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Željko LAKIĆ*[1]*, Marina ANTIĆ*[2]*, Vera POPOVIĆ*[3]**,
Dijana MIHAJLOVIĆ*[4]*, Vera RAJIČIĆ*[5]*,
***Dragana RADULOVIĆ*[6]*, Walter FRÖLICH*[7]**

EFFECT OF BIOSTIMULATOR ON YIELD COMPONENTS AND CRUDE PROTEIN CONTENT OF THE POPULATION OF WHITE LUPINE (*Lupinus albus* L.)

SUMMARY

Experimental studies of the impact of biostimulators on yield components, grain yield and crude protein content in white lupine grains were conducted in the period 2020-2022, in two variants: V1 - Control, variant without biostimulator and V2 - variant with biostimulator in the Agricultural Institute of the Republic of Srpska, Banja Luka. The goal of the research was to examine the effectiveness of the applied biostimulator on the analyzed properties of white lupine under production conditions. The sowing of lupine, in all years, was done by hand during March. The following parameters were analyzed: number of pods per plant, number of grains per pod, weight of grains per pod (g), weight of 1000 grains (g), grain yield (kg ha^{-1}), crude protein content in grains (g kg^{-1}) and alkaloid content in grains (%). The number of pods per plant in the control variant was on average 5.0, and the pods contained 3.0 grains, while in the V2 variant it was 7.8 with 3.6 grains. The average weight of 1000 grains in the control was 221.2-254.1 g, and in the V2 variant 259.5-279.3 g. The grain yield in the control was 1386.8 kg ha^{-1}, and in the biostimulator treatment 1806.6 kg ha^{-1}. Crude

[1]Željko Lakić, PI Agricultural Institute of Republic of Srpska, Banja Luka, B&H; and University of Banja Luka, Faculty of Agriculture, Banja Luka, BOSNIA AND HERZEGOVINA;
[2]Marina Antić, University of Banja Luka, Institute of Genetic Resources, B&H; and University of Banja Luka, Faculty of Agriculture, Banja Luka, BOSNIA AND HERZEGOVINA;
[3]Vera Popović (corresponding authors: drvvpopovic@gmail.com), Institute of Field and Vegetable Crops, Novi Sad, SERBIA; University of Bijeljina, Faculty of Agriculture, Bijeljina, BOSNIA AND HERZEGOVINA;
[4]Dijana Mihajlović, University of Banja Luka, Faculty of Agriculture, Banja Luka, BOSNIA AND HERZEGOVINA;
[5]Vera Rajičić, University of Niš, Faculty of Agriculture, Kruševac, SERBIA;
[6]Dragana Radulović, University of Banja Luka, Institute of Genetic Resources, Banja Luka, BOSNIA AND HERZEGOVINA;
[7]Walter Frölich, German Technical Cooperation, GERMANY; and PI Agricultural Institute of Republic of Srpska, Banja Luka, BOSNIA AND HERZEGOVINA;
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protein content varied from 254.0 g kg^{-1} DM in the control treatment to 279.8 g kg^{-1} DM in the variant with biostimulator. The white lupine population tested in the experiment had a high alkaloid content and is not suitable for animal and human consumption, but can be grown for green manure.

Keywords: white lupine, biostimulator, pods, grain yield, crude protein

INTRODUCTION

Lupinus albus L. - white lupine is an annual legume that can be grown for above-ground production of biomass or grains, as cover crops, for biological suppression of soil parasites and remediation of degraded lands (Fumagalli *et al.*, 2014). Nitrogen-fixing bacteria *Bradyrhizobium lupini* live on the roots of white lupine, which allows up to 240 kg ha^{-1} of nitrogen to accumulate in the soil during the growing season (Mateos *et al.*, 2015). The characteristic of this plant species is that can absorb hardly soluble phosphates from the soil. Lupine populations that have a high content of toxic alkaloids are suitable for growing on soils that are infected with nematodes because in this way these pathogenic organisms can be suppressed. The produced biomass of white lupine with a high alkaloid content in areas infected with nematodes is used as green manure. The cultivation of white lupine affects the restoration of the general condition of poor soils with a heavier mechanical composition. Thanks to its powerful root system, it can draw water from the deeper layers of the soil. According to the soil, lupins are not demanding. They thrive on different types of soil, from sandy to heavy clay soils (Gresta *et al*., 2017). Alkaline soils cause chlorosis and reduce growth, although some cultivars are more tolerant of soil salinity and heavy soils (Lim, 2012). Lupins are tolerant of growing in monoculture and can make better use of more difficult-to-access plant nutrients from acidic soils.

Producers use a variety of organic, mineral, and microbial fertilizers to attain the proper high yields (Božović *et al.,* 2018; Ugrenović *et al.,* 2021; Govedarica-Lučić *et al.,* 2020). The yield and quality of legumes depend mostly on balanced mineral nutrition. The quality of the yield can also be seen by the ratio of crude protein and crude fiber (Enchev and Bozhanska, 2024). The mineral nutrition of legumes depends on the type of soil, climatic factors of the region, and other agroecological factors (Popović *et al*., 2020; Popović *et al.*, 2022; Kosev *et al*., 2022; Vasileva *et al.,* 2023; Karthika *et al*., 2023). Biostimulators are plant extracts that contain a wide range of bioactive compounds capable of improving certain physiological processes that stimulate the growth and development of crops (Bulgari *et al*., 2015). The use of biostimulators is one of the promising alternative methods and solutions for improving the production of legumes (Parihar *et al.,* 2022). Biostimulators in the soil affect the microflora and thus can positively influence the growth and development of plants (Kunicki *et al.,* 2010). By applying biostimulator on lupine grains before sowing, it stimulates the processes of germination and sprouting of plants, and has a positive effect on the development of the root system and aerial parts of cultivated plants. The positive influence of seed treatment with the microbiological biostimulator on the yield of small-grained broad beans is reported by Lakić *et al*. (2022). The goal of the research was to test the effectiveness of the applied biostimulator on the analyzed features of white lupine under production conditions.

MATERIAL AND METHODS

Field experiment

The study of the impact of biostimulators on grain yield and crude protein content in white lupine grains was carried out in the period from 2020-2022. Field research was carried out on the experimental field of the Agricultural Institute of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina. The seeds of the autochthonous population of white lupine were used, taken from the gene bank of the Institute of Genetic Resources, University of Banja Luka. During 2018, the alkaloid content in the white lupine seeds was determined. The sowing rate was 160 kg ha^{-1}. A two-factorial experiment (factor A – treatment, factor B – year) was set up in four replicates. The size of the basic sample plot was 10 m^2. The distance between the rows was 50 cm, and in the row was 8 cm. No mineral fertilizers were used during these tests. Sowing of white lupine, in all years, was done mechanically, during March. White lupine seeds were treated before sowing with the microbiological biostimulator, fertilizer containing: nitrogen fixers - bacteria that perform the process of nitrogen fixation; phosphomineralizators - bacteria that break down organic phosphorus compounds (which make up 50-80 % of phosphorus in the soil) and convert them into an accessible form (P_2O_5) and growth regulators - auxins, which contains indole-3 acetic acid.

During these researches, the following parameters were analyzed: content and type of alkaloids in the grain (%), number of pods per plant, number of grains per pod, weight of grains per pod (g), 1000 grains mass (g), grain yield (kg ha^{-1}) and crude protein content in grain (g kg^{-1}). The alkaloid content of white lupine seeds was determined using Kapillar-GLC at the Institute für Pharmazie und Molekulare Biotechnologie, Ruprecht-Karls-Universität Heidelberg.

Thirty white lupine plants were counted and measured in each repetition for each treatment in order to ascertain the studied attributes, number of pods per plant, number of grains per pod, and weight of grains per pod.

The weight of 1000 grains (g) was determined from the pure seed fraction in the Laboratory for Seed Quality Control, Agricultural Institute of the Republic of Srpska in Banja Luka. With a seed counter, 100 seeds were sampled in 8 repetitions (ISTA Rules, 2018).

The yield of white lupine seeds was determined at the stage of full maturity per replicates, separately for each treatment, and then converted to a unit area. The white lupine harvest was performed in the first half of the seventh month.

The content of crude proteins in white lupine grains was determined according to the Kjeldahl method - BAS EN ISO 5983-2:2010.

Statistical analysis

Experimental data were analysed by descriptive and analytical statistics using the statistics module Statistica 12. All evaluations of significance were made on the basis of the ANOVA test at 0.05% and 0.01% significance levels. 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 analysis

The experiment was performed on the soil type determined as valley-brown soil on the alluvial substrate. Before setting up the experiment, soil samples were taken from the arable layer from a depth of up to 30 cm, and the results of the chemical analyzes are shown in Table 1. The pH value of the soil was neutral, the hummus content was average, the provision of soil with easily accessible phosphorus (10.2 mg/100) was moderate, and easily accessible potassium (30.6 mg/100) was very good. Based on the results of the chemical analysis of the soil sample the tested soil is suitable for growing white lupine.

Table 1. Agrochemical properties of soil

Depth (cm)	pH in KCl	pH in H_2O	Humus (%)	NH_4 (%)	NO_2 (%)	P_2O_5 mg/100 g	K_2O mg/100 g
0-30	5.8	6.8	3.2	1.5	3.3	10.2	30.6

Meteorological data

The analysis of mean monthly temperature (ºC) and sum of monthly precipitations (mm) was performed based on data obtained from the Republic Hydrometeorological Institute in Banja Luka. The analyzed climate elements were compared with the multi-year average for the Banja Luka area. Average monthly air temperatures and total precipitation by month for the period 2020-2022 and the ten-year average (2011-2020) are shown in Figures 1-4.

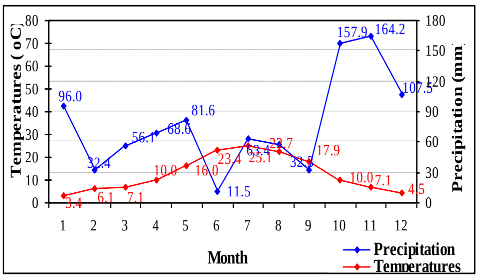

Figure 1. Temperatures (°C) and precipitation (mm) in Banja Luka, B&H (2020)

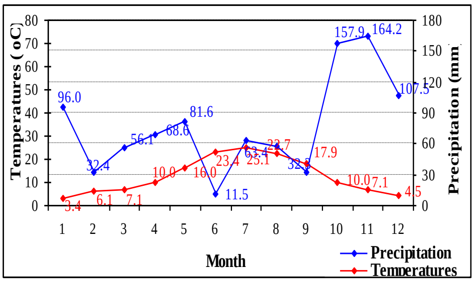

Figure 2. Temperatures (°C) and precipitation (mm) in Banja Luka, B&H (2021)

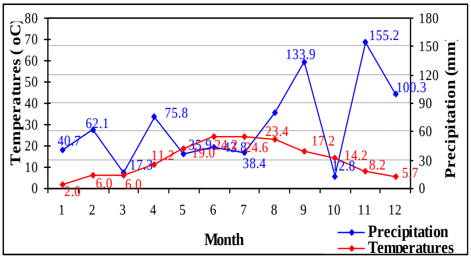

Figure 3. Temperatures (°C) and precipitation (mm) in Banja Luka, B&H (2022)

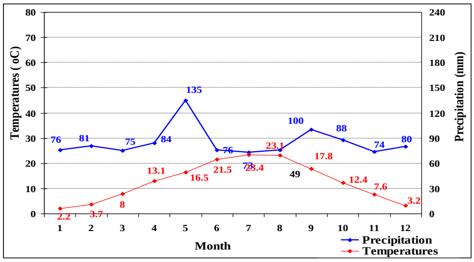

Figure 4. Temperatures (°C) and precipitation (mm) long-term average, Banja Luka, B&H (2011-2020)

In 2020, the amount of precipitation during the growing season was 322 mm, which is 121 mm less than the multi-year average. The maximum amount of precipitation was recorded in May (Figure 1).

Average monthly temperatures during the growing season in 2020 ranged from 7.8 ºC to 22.3 ºC in July. The average monthly temperature was lower than the ten-year average (Figure 4).

During the second year of the research, the amount of precipitation during the growing season (III-VII) of white lupine was lower compared to the previous multi-year period. The maximum amount of precipitation was recorded in April. The precipitation schedule in 2021 was less favorable (Figure 2). A dry period began in mid-May.

During 2021, the monthly temperatures during the growing season ranged from 6.1 ºC to 25.1 ºC in July (Figure 2).

In the period from March to the end of June 2022, a significantly lower amount of precipitation fell compared to the ten-year average from 2011-2020. The highest amount of precipitation during the growing season was in April (Figure 3).

Temperatures varied from 6.0 ºC in March to 24.6 ºC in July. The average monthly temperature during the growing season was about 2.5 ºC higher than the annual average (Figure 4).

During the duration of these studies, the most favorable conditions for growing white lupine were in 2020, and the worst in 2021 due to the dry period in the second part of the growing season.

RESULTS AND DISCUSSION

Yield components and crude protein content

The results of three-year tests on the impact of biostimulators on yield components and crude protein content in white lupine grains are shown in table 2.

The number of pods per plant in the control variant was an average of 5.0, and the pods contained 3.0 grains. In the V2 variant on the white lupine plants, there were an average of 7.8 pods, and in them there were an average of 3.6

grains. The V2 variant resulted in a higher number of pods/plant compared to the control during tested three years, Tab. 2.

Table 2. Average values for tested morpho-productive traits of tested genotypes, 2020-2022

Variant	Year	Number of pods/ plant – NPP	No of grains/ pod - NGP	Grain mass /pod- GMP (g)	1000 grains mass - TGM (g)	Grain yield - GY (kg ha^{-1})	Protein content - PC (g kg^{-1})
Control	2020	6.0	3.3	1.09	225.8	1 628.5	256.6
	2021	4.3	2.8	1.04	221.2	1 095.1	257.1
	2022	4.8	3.0	0.99	254.1	1 436.8	248.3
	Average	5.0	3.0	1.04	233.7	1 386.8	254.0
Biosti-mulator	2020	9.5	3.8	0.93	278.4	2 089.8	280.4
	2021	6.8	3.5	0.94	259.5	1 396.9	282.5
	2022	7.0	3.5	1.01	279.3	1 933.1	276.6
	Average	7.8	3.6	0.96	272.4	1 806.6	279.8

Table 3. Results of the provided analysis of variance

Parameter	LSD	G - Genotype	Y- Year	G x Y
NPP	0.05	0.92*	1.12*	1.59
	0.01	1.25**	1.54**	2.17
NGP	0.05	0.70	0.86	1.21
	0.01	0.96	1.17	1.66
GMP	0.05	0.08	0.10	0.15
	0.01	0.12	0.14	0.20
TGM	0.05	6.51*	7.97*	11.28*
	0.01	8.92**	10.92**	15.45**
GY	0.05	122.02*	149.45*	211.35
	0.01	167.15**	204.72**	289.51
PC	0.05	0.70*	0.86	1.21
	0.01	0.96**	1.17	1.66

A greater number of grains/pod, during the duration of these tests, was achieved in the V2 variant compared to the control. The influence of the biostimulator on the number of pods/plant was statistically highly significant. The average number of pods/plant in the white lupine Energy variety was 3.9-5.4, while in the Arabella variety it varied from 5.4-8.9 (Pospišil and Pospišil, 2015). According to Šatović (1992), the number of grains in a pod of white lupine varied from 2.4‑5.2. During the tests conducted by Gresta *at al*. (2023) the number of grains in a pod of white lupine was 3.5 on average, and the lowest number of grains was in the variety Volos (2.4). According to Mahfouze *et al.* (2018) during two-year trials the number of pods/plants varied from 3.67-12.0, and the number of grains/pods ranged from 3.66±0.33 - 5.67±0.32. The results of the mentioned authors are in agreement with the results we reached during these researches.

The mass of grains/pod, during testing years, was higher in the control. The average mass grains/pod in the control was 0.99-1.09 g, and in the V2 variant it varied from 0.93-1.01 g (Tab. 2).

During all years the 1000 grains mass was higher in the V2 variant compared to the control. In the control, the 1000 grains mass was 221.2-254.1 g, that is, on average 233.7 g. The 1000 grains mass in the V2 variant was 272.4 g, that is, it varied from 259.5-279.3 g. During these tests, variant, year, and variant x year interaction had a statistically highly significant effect on the 1000 grains mass (Tab. 3). According to the results of Pospišil and Pospišil (2015), the 1000 grains mass in the Energy variety was 290.7-378.9g. Šatović (1992) states that the 1000 grains mass can vary from 164-537 g. According to research by Gresta *et al.* (2023), the 1000 grains mass of white lupine was 426.3 g and 326.7 g on average. The average three-year yield in the control was 1386.8 kg ha^{-1}, that is, it varied from 1095.1 to 1628.5 kg ha^{-1}. In the biostimulator variant, the grain yield varied from 1396.9-2089.8 kg ha^{-1}, that is, on average, it was 1806.6 kg ha^{-1}. In these tests, a statistically highly significant effect of variant and year on the yield was determined. The achieved average yields of white lupine grains were from 1293 to 2591 kg ha^{-1} (Lakić *et al.*, 2018). The average grains yield varied depending on the year from 2130-3033 kg ha^{-1} (Pospišil and Pospišil, 2015).

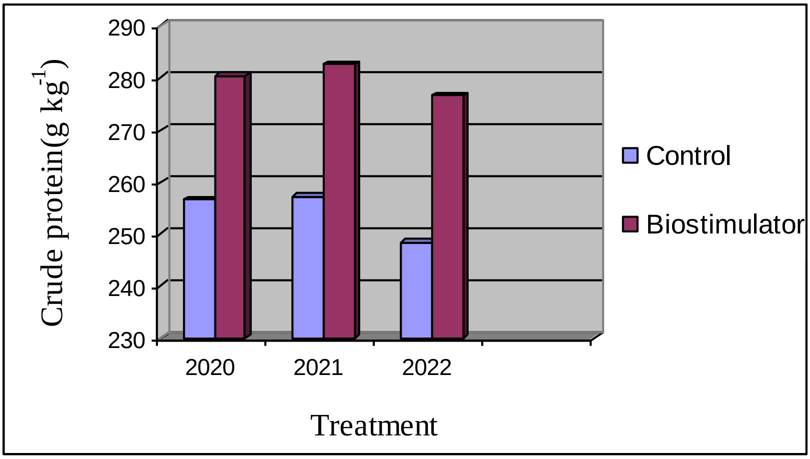

Figure 5. Crude protein content (g kg^{-1}) in the grains of white lupine, 2020- 2022

In the control, the average crude proteins content in white lupine grains was 254.0 g kg^{-1} DM, while in the V2 variant it was 279.8 g kg^{-1} DM, Figure 5. The highest crude protein content was determined in 2021 in V2 variant and is 282.5 g kg^{-1} DM or 28.25% of crude proteins. Treatment with biostimulator had a statistically highly significant effect on the content of crude proteins (Table 3). According to the results of Georgieva *et al.* (2018) crude protein content in white lupine grains ranged from 24.90% to 33.30%.

Alkaloid content

During these studies, the content of alkaloids in white lupine seeds was tested. The tested population of white lupine contains up to 2.5% alkaloids and belongs to bitter lupine. Lupanin was the most abundant alkaloid in lupine seeds, and secondary alkaloids were 13-Hydroxylupanin, Isolupanin, Multiflorin, Albin, Angustifolin, 13-Tigloyloxylupanin. According to research by Boschin *et al.* (2008), the alkaloid content of the Luxor (6.1 mg 100 g^{-1}) and Volos (5.2 mg 100 g^{-1}) varieties is much lower than the toxicity threshold (20 mg 100 g^{-1}) for human or animal consumption as announced by the health authorities of Australia, France, and the United Kingdom.

Gresta *at al.* (2023) state that the alkaloid content in the varieties Volos, Luxor and Lublanc is significantly lower (0.05–0.19 g kg^{-1}) compared to the variety Multitalia and local ecotypes of white lupine (2.0‑2.5 g kg^{-1}). Alkaloids are toxic to both humans and animals (Vishnyakova *et al*., 2020), so two general thresholds for the presence of alkaloids in food derived from lupine have been established, namely: 0.02% of dry matter for animal feed and 0.01% of dry matter for human consumption (Otterbach *et al*., 2019).

CONCLUSIONS

Treatment with biostimulator and the year influenced the increase in the number of pods per white lupine plant. The number of pods on lupine plants grown with the biostimulator treatment was on average 2.8 higher than the control. The effect of variant and year was not influenced on the number of grains/pods and weight of grains/pods. The 1000 grains mass, during all tested years, was higher in the treatment with the biostimulator compared to the control. Year and the interaction effect of treatment x year had a highly significant influence on this feature. The effect of the changed treatment with the biostimulator on the grain yield of white lupine, during all three years of testing, was highly significant. The grain yield in the treatment with the biostimulator was 419.8 kg higher in the three-year harvest compared to the control. During all three years of research, a highly significant influence of the biostimulator on the content of crude nutrients in white lupine grains was determined. The white lupine population tested in the experiment has a high alkaloid content and is classified as bitter lupine. It is not suitable to be used for animal and human consumption, but it can be grown for green fertilization.

AUTHORS' CONTRIBUTIONS

All authors have participated in this research. ŽL has designed, supervised and written the paper; VP, VR, MA, DM, RD and WG have participated in the experimentation and sample collection; Conceptualization: VP, VR, DM and AM have analyzed the data obtained; VP and VR have overseen the project and revised the manuscript. All authors have approved the final manuscript.

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