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Research Article

The influence of the biostimulator on the yield components and yield of faba bean (*Vicia faba* var. *minor*)

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Abstract

Faba bean is the third most important legume species after peas and beans in the world. The study on the influence of the biostimulator Slavol-S on the morphological and production characteristics of faba beans (*Vicia faba* var. *minor*) were carried out in the growing seasons of two successive years, in the agro-ecological conditions of Banja Luka. During the field research, the following parameters were analyzed: plant height (cm), number of pods per plant, pod length (cm), number of grains per pod, weight of grains per pod (g), harvest index (%) and grain yield (kg ha^{-1}). Two-year average height of the plants in the control treatment was 81.3 cm and under the treatment with biostimulator Slavol-S it was 105.1 cm. The effect of the treatment with the biostimulator on the height was highly significant in both years. Two-year average number of pods per plant in the control treatment was 9.25, while in the treatment with the biostimulator it was 12.38. The effect of biostimulator on the number of pods per plant was highly significant. In the control variant, two year average number of seed per plant was 1.97, while biostimulator treatment provided 2.41 seeds per plant. Two year average harvest index under the treatment with biostimulator was 9.33% higher than in the control variant. The impact of biostimulants was highly statistically significant and is recommended for improving beans production.

Keywords: faba bean; grain yield; harvest index; microbiological biostimulator; productivity parameters

Introduction

Faba bean is an annual legume from the botanic family Fabaceae. It is mainly grown for its grain, which is a source of raw protein. Faba bean is now the third most important legume species after peas and beans, making for 7.5% of European and 1.2% of the worldwide production of all legumes grown for seeds (Micek *et al*., 2015). It can be used as concentrated animal feed, and is also suitable for human consumption. The

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company for processing of faba bean "Nutris group" started activities in Croatia in 2022. In order to fulfill its processing capacity this company needs regional production of 70.000 tons of faba beans. They point on farmers in Croatia, Serbia, Bosnia and Herzegovina.

Faba bean belongs to the crops that have the ability to fix nitrogen from the air. Thanks to it, the need for nitrogen fertilizers for the next cultivated crop is significantly reduced (Jensen *et al*., 2010). A spindle root that penetrates deep into the soil has the ability to absorb more soluble nutrients from the deeper layers of the soil. During flowering, faba beans can be used as bee pasture. Among the cultivated honey plants, it is listed in the flora of the Kornati National Park (Pandža and Turčinov, 2021). This crop is suitable for feeding pigs in combination with maize and barley, and can also be used for feeding turkeys.

The grains are mainly composed of starch and protein, with starch fractions ranging from 37.0 to 50.5% and protein fractions between 26.1 and 38.0% (Duc *et al*., 1999; Lakić *et al*., 2018). According to other sources, the crude protein content of beans varies from 31.8% to 39.7%, and it consists of 18 amino acids (Alghamdi, 2009). The proteins found in faba beans have a high biological value, high content of the amino acid lysine and a neutral smell. The disadvantage is due to insufficient contents of sulphur - amino acids methionine and cystine (Cazzato *et al*., 2012). It should be noted that faba beans also show medicinal properties. It is used for the treatment of Parkinson's disease, but also for the treatment of gallstones and cirrhosis of the liver (Mohseni and Golshani, 2013).

Bob has a wide cultivation area. It can be grown in plains, but also in hilly areas, with and without irrigation. There are winter and spring forms. Winter faba beans are sown in the period from the end of October to the beginning of November, and spring beans are sown as early as possible in the spring (end of February-March). According to the results of research conducted by Sharifi (2018), the yield of faba beans was higher in earlier sowing dates.

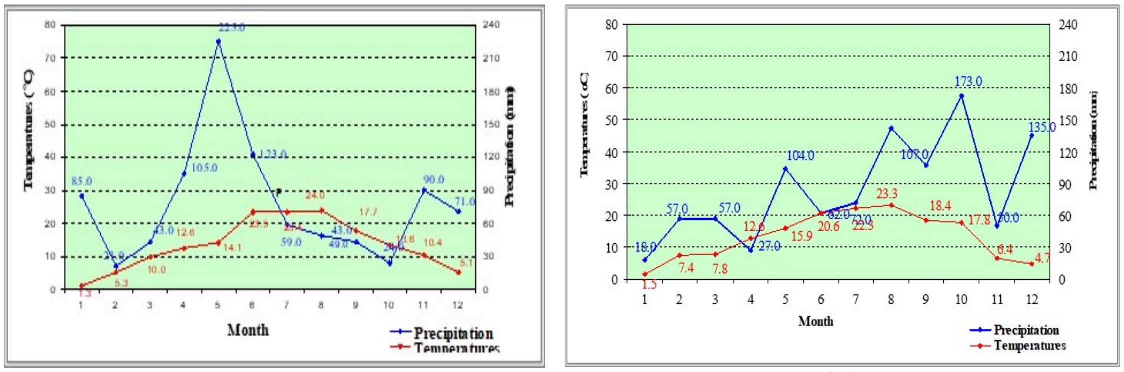
This plant species tolerates low temperatures and posses higher moisture requirements. It thrives best on soils with a neutral to slightly acidic reaction. The physical properties of the soil (structure), the content of organic matter and the water holding capacity of the soil are important for good bean growth. In our country, it is grown on small areas, but due to the proximity of processing facilities that extract proteins from beans, there is a need for its expansion. In 2020, the areas sown with broad beans in the world amounted to about 2.67 million hectares and in Europe it is estimated around 638.950 ha (Faostat, 2021). The production of beans in the world in 2017 amounted to 4.8 million tons, and the largest producers are China 1.8 million tons, Ethiopia 0.9 million tons and Australia 0.37 million tons (Alharbi and Adhikari, 2020). The average yield of beans in the world is about 1.8 t ha^{-1}, and in Europe 2.7 t ha^{-1} (Erić *et al*., 2007).

The goal of this research was to investigate the impact of biostimulator Slavol-S on certain agronomic properties of the local population of faba beans in the agro-ecological conditions of the Banja Luka area, to point out the importance of this neglected plant species, and to contribute to the promotion and expansion of its production in our country.

Materials and Methods

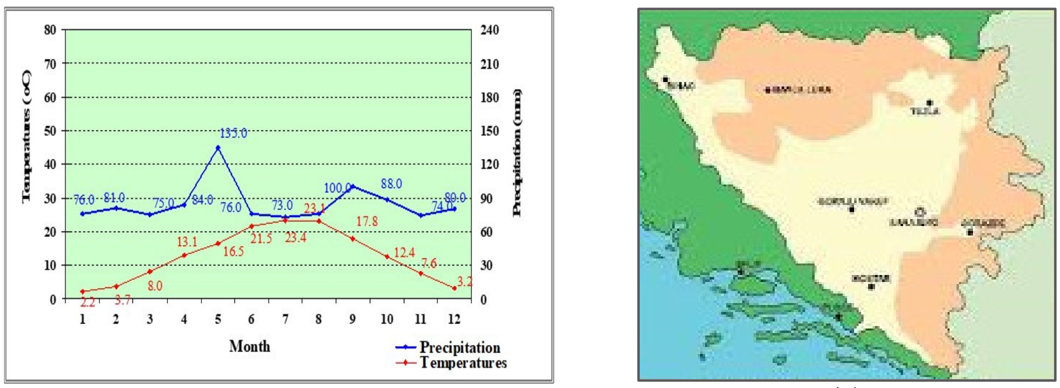
Study conditions

Tests on the influence of the biostimulator Slavol-S on the morphological and production characteristics of faba beans were carried out in the growing seasons in 2019 and 2020, Figures 1a and 1b. Filed research was carried out on the experimental field of the Agricultural Institute of the Republic of Srpska, Banja Luka, Figure 2b. For these tests, a local population of faba bean from the Institute's collection was used. A two-factorial experiment (factor A – treatment, factor B – year) was set up in four replications. The size of the basic sample plot was 10 m^2. During the test, faba beans were grown on the plots where the treatment with the biostimulator Slavol - S was applied and in the control plots. No mineral fertilizers were used during the field research.



(a) (b)

Figure 1. Precipitation and temperature in 2019, Banja Luka, B&H (**a**) Precipitation and temperature in 2020, Banja Luka, B&H (**b**)



(a) (b)

Figure 2. Precipitation and temperature, Banja Luka, 2011-2020: (**a**) Map of Banja Luka, B&H (**b**)

During the field experiment, the usual agrotechnical measures were applied, namely: basic cultivation, pre-sowing soil preparation, sowing, protection against weeds, diseases and pests. Sowing was done manually in rows at the beginning of March. For sowing the trial, 150 kg ha^{-1} of seed was used (about 350.000 plants ha^{-1}).

The sowing distance between rows was 50 cm. The sowing depth was 5-6 cm. Herbicides Corum (a.m. 22.4 g l^{-1} imazamox, 480 g l^{-1} bentazon) and Basagran (a.m. 480 g l^{-1} bentazon) were used for weed control. The first treatment against weeds was carried out at the beginning of April with the herbicide Corum (0.6 l ha^{-1}) + Basagran (0.6 l ha^{-1}). The second treatment was carried out in the last decade of April with 0.6 l ha^{-1} of Corum. It was also treated against the diseases with the fungicide Folicur EW 250 (a.m. tebuconazole 250 g l^{-1}) with a dose of 1 l ha^{-1} at the first stage of flowering. The treatment was carried out in mid-May. The insecticide Karate Zeon (a.m. 50 g kg^{-1} lambda cyhalothrin) was used against the black bean aphid (Aphis fabae Scop.) in a dose of 0.2 l ha^{-1}. The seeds were treated with a biostimulator Slavol-S (which contains auxin (IAA) as a secondary metabolite of bacteria) in a dose of 250 ml ha^{-1}. It was done immediately before sowing in conditions without direct sunlight. Determining the effect of the applied treatment with the biostimulator Slavol-S on the morphological and production characteristics of faba bean was performed by comparing the results achieved with the treatment where the biostimulator Slavol-S was applied and the control. The harvest was at the beginning of July. Immediately before harvest, 40 plants were taken from each repetition for each treatment, that is 120 plants for each treatment. The selected faba beans plants were a representative sample. After harvesting the faba bean plants from each replicate, they were placed in labeled paper bags for each treatment. After drying, the pods were manually separated from the stems, then the seeds separated from the pods. After that, the following parameters were analyzed: plant height (cm), number of pods per plant, pod length (cm), number of grains per pod, weight of grains per pod (g), harvest index (%) and grain yield (kg ha^{-1}).

Statistical analysis

The obtained research results were processed using the statistical method of analysis of variance (ANOVA). In cases where a significant difference was found, the LSD test was used. The significance of the differences was established at the level of $p<0.05$ for significant differences and $p<0.01$ for highly significant differences.

Soil and climatic conditions

The trial was set up on the valley-brown soil on the alluvial substrate close to the river Vrbas. According to its mechanical composition, it belongs to the group of loamy-clay soils. This soil has a moderate capacity for water, while the capacity for air is unfavourable, and with inappropriate agricultural techniques there is a danger of excessive occurrence of CO_2 and its unfavourable impact on crops.

Soil sample was taken from arable layer before setting up the experiment. The results of chemical analyzes are shown in Table 1.

Table 1. Results of soil fertility test

Depth (cm)	Humus (%)	pH in H_2O	pH in KCl	P_2O_5 mg/100 g	K_2O mg/100 g
0-30	2.3	7.78	6.81	32.5	26.3

Weather conditions have a significant influence on biomass production and plant yield (Popović *et al.*, 2012; 2013; 2018; 2020; Milanović *et al.*, 2020, Tomas-Simin *et al.*, 2020; Dražić *et al.*, 2021; Zejak *et al.*, 2022; Bojović *et al.*, 2022; Popović *et al.*, 2022; Petrović *et al.*, 2022; Lakić *et al.*, 2022; Milunović *et al.*, 2022). Environmental and climate studies have long been of practical importance to support agricultural monitoring for the effective land management and development of the sustainable growth and wellbeing existing of local population. Elevation has often been used to study the effects of climatic elements on change organic matter in the soil (Lemenkova, 2021; Pekeć *et al.*, 2021; Vidojević *et al.*, 2021). The analysis of the sum of monthly precipitation (mm) and mean monthly temperatures (°C) was carried out on the basis of data obtained from the Hydrometeorological Institute of the Republic of Srpska for the location of Banja Luka (44° 46' 00 N; 17° 11' 00'E, Figure 2b). Th e analyzed climatic elements were compared with the ten-year average for the Banja Luka (2011-2020). The chemical analysis of the soil was carried out in the laboratory for soil fertility control in the Agricultural Institute of the Republic of Srpska in Banja Luka. Harvest index (HI) was calculated by measuring the total mass of the faba bean plant and pods mass. According to the data in Table 1, it can be concluded that the pH value of the soil was weakly alkaline. The humus content was quite low due to absence of organic manures. The provision of soil with easily accessible phosphorus (32.5 mg 100^{-1}) and easily accessible potassium (26.3 mg 100^{-1}) was very high. Based on the results of the chemical analysis of the soil sample, it can be concluded that the soil is suitable for growing faba beans.

Average monthly temperatures and precipitation in 2019 and 2020 and the ten-year average (2011-2020) are shown in graphs 1-3. More detailed climatic elements are presented in the Tables 2 and 3. In the period from March to the end of June 2019, it was measured significantly higher amount of precipitation than the average for the past decade period (2011-2020). Extremely high precipitation of 225 mm was registered in May 2019 (Figure 1a). The amount of precipitation during the growing season was 112 mm higher than the past decennium average. The average monthly temperature during the growing season was higher by about 1 °C compared to the past decennium average.

During the second year of the study, the precipitation sum was lower compared to the previous year (Tables 2 and 3, Figures 1a and 1b). In both years, maximum amount of precipitation in the vegetation season was recorded in May. Very dry period was recorded in April 2020. The average monthly temperature during the vegetation period in the second year of the study was lower than in 2019, but also lower than the ten-year average (Figure 2a).

Table 2. Weather data, Banja Luka, 2019

Month	I	II	III	IV	V	VI	VII
Mean temp. (°C)	1.3	5.3	10.0	12.6	14.1	23.5	23.4
Maximum temp. (°C)	14.8	24.4	26.0	29.9	27.2	35.3	36.8
Min. temp. at 2 m (°C)	-7.6	-8.9	-2.8	0.8	0.8	13.0	9.6
Min. temp. at 5 cm (°C)	-8.9	-9.6	-3.0	-0.6	0.5	12.1	8.6
Days with frost	21	21	4	-	-	-	-
Precipitation, l/m^2	85	21	43	105	225	123	59
Days with rain	22	7	8	15	19	10	11
Rel. humidity (%)	83	73	63	70	78	74	70
Insolation	54	141	210	203	154	298	304

Table 3. Weather data, Banja Luka, 2020

Month	I	II	III	IV	V	VI	VII
Mean temp. (°C)	1.5	7.4	7.8	12.6	15,9	20.6	22.3
Maximum temp. (°C)	18.3	20.6	26.5	28.5	30.0	35.1	35.7
Min. temp. at 2 m (°C)	-8.2	-4.0	-3.5	-5.0	2.8	10.3	9.3
Min. temp. at 5 cm (°C)	-8.4	-6.2	-5.4	-6.6	1.2	9.1	8.6
Days with frost	27	10	7	8	-	-	-
Precipitation, l/m^2	18	57	57	27	104	62	72
Days with rain	8	7	12	7	20	14	11
Rel. humidity (%)	84	67	67	56	70	70	68
Insolation	175	143	153	299	197	259	321

Results and Discussion

The results of the study of the influence of biostimulators on the morphological and production characteristics of faba beans are shown by years of research in the Table 4, Figures 3-9. The plant height is an important property that affects the yield of green and dry biomass. This property is influenced by numerous factors, such as: genotype, environmental conditions, applied agricultural techniques, plant nutrition, etc.

Table 4. Plant height, number of pods, length of pods, number of seeds in pod, mass of grain in pod, harvest index and grain yield

Treatment	Year	Plant height (cm)	No. of pods per plant	Length of pod (cm)	Number of grains in pod	Mass of seeds per pod (g)	Harvest index (%)	Grain yield (kg ha^{-1})
Control	2019	81.50	9.50	5.33	2.01	0.50	30.60	1289.0
	2020	81.00	9.00	5.10	1.94	0.48	30.29	1266.8
	Average	81.25	9.25	5.21	1.97	0.49	30.45	1277.9
Biostimulator Slavol-S	2019	102.50	12.00	5.48	2.18	0.79	39.40	1984.8
	2020	107.75	12.75	5.80	2.64	0.84	40.15	2090.0
	Average	105.12	12.38	5.64	2.41	0.82	39.78	2037.4
Average	2019	92.00	10.75	5.41	2.09	0.64	35.00	1636.9
	2020	94.37	10.88	5.45	2.29	0.66	35.22	1678.4
	Average	93.18	10.82	5.43	2.19	0.65	35.11	1659.2

The average plants height for two-year study in the control treatment was 81.25 cm while the treatment with biostimulator Slavol-S resulted with 105.12 cm average height (Table 4, Figure 3). In the first year, the average plants height treated with the biostimulator was 21 cm higher compared with the control treatment (Table 4). Biosimulator treatment in the second year resulted with even 26.75 cm higher plants than in the control treatment (Table 4). The average plants height of faba bean determined on 25 tested genotypes was 105 cm (Singh *et al*., 2017). The plants height of faba bean varied from 74.25 to 88.50 cm (Sharifi, 2015). Depending on the variety and the influence of the year, the average plants height ranged from 88-129 cm (Alghamdi, 2007). The effect of treatment with the biostimulator Slavol-S on the plants height was highly significant during both years (Table 5).

Table 5. ANOVA parameters for plant height, number of pods per stem, length of pods, number of seeds per pod, harvest index and grain yield

Parameter	LSD	Treatment	Year	T × Y
Plant height (cm)	0.05	6.18**	6.18	8.74
	0.01	8.88**	8.88	12.56
Number of pods per plant	0.05	2.86**	2.86	4.05
	0.01	4.12**	4.12	5.82
Length of pod (cm)	0.05	0.99	0.99	1.40
	0.01	1.42	1.42	2.01
Number of seeds per pod	0.05	0.29**	0.29	0.41**
	0.01	0.42**	0.42	0.60**
Mass of seeds per pod (g)	0.05	0.25**	0.25	0.35
	0.01	0.36**	0.36	0.51
Harvest index (%)	0.05	2.58**	2.58	3.65
	0.01	3.70**	3.70	5.24
Grain yield (kg ha^{-1})	0.05	120.96**	120.86	171.07
	0.01	173.80**	173.80	245.79

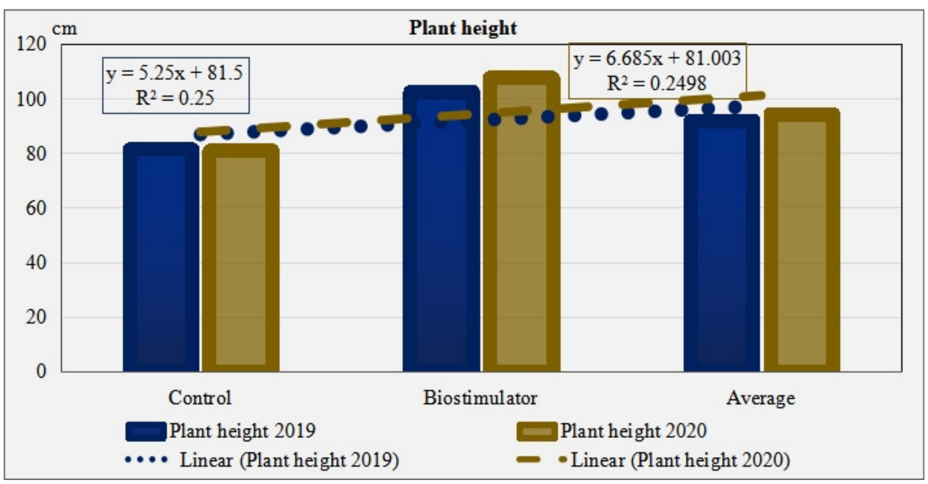


Figure 3. Effect of biostimulator of plant height, 2019-2020

Two years average number of pods per plant was 9.25 in the control variant while under the treatment with biostimulator it was 12.38 pods (Table 4). Biostimulator treatment in the first year increased the number of pods (plus 2.5 pods per plant) compared with control variant (Table 4). The difference in the number of pods betwen control and biostimulator variant was more emphasized in the second year (Table 4). According to the results by Li-Yuan (1988), the number of pods per plant varied from 17 to 30. The effect of the applied biostimulator Slavol-S during the second year was slightly higher compared to the first year. During the study, the influence of biostimulator Slavol-S on the number of pods per plant was highly significant while the year and the interaction effect did not have a significant effect on the number of pods per plant (Table 5, Figure 4).

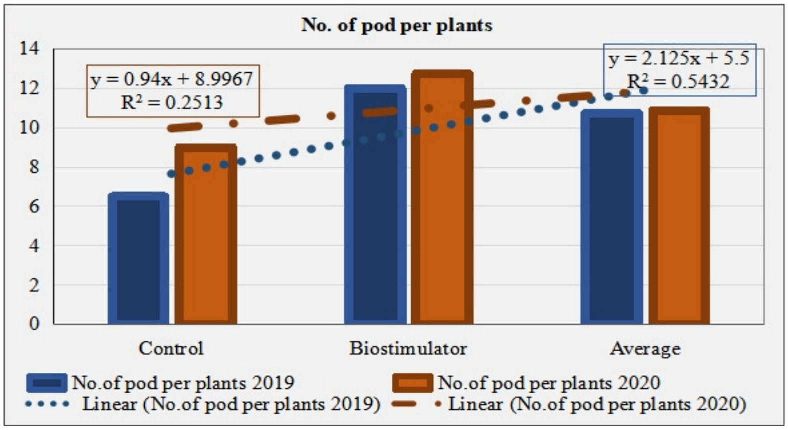


Figure 4. Effect of biostimulator of number of pods, 2019-2020

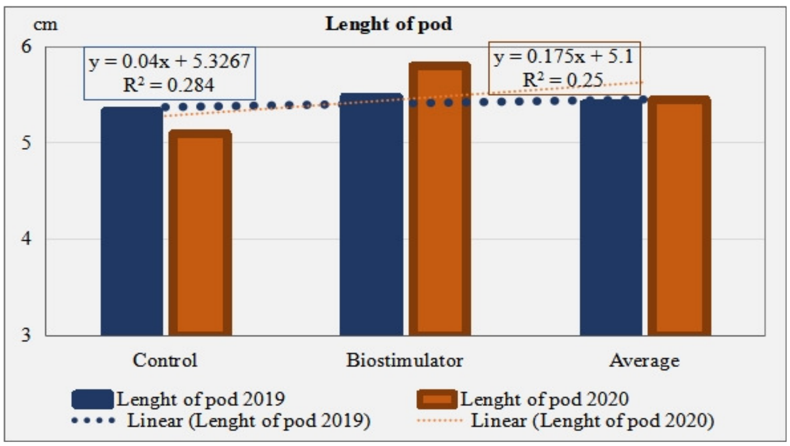


Figure 5. Effect of biostimulator of lenght of pods, 2019-2020

Two years average length of pod in the control treatment was 5.21 cm, while in the treatment with the biostimulator Slavol-S it was 5.64 cm (Table 3). During 2019, the average length of the pods in the control plot was 5.33 cm, while in the second year it was smaller by 0.23 cm. The plants grown in the treatment with the biostimulator Slavol-S obtained higher length of the pods in the second year. In both years, the treatment with the biostimulator provided higher length of the pods than control variant, but no statistically significant differences were found between the control treatment and the treatment with biostimulator (Table 4, Figure 5).

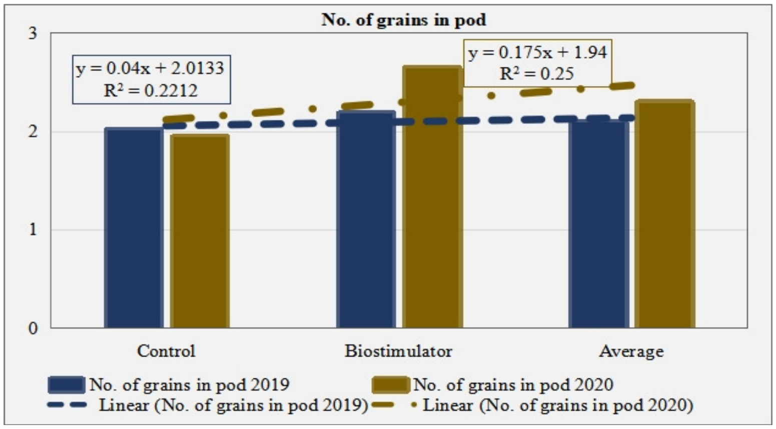


Figure 6. Effect of biostimulator of faba bean No. of grain in pod, 2019-2020

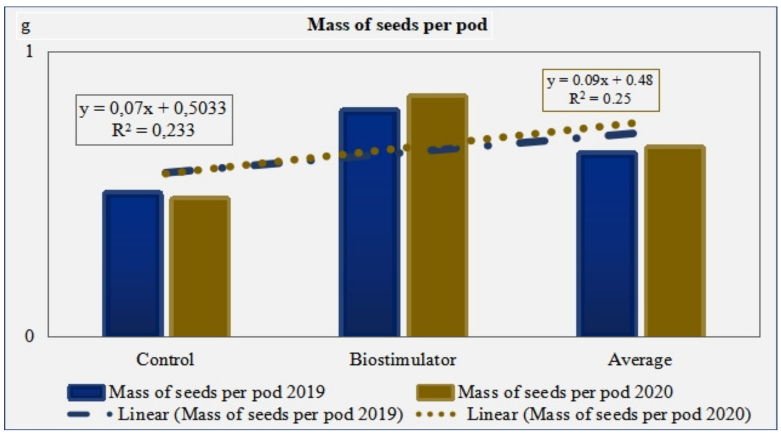


Figure 7. Effect of biostimulator of faba bean mass of grain per pod, 2019-2020

In the first year, the number of grains per pod in the control treatment ranged from 1-3, and in the second one from 2-3. When treated with a biostimulator, the number of grains per pod in the first year ranged from 1-4, and in the next year from 2-5. In both years, the number of grains per pod was higher under the treatment with the biostimulator (Table 3, Figure 6). During 2020, the positive effect of the biostimulator was more emphasized. The treatment with biostimulator and the interaction effect (treatment x year) showed statistically highly significant difference due to number of grains in per pod compared to control variant (Table 4). Kubure *et al.* (2016) stated that faba bean variety 'Walki' obtained 18.6 pods per plant, pod length of 6 cm, and 2.83 grains per pod. At the same time, the same authors, on the variety Local determined 21.56 pods per plant, pod length of 4.58 cm, and 2.94 grains per pod.

Mass of seed is a property that, together with the number of pods on stem affects the faba bean grain yield. The mass of grains per pod in the control treatment in 2019 varied from 0.25-1.32 g, and in the following year it was from 0.31-1.22 g. In the first year, the mass of grains per pod in plants grown in the treatment with the biostimulator Slavol-S varied from 0.34-1.47 g while in the second year it ranged from 0.42-1.49 g. The two-year average mass of grains per pod in the control treatment was 0.49 g, while in the treatment with the biostimulator it was higher by 0.33 g (Table 3, Figure 7). The treatment with the biostimulator had very positive effect on the mass of grains per pod in both years, and the differences compared to the the control variant were statistically highly significant (Table 4). The influence of year and interaction effects (treatment x year) on grain mass per pod were not statistically significant.

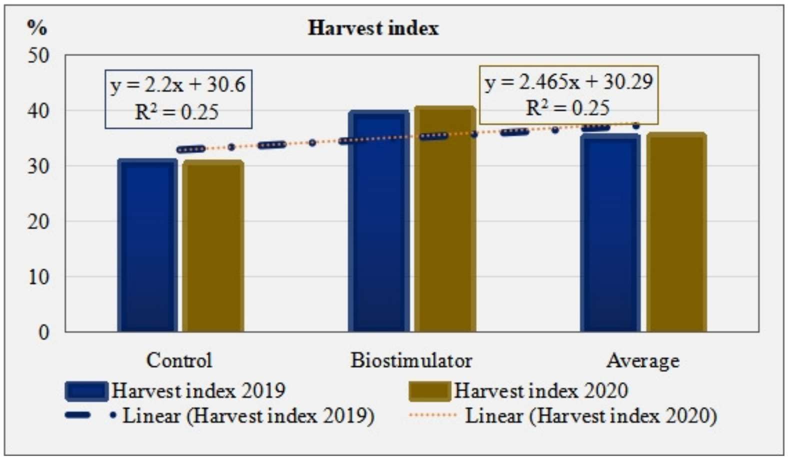


Figure 8. Effect of biostimulator of faba bean harvest index, 2019-2020

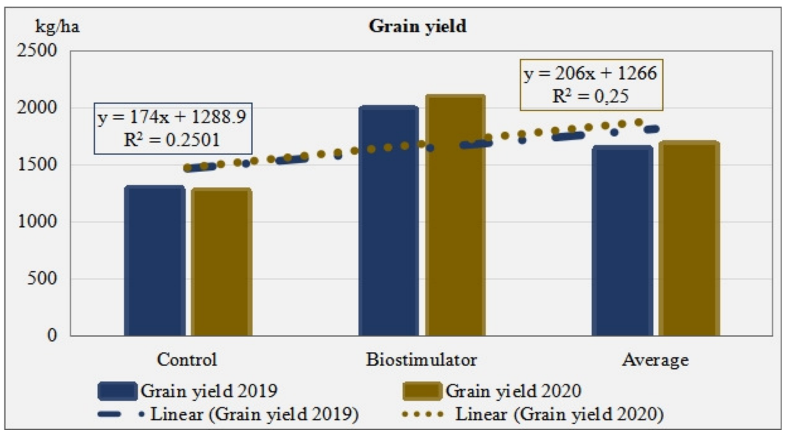


Figure 9. Effect of biostimulator of faba bean grain yield, 2019-2020

The harvest index represents the relative relationship between the grain yield and the total mass of the whole plant, reduced to standard moisture. For the most plant species, especially fodder crops, vegetative development is favored. Because of this fact, the ratio of total biomass to grain yield is two to three times higher. During two-year tests, the average value of the harvest index of faba bean grown in the control plot was 30.45% (Table 3). The average harvest index of plants grown in the treatment with biostimulator Slavol-S was 39.78%. The differences in the harvest index between the treatment with the biostimulator and the control were statistically highly significant (Table 4, Figure 8). The significance of the influence of the year and interaction (treatment × year) on this parameter was not determined.

According to the results of the two-year research, the average yield in the control plot was 1277.9 kg ha^{-1}, while under the treatment with the biostimulator Slavol-S it was 2037.4 kg ha^{-1} (Table 4; Figure 9). The yield in both years in the control treatment was quite similar (Table 4). Highly significant difference in the yield between biostimulator treatment and control variant occurred in both years (Table 5). In the agro-ecological conditions of Eastern Slavonia (Croatia), faba bean obtained a grain yield from 2.94 to 4.06 t ha^{-1} (Popović *et al*., 1988). The average faba bean yield in 2020 in the world was 2.21 t ha^{-1} while in Europe it was 2.86 t ha^{-1} (FAOSTAT, 2021). According to the results at four locations in Serbia, where two varieties ('Gema' and 'Šarac') were compared with the standard variety 'Uran', the highest grain yield (2.905 kg ha^{-1}) was obtained with the variety Gema (Mikić *et al*., 2009).

In recent years, local scientists pay more attention on global warming, as well as more and more frequent occurrence of extreme weather events (Nožinić *et al*., 2022). Though cold tolerance was not a main subject of this research, it is important to mention that the frosts which appeared even eight times in April 2020 did not make damage on the young faba bean plants. In a cold tolerance screening experiment of faba bean, white flower accessions without tannins were found to be the most susceptible, whereas cold-tolerant accessions of faba bean and wild relatives had high pigmentation and related high tannin content (Inci and Toker, 2011; Braaten, 2012). One possible mechanism explaining the role of tannins in frost protection is their activity as a supercooling promoting agent or anti-ice nucleating agent (anti-INA), which prevents intracellular ice formation and subsequent damage (Koyama *et al*., 2014). Due to the higher amount of precipitation during the first year, the appearance of diseases was more pronounced, namely the appearance of bean rust (*Uromyces viciae-fabae*) and root rot (*Rhizoctonia solani, Fusarium avenaceum*).

Conclusions

The soil conditions for growing faba beans (*Vicia faba* var. *minor*) at the location where the field research was carried out were favourable. Based on the present findings it can be concluded that in both years, the treatment with biostimulator Slavol-S resulted with significantly higher plants compared with control variant. During the first year, the number of pods per plant under the treatment with biostimulator was higher by 2.5, and in the second year by 3.7 pods per plant compared to the control variant. The average length of pods was longer in the treatment with the biostimulator compared to the control, but these differences were not significant. The treatment with the biostimulator resulted with the increase in the number of grains per pod. The interaction had a highly significant influence on this property. The mass of grains per pod was higher in the treatment with the biostimulator during both years of the trial. The average harvest index under the treatment with biostimulator was higher by 9.33% than the average in the control treatment. In both years, obtained grain yield was significantly higher under the treatment with biostimulator than in the control variant. Though the frost occurred eight times in April 2020, low temperatures did not cause damage on the young plants. Therefore, information in this regard would help producers to select appropriate measure and treatments for improving these traits of yield of faba beans.

Authors' Contributions

Conceptualization, Ž.L., V.P., D.N.; methodology, M.A., Ž.L.; software, V.P.; validation, Ž.L., V.P.; formal analysis, V.P., M.A.; investigation, D.N.; resources, Ž.L.; data curation, V.P.; writing–original draft preparation, V.P.; Ž.L; M.A.; writing–review and editing, V.P; Ž.L., M.A, M.N.; visualization, V.P.; supervision, V.P.; Ž.L., M.A.; project administration, V.P.; Ž.L. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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