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Effect of biofertilizers on the content of bioactive components of common bean under open field and greenhouse cultivation conditions

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ABSTRACT

Background: Biofertilizers are important in improving the qualitative and biochemical properties of plants by increasing the quality of the soil, which is directly related to obtaining quality and functional food. Their use is particularly valuable in the cultivation of common beans, which serve as an alternative protein source, that are rich in micronutrients, antioxidants, and vitamins, and possess many medicinal values. By enhancing these qualities through biofertilizer application, common beans can become an important functional food in people's daily diets.

Objective: The main objective of the study was to evaluate the effectiveness of biofertilizer application in the context of improving food functionality on the content of bioactive components (dry matter, total sugars, ascorbic acid, total proteins) of green bean pods and grains.

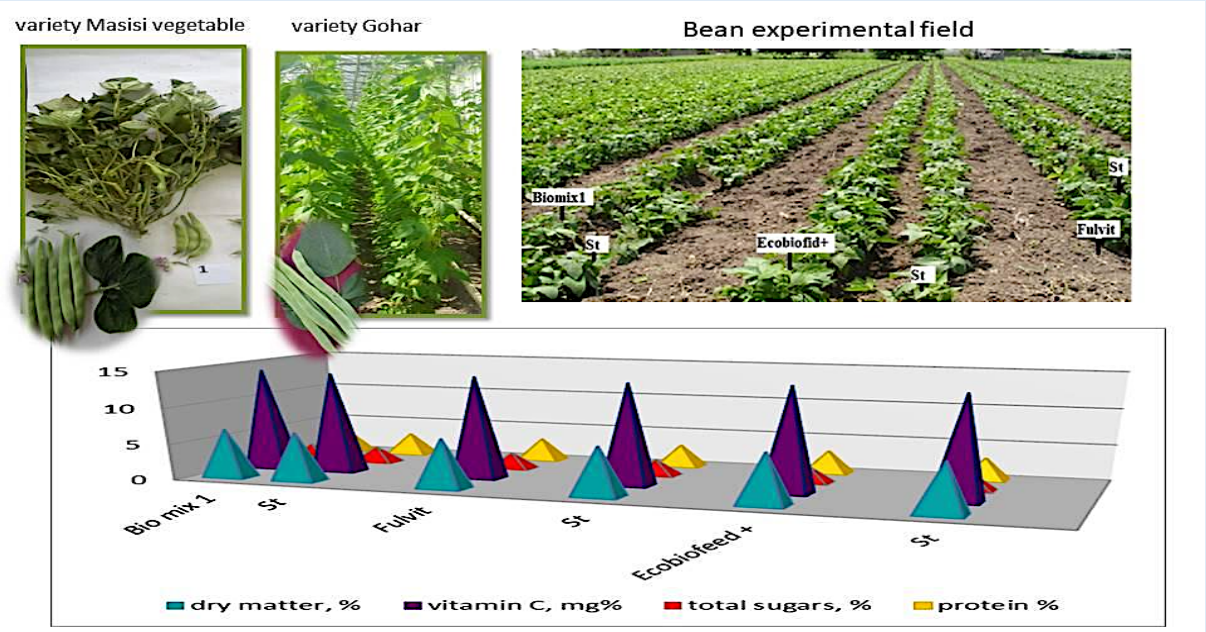
Methods: The study evaluated the effects of three biofertilizers —Fulvomix and Ecobiofid+ (of Armenian origin) and Biomix 1 (of Indian origin)—on the biomorphological, biochemical properties and functional processes of bean crops. Fertilizers were applied during pre-sowing treatments, early intensive growth, and fruiting stages. The experiments

were carried out in both open fields and greenhouses, with four repetitions on experimental plots measuring 20 square meters each. Soil analysis, along with the biochemical composition of bean pods and grains, was performed in accordance with the accepted methodology.

Results: As a result of the applied biofertilizers, an increase in the content of dry matter, vitamin C, total sugars, and proteins was recorded in green pods and beans. The highest results were recorded with the use of Ecobiofid + biofertilizer.

Conclusion: The application of three biofertilizers contributes to increasing the functional properties of green pods and grain, due to the improvement of the structural quality of the soil, as well as the increase of the indicators of biochemical, bioactive compounds. The positive effect of the applied biofertilizers on the quality indicators and functionality of green pods and grains, as well as on the productivity of the plant, allows the tested semi-fertilizers to be offered to production as a basis for organic farming and the production of healthy, functional food.

Key words: Common beans, Phaseolus vugaris L., biofertilizers, biochemical, organic agriculture, cultivar, seeds.



The change of semi-chemical indices of green beans and grains depending on applied biofertilizers

INTRODUCTION

Currently, the production of safe and sustainable food through the implementation of organic agriculture is widespread and in high demand worldwide, especially in the leading countries of Europe and the United States [1-3]. Organic agriculture is a system that promotes the use of safe materials to obtain highly efficient,

environmentally friendly products without harming human health and the environment [4-5].

A significant aspect of organic agriculture is the inclusion of safe and nutrient-rich foods in categories like functional foods, which include common beans. Since legumes are a source of human nutrition they play a critical role in functional food science, which is an interdisciplinary field dedicated to the discovery and development of bioactive compounds in legumes for the management of symptoms and risk reduction of chronic diseases, and the application of healthy and easily digestible foods [6-8]. Functional foods are characterized by their bioactive compounds, which are biologically active molecules that improve health through physiological mechanisms. These foods are natural or processed food products that contain biologically active compounds [9-10]. To further explore the effects of functional foods, researchers in the field of science can study the effects of different biofertilizers on the quality of specific foods [11]. The strengthening and development of organic agriculture began in 1972 when the International Federation of Organic Agriculture Movement (IFOAM) was founded in Versailles [12-13]. The purpose of IFOAM was to disseminate and implement organic agriculture information in all countries of the world. Since the beginning of the 1990s, the global share of organic agriculture has reached 20% annually [14].

Unfortunately, Armenia is not fully integrated into this significant movement, despite having significant opportunities and potential for the development of organic agriculture. Nevertheless, substantial efforts to realize this potential have been, with a central focus on incorporating inorganic materials, including biofertilizers and other organic preparations [15-16].

To achieve competitive, stable, and safe agricultural products, widely used fertilizers and organic materials obtained through various methods serve as alternatives to mineral fertilizers. The adoption of biofertilizers or organic preparations leads to enhanced vegetative growth in plants, increased yield, and improved quality characteristics. Notably, the nutrient content in vegetables is three times higher when using biofertilizers compared to traditional mechanical fertilizers [17-18].

The introduction and use of the Legume family (Fabaceae, formerly Leguminosae) vegetables are essential in organic farming, due to their ability to enrich the soil with natural nitrogen. This is achieved through nitrogen-fixing tuber bacteria, which synthesize organic nitrogen, reducing the dependency on nitrogen fertilizers, which is particularly important in cover soil cultivation. Beans, belonging to the Fabaceae family, exhibit three key properties relevant to food, agrotechnics, and soil health [19-20].

Food: Green bean pods, unripe beans, and ripe beans are all used as nutrient-dense foods. They provide essential proteins, amino acids, carbohydrates, vitamins, fats, and other chemical substances necessary for the human body [21].

Agrotechnical - From an agrotechnical perspective, leguminous plants play a crucial role in enriching the soil with nitrogenous compounds. This accumulation contributes to increased yields in subsequent crops. Specifically, legumes, including beans, have the capacity to enrich the soil with 50-100 kg/ha of nitrogen during a single vegetation period. To put this in perspective, that's equivalent to 20 tons of manure [22-23].

Beans are valuable in crop rotation, serving as effective predecessors for nearly all crops. While slightly less advantageous than black spelt (noted for winter wheat), beans excel due to their early maturity, abundant green biomass, and high nitrogen content. Additionally, beans are employed as a form of green fertilization [24-25].

Considering the information above, this research focused on studying the impact of various organic fertilizers on common bean crops grown under both open and greenhouse cultivation conditions. Specifically, we investigated the effects of several biofertilizers on bean crops in these different environments.[26].

Objective: During the research, the influence of two biofertilizers of Armenian origin, Fulvomix and Ecobiofid+, on the biomorphological, biochemical properties and functional processes of beans was studied.

MATERIALS AND METHODS

Materials: Fulvomix is an organic biostimulant liquid fertilizer manufactured by Ecorise. This Armenian-made liquid biofertilizer contains several biologically active substances, including humic acids, fulvic acids, and micronutrients chelated with fulvic acids using specially applied technologies. This Armenian biofertilizer is based on biohumus and was applied via root feeding at a 1/100 dilution rate, three times during the growing season at intervals of 10–15 days.

Ecobiofeed+ is a complex biofertilizer that consists of highly active nitrogen-fixing microorganisms, natural minerals, plant-based components, macro- and microelements (phosphorus, potassium, magnesium, manganese, iron, and copper), vitamins, and amino acids. The zeolite content in this biopreparation facilitates the release of nitrogen, potassium, phosphates, and other nutrients from the soil to the plant. The biofertilizer is applied as a pre-sowing seed treatment and nutrient application at a 1/100 dose, both before seed sowing and at the beginning of plant fruiting.

"Bio mix 1" biological fertilizer of Indian origin is based on different types of bacteria: Azospirillium brasilense, Azotobacter vinelandil, Pseudomonas Fluorescens, Trichoderma viride, Bacillus polymyxa (cfu> 2x106gm). The solution of bio-fertilizer "Bio Mix 1" was prepared in the following way: 0.05 kg of sugar and 0.05 kg of flour were mixed with 100 g of fertilizer powder, and the resulting mixture was dissolved in 400 l of water. The solution was, then, kept for 24 hours and used during pre-sowing cultivation, soil watering method, and root feeding during vegetation.

Experimental site and plots: The research was carried out at the experimental station of the Scientific Center of Horticulture and Technical Crops of the RA, both in the open field and under the conditions of greenhouse cultivation in 2021-2023. The researchers focused on local varieties of beans belonging to *Phaseolus vulgaris* species by examining both determinate and indeterminate growth patterns. In particular, the Masis vegetable variety was chosen from the decisive forms for cultivation in the open field. For greenhouse cultivation, the indeterminate variety known as Gohar was chosen.

The experiments were carried out both in the open field and in the greenhouse with four repetitions using 20 square meters plots. The planting scheme was different for bushy (90+70/2x15 cm) and climbing forms (90+70/2x20 cm). Sowing was done in accordance with agrotechnical requirements, which included an open field in the first ten days of May, and in the greenhouse (without heating) in the second ten days of March. The seeds were sown by hand at a depth of 3-5 cm, and the direction of the experiments was from north to south. Autumn wheat was sown in the open field, tomatoes in the closed field. The testing of varieties followed the methodology of testing state varieties of agricultural crops and international varieties of beans [27-28].

Treatment and analyses: Prior to sowing, the mechanical composition of the experimental plot was studied. In addition, the pre-sowing treatment of the

soil was carried out in the biochemistry laboratory of the State Research Institute.

Our data analysis revealed the following soil characteristics:

- Humus content in tillage and subsoil ranged from 2.25% to 2.25%.
- Total nitrogen content was 0.149% to 0.09%.
- Easily hydrolysable nitrogen content was 8.45 mg to 5.39 mg, which corresponds to acceptable levels for organic farming.

Under greenhouse conditions, we performed soil analysis using express methods. Soil electrical conductivity (EC) was determined as 0.77, pH 6.4, NO3-N as 0 mg/L, P as 40 mg/L, K as 118 mg/L, and Ca+Mg-as19.6 mg/L. These results confirm that greenhouse soils are suitable for organic farming. All agrotechnical measures, including care work, were carried out in accordance with the characteristics of the given zone and crops. The effects of three biofertilizers on biological, biochemical, phenological, and yield parameters of bean crops were studied. The amount of dry matter, ascorbic acid, and total sugar was determined in green beans pods. In addition, the number of total proteins was determined in beans. The content of dry matter in the fruits was determined by the refractometric method. Vitamin C content was determined according to Moore's method, and sugar content was determined by Bertrand's permanganate method. To calculate the number of proteins, the nitrogen content, which was determined by the Kjeldahl method, was multiplied by a factor of 6.25. In the control version, the plants were fed with mineral fertilizer. Nutrient uptake (mainly N) was estimated as % nutrient x plant productivity (vegetative biomass and number of pods per plant).

Statistical analysis: Data were expressed as mean ± standard error with a sample size (n) of 4. Comparison of means was performed using the Fisher LSD test ($P \leq 0.05$) after multifactorial ANOVA analysis. The data were analyzed by F-test at 5% level to determine the variance effects of the tested variants on bean yield, and biochemical elements of soil and bean pods and grains were analyzed. Excel and GraphPad software were used for statistical analysis [29-32] .

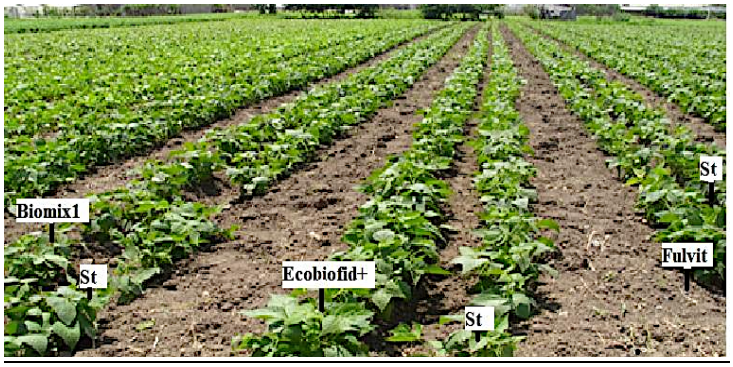


Figure 1. Beans experimental field.

RESULTS

During the vegetation period, phenological observations were made to find out the effects of the studied biofertilizers on the transition of plants' phenophases, the maturation of green underbelly, and the duration of fruiting under the conditions of open and greenhouse cultivation. As can be seen from the analysis of the studies (Fig. 2), in the open field and greenhouse cultivation sites, all phenopools outperformed the control options when applied with biofertilizers. In open

field cultivation conditions, Biomix 1 biofertilizer outperformed the control version (sowing-germination, germination-flowering, germination-technical maturation) by 1, 5, and 6 days, Fulvomix outperformed the control version by 2, 8, and 5 days, and Ecobiofeed + outperformed the control version by 2, 6, and 8 days, respectively.

As a result of biological studies, the following indicators were evaluated: the degree of branching of the stem, the degree of leafing, the color of the flowers, the shape of the stem, and the size. The studies revealed that cultivars had different biological characteristics depending on the applied biofertilizer. Stem branching and degree of leafing were calculated according to accepted methodology. The variety under study in the conditions of cultivation in the open field, depending on the applied biofertilizers, recorded quite large fluctuations.

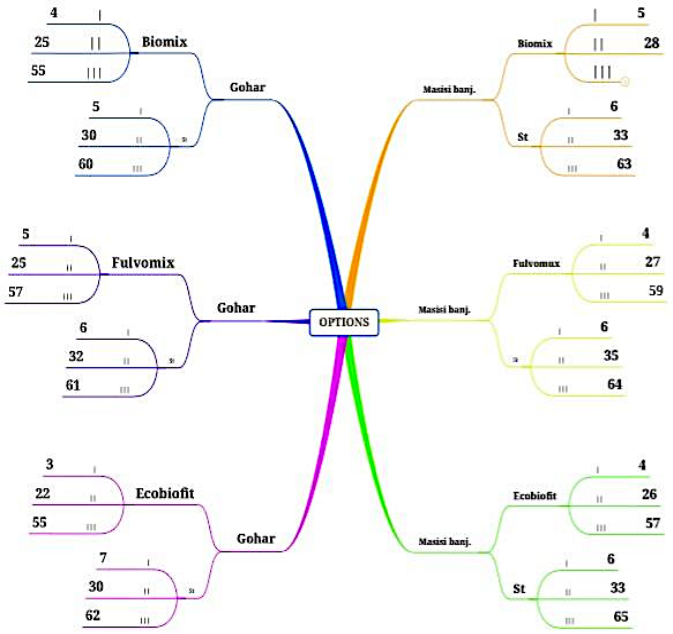


Figure 2. Duration of bean phenological stages depending on the effect of different biofertilizers.

A strong degree of branching was recorded in the Masis vegetable variety under the influence of Ecobiofeed + and Fulvomix fertilizers. In the case of the utilization of Bio mix 1, a higher-than-average degree of branching was recorded. In green house conditions of the bean Gohar variety, all three biofertilizers recorded very strong degrees of branching (fig. 3).

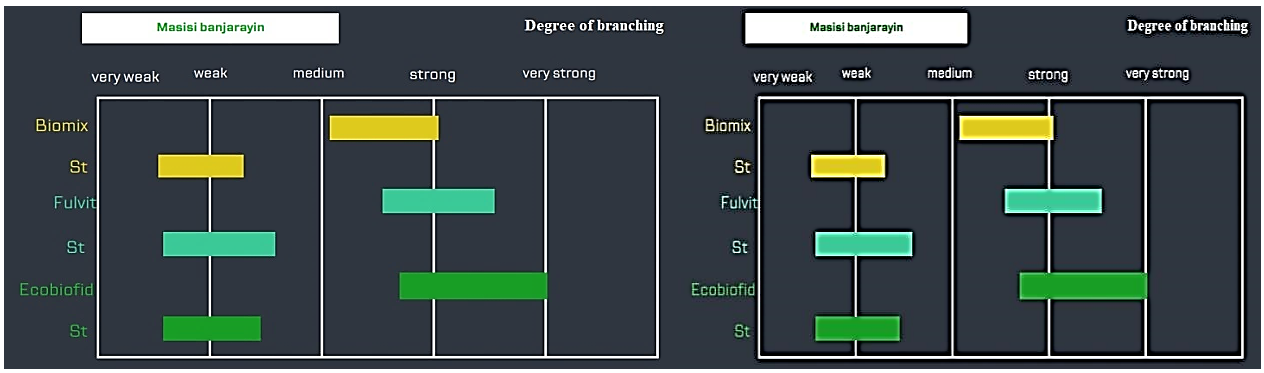


Figure 3. Depending on the applied biofertilizer, the degree of branching of bean plants.

According to our studies, the Fulvomix and Ecobiofeed + preparations had a higher-than-average degree of leafing under the conditions of open field cultivation. On the other hand, the Bio mix 1 provided an average degree of leafing. Under the greenhouse conditions, the Gohar variety recorded a strong degree of leafing when using all three preparations (fig. 4).

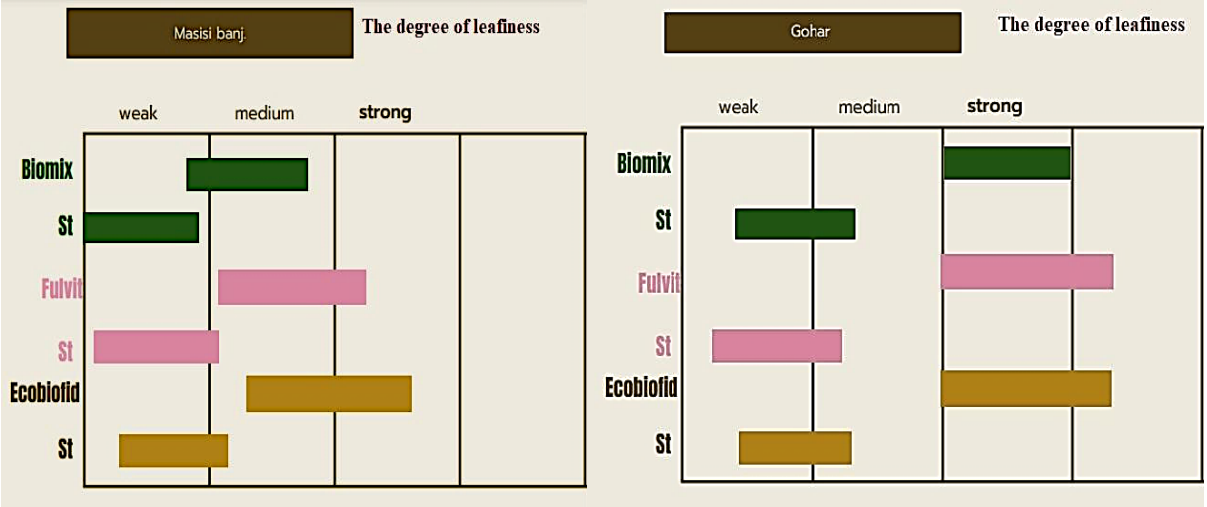


Figure 4. Depending on the applied biofertilizer, the degree of leafiness of bean plants.

It should also be noted that the plants treated with bean preparations had more intense green coloring compared to the controls, which indicates the active activity of photosynthesis. No significant changes were recorded regarding the length, shape, and width of the green underbelly. The effects of different semi-fertilizers on the structural years of the crop were also studied through the number of green pods per plant, pod weight, pod number, 1000 pod weight, and most importantly, yield indicators. The high yield of vegetable bean varieties is determined by yield elements such as the number of pods on the plant, the mass, and the number of grains in the pod. These plans are mainly determined by the genetic characteristics of the variety.

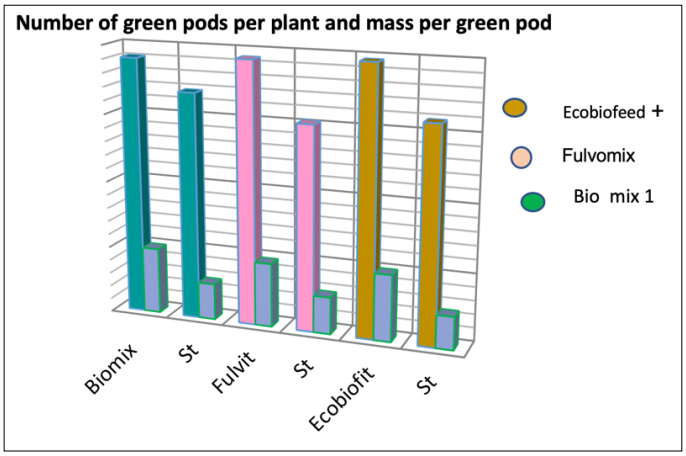


Figure 5. Number of green pods per plant and mass per green pod.

From the results of research in this direction, an inverse relationship between the number of plants and their mass has been observed. Specifically, as the number of plants increases, the individual plant mass decreases. This trend was observed across almost all test options, but it was particularly pronounced in the

case of biofertilizers. After analyzing the data related to yield elements, it becomes evident that the number of grains per undum (seed pod) is one of the least fluctuating indicators. Furthermore, this parameter remained nearly unchanged for each bean variety throughout the study years. The most significant difference was observed in grain mass, including the mass of 1000 grains exhibited considerable variations compared to the control group. For the Masisi vegetable variety cultivated in open field conditions, Ecobiofeed+ (405.1 g) and Fulvomix (405.5 g) biofertilizers showed a difference of 2.5% and 2.2%, respectively, compared to the controls (395.2 g and 396.4 g). Bio Mix 1 (401.8 g) exhibited a difference of 1.7%. In the case of the Gohar variety grown in greenhouse conditions, significant changes were again observed for all three biofertilizers compared to the controls (480.1 g, 479.9 g, and 479.7 g). Ecobiofeed+ (489.8 g) showed a difference of 2.04%, Fulvomix (490.2 g) exhibited a difference of 2.1%, and Bio Mix 1 (489.6 g) had a difference of 2.06% (see Fig. 6).

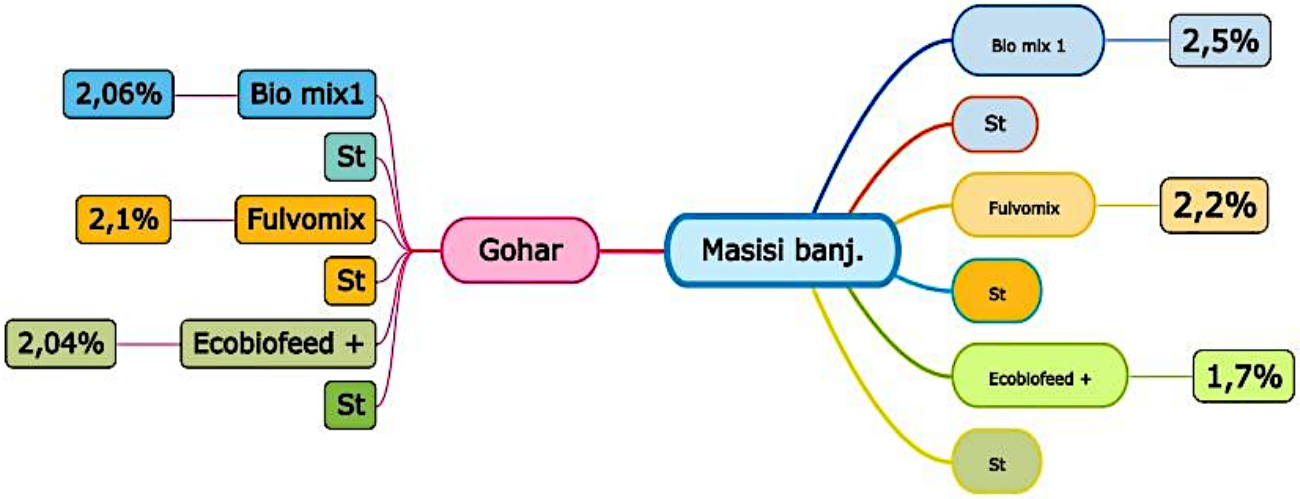


Figure 6. The mass of 1000 seeds.

Bean green pods are valued for the content of important and available biochemical substances for the human body's vital activity: total sugars, dry substances ascorbic acid, and the grains for the content of proteins. It is interesting to study the quantitative change in the content of these substances depending on the applied organic biofertilizers. The results of the studies in this direction document (Tab. 1) that the number of biochemical substances in the green bean pods exceeded the control version depending on the applied biofertilizer (Biomix, Fulvomix, or Ecobiofeed) in the conditions of open field cultivation. The amount of dry matter was found to be 1.01% 1.12%, and 1.25%, ascorbic acid was found at levels of 1.5%, 1.6%, and 1.9%, and the total sugars were found to be 0.5%, 0.7%, and 0.9% for the Biomix, Fulvomix, or Ecobiofeed respectively. In the grains, the protein content was found at 2.1%, 2.1%, and 2.3% for Biomix, Fulvomix, or Ecobiofeed respectively. Quantitative changes of biochemical substances were also recorded under the conditions of greenhouse cultivation. Against the background of the applied biochemical fertilization, the contents of substances in greens and grains exceeded the control versions. The content of dry matter in green manure exceeded 1.2% in the case of Biomix, exceeded 1.1% when Fulvomix was utilized, and 1.5% when

Ecobiofeed was utilized. The ascorbic acid contents were found at 1.5%, 1.4%, and 1.8 % and the total sugars were found to be 0.7%, 0.6%, and 0.9% for Biomax, Fulvomix, and Ecobiofeed respectively. The grain protein content was found to be 1.9%, 1.7%, and 1.9% for Biomax, Fulvomix, and Ecobiofeed respectively.

Table 1. The change of semi-chemical indices of green bean pods and grains depending on applied biofertilizers

The name of the variety	Name of biofertilizer	Beans in green pods			In bean grains gr/100 gr
		dry matter, %	vitamin C, mg%	total sugar, %	protein
Masisi vegetable	Bio mix 1	6.87	14.24	2.30	3.17
	St	6.80	14.02	2.28	3.10
	Fulvomix	6.86	14.20	2.28	3.18
	St	6.78	13.98	2.26	3.11
	Ecobiofeed +	6.96	14.29	2.30	3.19
	St	6.87	14.01	2.27	3.11
Gohar	Bio mix 1	8.92	18.92	3.43	3.86
	St	8.81	18.64	3.40	3.80
	Fulvomix	8.90	18.87	3.41	3.85
	St	8.80	18.60	3.38	3.80
	Ecobiofeed +	8.96	19.01	3.45	3.96
	St	8.82	18.65	3.41	3.90

The most crucial criterion for evaluating the efficiency of vegetable crops is yield, which reflects all aspects of plant vitality. Among the studied varieties, both in open field and greenhouse cultivation conditions, the variants treated with all three biofertilizers exhibited high yield indicators, surpassing the control variants (see Table 2 and 3).

Table 2. The yield of green bean pods depending on applied biofertilizers under open field conditions

The name of the variety	Name of biofertilizer	The yield of green bean pods, c/ha			Average harvest, c/ha	Dispersion	Crop supplement, %
		2021	2022	2023			
Masisi vegetable	Bio mix 1	132.8	132.4	134.2	133.133	0.893333	3.3
	St	129.6	128.8	128.1	128.833	0.563333	-
	Sx% $LSD_{o,5}$			2,2 0,7			
	Fulvomix	133.7	134.1	133.3	133.7	0.16	4,0
	St	128.2	127.6	129.8	128.53	1.2933	-
	Sx% $LSD_{o,5}$			0.5 0.7			
	Ecobiofeed +	136.2	135.5	137.2	136.3	0.73	6.3
	St	129.3	126.7	128,5	128.16	1.773	-
	Sx% $LSD_{o,5}$			0.3 0.5			

Table 3. The yield of green bean pods depending on applied biofertilizers under cover soil cultivation conditions

The name of the variety	Name of biofertilizer	The yield of green bean pods, c/ha			Average harvest, c/ha	Dispersion	Crop supplement, %
		2021	2022	2023			
Gohar	Bio mix 1	403.2	402.3	401.9	402.466	0.443333	2.5
	St	397.6	390.6	389.1	392.433	20.58333	-
	Sx%			1.3			
	$LSD_{o,5}$			1.9			
	Fulvomix	404.5	405.9	405.4	405.26	0.503333	2.0
	St	398.5	397.6	395.7	397.26	2.043333	-
	Sx%			0.6			
	$LSD_{o,5}$			0.6			
	Ecobiofeed +	406.8	408.8	407.4	407.66	1.053333	2.5
	St	396.6	399.0	397.3	397.63	1.523333	-
	Sx%			0.07			
	$LSD_{o,5}$			0.1			

As can be seen from the analysis of the data in the table, Biomix 1, Fulvomix, and Ecobiofeed + biofertilizers stood out with a high yield of green under the conditions of open field cultivation. Each version, respectively, exceeded the testers by 4.3, 5.17, and 8.14 c/ha. All biofertilizers were distinguished by the high yield of green bean pods under the greenhouse condition; the increase in yield compared to the controls was 10.0, 8.0, and 10.0 t/ha for Biomix 1, Fulvomix, and Ecobiofeed + respectively.

CONCLUSION

From the analysis of the results obtained, it can be concluded that the application of all three biofertilizers leads to an increase in the functionality of green bean pods and dry grains by increasing the biochemical indicators. The high content of vitamin C, dry matter, and total sugar in green bean pods is important for human health, by offering easy-to-digest foods that can be incorporated into a balanced diet. The high amounts of proteins in dry grains are another important improvement, because proteins are indispensable in the human diet, contributing to the digestion of food, restoration of tissues, and growth of the body. These enhanced nutritional values position bean crops as functional foods capable of addressing malnutrition, supporting recovery during illnesses, and promoting a healthy lifestyle by offering green bean pods as a readily digestible dietary option.

In addition to these highly valuable functional nutritional properties, the application of biofertilizers also had a positive effect on the physicochemical properties of the soil, as well as the biomorphological properties of the bean crop. These indicators are important for making recommendations to farmers.

Based on the results of the research, it can be concluded that the bean crop shows excellent results when biofertilizers are applied. All three biofertilizers used in the study showed a significant positive effect compared to the control options, under both open field and greenhouse conditions.

List of abbreviations: I-sowing-sprouting, day, II-budding-blooming, day, III-germination-technical ripening, day.

Competing interests: The authors declare that they have no competing interests.

Author Contributions: G. K., K.S. designed the study. G.K. provided the bean Masisi vegetable, Gohar varieties for research. G.K. performed biochemical analysis., G.K.

V.V. performed statistical analyses. G.K. wrote the manuscript. G.K., G.M. edit the article. All authors read and approved the final version of the manuscript.

Competing interests: There are no conflicts of interest to declare.

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