



# A functionally sustainable approach to producing pure agricultural products

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**Submission Date:** October 23rd, 2025; **Acceptance Date:** January 11th, 2026; **Publication Date:** January 13th, 2026

**Please cite this article as:** Jhangiryan T., Gasparyan G., Barseghyan M., Beglaryan I., Daveyan S., Hunanyan S. A Functionally Sustainable Approach to Producing Pure Agricultural Products. *Functional Foods in Health and Disease* 2026; 16(1): 42 – 54.

DOI: <https://doi.org/10.31989/ffhd.v16i1.1775>

## ABSTRACT

**Background:** Functional foods address dietary needs and promote health by harnessing bioactive compounds. Peppers, a staple crop worldwide, are vital for nutrition and the economy. Biohumus enhances soil microbial activity, improves plant nutrition, and stimulates the activation of bioactive components in crops.

**Objective:** This study evaluates the effectiveness of microbial biohumus, developed by the "Hrant Petrosyan Scientific Center," in increasing the yield and quality of three pepper varieties: Loshtak, Arajnek, and Jermatnayin Hska.

**Methods:** Seedlings of the pepper varieties, provided by the "Vegetable and Technical Crops Scientific Center" CJSC of the RA Ministry of Economy, were cultivated in vegetative containers with 85 kg of soil at the "Hrant Petrosyan Scientific Center of Soil Science, Agrochemistry and Melioration," a branch of the ANAU Foundation. Growth and development parameters were measured throughout the experiments, and the contents of ascorbic acid and chlorophylls a and b were determined.

**Novelty:** This study introduces an organic fertilizer tailored for sustainable agriculture. Biohumus not only boosts yield but also enhances the functional and nutritional quality of crops, aligning with global trends in functional food production.

**Results:** The application of biohumus as a biofertilizer significantly increased the yield of the three pepper varieties:

- Arajnek: 33.3% increase (304 c/ha) compared to the control (228 c/ha).
- Jermatnayin hska: 51.3% increase (237 c/ha) compared to the control (191 c/ha).
- Loshtak: 50.04% increase (360 c/ha) compared to the control (239.8 c/ha).

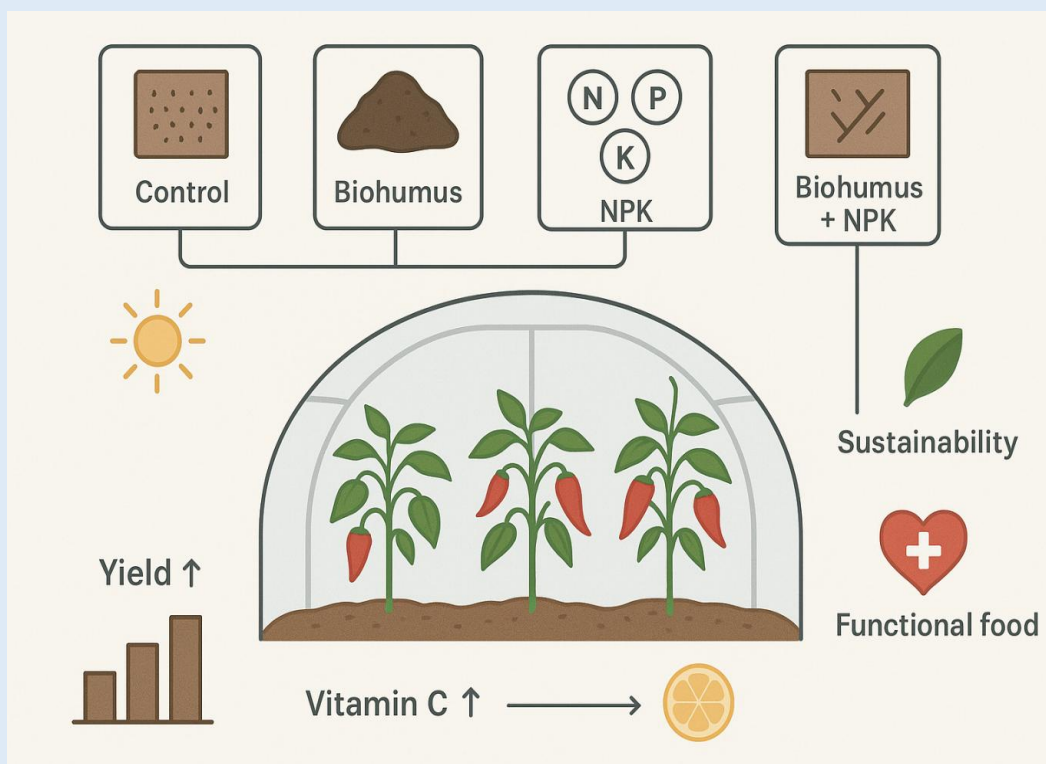
Combining biohumus with inorganic fertilizers (NPK) resulted in even higher yields:

- Arajnek: 82% increase (415 c/ha).
- Jermatnayin hska: 75.05% increase (342 c/ha).
- Loshtak: 81.8% increase (436 c/ha).

Additionally, biohumus application enhanced chlorophyll content (a+b: 18.78 mg/l) compared to the control (15.0 mg/l) and increased ascorbic acid levels, particularly in Jermatnayin hska fruits treated with N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>.

**Conclusion:** The application of biohumus offers a sustainable approach to agricultural practices. It improves yield while reducing the need for chemical fertilizers, thereby supporting the production of functional foods.

**Keywords:** Pepper varieties, biohumus, food functionality, yield, quality



**Graphical Abstract:** Functionally sustainable approach to producing pure agricultural products.

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## INTRODUCTION

In the face of a growing global population, increasing diversification of dietary needs, and a heightened

emphasis on healthy lifestyles, there is a pressing demand for agricultural practices that ensure not only high yields but also improved nutritional and functional

quality of produce [1–4]. Within this context, considerable attention has been directed toward the development of functional foods that contribute to maintaining human health through diet [5–8]. Such products are typically enriched with biologically active compounds, including antioxidants, vitamins, trace elements, and phytochemicals [9–10].

Among vegetable crops, sweet pepper (*Capsicum annuum*) is recognized as a key source of functional foods. It is rich in ascorbic acid (vitamin C), carotenoids, flavonoids, and other bioactive compounds that collectively underpin its antioxidant, anti-inflammatory, and immunomodulatory properties. Beyond its nutritional significance, sweet pepper is also one of the most economically important horticultural crops worldwide [11–15].

However, under intensive farming conditions, the extensive use of chemical fertilizers and mulching materials, while temporarily enhancing yields, often results in soil degradation, reduced biodiversity, and environmental pollution. In response, sustainable agricultural approaches—particularly the application of organic and biological fertilizers—have gained increasing prominence. Among these, biohumus, an organic fertilizer derived from microbial decomposition of organic residues, has emerged as a promising alternative [16–20, 26–29].

Biohumus not only enhances soil structure and fertility but also stimulates plant growth by promoting rhizospheric microbial activity, thereby improving nutrient availability, fostering phytohormone synthesis, and strengthening plant resistance. Importantly, recent evidence suggests that biofertilizers can also influence the accumulation of bioactive compounds in crops, thereby enhancing their nutritional and functional properties [16, 21–29].

The present study aims to evaluate the effectiveness of biohumus in improving yield performance, physiological development, and nutritional quality traits of three sweet pepper cultivars (*Loshtak*, *Arrajnek*, and *Jermatnayin Hska*). Specifically, the study examines the effects of both pure biohumus and its combination with mineral fertilizers (NPK) on the accumulation of biologically active compounds, with a focus on ascorbic acid and chlorophylls (a and b).

The novelty of this research lies in its assessment of biofertilizer application on the functional quality of distinct sweet pepper cultivars under localized conditions. The findings are expected to provide valuable insights to enhance local crop productivity and support the development of health-promoting foods, aligning with global trends in sustainable agriculture and nutrition.

## MATERIALS AND METHODS

**Plant Material:** The experimental material consisted of seedlings from three pepper cultivars—*Loshtak*, *Arajnek*, and *Jermatnayin Hska*—all members of the Solanaceae family. Seedlings were provided by the Vegetable and Industrial Crops Scientific Center (CJSC) under the Ministry of Economy of the Republic of Armenia. Cultivation was carried out in containers, each filled with 85 kg of soil, prepared by the Hrant Petrosyan Scientific Center of Soil Science, Agrochemistry, and Melioration (a branch of the Armenian National Agrarian University). Growth and developmental traits of the plants were systematically monitored during the entire experimental period.

**Soil Physicochemical and Agrochemical Properties:** To evaluate the impact of biohumus on soil quality, both physical and chemical parameters were analyzed before and during the experiment using widely accepted

methods [15]. Soil humus was quantified using the Tyurin procedure, and pH was measured potentiometrically. Soil texture was classified according to Kachinskiy's method. The availability of primary nutrients was assessed as follows: nitrogen (N) following Tyurin and Kononova, phosphorus ( $P_2O_5$ ) using Machigin's method, and potassium ( $K_2O$ ) according to Maslova. The sand fraction was determined following the approach proposed by Savinov [29-30].

**Determination of Photosynthetic Pigments:** Chlorophyll *a*, chlorophyll *b*, and carotenoids were quantified spectrophotometrically. Pigment extraction was performed with acetone and ethanol, and absorbance was measured at the appropriate wavelengths as described in [17].

**Determination of Ascorbic Acid Content:** Ascorbic acid concentration in pepper samples was determined by iodometric titration, following the standard procedures outlined in [32].

**Statistical Analysis:** All measurements were conducted in triplicate, and results were expressed as mean  $\pm$  standard deviation (SD). One-way analysis of variance (ANOVA) was used to evaluate treatment effects. Statistical significance was determined at the 95% confidence level using the statistical significance difference (SSD) test, and relative experimental error ( $S_x\%$ ) was also calculated.

## RESULTS AND DISCUSSION

The results of this study demonstrate that the application of biohumus substantially improved the nutrient composition of the soil, particularly by increasing the availability of nitrogen, phosphorus, and potassium. The most pronounced effects were recorded in the combined

treatment of biohumus with mineral fertilizers, which revealed a synergistic interaction.

These findings are consistent with previously reported evidence showing that vermicompost and related organic amendments enhance soil fertility by increasing organic matter content and improving nutrient retention. Numerous studies have highlighted their role in improving soil physical, chemical, and biological properties, including pH stabilization, stimulation of microbial activity, and enhancement of nutrient availability, ultimately contributing to better plant growth and productivity. Other research has shown that vermicompost improves soil structure, aggregate stability, water-holding capacity, and enzymatic activity, thereby strengthening long-term soil resilience. Furthermore, studies investigating vermicompost derived from liquid waste via vermifiltration have confirmed significant increases in total nitrogen and carbon content, as well as in soil enzymatic activity, particularly at higher application rates, leading to improved soil health and enhanced crop nutrition [33–38].

Overall, the present findings align with contemporary scientific evidence, reinforcing the conclusion that biohumus is an effective organic amendment that improves both soil quality and crop productivity. The combination of biohumus with mineral fertilizers proved remarkably effective, ensuring optimal nutrient availability and supporting both the physical and biological improvement of soils.

The experiment included the following four treatment groups:

1. Control (no amendments)
2. Biohumus only
3. Mineral fertilizers ( $N_{60}P_{60}K_{60}$ )
4. Combined treatment (Biohumus +  $N_{60}P_{60}K_{60}$ )

**Table 1.** Effect of biohumus, mineral fertilizers, and their combined application on the timing of growth stage transitions in different sweet pepper cultivars (2021–2023).

a	Seedling establishment	Flower cluster formation	Lateral branch development	Fruit set	Primary fruit formation	First harvest	Second harvest	Third harvest	Fourth harvest	Fifth harvest	Sixth harvest	Days from planting to harvest
<b>Loshtak</b>												
Control	25. 04	19. 05	05. 06	20. 06	31. 06	18. 07	24. 07	31. 07	06. 08	12. 08	18. 08	116
Biohumus 20t/ha	25. 04	18. 05	04. 06	18. 06	25. 06	12. 07	18. 07	24. 07	30. 07	06. 08	12. 08	110
NPK 60 kg/ha	25. 04	23. 05	07. 06	25. 06	21. 07	20. 07	26. 07	02. 08	08. 08	19. 08	20. 08	120
Biohumus 20t/ha+NPK 60 kg/ha	25. 04	18. 05	04. 06	16. 06	30. 06	26. 07	22. 07	26. 07	02. 07	08. 08	14. 08	112
<b>Arajnek</b>												
Control	25. 04	17. 05	06. 06	20. 06	11. 07	22. 07	28. 07	03. 08	09. 08	15. 08	21. 08	119
Biohumus 20t/ha	25. 04	15. 05	05. 06	21. 06	29. 06	17. 07	23. 07	29. 07	04. 08	10. 08	16. 08	114
NPK 60 kg/ha	25. 04	19. 05	08. 06	23. 06	07. 07	26. 07	02. 08	10. 08	16. 08	22. 08	26. 08	124
Biohumus 20t/ha+NPK 60 kg/ha	25. 04	16. 05	06. 06	19. 06	31. 06	13. 07	20. 07	26. 07	10. 08	16. 08	22. 08	120
<b>Jermatnayin hska</b>												
Control	25. 04	15. 05	03. 06	16. 06	25. 06	14. 07	20. 07	26. 07	01. 06	07. 06	13. 08	111
Biohumus 20t/ha	25. 04	13. 05	31. 05	15. 06	24. 06	12. 07	18. 07	24. 07	30. 07	06. 08	12. 08	110
NPK 60 kg/ha	25. 04	17. 05	04. 06	18. 06	28. 06	16. 07	23. 07	29. 07	04. 08	10. 08	16. 08	114
Biohumus 20t/ha+NPK 60 kg/ha	25. 04	14. 05	30. 05	12. 06	20. 06	04. 07	12. 07	19. 07	25. 07	31. 07	06. 08	104

Analysis of the data presented in Table 1 reveals that the duration of growth stages in sweet pepper (from planting to harvest) is primarily influenced by two significant factors: the biological characteristics of the cultivars and the mode of fertilizer application (either individually or in combination). For example, in the cultivar *Loshtak*, the total duration from planting to harvest in the control treatment was 116 days. When treated with biohumus at 20 t/ha, the duration was shortened by 6 days, and in the combined therapy of biohumus + NPK (60 kg/ha), it was reduced by 4 days. In contrast, the application of mineral fertilizers alone (NPK 60 kg/ha) extended the growth period by 6 days compared to the control. A similar trend associated with fertilization practices was observed in the cultivars *Arajnek* and *Jermatnayin Hska*.

Interestingly, the sole application of mineral fertilizers consistently prolonged the duration of growth stages. This effect is likely due to the nutrient availability in mineral fertilizers, which promotes more intensive vegetative growth while delaying the development of generative organs. Such patterns have also been reported in other studies [9,19,36].

Among the studied cultivars, the shortest growth duration was observed in *Jermatnayin Hska*. In the control treatment, the growth period was 1 day shorter than that of *Arajnek* and 5 days shorter than that of *Loshtak*. Under the combined therapy of biohumus + NPK (60 kg/ha), the growth period in *Jermatnayin Hska* was reduced by 16 and 8 days compared to *Arajnek* and *Loshtak*, respectively. A similar tendency was observed across the other treatments as well (Table 1).

Overall, the length of the growth cycle in sweet pepper is determined by two primary factors: the

cultivar's biological characteristics and the type or combination of fertilizers applied. For instance, within the same cultivar, the shortest duration from planting to final harvest was consistently observed in the combined biohumus + NPK (60 kg/ha) treatment, which was 6 days shorter than the control and 9 days shorter than the mineral fertilizer treatment alone. This pattern was consistently evident in all three cultivars under study (*Loshtak*, *Arajnek*, and *Jermatnayin Hska*) (Table 1).

From a practical perspective, these results suggest that integrating biohumus with mineral fertilizers not only accelerates the transition between phenological stages but also shortens the total growth cycle, enabling earlier and multiple harvests. This approach may improve resource-use efficiency, optimize harvest schedules, and enhance overall crop productivity, making it highly relevant for sustainable pepper production systems.

The evaluation of yield performance in the cultivars *Arajnek*, *Jermatnayin Hska*, and *Loshtak* revealed distinct varietal differences. Overall, the application of biohumus significantly enhanced yield across all studied cultivars.

For *Arajnek*, the obtained yields were 228, 304, 276, and 415 c/ha under control, biohumus, mineral fertilizer (N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>), and combined biohumus + NPK treatments, respectively. Compared to the control, this represented increases of 33.3%, 21.05%, and 82.0% for the biohumus, NPK, and combined treatments, respectively.

In the case of *Jermatnayin Hska*, yields of 191, 237, 240, and 342 c/ha were recorded for the respective treatments. The yield advantage over the control amounted to 51.3%, 25.65%, and 75.05% for biohumus, NPK, and the combined treatment, respectively.

For *Loshtak*, the response pattern differed somewhat from the other two cultivars. Here, yield

increases reached 50.04% with biohumus, 20.5% with NPK, and 81.8% with the combined application,

highlighting the particularly strong effect of integrating biohumus with mineral fertilizers (Table 2).

**Table 2.** Biohumus and chemical fertilizer effects on the yield and crop supplement of pepper plant varieties

Plant varieties	Variant	Yield, c/ha, 2021-2023	Crop supplement	
			c/ha	%
Arajnek	Control	228		
	Biohumus	304	76.0	33.3
	N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	276	48.0	21.05
	Biohumus+ N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	415.0	187.0	82.0
S <sub>x</sub> %	1.76 ± 0.24			
SSD <sub>0.95</sub> c/ha	34.23 ± 3.1			
Jermatnayin hska	Control	191		
	Biohumus	237	46.0	24.1
	N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	240	49.0	25.65
	Biohumus+ N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	342	151.0	79.05
S <sub>x</sub> %	1.93 ± 0.11			
SSD <sub>0.95</sub> c/ha	39.7 ± 2.9			
Loshtak	Control	239.8		
	Biohumus	360.0	120.2	50.12
	N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	289.0	49.2	20.5
	Biohumus+ N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	436.0	196.2	81.8
S <sub>x</sub> %	2.06 ± 0.14			
SSD <sub>0.95</sub> c/ha	23.9 ± 3.4			

**Table 3.** Physico-chemical parameters of soils in vegetation pots after the end of the vegetation period

Variant	Humus content, %	p <sup>H</sup>	Mechanical composition: <0,01mm, %
Control	3.1	7.5	31.7
Biohumus	5.4	7.8	30.8
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	3.1	7.4	31.7
Biohumus+ N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	5.4	7.6	35.0

The presented data highlight the positive impact of biohumus on key soil physicochemical properties:

- **Humus content** increased significantly with the application of biohumus—from 3.1% in the control to 5.4%, indicating improved soil fertility.

- **Soil pH** showed a slight rise, especially in the biohumus treatments, making the soil environment closer to neutral and more favorable for nutrient uptake.

- **Mechanical composition** (particles <0.01 mm)

slightly increased in the combined treatment (Biohumus + N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>), suggesting improved soil texture and moisture retention capacity.

In summary, both standalone and combined applications of biohumus improved soil quality, enhancing soil structure and nutrient status (Table 3).

**Table 4.** Physico-chemical parameters of soils in vegetation pots after the end of the vegetation period

Variant	Available nutrients, mg/100g in the soil		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Control	4.16	6.9	32.4
Biohumus	6.24	15.0	71.0
N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	5.20	16.3	35.0
Biohumus + N <sub>60</sub> P <sub>60</sub> K <sub>60</sub>	7.28	24.0	63.8

The results demonstrate that biohumus application significantly improved the availability of essential nutrients in the soil:

- **Nitrogen (N):** Increased from 4.16 mg/100g in the control to 6.24 mg/100g with biohumus, and up to 7.28 mg/100g in the combined treatment, showing enhanced nitrogen enrichment.

- **Phosphorus (P<sub>2</sub>O<sub>5</sub>):** The most notable increase occurred with the combined application (24.0 mg/100g), indicating strong synergistic effects between biohumus and mineral fertilizers.

- **Potassium (K<sub>2</sub>O):** Biohumus alone led to a substantial rise in potassium availability (71.0 mg/100g), much higher than either the control or the mineral fertilizer treatment, suggesting its high potassium-releasing potential (Table 4).

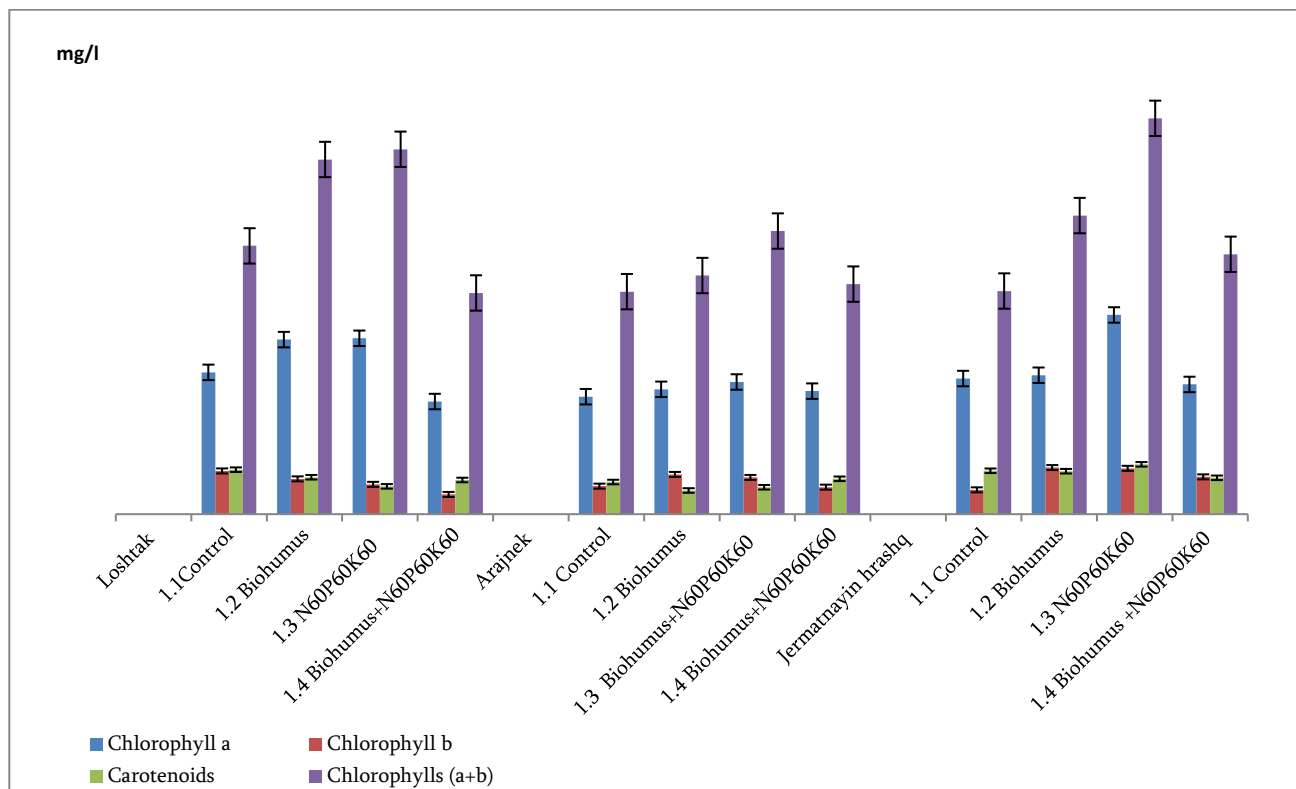
Biohumus application, particularly in combination with mineral fertilizers, substantially improved soil nutrient status, thereby enhancing conditions for plant nutrition and crop productivity. Analysis of soil samples taken from the vegetation cabin after the growing season showed that, under the 20 t/ha biohumus treatment, the humus content in the 0–20 cm layer increased 1.74-fold compared with the control. In the same treatment, the

levels of plant-available nitrogen, phosphorus, and potassium increased by 1.5, 2.17, and 2.19 times, respectively. The soil remained strongly carbonated under these conditions (Tables 3-4).

In the mineral fertilizer treatment (N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>), humus content rose 1.3-fold relative to the control, while available nitrogen, phosphorus, and potassium increased by 1.25, 2.36, and 1.08 times, respectively. The was, and the soil's carbonate status remained unchanged (Tables 3-4).

The combined treatment of biohumus (20 t/ha) with mineral fertilizers (N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>) produced the most pronounced improvements. In this case, humus content in the 0–20 cm layer increased 1.74-fold compared with the control, while available nitrogen, phosphorus, and potassium rose by 1.75-, -, 3.4-, and 1.9-fold, respectively. No significant changes were observed in soil reaction or in the proportion of water-stable aggregates, and the soil remained strongly carbonated (Tables 3-4).

These results indicate that the integrated use of biohumus and mineral fertilizers represents an effective strategy for improving the agrochemical properties of soils, ensuring greater nutrient availability, and supporting sustainable crop production.



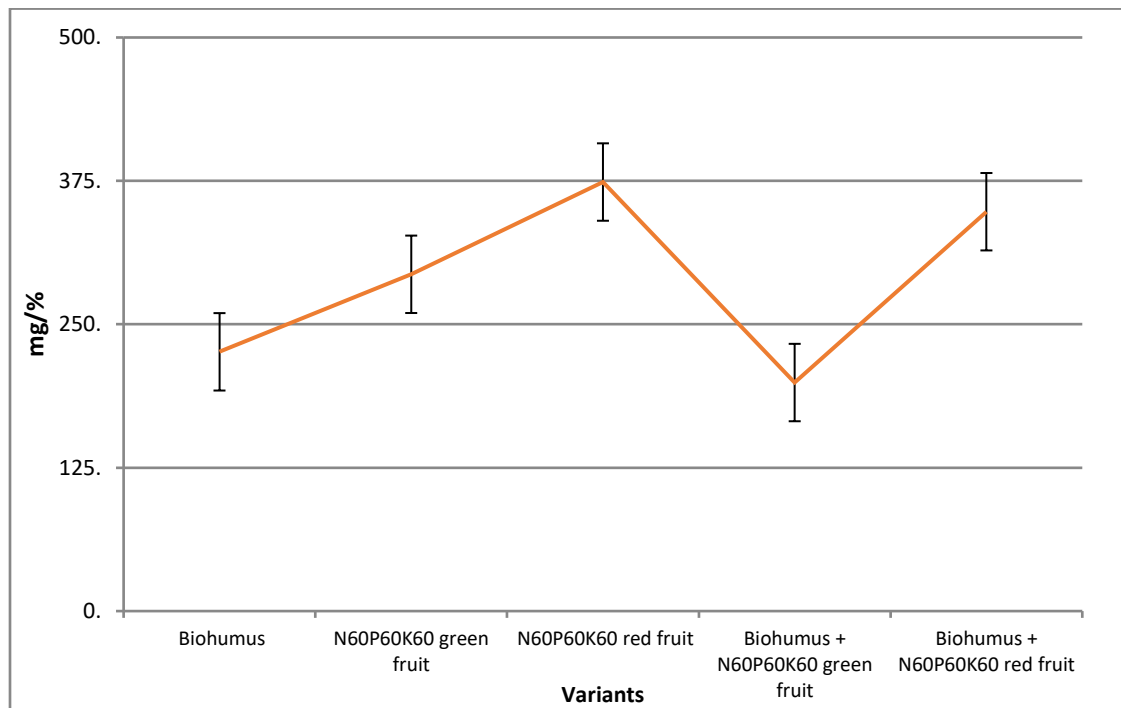
**Figure 1.** The effect of organic versus chemical fertilization on the accumulation of photosynthetic pigments—chlorophyll a, chlorophyll b, and carotenoids—in the leaf tissues of pepper cultivars Loshtak, Arajnek, and Jermatnayin Hska.

Elevated temperature and water scarcity have a considerable effect on plant photosynthetic performance. Under such stress conditions, chlorophyllase activity tends to increase, leading to the breakdown of the chlorophyll–lipoprotein complex. Tracking these biochemical changes enables evaluation of the effects of nutrient supply and drought stress on plant adaptability. Earlier reports also indicate that inoculation with rhizobacteria can be an effective strategy to improve growth, enhance antioxidant capacity, and maintain photosynthetic pigments in basil under limited water availability [38-39].

The spectrophotometric analysis of acetone extracts from experimental pepper plants showed that photosynthetic pigment concentration was higher in

fertilized treatments than in the control. The total chlorophyll (a + b) content reached 18.8 mg/L, while chlorophyll “a” was 9.353 mg/L, exceeding the control values of 15.0 mg/L and 7.795 mg/L, respectively. Similarly, carotenoid levels increased in all fertilization treatments. An elevated chlorophyll a/b ratio was observed across all variants, indicating improved tolerance to elevated temperature and drought stress when grown under 1) Biohumus, 2) N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>, and 3) Biohumus + N<sub>60</sub>P<sub>60</sub>K<sub>60</sub> conditions (Fig. 1) [39].

Overall, the results suggest that biohumus and mineral fertilizers, both separately and in combination, contribute to higher pigment accumulation. This, in turn, supports better photosynthetic efficiency and enhances plant resilience under water-deficient environments.



**Figure 2.** Comparative impact of organic and synthetic fertilizers on ascorbic acid (vitamin c) levels in pepper plant tissues

Several factors influence ascorbic acid accumulation in fruits, including climatic conditions, temperature fluctuations, and soil nutrient availability. Since temperature regimes vary across regions, they play a decisive role in shaping the biochemical composition of plant tissues during growth and development. In this study, ascorbic acid content was determined by iodometric titration. The results revealed that fruits harvested at the stage of technical maturity contained higher levels of ascorbic acid compared to those collected at biological maturity. Among the tested variants, the highest concentration of ascorbic acid was recorded in *Jermatnayin hska* fruits cultivated under  $N_{60}P_{60}K_{60}$  treatment (Fig. 2).

**Scientific Innovation and Practical Implications:** This study introduces a novel approach to enhancing the nutritional and ecological value of pepper cultivation by utilizing microbial biohumus derived from non-toxic organic wastes. The innovative aspect lies in the

integration of biohumus with traditional chemical fertilizers, demonstrating that this combination can significantly improve pepper yield and ascorbic acid content compared to conventional methods.

From a scientific perspective, the research contributes to the growing body of knowledge on sustainable agricultural practices. It provides empirical evidence supporting the use of biohumus as a viable alternative or supplement to chemical fertilizers, aligning with global trends towards organic farming and soil health restoration.

Practically, the findings have several implications:

**Sustainable Agriculture:** The use of biohumus promotes soil fertility and structure, reducing dependence on synthetic fertilizers and mitigating environmental pollution.

**Economic Viability:** For farmers, especially in developing regions, biohumus offers a cost-effective solution by recycling local organic waste into valuable soil

amendments, potentially lowering input costs and increasing profitability.

**Public Health:** By enhancing the nutritional profile of peppers, particularly their vitamin C content, the study supports the development of functional foods that can contribute to improved human health outcomes.

## CONCLUSION

It was shown that applying biohumus as a biofertilizer increased the bulk yield of three pepper varieties and contributed to the recycling of farm waste. It also allowed achieving the specific yield (per plant) level provided by traditional fertilizers. As the difference between the pepper plant variants and the control exceeds the SSD, the difference is real, and there is a crop supplement. Our study clearly demonstrated the multiple benefits of combining biohumus with inorganic fertilizer (NKP). The results indicate the possibility of partial replacement of chemical fertilizers with biofertilizers (microbial biohumus) in vegetable crop culture farming.

**Abbreviations:** NPK- nitrogen (N), phosphorus (P), and potassium (K)—the main combination of nutrients essential for plant growth, Sx%- Standard error expressed as a percentage, SSD<sub>0.95</sub>- Significant Statistical Difference at the 0.95 confidence level.

**Acknowledgement:** The authors express their sincere gratitude to the administration of the «H. Petrosyan Scientific Center of Soil Science, Agrochemistry and Melioration» branch at the Armenian National Agrarian University Foundation for their invaluable support and assistance throughout this research.

**Contributions:** TJ-conceptualization, methodology, data, Authors' curation, resources, writing-original draft preparation, writing-review, editing, and supervision,

GG-conceptualization, methodology, validation, resources, data curation, writing-original draft preparation, writing-review, and editing, MB-resources, writing-review and editing; IB- resources, writing review and editing; SD-conceptualization, validation, resources, data curation, editing and supervision, SH-resources, writing review and editing, data curation.

All authors read and approved the final version of the manuscript.

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