of pods per plant were determined. The research data obtained in the experiment were evaluated statistically using the ANOVA program. The statistical reliability of the experimental data was assessed by the minimum threshold of significant difference R_{05}.

RESEARCH RESULTS AND DISCUSSION

According to the data obtained in the experiment, coating the seeds of different varieties of fava beans with a biological product and coating the seeds + spraying the plants with the product affected the height of the plants (Figure

2), i.e. the fava beans of both varieties 'Tiffany' and 'Bobas' grew taller, compared to the plants of the control variant. However statistical differences were insignificant. Plants of the variety 'Bobas' grew larger comparing the effects of the biological product on fava beans 'Bobas' and 'Tiffany'.

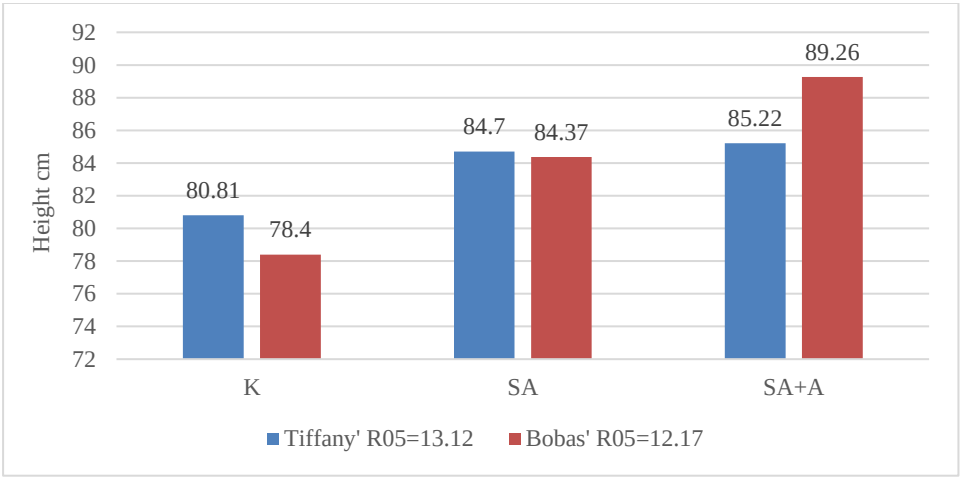

Figure 2. Effect of the biological product on average height of fava beans of different varieties.

According to the research data, the average height of the fava beans 'Tiffany' of the SA variant was almost in 4 cm higher comparing to the control variant, and the fava beans of the SA+A variant compared to the control variant were in 4.41 cm taller. This fact shows that the bioproduct increased the height of 'Tiffany' fava beans, however there were no significant differences in the height of the fava beans observed comparing to the control variant. The highest average plant height was determined in the SA+A variant comparing the fava beans of different variants of the variety 'Bobas'. There the fava beans were almost in 11 cm taller than the plants of the control variant. In the SA variant, the fava beans were almost in 6 cm taller than in the control. Based on the results of the experiment, it can be stated that the biological product had an effect on the average height of the fava beans, nevertheless there were no significant differences in the height of the fava beans of the 'Bobas' variety compared to the control plants. The smallest fava beans grew in the control variant of the 'Bobas' variety, i.e. the height of the plants reached on average 78.4 cm. The height of the fava bean stems of both varieties was the highest in the variant in which both the fava bean seeds were treated with the bioproduct and the plants were sprayed with the bioproduct: 'Tiffany' - on average 85.22 cm, 'Bobas' - on average 89.26 cm.

In conclusion, it can be stated that seed treatment and spraying with biological product of fava beans had a positive effect on stem height in both 'Tiffany' and 'Bobas' fava beans.

The results of the experiment showed that the application of the bioproduct influenced the average root length of fava beans of different varieties (Figure 3), although no statistically significant differences were observed when comparing the obtained results.

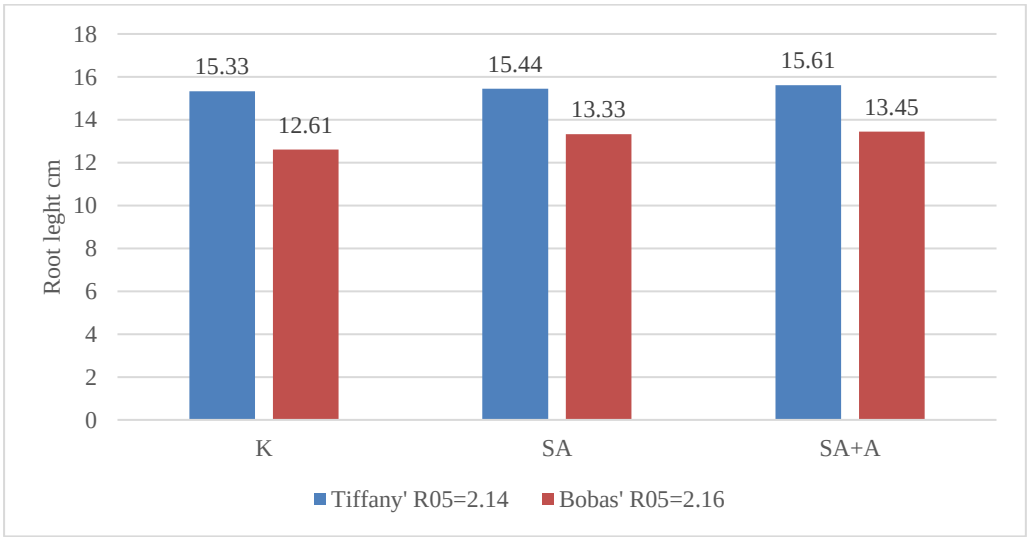

Figure 3. Effect of the biological product on average root length of different varieties of fava beans.

Compared to the control variant, the average root length of the 'Tiffany' variety fava beans of the SA variant was slightly higher - only in 0.11 cm, while the root length of the SA+A variant compared to the control variant was in 0.28 cm longer. The situation in fava beans of the variety 'Bobas' was similar. The highest average root length was determined in the variant SA+A, where the fava bean roots were in 0.84 cm longer than in the control variant. Bean roots were on

average 0.72 cm longer in the SA variant than in the control variant. The lowest average length of fava bean roots was detected in the control variant of the 'Bobas' variety – on average 12.61 cm. Meanwhile, the average root length of 'Tiffany' fava beans in the control variant was in 2.72 cm longer. The root length of both varieties of fava beans was the highest in the variant in which the bioproduct was applied both for seed treatment and for spraying the plants during the growing season. The root length of the fava beans reached: 'Tiffany' - on average 15.61 cm, and 'Bobas' - on average 13.45 cm.

In conclusion, it can be stated that the average root length of fava beans of both 'Tiffany' and 'Bobas' varieties in the variants in which the biological product was applied reached higher results than in the control variant in which the biological product was not used, although the differences were not statistically significant. Also, fava beans of the variety 'Tiffany' produced longer roots than fava beans of the variety 'Bobas'.

Application of the biological product in fava beans of different varieties had a positive effect on the number of nodules formed on the roots of the plant (Figure 4). The roots of both fava bean varieties 'Tiffany' and 'Bobas' formed more nodules than in the control variant in both variants where the biological product was applied. The highest average number of nodules was found in fava beans of the 'Tiffany' variety.

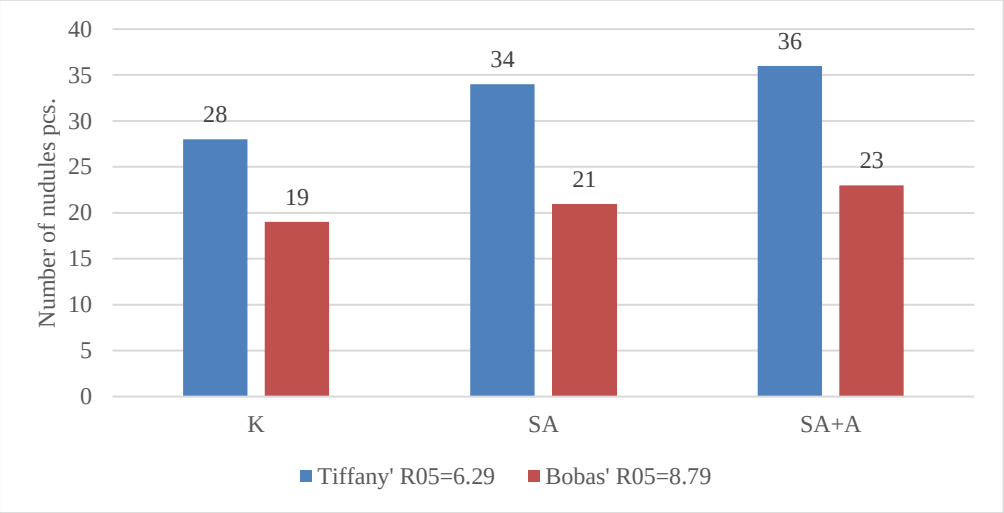

Figure 4. Effect of the biological product on the average number of root nodules of different varieties of fava beans.

The roots of 'Tiffany' fava beans of the SA variant formed on average by 6 nodules more than plants of the control variant, although this was not a statistically significant difference. Meanwhile, the fava beans of SA+A variant showed a result on average by 8 nodules better compared to the control, and this difference was statistically significant. Fava beans of the variety 'Bobas' produced on average the largest (23 pcs.) number of nodules on their roots when their seeds were treated with the bioproduct and the plants were sprayed during the growing season as well. However, compared to the control variant, the identified differences were insignificant. Insignificantly (2 pcs.) higher, compared to the control variant, the average number of nodules was determined on the roots of the fava beans when only the seeds were treated with the bioproduct.

In conclusion, it can be stated that seed treatment and spraying of plants with a biological product during the growing season had the greatest influence on the formation of root nodules for both 'Tiffany' and 'Bobas' fava beans, although statistically significant differences were not obtained in all cases.

The results obtained during the experiment showed that the use of the biological product encouraged the formation of a larger amount of pods in the fava beans of both investigated varieties (Figure 5). 'Tiffany' fava beans formed more pods than fava beans of the variety 'Bobas'. In addition, in fava beans of the 'Tiffany' variety, the average number of pods in the SA variant reached on average 4.8 pcs., i.e. in 0.61 pcs. more than in the control variant, and in the variant SA+A on average in 1.54 pcs. more compared to the control. The differences obtained were statistically significant comparing to the result of the control variant. Thus, it can be stated that the used biological product had a significant influence on the formation of a larger number of fava bean pods of the 'Tiffany' variety.

Meanwhile, in the fava beans of the 'Bobas' variety, the highest number of pods was determined in the variant in which the bioproduct was used both as a seed treatment and the fava beans were sprayed later during the growing season. The average number of pods per plant was 5.20 pcs. In the SA variant were only seeds of fava bean were covered with the product, the number of pods was higher by 1.15 pcs. compared to the control variant. Based on the obtained results, it can be stated that the application of the biological product had an effect on the formation of a higher number of fava bean pods in this variety, as significant differences were observed. The lowest number of pods was formed in the control variant of the variety 'Bobas', while the number of pods in the 'Tiffany' variety was on average in 0.14 pcs higher. In the variant, in which seeds of both fava bean varieties were treated with a bioproduct, the number of pods reached: 'Tiffany' - on average 4.80 pcs., and 'Bobas' - on average 4.51 pcs. The number of fava bean pods of both varieties was the highest in the variant in which both the fava bean seeds were treated with the biological product and the plants sprayed later during the growing season. The number of pods formed reached: 'Tiffany' - on average 5.73 pcs., and 'Bobas' - on average 5.20 pcs.

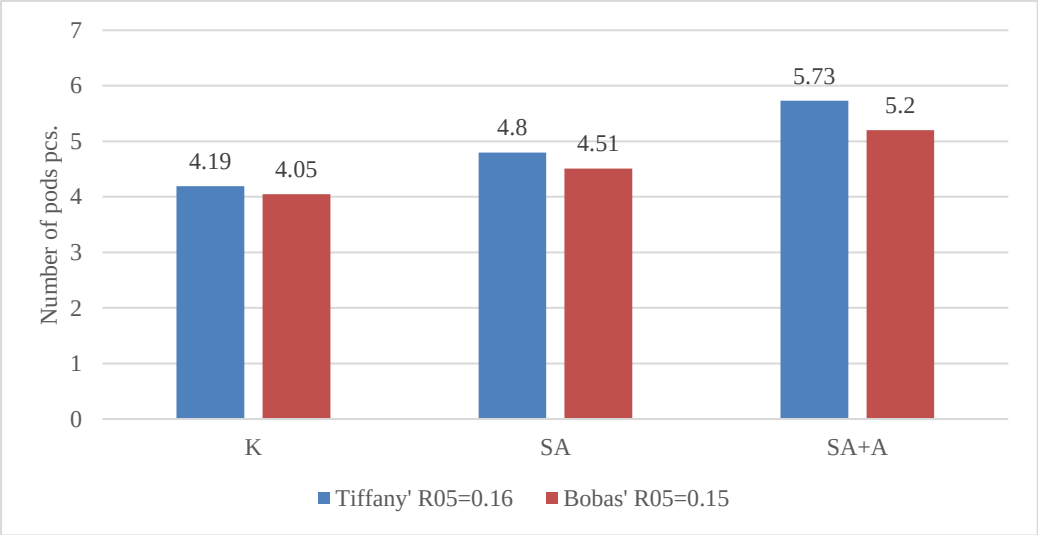

Figure 5. Effect of the biological product on the average number of pods per plant of different varieties of fava beans.

In conclusion, it can be indicated that in order to make form the fava beans of the varieties 'Tiffany' and 'Bobas' larger amounts of pods, the application of the bioproduct is undoubtedly appropriate, especially in the case when the product is applied as a seed treatment and the growing plants are sprayed with the bioproduct later during the growing season.

Ascochyta blight (*Ascochyta fabae*) is a disease that spreads with infected seeds and infected plant debris in the fields. Spores the fungus are also carried by wind and rain to other crops (Deveikytė et al., 2020). The disease affected fava bean leaves, stems, flowers and pods. Fava beans of the variety 'Bobas' were more affected by the disease, while fava beans of the variety 'Tiffany' were less injured (Figure 6).

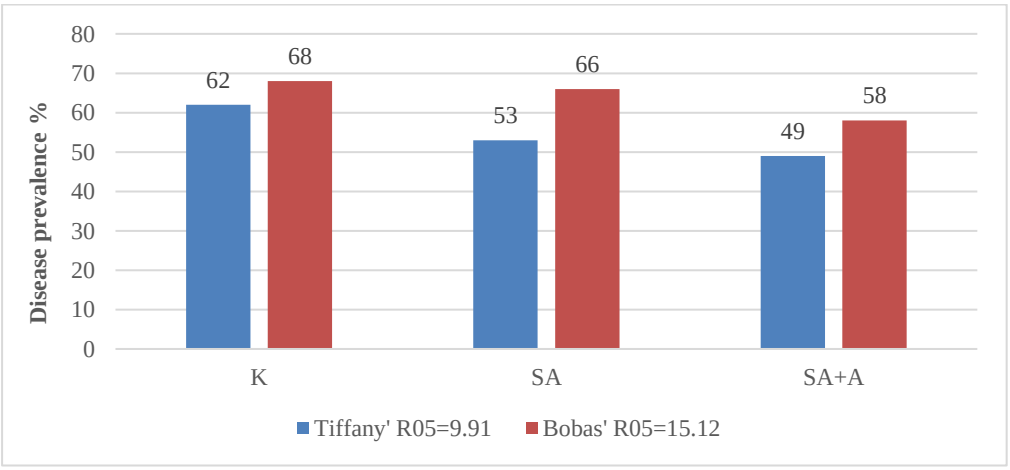

Figure 6. The influence of the biological product on the prevalence of Ascochyta blight disease in fava beans of different varieties.

Fava beans of the variety 'Bobas' were most affected by Ascochyta blight disease in the control variant (68%). In the variant, in which the fava bean seeds were treated with a biological product, plants in 2% less affected by the disease were found. Therefore, Ascochyta blight disease damaged fava beans of this variety in 10% less than in the control variant, if fava bean seeds were covered and plants were sprayed with a biological product. Based on the obtained results, it can be defined that the biological product had an effect on strengthening the immunity of the fava beans, however there were no significant differences observed among the results of the prevalence of the disease in 'Bobas' variety plants, compared to the control variant. Assessing disease prevalence in fava beans of the variety 'Tiffany' the highest result was detected also in the control variant. The assessment of the prevalence of the disease showed that 62% of the plants were affected by Ascochyta blight. Fava bean seed treatment (SA variant) reduced the prevalence of the disease by 9%. However, the difference was not statistically significant. Meanwhile, in the variant in which the bioproduct was not only applied to the fava bean seeds, but also the plants were sprayed during the growing season, fava beans were damaged by 13% less compared to the control, and this difference was statistically significant. Based on the obtained results, it can be stated that the biological product had a significant effect on strengthening the immunity of beans.

Finally, it can be indicated that both the seed treatment and plant spraying with a biological product had an effect on the prevalence of Ascochyta blight in both 'Tiffany' and 'Bobas' varieties of fava beans. Less plants than in the control were damaged in those cases where the biological product was used. Better results were achieved when the bioproduct

was both applied for seed treatment and later sprayed on the plants during the growing season. It is important to mention that fava beans of the variety 'Tiffany' were more resistant to Ascochyta blight than 'Bobas' variety fava beans.

CONCLUSIONS

The results of the experiment showed that fava bean seeds treatment and spraying of the plants with a biological product had a positive effect on stem height in both varieties 'Tiffany' and 'Bobas' fava beans. The average root length of fava beans was longer of both 'Tiffany' and 'Bobas' varieties when the biological product was applied, although the differences were not statistically significant. Seed treatment together with spraying of plants with a biological product during the growing season had the greatest influence on the formation of root nodules for both 'Tiffany' and 'Bobas' fava beans, although statistically significant differences were not obtained in all cases. In order for the plants to form more pods, the application of the bioproduct is undoubtedly appropriate for both 'Tiffany' and 'Bobas' fava beans, especially if the product is applied as a seed treatment and also the growing plants are sprayed with the bioproduct later during the growing season. Fava beans of the variety 'Tiffany' were more resistant to Ascochyta blight than 'Bobas' variety fava beans. Seed treatment together with the spraying of growing plants with a biological product had an effect on the prevalence of Ascochyta blight in both 'Tiffany' and 'Bobas' varieties of fava beans.

REFERENCES

1. Blake, C., Christensen, M. N., Kovács, Á. T. 2021. Molecular aspects of plant growth promotion and protection by Bacillus subtilis. *Molecular Plant-Microbe Interactions*, 34(1), 15-25. https://doi.org/10.1094/MPMI-08-20-0225-CR
2. Dasgupta D., Kumar K., Miglani R., Mishra R., Panda A. K., Bisht S. S. 2021. Chapter 1 - Microbial biofertilizers: Recent trends and future outlook / Editor(s): Surajit De Mandal, Ajit Kumar Passari, Recent Advancement in Microbial Biotechnology, P. 1-26. https://doi.org/10.1016/B978-0-12-822098-6.00001-X
3. Deveikytė, I.; Semaškienė R.; Sabeckis A. 2020. Lietuvos agrarinių ir miškų mokslų centras. Pupos. Integruotos kenksmingųjų organizmų kontrolės gairės. 3 p. Prieiga per internetą: https://www.lammc.lt/data/public/uploads/2020/05/d1_ikok_gaires_pupos.pdf [in Lithuanian]
4. Elkoca, E., Kantar, F., Sahin, F. 2007. Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *Journal of plant nutrition*, 31 (1), 157-171. https://doi.org/10.1080/01904160701742097
5. Falardeau, J., Wise, C., Novitsky, L., Avis, T. J. 2013. Ecological and mechanistic insights into the direct and indirect antimicrobial properties of Bacillus subtilis lipopeptides on plant pathogens. *Journal of chemical ecology*, 39, 869-878. https://doi.org/10.1007/s10886-013-0319-7
6. FAO STAT. 2021. Production stat: crops. FAO statistical databases (FAO stat), food and agriculture organization of the United Nations (FAO). Available at:: http://faostat.fao.org
7. Gaurilčikienė, I., Šurkus J. 2002. Žemės ūkio augalų kenkėjai, ligos ir jų apskaita : mokslinis metodinis leidinys. Akademija (Kėdainių r.): Lietuvos žemdirbystės institutas. Print. 345 p. [in Lithuanian]
8. Hayat, R., Safdar, A., Ummay, A., Rabia, K., Iftikhar, A. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of microbiology*, 60(4) 579-598. https://doi.org/10.1007/s13213-010-0117-1
9. Hossain, M. I.; Sadekuzzaman, M.; HA, S.-Do. 2017. Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. *Food research international*, 100, 63-73. https://doi.org/10.1016/j.foodres.2017.07.077
10. Kaur, P. K., Joshi, N., Singh, I.P., Saini, H.S. 2017. Identification of cyclic lipopeptides produced by Bacillus vallismortis R2 and their antifungal activity against Alternaria alternata. *Journal of applied microbiology*, 122(1), 139-152. https://doi.org/10.1111/jam.13303
11. Muras, A., Romero, M., Mayer, C., Otero, A. 2021. Biotechnological applications of Bacillus licheniformis. *Critical Reviews in Biotechnology*, 41(4), 609-627. https://doi.org/10.1080/07388551.2021.1873239
12. Ratul, M. Ram, Chetan, K., Kartikay, B., Ruchi, T., Surya, P. Singh, Harikesh, B. Singh. 2018. Biocontrol technology: eco-friendly approaches for sustainable agriculture. In: *Omics technologies and bio-engineering*. Academic Press, p. 177-190. https://doi.org/10.1016/B978-0-12-815870-8.00010-3
13. Reganold, J. P.; Wachter, J. M. 2016. Organic agriculture in the twenty-first century. *Nature Plants*, 2(2), 1-8. https://doi.org/10.1038/nplants.2015.221
14. Šiuliauskas, A. 2015. Praktinė augalininkystė. Javai ir rapsai. Vilnius. p. 449. [in Lithuanian]
15. Sukkasem, P., Kurniawan, A., Kao, Tzu-Ch., Chuang, H. 2018. A multifaceted rhizobacterium Bacillus licheniformis functions as a fungal antagonist and a promoter of plant growth and abiotic stress tolerance. *Environmental and Experimental Botany*, 155, 541-551, https://doi.org/10.1016/j.envexpbot.2018.08.005.

16. Tendulkar, S.R., Saikumari, Y.K., Patel, V., Raghotama, S., Munshi, T.K., Balaram, P., Chattoo, B.B. 2007. Isolation, purification and characterization of an antifungal molecule produced by Bacillus licheniformis BC98, and its effect on phytopathogen Magnaporthe grisea. *Journal of applied microbiology*, 103(6), 2331-2339. https://doi.org/10.1111/j.1365-2672.2007.03501.x
17. Vasilevski, G. 2003. Perspectives of the application of biophysical methods in sustainable agriculture. *Bulgarian Journal of Plant Physiology*, 29(3), 179-186.
18. Wang, X.Q., Zhao, D.L., Shen, L.L., Jing, C.L., Zhang, C.S. 2018. Application and Mechanisms of Bacillus subtilis in Biological Control of Plant Disease. In: Meena, V. (eds) *Role of Rhizospheric Microbes in Soil*. Springer, Singapore. https://doi.org/10.1007/978-981-10-8402-7_9
19. Zhao, Z., Wang, Q., Wang, K., Brian, K., Liu, C., Gu, Y. 2010. Study of the antifungal activity of Bacillus vallismortis ZZ185 in vitro and identification of its antifungal components. *Bioresource technology*, 101(1), 292-297. https://doi.org/10.1016/j.biortech.2009.07.071
20. Žemės ūkio augalų veislių ūkinio vertingumo tyrimų metodikos. 2002. Ankštinių javų (žirnių, pupų, vikių, lubinų, seradelėsseradėlės, lešiųlęšių, sojos) veislių ūkinio vertingumo tyrimų metodika. Lietuvos Respublikos Žemės ūkio Ministerija. Lietuvos valstybinis augalų veislių tyrimo centras, pp.16-23. [in Lithuanian]