

Effects of Bioactive Compounds on the General and Active Symbiotic Potential and Biological Nitrogen Fixation of *Phaseolus vulgaris* L. Varieties

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Abstract

This article discusses the effects of seed treatment with bioactive substances on the symbiotic activity of plants during growth and development through foliar feeding in the Samarkand region of the Republic of Uzbekistan, when growing the “Ravot” and “Makhsuldor” varieties of beans. The variants in which bioactive substances were applied to seeds and leaves showed an increase in the potential for general and active symbiosis, and the superiority of the Mahsuldor variety over the Ravot variety. The increase in the total and active symbiotic potential in biological nitrogen fixation under the influence of the drugs used in both varieties. It is stated that the Gumel Lyuks preparation is superior to other preparations in terms of its effect, and that the active symbiosis potential lasts for 1 - 5 days. Bioactive preparations are used to activate the nitrogenase enzyme, to increase the concentration of leghemoglobin in the nodules, and increase biological nitrogen fixation. The biological nitrogen content in bean varieties ranged from 39.34 to 54.03 kg, which led to the activation of the nitrogenase enzyme under the influence of the drugs used and an increase in the amount of biological nitrogen fixation. The leghemoglobin content was 2.86 - 3.65 mg/g, the fixed biological nitrogen content was 39.34 - 45.87 kg/ha in the Ravot variety variants, and 50.24 - 54.03 kg/ha in the Mahsuldor variety.

Keywords

Symbiosis, Leghemoglobin, Choleglobin, Enzyme, Biological Nitrogen,

1. Introduction

Phaseolus vulgaris L. is one of the major food crops for human consumption and is a major source of dietary protein in developing countries worldwide [1].

The symbiotic interaction between Fabaceae and rhizobia is called fabacean-rhizobia symbiosis. Rhizobial symbiosis is established between legume species (Fabaceae) and nitrogen-fixing soil bacteria called rhizobia. This interaction usually occurs in nitrogen-deficient soils when the roots release flavonoids into the rhizosphere that are specifically recognized by the rhizobia. Specific recognition of flavonoids triggers the synthesis and secretion by the rhizobia of lipochitooligosaccharides, known as nodulation factors, which are taken up by receptors on the plasma membrane of root hairs. The introduction of nitrogen-fixing organisms into crops reduces the need for fertilizers [2]-[4].

More than 70% of legumes develop symbiotic relationships with rhizobia, some fixing up to 200 kg N/ha per year, and *Phaseolus vulgaris* L. fixes 16.3 to 71.9 kg of biological nitrogen. However, the efficiency of this process varies depending on the rhizobia species and the variety of legumes, as well as environmental conditions. The minimum temperature for nitrogen fixation in legumes varies between species, from 2°C to 10°C. Nitrogenase activity increases and reaches a maximum at around 12°C - 35°C [5].

The symbiosis begins with infection of the roots of legume plants by *Rhizobium*, which forms root nodules where nitrogen fixation occurs. The nitrogen fixation process is catalyzed by the enzyme nitrogenase, which takes up the nitrogen molecule and converts it to ammonia [6].

The most common route of rhizobial infection is through the formation of a tubular structure known as an infection thread at the tip of the root hairs. The bacteria migrate to the growing nodule via the infection thread and develop into bacteroids, which produce nitrogen-fixing organelles called symbiosomes. In the nodules, the nitrogen (in the form of ammonia) fixed by the bacteroids is exchanged for carbon produced by the plants, providing an energy source for the bacteria [7].

These symbiotic structures are essential for providing the rhizobia with a carbon source and creating an optimal cellular environment. Within the nodule, the rhizobia use the host plant's energy to convert nitrogen to ammonium (NH₄⁺) under the action of the bacterial enzyme nitrogenase. This NH₄⁺ is then absorbed and utilized by the host plant, a fundamental process known as biological nitrogen fixation [8].

Since biological nitrogen fixation is considered an environmentally friendly technology for feeding plants with nitrogen, ensuring plant growth and development in nitrogen-deficient soils, plants are inoculated with *Rhizobium* strains,

which reduces the use of mineral fertilizers, which helps to obtain satisfactory yields at low costs [9] [10]. These limitations are mainly a result of the low efficiency of nodulation in this plant, as well as the high sensitivity of the symbiosis to abiotic stresses [11] [12]. Drought is recognized as an important environmental factor that is expected to have an increasingly serious impact on agricultural areas due to climate change [13] [14].

Drought is estimated to affect more than 60% of common bean production worldwide [15] [16]. Although various bean genotypes have been identified that exhibit high performance under drought conditions, drought stress significantly impairs biological nitrogen fixation in this legume species [17] [18].

However, drought stress disrupts plant metabolic processes, especially biological nitrogen fixation. It is known that nitrogenase activity decreases in plant nodules under drought-stress conditions and amide metabolism is disrupted. In bean, drought-stressed nodules exhibit lower water potential and lead to premature senescence, reducing biological nitrogen fixation activity [19] [20].

Signaling molecules, which are received by specific receptors located on the plasma membrane of root hair cells, trigger a signaling cascade that leads to infection by rhizobia and cortical cell division [21] [22].

Temperature has a significant effect on the growth and physiological activity of rhizobia [23].

In this study, the optimum temperature for the growth of the isolates was assumed to be 35°C. Accordingly, Benselama *et al.* reported a temperature similar to the optimum temperature for rhizobia [24]. It is estimated that in 2019, approximately 34 million tons of nitrogen were fixed worldwide, considering only four major legumes [24]. Worldwide, and Brazil is the global leader in nitrogen fixation with legumes [25] [26]. It penetrates the root surface and cell walls without being bound by the plant's immune system [27].

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Common beans can effectively fix atmospheric nitrogen when associated with Rhizobia. There is no doubt that the use of chemical nitrogen fertilizers has increased significantly in the last few decades. Nitrogen fertilizers are effective in increasing yields; however, they are also subject to various losses such as leaching, denitrification, and ammonia volatilization [22] [28]-[30]. However, biologically derived nitrogen is less susceptible to these losses [31].

It was apparent that *Pantoea agglomerans* actively grew wheat coleoptiles by 2.6 mm and maize coleoptiles by 2.3 mm compared with the control. Observable evidence also showed that sorghum coleoptile actively grew by 1.7 mm compared with the control treatment by 2.9 mm [32] [33].

Rhizobium, a group of gram-negative soil bacteria, promotes the formation of root nodules in legumes. In addition to nitrogen fixation, *Rhizobium* can protect plants from pathogens and diseases. Considering the advantages of *Rhizobium*, it

can be used as a biofertilizer to improve soil fertility and crop yield, especially in legume systems. In addition, the nitrogen enriched by *Rhizobium* can be used for subsequent crops [34].

We did not observe any data on the physiological properties of plants, symbiotic activity, or biological nitrogen accumulation as a result of the exposure of *Phaseolus vulgaris* L. varieties Ravot and Mahsuldor to the bioactive substances Uzgumi, Edagum SM, and Gumel Lyuks. Therefore, we focused our research on the study of the increase in the general and active symbiosis potential, nitrogenase enzyme activity, leghemoglobin concentration in the nodules, and the amount of fixed biological nitrogen of *Phaseolus vulgaris* L. varieties under the influence of the above-mentioned bioactive substances. When the *Phaseolus vulgaris* L. varieties Ravot and Mahsuldor are treated with the bioactive substances Uzgumi, Edagum SM, and Gumel Lyuks, the physiological processes of the plants are accelerated, the symbiotic potential, and the biological nitrogen-fixing properties increase.

2. Materials and Methods

In field experiments, seeds of the varieties “Ravot” and “Mahsuldor”, zoned in the republic, were sown as a repeated crop in areas cleared of grain according to the $60 \times 10 \times 1$ scheme. The total area of each variant was 120 m², of which the area under consideration was 60 m², the systematic variants were placed in 4 replicates. In field experiments, the following variants were tested: N₃₅P₆₀K₃₀ (Fon) + without bioactive substances (distilled water) and Fon + Uzgumi; Fon + Edagum SM; Fon + Gumel Lyuks with biologically active substances* (Table 1).

Table 1. Experimental scheme (structure).

№	Varieties	Biologically active substances	Application period and criteria	
			germinating seeds	spraying on the leaf during the growing season (branching)
1	Ravot	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)	-	-
2		Fon + Uzgumi	1.4 l/t	0.7 l/ha
3		Fon + Edagum SM	0.6 l/t	0.6 l/ha
4		Fon + Gumel Lyuks	2 l/t	0.6 l/ha
5	Mahsuldor	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)	-	-
6		Fon + Uzgumi	1.4 l/t	0.7 l/ha
7		Fon + Edagum SM	0.6 l/t	0.6 l/ha
8		Fon + Gumel Lyuks	2 l/t	0.6 l/ha

In this case (Table 1), the seeds were incubated in a solution of bioactive substances without bioactive substances (distilled water) and in a solution of bioactive

substances with a concentration specified in the experimental scheme, according to the options. Also, during the branching phase of the plants, a solution was prepared at the concentration specified in the experimental scheme and sprayed on the leaves of the plants (**Table 2**).

Table 2. *Chemical composition of bioactive substances.

Uzgumi	Edagum SM	Gumel Lyuks
humic and fulvic acid	amino acids, carboxylic acids and vitamins	humic and fulvic acid, complex microelements in chelated form
B	-	B
Mn	Mn	Mn
Mg	Mg	Mg
Cu	Cu	Cu
Zn	Zn	Zn
Co	-	Co
-	-	Mo
-	Si	Si
-	S	S
-	Fe	Fe
-	Ca	Ca
-	-	Na
-	-	J
Humic acids	Humic and fulvic acids	
organic matter residue		
N	N	N
P	P	P
K	K	K

Laboratory and field experiments, phenological observations, and biometric measurements were carried out using generally accepted methods [35].

Also, when studying the symbiotic activity of the crop, nodule formation, the appearance of leghemoglobin, and its transition to choleglobin were recorded, and on this basis, the total and active symbiotic potential was calculated according to G.S. Posipanov [36]. The calculation of biological nitrogen content was carried out based on nitrogenase enzyme activity and leghemoglobin concentration. In this case, the acetylene reduction method (ARA - Xromatek Kristall 9000) was mainly used to determine nitrogenase enzyme activity in $\mu\text{g N}_2/\text{plant per hour}$. This method helps to indirectly assess nitrogenase activity. In this case, a plant

sample (tuber) was first placed in a hermetic container. 10% acetylene (C₂H₂) was added to it (in the chamber). In the process, nitrogenase converts acetylene to ethylene (C₂H₄). The amount of ethylene was measured by gas chromatography. The amount of ethylene produced is an indirect indicator of nitrogenase activity. The concentration of leghemoglobin (mg/g wet weight) at the end of vegetation (before ripening, when the tubers decay during the ripening phase) was determined based on the following equation:

$$S = \frac{0.5 * A * V * M}{9.25 * P * 1000} = \frac{\text{mg}}{\text{g}} \text{ wet nodules}$$

0.5—correction factor for the density of the solution, multiplied by two;

A—optical density of the solution (wavelength – 525 nm);

V—volume of the solution to be colorimetrically measured, ml;

M—molecular mass of leghemoglobin;

P—mass of the analyzed particle, g;

9.25—millimolar extinction coefficient (at wavelength – 525 nm);

1000—conversion coefficient from milligrams to grams.

3. Results and Discussion

The results of the study conducted in 2023-2024 showed that bioactive preparations (Uzgumi, Edagum SM, Gumel Lyuks) have a positive effect on the symbiotic nitrogen fixation processes of the bean plant. Two varieties—Ravot and Mahsuldor—were used in the study, and their responses were evaluated using biochemical and agrobiological indicators.

According to the results of the study [38], 7 days after the emergence of the grasses of the Ravot and Mahsuldor varieties, the first nodules began to appear in all variants. After 3 days, on July 14, it was determined that the nodules in all variants of both varieties had passed into the leghemoglobin state, that is, the nodules had turned reddish. In the control variant of the Ravot variety, the period of transition from leghemoglobin to choleglobin, that is, the active symbiosis potential, was 56 days. The active symbiosis potential was 1 day longer in the Fon + Uzgumi variant than in the control, 3 days in the Fon + Edagum SM, and 7 days in the Fon + Gumel Lyuks variant. After that, the nodules turned pale, and after 5 - 6 days, depending on the variants, the nodules began to die. In both varieties, the period of root nodule formation and leghemoglobin formation coincided with the same period. However, in the Mahsuldor variety, the duration of active symbiotic potential was longer than in the Ravot variety. In both varieties, the variant in which the Fon + Gumel Lyuks preparation was used showed the highest indicator of active symbiotic potential.

The studies conducted in 2024 also recorded similar results to those obtained in 2023. In the studies conducted in 2024, slight differences were found in the total and active symbiosis potential compared to 2023. However, nodular bacteria were formed 7 - 8 days after the grasses sprouted, and after 4 - 5 days the nodules

transitioned to the leghemoglobin state. The active symbiosis potential was 57 days in the control and Fon + Uzgumi, 61 days in Fon + Edagum SM, and 63 days in Fon + Gumel Lyuks. In the productive variety, the active symbiosis potential was noted to be longer, being 76; 78 days in Fon + Edagum SM and Fon + Gumel Lyuks.

Table 3. General and active symbiotic potential of *Phaseolus vulgaris* L. varieties (2023-2024), n = 10, P < 0.05%.

Varieties	Biologically active substances	The appearance of nodules	The appearance of leghemoglobin	Transition from leghemoglobin to choleglobin	Erosion of the nodules
2023-y					
Ravot	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)	*7/11.VII**	3/14.VII	56/9.IX	5/14.IX
	Fon + Uzgumi	7/11.VII	3/14.VII	57/10.IX	5/15.IX
	Fon + Edagum SM	7/11.VII	3/14.VII	59/12.IX	6/18.IX
	Fon + Gumel Lyuks	7/11.VII	3/14.VII	63/16.IX	6/22.IX
Mahsuldor	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)	7/11.VII	3/14.VII	71/24.IX	6/30.IX
	Fon + Uzgumi	7/11.VII	3/14.VII	72/25.IX	6/31.IX
	Fon + Edagum SM	7/11.VII	3/14.VII	74/27.IX	7/03.IX
	Fon + Gumel Lyuks	7/11.VII	3/14.VII	74/27.IX	8/04.IX
2024-y					
Ravot	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)	7/14.VII	5/19.VII	57/14.IX	6/20.IX
	Fon + Uzgumi	8/15.VII	4/19.VII	57/14.IX	6/20.IX
	Fon + Edagum SM	8/15.VII	4/19.VII	61/18.IX	6/24.IX
	Fon + Gumel Lyuks	8/15.VII	4/19.VII	63/20.IX	7/27.IX
Mahsuldor	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)	7/14.VII	5/19.VII	73/01.X	6/07.X
	Fon + Uzgumi	8/15.VII	4/19.VII	73/01.X	6/07.X
	Fon + Edagum SM	8/15.VII	4/19.VII	76/04.X	7/11.X
	Fon + Gumel Lyuks	8/15.VII	4/19.VII	78/06.X	8/14.X

*-days; **-calendar period.

In the studies (**Table 3**), along with determining the total and active symbiotic potential of the nodules, the concentration of leghemoglobin in the nodules and the activity of the nitrogenase enzyme in the nodules were analyzed, and based on this, the amount of fixed biological nitrogen was also determined (**Table 4**).

Table 4. Nitrogenase enzyme activity, leghemoglobin concentration in tubers and fixed biological nitrogen in *Phaseolus vulgaris* L. varieties (2023-2024), n = 10, P < 0.05%.

Varieties (A factor)	Biologically active substances (B factor)		Nitrogenase enzyme activity, $\mu\text{g N}_2/\text{plant. hour}$	Leghemoglobin concentration in nodules, mg/g wet weight	Fixed biological nitrogen, kg/ha
2023-y					
Ravot	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)		412.3 ± 36.8	2.86 ± 0.25	39.34 ± 3.71
	Fon + Uzgumi		425.1 ± 37.3	2.93 ± 0.26	41.29 ± 3.83
	Fon + Edagum SM		426.4 ± 38.0	3.12 ± 0.29	42.87 ± 3.99
	Fon + Gumel Lyuks		427.3 ± 36.9	3.26 ± 0.28	45.87 ± 4.01
Mahsuldor	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)		415.3 ± 35.1	3.15 ± 0.27	50.24 ± 4.81
	Fon + Uzgumi		426.7 ± 37.3	3.34 ± 0.31	52.35 ± 4.91
	Fon + Edagum SM		427.6 ± 35.8	3.57 ± 0.33	53.92 ± 5.01
	Fon + Gumel Lyuks		428.5 ± 37.6	3.65 ± 0.34	54.03 ± 3.81
1	LSD ₀₅	A factor	3.47	0.27	11.40
2	LSD ₀₅	B factor	15.54	0.30	4.21
3	LSD ₀₅ AB factor		7.31	0.28	7.44
4	S _{x̄} %		3.12	3.47	3.25
2024-y					
Ravot	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)		423.7 ± 38.1	3.23 ± 0.29	41.15 ± 3.87
	Fon + Uzgumi		436.5 ± 39.8	3.31 ± 0.30	42.40 ± 3.89
	Fon + Edagum SM		437.8 ± 38.2	3.47 ± 0.32	45.51 ± 4.23
	Fon + Gumel Lyuks		438.7 ± 38.0	3.63 ± 0.34	47.10 ± 4.41
Mahsuldor	N ₃₅ P ₆₀ K ₃₀ (Fon) – Without biologically active substances (distilled water)		427.9 ± 40.1	3.54 ± 0.33	53.23 ± 4.87
	Fon + Uzgumi		439.3 ± 39.6	3.71 ± 0.35	54.65 ± 5.01
	Fon + Edagum SM		440.2 ± 40.8	3.96 ± 0.37	57.01 ± 5.22
	Fon + Gumel Lyuks		441.1 ± 41.4	4.05 ± 0.39	58.63 ± 5.56
1	LSD ₀₅	A factor	3.86	0.36	11.24
2	LSD ₀₅	B factor	13.24	0.47	5.36
3	LSD ₀₅ AB factor		8.46	0.41	7.49
4	S _{x̄} %		3.41	3.26	3.72

Nitrogenase enzyme is the main enzyme produced by endothermic bacteria and

converts molecular nitrogen (N_2) into ammonia (NH_3). In the non-preparation $N_{35}P_{60}K_{30}$ (Fon)– Without biologically active substances (distilled water) variants, this indicator was relatively low, and in the Ravot variety in 2023 it was 412.3 μg , and in 2024 it was 423.7 μg . When bioactive preparations were used, the enzyme activity was found to increase consistently. For example, in the variant where the Gumel Lyuks preparation was used, this indicator was 438.7 μg in the Ravot variety in 2024.

This increase is mainly explained by the fact that humic and fulvic acids, microelements contained in the preparation activate plant metabolism, improve the root environment and stimulate the rhizosphere microflora. As a result, the enzymatic activity of nodule bacteria increases.

Bioactive preparations increased the vital activity of symbiotic microorganisms that carry out nitrogen fixation in the rhizosphere and enhanced the production of the nitrogenase enzyme.

Leghemoglobin is a pigment with a heme structure found in nodules, which binds oxygen and protects nodule bacteria from the toxic effects of oxygen. The abundance of this substance indicates high activity of the nodules.

Bioactive preparations activated metabolic flows in the plant, resulting in increased synthesis of leghemoglobin.

Leghemoglobin concentration is a physiological indicator of nitrogen fixation in nodules, which increased due to the balancing of the internal environment of the plant through bioactive preparations, and the concentration of leghemoglobin in root nodules ranged from 2.86 to 3.65 mg/g depending on the variety. Of these, significant differences were found in the variants where preparations were used compared to the variant without preparations (distilled water) for the Ravot variety $N_{35}P_{60}K_{30}$ (Fon) – Without biologically active substances (distilled water), in which the Fon + Gumel Lyuks variant prevailed. In the productive variety, the concentration of leghemoglobin in nodules was 3.15 - 3.65 mg/g. The concentration of leghemoglobin in the tubers of the productive variety was slightly higher than that of the Ravot variety. This increase in leghemoglobin indicates the effective course of symbiosis processes and the improvement of microaerobic conditions in the nodules. According to the results of our research (2023), it was determined that the varieties fixed biological nitrogen up to 39.34 - 54.03 kg/ha during the growing season. In particular, it was noted that the Ravot variety variants accumulated biological nitrogen up to 39.34 - 45.87 kg/ha. At the same time, slightly higher indicators were determined in the variants where the preparations were used than in the $N_{35}P_{60}K_{30}$ (Fon) – Without biologically active substances (distilled water) variants. Among the variants, the Fon + Gumel Lyuks variant was found to have the highest biological nitrogen fixation. The productive variety recorded a slightly higher biological nitrogen fixation of 50.24 - 54.03 kg/ha compared to the Ravot variety.

4. Conclusions

In the conditions of the Republic of Uzbekistan, various repeated crops are grown

in the fields that are freed from grain in the summer, but due to global climate change and water shortages, special attention is paid to the cultivation of legumes in the republic, however, the technology for growing legumes, especially beans, in production is not sufficiently scientifically substantiated, and yields remain low.

In this regard, the use of growth stimulants and biologically active substances is of great importance.

The use of bioactive substances such as humic and fulvic acids, imported from abroad and synthesized from local raw materials, stimulates the growth and development of plants.

In particular, the microelements contained in biologically active substances have a positive effect on plant metabolism, and in the variants where the Gumel Lyuks preparation is used, the general and active symbiosis period is longer in both varieties. The Gumel Lyuks preparation is superior to other preparations in terms of the effect of the preparations, and the active symbiosis potential lasts 1 - 5 days.

Bioactive preparations have a positive effect on the activation of the nitrogenase enzyme, an increase in the concentration of leghemoglobin in the nodules and, in turn, on biological nitrogen fixation. If the nitrogenase enzyme in the nodules increased under the influence of the preparations, the highest indicator was determined in both varieties in the variant where Gumel Lyuks was used.

Nitrogenase enzyme activity was higher in the Mahsuldor variety than in the Ravot variety. When comparing the experimental variants, the amount of accumulated biological nitrogen and leghemoglobin concentration in the variants where bioactive substances were used compared to the $N_{35}P_{60}K_{30}$ (Fon) – Without biologically active substances (distilled water) variant increased along the $N_{35}P_{60}K_{30}$ (Fon) – Without biologically active substances (distilled water), Fon + Uzgumi, 4-Fon + Edagum SM, Fon + Gumel Lyuks variants, and prevailed in the Mahsuldor variety over Ravot. In both varieties, the highest results were recorded in preparations where Gumel Lyuks was used.

Biological nitrogen in bean varieties ranged from 39.34 to 54.03 kg, which led to an increase in the amount of biological nitrogen fixation due to the activation of the nitrogenase enzyme under the influence of the applied preparations.

Bioactive preparations (especially Gumel Lyuks) effectively activated the processes of nitrogen fixation. The preparations increased the activity of the nitrogenase enzyme and the concentration of leghemoglobin, increasing biological nitrogen fixation by 8% - 10%. Among the varieties, the Mahsuldor variety showed a high response to the preparations. The use of humus-based bioactive preparations is important in increasing soil fertility using legumes in an environmentally friendly way.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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