IMPROVEMENT OF QUALITATIVE COMPONENTS AND YIELD WITH THE CONTRIBUTION OF BIOLOGICAL FERTILIZERS IN ORGANIC PEPPER PRODUCTION

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Abstract. The European Green Directive includes a series of requirements in food production aimed at reducing the emission of gases that contribute to increased climate change, as well as standards that define products with high biological value. Awareness of the need to use health-safe food in nutrition is steadily growing. The aim of this research was to determine the effects of applying microbiological preparations – effective microorganisms (EM) in the form of the EM Aktiv preparation and *Trichoderma spp*. (Vital Tricho – VT preparation) – on the yield and quality of pepper fruits. The research was conducted in a protected environment in 2016, 2017, and 2018 using an organic cultivation system for the Blondy F_1 pepper hybrid. The application of microbiological fertilizers included the following variants: T1 – control, T2 – EM preparation, T3 – VT preparation, T4 – EM + VT. The highest statistically significant results were obtained in treatment T4. Compared to the control, pepper yield increased by 28.38%, dry matter content by 14.05%, protein content by 28.04%, carbohydrate content by 7.16%, and vitamin C content by 10.22%. The use of these preparations makes it possible to achieve stable pepper production in organic systems, while adhering to all principles of sustainability and the production of health-safe fruits.
Keywords: *Capsicum annuum, organic production, effective microorganisms, Trichoderma sp., yield, chemical components of the fruit*

Introduction

Pepper (*Capsicum annuum* L.) is a herbaceous plant from the family Solanaceae, genus *Capsicum*. Due to its unique taste and high nutritional value, pepper is a commercially important crop that is extensively cultivated worldwide (Buczkowska et al., 2016; Sini et al., 2024). In Serbia, the pepper that is cultivated in the north is originally from Hungary, and in central and southern Serbia, the pepper was brought from Turkey in the 17th century. In the modern diet, pepper is a highly valued and useful vegetable. It can be used fresh and processed or as a herb, and it is a raw material for various types of industrial processing. In terms of nutritional value, the pepper fruit is rich in micronutrients and bioactive compounds, which can contribute to the prevention of various diseases (Baenas et al., 2019). Also, the high nutritional and biological value is reflected in the rich content of carbohydrates, proteins, vegetable fibers, oils, organic acids and mineral substances. The mineral content of peppers is of great importance, because peppers are consumed

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fresh. The minerals it contains include K, Na, Ca, Mg and others (Żurawik et al., 2020). The high biological value is reflected in the content of vitamins, capsaicin, pigments and essential oils.

With the help of a protected space (greenhouses and hothouses), the ripening period of peppers is significantly extended, so fresh peppers can be available for more than half a year. Apart from the extended ripening season, the advantages of growing peppers in a protected area are significantly higher yields per unit area. The discerning market and the high standards of the food industry encouraged breeders to develop new varieties and hybrids of peppers with required characteristics (Lozada et al., 2022). On the other hand, the increase in consumer demand for health-safe food also increased the interest of producers and experts in those methods of plant production that will enable the reduction of the consumption of synthetic inputs in modern plant production (primarily pesticides and mineral fertilizers). One of the most promising possible solutions is the use of different effective microorganisms (EM) for the development of production in agriculture of the XXI century (Bajagić et al., 2024). Pszczółkowski et al. (2023) states that EM can produce hormones and growth stimulators that affect the cell division of microorganisms and can change the structure of the rhizosphere microbial community and stimulate plant growth. Also, according to Zaini et al. (2023), EM can act as biostimulants and biodegraders, which fix nutrients and provide protection against various undesirable influences. They influence the increase of plants' resistance to stress and the content of bioactive components in the fruits. EM biotechnology is currently used in 140 countries on most continents because effective microorganisms have been shown to be the most suitable for sustainable food production systems (Cvijanović et al., 2022). According to research by Rajičić et al. (2025), microbiological fertilizers make a great contribution to increasing the productive properties of cultivated crops, as they support and strengthen existing microorganisms in the soil and maintain a strong symbiosis with the root system in the rhizosphere, where the exchange of food and other biologically active substances takes place, benefiting both microorganisms and plants. Microorganisms are fungi from the genus Trichoderma that affect the protection of plants from pathogens and are recommended for use in organic plant growing systems. Numerous experiments have shown that most strains from this genus have a positive effect not only on plant growth and development, but also on yield (Di Mola et al., 2023), antagonism with some pathogenic microorganisms and some pests that attack cultivated plant species (Yang et al., 2010). Effective research results were obtained by Ismail et al. (2024), where five different Trichoderma isolates showed a certain level of tolerance to insecticides and potential for their removal or biodegradation, implying the use of *Trichoderma* as an important tool in pesticide degradation.

Modern agriculture is once again returning to organic agrotechnical methods and the application of alternative preparations that are allowed for use in integral and organic production. Sajid et al. (2024) state that the use of various growth promoters permitted for sustainable production, when applied foliarly, increases crop productivity, improves the nutritional quality of the fruit, and reduces the need for conventional fertilization. The tendency is to introduce into production varieties that are tolerant to diseases and pests and to achieve higher yields in an environmentally friendly way. Therefore, there is an immediate need to find environmentally acceptable solutions, such as the wider application of biocontrol agents (Zin and Badaluddin, 2020; Alaylar, 2022). Defining organic agriculture is different, but the main association is that it is the production of food without the use of pesticides and mineral fertilizers.

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The aim of the research is to determine the effects of the application of microbiological preparations - effective microorganisms (EM) (preparation EM Aktiv) and *Trichoderma spp.* (preparation Vital Tricho) and variable agroecological components in the protected area (marked as years) on the yield and quality of pepper fruits in the organic production system.

Materials and methods

Experimental research and planting

Research was carried out during 2016, 2017 and 2018 (factor A) in a high-rise greenhouse in Bačka Topola (secondary agricultural school), Serbia on carbonate chernozem. The trial was set up in a randomized design with 3 replications. The area of the experimental field in the protected area was 97.94 m^2 (including the frame paths), the area of the block was 16.60 m^2, and the area of the basic experimental plot was 5.46 m^2. Treatments with microbiological preparations (factor B) were in variants: T1 control (no treatment), T2 – treatment with a liquid microbiological preparation containing effective microorganisms (EM) - EM Aktiv, T3 – treatment with a preparation Vital Tricho containing specific strains of fungi, *Trichoderma sp*. T2 and T3 were applied to the soil and foliarly 3 times during the growing season at different stages of growth and development. T4 - a combination of these two preparations - soil treatment with Vital Tricho and foliar treatment 3 times during the growing season in different stages of growth and development with a liquid microbiological preparation containing effective microorganisms (EM).

Sowing of the Blondy F_1 pepper variety/hybrid was carried out in crates filled with Klasman TS1 commercial substrate, which is on the national list of Serbia’s registered plant nutrition and soil conditioners intended for organic production. After sprouting and the onset of the phase of the appearance of the first true leaves, pricking was carried out in pots filled with organic substrate. Sheep and cattle manure was used for the basic treatment, in the amount of 3 kg/m^2, and before planting the pepper, additional soil treatment and soil preparation for planting was carried out with the help of a rotary tiller. Pepper planting was done manually in double rows 40 cm wide with a distance of 25 cm between the plants in the rows. The elementary plots were 90 cm apart from each other within the block, and the isolation zone between the blocks was 1 m. During planting, experimental plots were treated (soil treatment) with a 1.0% solution of preparation (EM Aktiv) based on effective microorganisms (EM) and a 0.4% solution of preparation Vital Ticho based on *Trichoderma sp.* Foliar treatments were applied in the amount of EM Aktiv 2% and Vital Tricho 0.04%, the first foliar treatment after 15 days from planting, the second and third foliar treatments were performed at the beginning of the flowering phase and in the full flowering phase. After planting, an irrigation system was installed. Weed control in the experimental plot was done manually. No attacks from diseases and pests were registered. The first vegetable in each year of the study was lettuce.

A variety of pepper

In the experimental field trial, the hybrid pepper Blondy F_1 was used (*Photo 1*). Extremely large and native light yellow babura. An early hybrid with very strong roots and a powerful plant of unlimited growth and high fertility potential. The average weight of the fruit is over 250 g, the length is 10 cm and the width is about 8 cm. It is very

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resistant to fruit top rot (spotting). It behaves well in transport and retains freshness the longest of all hybrids. For the cultivation of this pepper, typical agrotechnical measures are used, without special conditions. The agroclimatic conditions are, as with most hybrids, a heat-loving plant, the optimal temperature for growth and development is 22-25°C. It is a big consumer of water, which is ensured by precipitation and irrigation per turn, minimum 30-40 mm, that is 30-40 l m^2.

***Photo 1.** Peppers in the planting stage and after picking (Source: Authors)*

Microbiological preparations

In an experiment in a protected area - a greenhouse, a microbiological fertilizer with effective microorganisms (EM Aktiv) and *Trichoderma sp*. (Vital tricho), which are on the official list of permitted means for use in organic production in the Republic of Serbia (http://www.uzb.minpolj.gov.rs/attachments/280_LIsta_SIB_organska_24mart2016.pdf). EM active is a concentrate in a liquid state, in which more than 80 strains of the main anabiotic organisms that are naturally found in the soil have been grown (photosynthetic bacteria *(Rhodopseudomonas palustris*, *Rhodobacter sphaeroides)*, milk bacteria *(Lactobacillus plantarum, L. casei, Streptococcus lactis)*, yeasts *(Saccharomyces cerevisiae, Candida utilis)*, actinomycetes *(Streptomyces albus, Streptomyces griseu)* and fungi *(Aspergillus oryzae*, *Mucor hiemalis)*. The preparation does not contain genetically modified microorganisms, but a solid community of aerobic and anaerobic microorganisms. *Trichoderma sp*. is an integral part of the commercial bio preparation Vital Tricho, which serves to control pathogenic fungi (eg *Phytophtora spp*., *Phytium spp., Fusarium spp*., *Rhizoctonia spp*.). Vital Tricho is a microbiological preparation that contains antagonistic fungi *Trichoderma viride* and *Trichoderma asperellum* in a concentration of 5×10^9, which are grafted onto an inorganic soil improver. It is used as a means of biological control of pathogenic microorganisms, to improve soil structure and create favorable conditions for plant growth and development. Vital Tricho does not contain genetically modified organisms, nor was it obtained with the help of genetically modified organisms.

The sampling

Successive harvesting was done in five terms. Fruits were picked at technological maturity, pale yellow in color. The total yield (kg) per unit area (m^2) was measured on ten plants per elementary plot. Based on the collected sample, the average weight of the fruit was determined (per repetition, within one treatment), which was used to prepare average samples for determining the chemical properties of the fruit. The chemical properties of the fruit were performed in the laboratory of the Superlab Institute, Belgrade. Chemical properties of the fruit: dry matter content (%), protein content (%), carbohydrate content (%), vitamin C (ascorbic acid) content (mg/100 g).

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Methods of research

The yield was determined as the number of fruits per unit area per m^2. The chemical properties of the fruit were performed in the laboratory of the Superlab Institute, Belgrade. Dry matter was determined using the method proposed by Džamić et al. (1999). The sample in the amount of 3-5 g was placed in vege glasses, and then dried at a temperature of 100°C for a period of 4 to 6 h. After re-measurement, the proportion of dry matter in the fruit was calculated (%).

Vitamin C content was determined with the help of “HPLC” liquid chromatography. The collected samples were crushed with a blender, and metaphosphoric acid (20 ml) was added to an amount of 1 to 5 g of each sample with intensive mixing. The extract was centrifuged for 15 mins. Then, filtered and diluted to 25 ml with metaphosphoric acid. According to the standard procedure, the samples were analyzed with the help of an HPLC apparatus at a wavelength of 245 nm. Crude protein amount was calculated from total nitrogen content using Kjeldahl method (MFDS, 2019). Carbohydrates were calculated by 100% minus sum of % protein, % lipid, and % ash.

Weather conditions

During the three-year experimental trial, temperature (°C) and relative air humidity (%) were measured for each year with a digital thermometer or hygrometer (type Class 303c, producer: China) (*Table 1*). The temperature was affected by the temperature of the air outside the greenhouse, as well as the relative humidity of the air, as well as timely ventilation of the greenhouse. The irrigation process and watering rates were the same for each year (572 l/m^2) (*Table 2*).

Table 1. *Average values of mean monthly air temperatures (°C) and relative humidity air (%)*

Year	Temperatures (°C)						Relative humidity air (%)					
	April	May	June	July	August	Avg.	April	May	June	July	August	Sum
2016	21.2	22.8	20.8	26.9	24.1	**23.2**	49.0	37.7	38.7	39.6	39.6	**40.9**
2017	22.7	24.3	22.2	28.2	25.7	**24.7**	58.1	53.0	54.2	54.9	54.9	**55.0**
2018	24.5	29.2	26.2	33.4	31.2	**28.9**	56.7	51.7	53.2	54.5	54.5	**54.1**

Table 2. *Total sums of irrigation (l/m^2) per month per year of research*

Part of the month	April	May	June	July	August
I	7	25	55	50	40
II	10	40	55	50	40
III	15	40	55	50	40
Sum	**32**	**105**	**165**	**150**	**120**

The average monthly temperature for the entire vegetation period of pepper in 2016 was 23.2°C, in 2017 24.7°C and 28.9°C in 2018, while the relative humidity in 2016 was 40.9%, in 2017 55.0% and 2018 54.1%. The most favorable year in terms of measured values of temperature and relative air humidity, which were measured three times and the average values were shown, was 2017. The reason for this is that the experiment was set

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up in semi-controlled conditions - a high block greenhouse system, so it was also influenced by the level of the external temperature.

Statistical analysis

Statistical data analysis was done with the help of IBM SPSS Statistics software, version 26.0. An analysis of variance (ANOVA) was applied to assess the impact of the factors with significance levels set at $p < 0.05$ and $p < 0.01$. To compare mean values between treatments, the Tukey test (Tukey's Honestly Significant Difference test, Tukey HSD) was performed. Minitab Statistical Software (Trial version) used for Pearson's correlation analysis was conducted to determine the relationship between the studied traits, as well as for visualizing the relative importance of different factors affecting the variables and their interactions. The results are presented tabularly and graphically.

Results

The average pepper yield ranges from 17.17 kg m^2 to 23.40 kg m^2. *Figure 1* shows how the average yield value changes depending on the factors of the year and applied different treatments. Each point on the graph represents the average yield for a specific combination of year and treatment. Lines extending above and below each point represent the 95% confidence interval for the mean. It tells about the certainty (95%) that the actual mean return value for that combination lies within that interval. The wider the interval, the greater the variability of those combined factors. Confidence intervals were calculated based on the standard deviation of the data for each individual combination of factors, variability within each group was taken into account. Treatments with higher pepper yields and narrower confidence intervals are more effective.

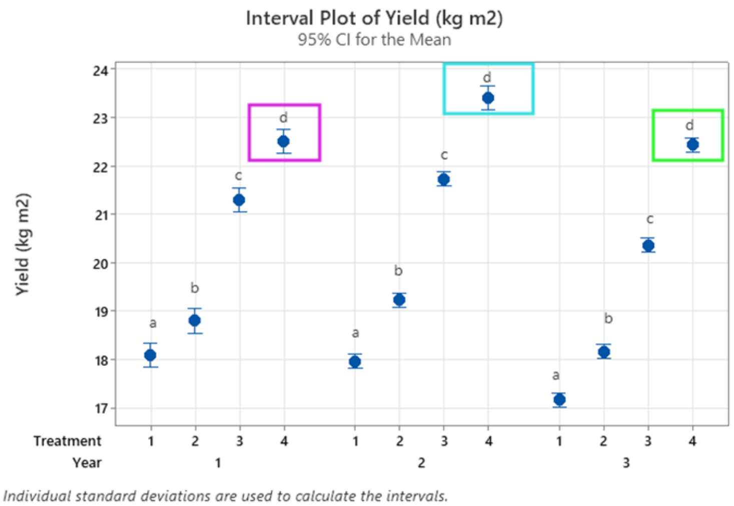

Figure 1. *Interval plot of yield (kg m^2) with 95% confidence interval for the mean value. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – Vital Tricho + EM Aktiv - treatments with different combinations of fertilizers applied during the study period (2016-2018). Letters above dots indicate statistically significant differences between treatments. Different letters indicate significant differences, while the same letters indicate no significant difference between treatments*

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In the first year of research, the highest average yield was achieved in treatment 4 (22.50 kg m^2), that trend continued for the same treatment 4 in the second year (23.40 kg m^2), then in the third (22.43 kg m^2). It is observed that in the third year of the research, the yield is slightly lower compared to the first two, but with a narrower confidence interval, which indicates a better effectiveness of the treatment. Significantly lower yields were achieved in all three years of research in control treatment 1 (17.17 kg m^2 - 18.10 kg m^2) and treatment 2 (18.17 kg m^2 -19.23 kg m^2). *Figure 2* clearly provides a visual representation of the complex yield analysis, allowing to compare the impact of different years on yield height and the effectiveness of different treatments. The yield of peppers is influenced by complex relationships between factors (*Table 3*) and the optimal yield is achieved by combining individual treatments in a given year.

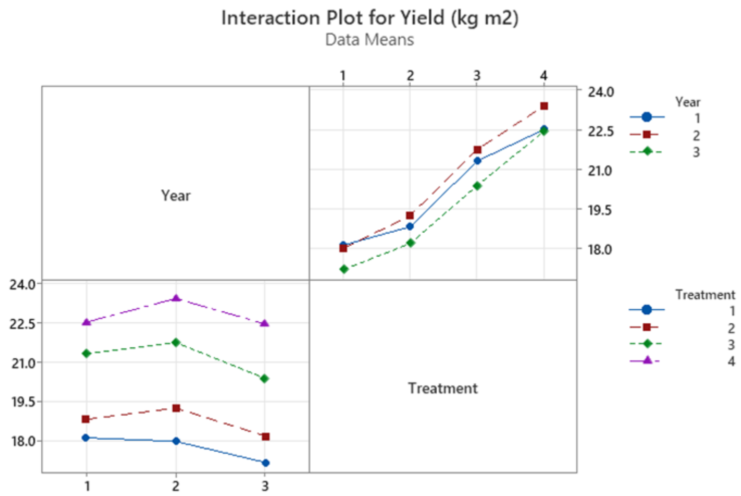

Figure 2. *Interaction plot of yield (kg m²). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018)*

Table 3. *Analysis variance for yield (kg m²)*

Source	df	SS	MS	F-Value	P-Value
Year (A)	2	6.724	3.3619	550.14**	0.000
Treatment (B)	3	140.892	46.9640	7685.02**	0.000
Year*Treatment (AB)	6	1.207	0.2012	32.92**	0.000
Error	24	0.147	0.0061		
Total	35	148.970			
St.Dev. 2.0631 C.V. (%) 0.27					

**Highly significant at $p < 0.01$ probability level; df-Degree of freedom; SS-Sum of squares; MS-Mean Squares; F-F value calculated; p-level-p value calculated. St.Dev.-Standard Deviation, C.V.- Coefficient of variation

The factor Year (A) has a statistically significant influence on the achieved grain yield values. Average yield values differ significantly between survey years. The treatment (B) factor also has a statistically very significant influence on the achieved values for this trait. With the application of different treatments, different pepper yields are achieved.

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The interaction of factors (*Table 3*) year and treatments (AB) shows statistically very significant differences in the yield values obtained. The influence of one factor on grain yield changes depending on the level of another factor (*Fig. 2*).

The quality of the fruit was based on the analysis of the chemical properties of the fruit, for the following contents: dry matter, proteins, carbohydrates and vitamin C (ascorbic acid).

Dry matter content generally increased with treatments (*Fig. 3*). In the first year of research, control treatment (1) and treatment 2 have approximate values (0.31%, 0.33%). The highest content was achieved in treatment 4 (0.46%). A similar trend continues in other years, where the highest content was 0.56% (in the third year in treatment 4). A statistically significant difference between the treatments was found, which means that different treatments affect the content of dry matter (*Fig. 4; Table 4*). Variations between years were also found, which indicates that external factors (such as climate conditions, soil) influence the obtained values.

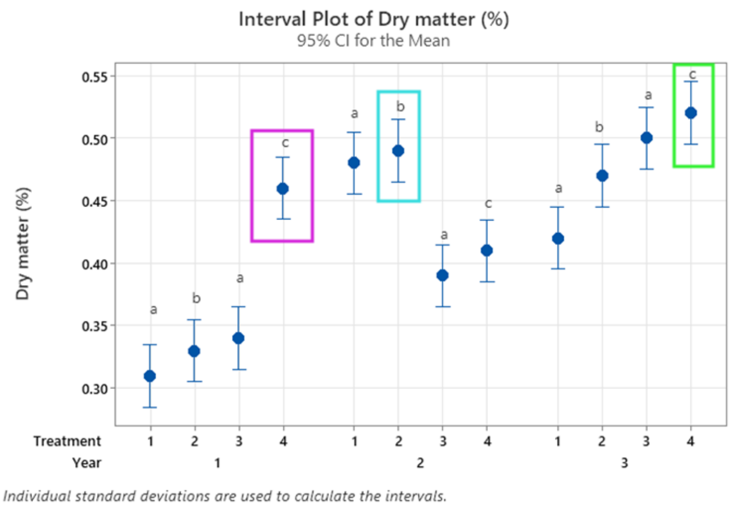

Figure 3. *Interval plot of dry matter (%) with 95% confidence interval for the mean value. Different letters (a, b, c) indicate significant differences (p < 0.05). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018). Letters above dots indicate statistically significant differences between treatments. Different letters indicate significant differences, while the same letters indicate no significant difference between treatments*

Protein content. Visualization of the relative importance of different factors that influence the protein content of pepper fruit are shown in *Figure 5,* with average values for different treatments in the examined years (*Table 5*). The protein content in treatment 1 is consistently lower compared to the other treatments. In treatments 2, 3 and 4, the protein content increases sharply compared to treatment 1. In the first year of the study, treatment 1 has the lowest protein content (0.94%), while treatment 4 has the highest (1.21%). Years 2 and 3 (red and green lines) follow a similar trend with slightly higher values compared to year 1. In the second year, treatment 1 again has the lowest protein content, while treatments 3 (1.21-1.23%) and 4 (1.23-1.28%) are significantly higher in protein content. In the third year, treatment 1 again has the lowest protein content, while the other treatments are at a higher level.

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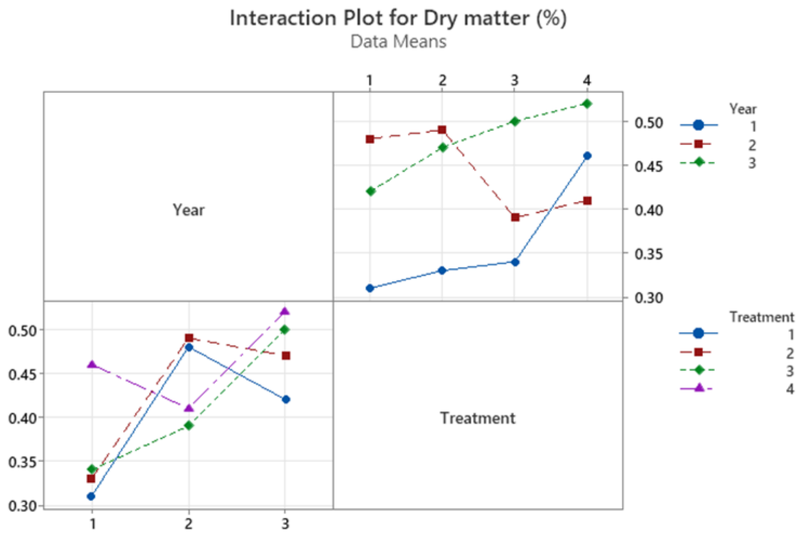

Figure 4. *Interaction plot of dry matter (%). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018)*

Table 4. *Analysis variance for content dry matter (%)*

Source	df	SS	MS	F-Value	P-Value
Year (A)	2	0.087350	0.043675	436.75**	0.000
Treatment (B)	3	0.019600	0.006533	65.33**	0.000
Year*Treatment (AB)	6	0.061250	0.010208	102.08**	0.000
Error	24	0.002400	0.000100		
Total	35	0.170600			
St. Dev. 0.06982 C.V. (%) 2.14					

**Highly significant at $p < 0.01$ probability level; df-Degree of freedom; SS-Sum of squares; MS-Mean Squares; F-F value calculated; p-level-p value calculated. St.Dev.- Standard Deviation, C.V.- Coefficient of variation

Table 5. *Analysis variance for content protein (%)*

Source	df	SS	MS	F-Value	P-Value
Year (A)	2	0.034350	0.017175	772.88**	0.000
Treatment (B)	3	0.470589	0.156863	7058.83**	0.000
Year*Treatment (AB)	6	0.001628	0.000271	12.21**	0.000
Error	24	0.000533	0.000022		
Total	35	0.507100			
St.Dev. 0.12037 C.V. (%) 0.37					

**Highly significant at $p < 0.01$ probability level; df-Degree of freedom; SS-Sum of squares; MS-Mean Squares; F-F value calculated; p-level-p value calculated. St.Dev.-Standard Deviation, C.V.- Coefficient of variation

In *Figure 6,* we observe the trends of the lines, which are parallel, indicating the absence of interactions between the factors. The effect of one factor is the same

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regardless of the level of the other factor. Lines that are not parallel indicate that there are interactions between factors and that the effect of one factor depends on the level of another factor. The interaction of the factor AB had a statistically very significant effect on the obtained values of the protein content in the pepper fruit. Considering the influence of factors and their statistical significance (*Table 5*), the production process can be optimized with the application of appropriate agrotechnical measures in a given year.

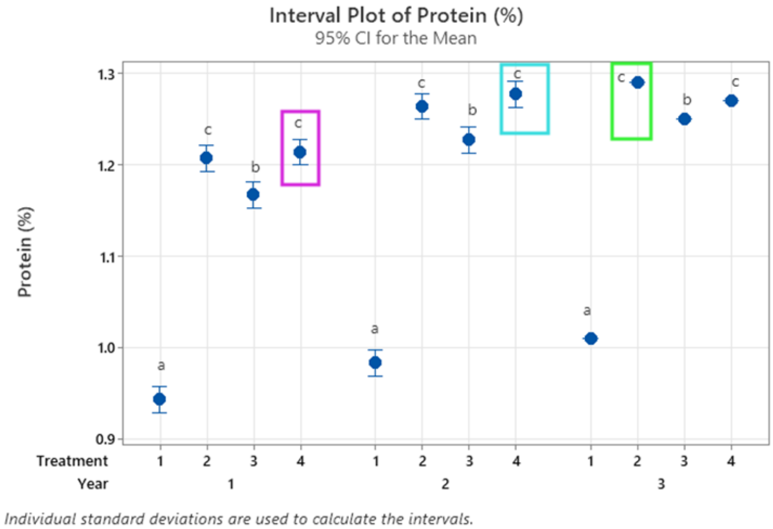

Figure 5. *Interval Plot of protein (%) with 95% confidence interval for the mean value. Different letters (a, b, c) indicate significant differences (p < 0.05). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018). Letters above dots indicate statistically significant differences between treatments. Different letters indicate significant differences, while the same letters indicate no significant difference between treatments*

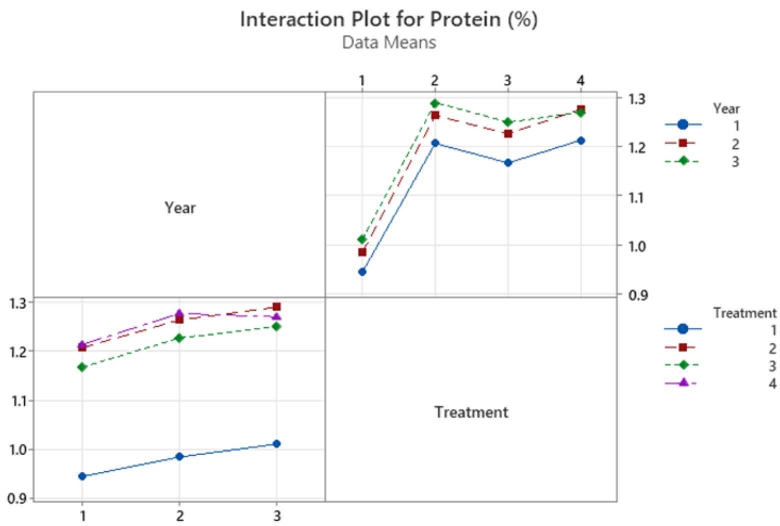

Figure 6. *Interaction Plot of protein (%). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018)*

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The content of carbohydrates. In the first year of research, the lowest carbohydrate content was measured in treatment 1 (3.50%), while in treatment 4 the content was 3.74%. The obtained values of carbohydrate content (*Fig. 7*) in all treatments have a higher carbohydrate content than in the first year. Statistically significant differences were present between all treatments (*Table 6*).

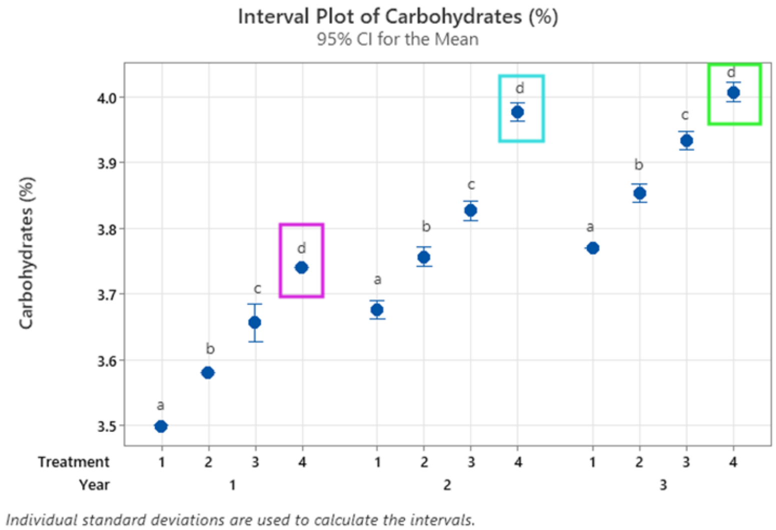

Figure 7. *Interval Plot of carbohydrates (%) with 95% confidence interval for the mean value. Different letters (a, b, c, d) indicate significant differences (p < 0.05). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018). Letters above dots indicate statistically significant differences between treatments. Different letters indicate significant differences, while the same letters indicate no significant difference between treatments*

Table 6. *Analysis variance for content carbohydrates (%)*

Source	df	SS	MS	F-Value	P-Value
Year (A)	2	0.466289	0.233144	7630.18**	0.000
Treatment (B)	3	0.328297	0.109432	3581.42**	0.000
Year*Treatment (AB)	6	0.006644	0.001107	36.24**	0.000
Error	24	0.000733	0.000031		
Total	35	0.801964			
St. Dev. 0.15137 C.V. (%) 0.14					

**Highly significant at p < 0.01 probability level; df-Degree of freedom; SS-Sum of squares; MS-Mean Squares; F-F value calculated; p-level-p value calculated. St.Dev.-Standard Deviation, C.V.- Coefficient of variation

The biggest statistical difference is between treatments 1 and 4 (*Fig. 8*). Altered treatment 4 consistently shows the highest carbohydrate content across all years. The overall trend shows an increase in carbohydrate content over the years for all treatments. There are significant differences between the treatments, which means that the treatments have a strong influence on the carbohydrate content. In the third year, the carbohydrate content continues to increase for all treatments (3.77-4.01%).

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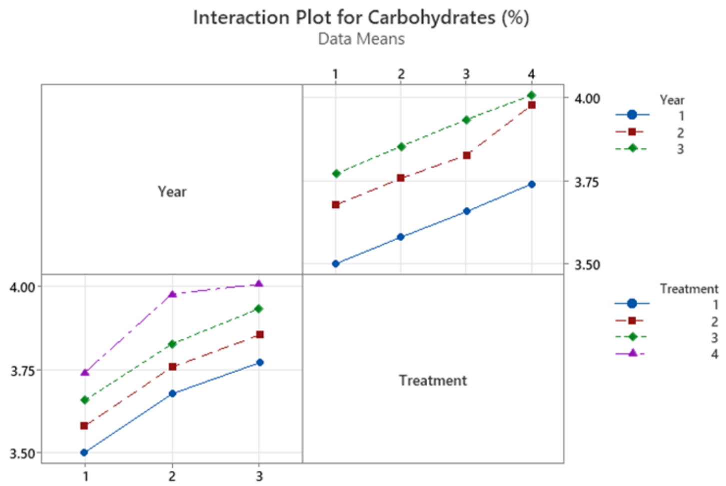

Figure 8. *Interaction plot of carbohydrates (%). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018)*

The content of vitamin C (ascorbic acid). Pepper fruits are extremely rich in ascorbic acid, i.e. vitamin C. In fresh pepper fruits, the content of vitamin C varies greatly because it depends on many factors (genotype, environmental conditions (temperature, light intensity and agrotechnical measures) and usually ranges from 100-300 mg in 100 g of fruit. Significant variability in the amount of vitamin C is observed due to different treatments and years (*Fig. 9; Table 7*). The vitamin C content increases over the years, especially in treatment 4 (199.43-214.30 mg^{-1}). The obtained values are statistically significant compared to the control treatment 1. The level of vitamin C changes depending on the combination of treatment 1 and treatment 2. The highest average content of vitamin C is in the third year (214.30 mg 100 g^{-1}) with by a very narrow confidence interval, which indicates a small variability in the results and greater certainty of the assessment.

Table 7. *Analysis variance for content vitamin C (mg 100 g^{-1})*

Source	df	SS	MS	F-Value	P-Value
Year (A)	2	1300.62	650.308	53207.00**	0.000
Treatment (B)	3	2041.63	680.543	55680.76**	0.000
Year*Treatment (AB)	6	1.41	0.235	19.21**	0.000
Error	24	0.29	0.012		
Total	35	3343.95			
St. Dev. 9.7745 C.V. (%) 0.05					

**Highly significant at $p < 0.01$ probability level; df-Degree of freedom; SS-Sum of squares; MS-Mean Squares; F-F value calculated; p-level-p value calculated. St.Dev.-Standard Deviation, C.V.- Coefficient of variation

In *Figure 10,* the largest statistical difference is between treatments 1 and 4. In all years of the study, treatment 4 had the greatest effect on increasing vitamin C, as did all treatments compared to the control. Significant differences were observed among the treatments, implying a strong effect on vitamin C content.

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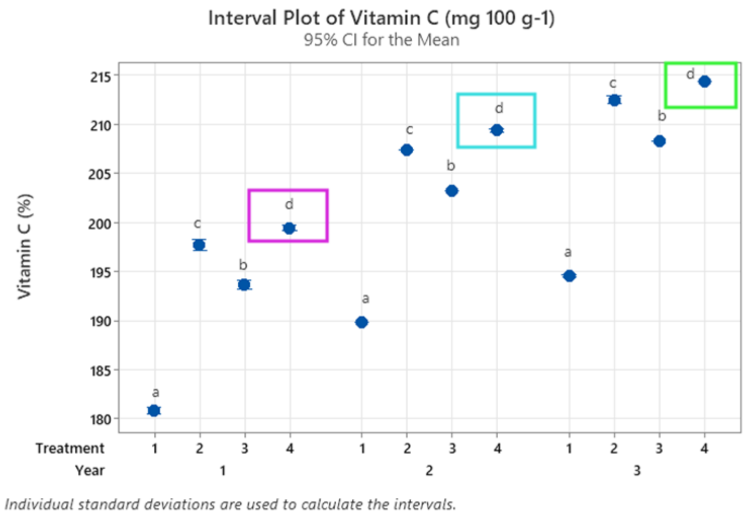

Figure 9. *Interval Plot of vitamin C (mg 100 g*$^{-1}$*) with 95% confidence interval for the mean value. Different letters (a, b, c, d) indicate significant differences (p < 0.05). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018). Letters above dots indicate statistically significant differences between treatments. Different letters indicate significant differences, while the same letters indicate no significant difference between treatments*

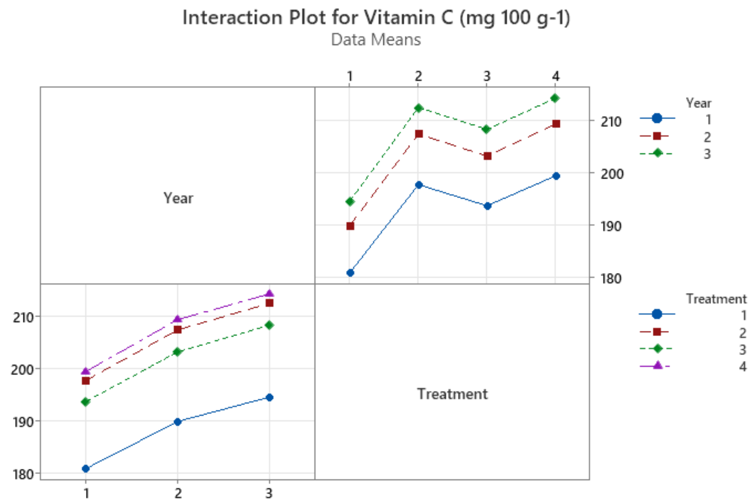

Figure 10. *Interaction plot of vitamin C (mg 100 g*$^{-1}$*). Year: 1 – (2016); 2 – (2017); 3 – (2018). Treatment: 1 – control; 2 – EM Aktiv 3 – Vital Tricho; 4 – EM Aktiv + Vital Tricho - treatments with different combinations of fertilizers applied during the study period (2016-2018)*

The correlation coefficient gives a clear insight into the degree of association between traits and indicates whether simultaneous improvement of correlated traits is possible or not (*Fig. 11*).

A positive and moderately high correlation was found between yield and vitamin C (r = 0.47**), between yield and carbohydrates (r = 0.55**), as well as yield and protein (r = 0.61**), while a positive but very low correlation was also found between yield and dry matter (r = 0.13).

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The correlation between the properties of dry matter and vitamin C is positive and of medium strength (r = 0.65**), as well as for dry matter and carbohydrates (r = 0.69**). In contrast, in the dry matter and protein properties, where a low correlation was established (r = 0.39*). A positive and very high correlation was established between the properties of protein and carbohydrates (r = 0.90**), as well as between carbohydrates and vitamin C (r = 0.87**).

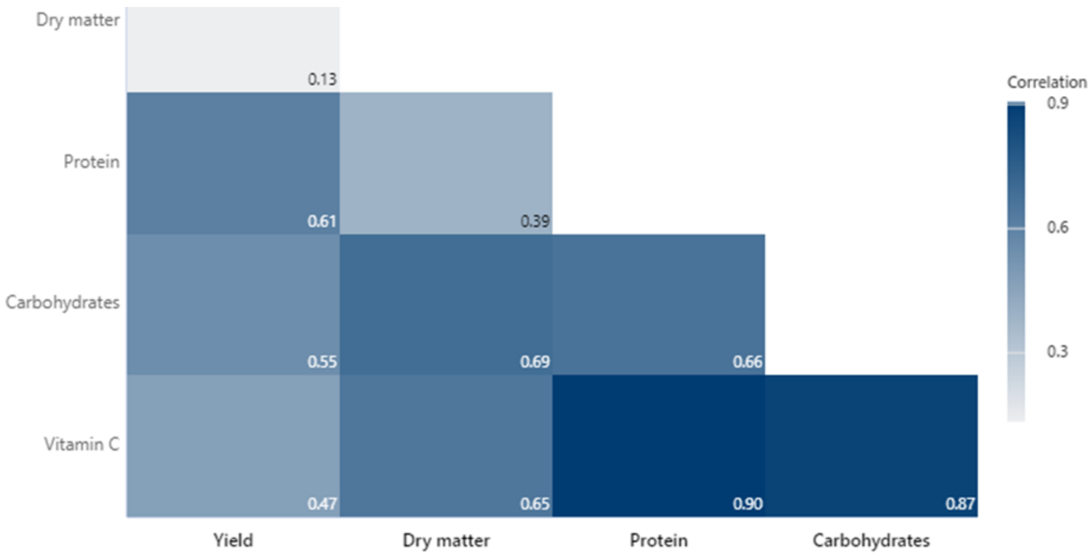

Figure 11. *Correlation coefficient between the studied traits (yield, dry matter, protein, carbohydrates, vitamin C). **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level*

Discussion

Considering that in our research the year had a statistically significant influence on the pepper yield and the biological components according to the given results, they are correlated with the research of Caruso et al. (2019) also noted that the year, as an investigated factor, had a statistically significant effect on the pepper yield, and that was greater than the variety. Additionally, using two strains of endophytic bacteria, characterized as *Bacillus subtilis* and *Paenibacillus illinoinensis*, on pepper plants, under conditions of drought stress, Vigani et al. (2019) found a significant increase in the production of fresh biomass and dry matter of treated plants.

The application of different types and variants of microbiological fertilizers compared to the control showed a positive effect on the tested properties of the pepper, which may imply a change in the amount of regular fertilizer, as well as a reduction in the amount for the needs of pepper cultivation. These studies are in accordance with long-term research on peppers by Gupta et al. (2021) showed that by applying some strains of endophytic bacteria that have the property of promoting plant growth, it is possible to reduce the application of mineral fertilizers by 20% without any loss of yield. At full doses of fertilization, they recorded statistically significantly higher average fruit yields by 37% using individual strains or their combined application. Using different organic matter treatments such as cattle manure, earthworms and poultry manure with the addition of *Trichoderma viridae*, Gopinath et al. (2009) recorded average yields from 6.2 to 32.3 t ha^{-1} in a two-year experiment. These studies are in comparison with our research, because for most of the

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tested parameters the best effect was in the variants of application of biological preparations with *Trichoderma sp*. and the combination of EM Aktiv + *Trichoderma sp*.

The factors examined had a positive effect on dry matter content, especially biopreparations, and similar results were obtained by Sini et al. (2024) concluded from their studies in a protected area that dry matter and yield were higher compared to the variant where microbiological fertilizer was applied.

Microbiological preparations had a statistically significant effect on the protein content, and the variant with EM Aktiv and the combination of the two preparations the most. On the other side, examination of the effect on the protein content with the use of preparations with *Trichoderma sp.* indicate a significant increase compared to control (Duan et al., 2023). Similarly, in research by Kefale et al. (2023), red bell peppers had the highest percentage of protein (14.7%) compared to the protein content of garlic and onions.

In our research, treatment variants had a strong impact on the carbohydrate content, which increased further from the year. In the research of Soare et al. (2017) came to the conclusion that the genotype has a highly significant influence on the content of carbohydrates in the fruit of peppers. Guil-Guerrero et al. (2006) concluded that in examining the nutritional composition of 10 pepper varieties, the results were significantly higher from the organic system compared to the conventional system, where, for example, a high amount of available carbohydrates and crude protein (4.82% and 1.20%, respectively) was found in the Red Italian variety.

The total average vitamin C content, in the presented studies, was from 188.44 - 207.70 mg 100 g^{-1}, where the hybrid has a potential influence. The results are correlated with the research of Szwejda-Grzybowska et al. (2016) who explain that in the hybrid pepper Blondi, during three seasons, the largest number of samples had between 102 and 134 mg of vitamin C per 100 g of fresh fruits. According to the results of a two-year study carried out in the agroecological conditions of western Turkey, carried out by cluster analysis on 48 populations, i.e. 94 genotypes of pepper classified into seven groups, the average content of dry matter in fruits for different groups of genotypes varied from 9.56 to 14.3%, the average content of vitamin C for different groups varied from 87.61 to 141.93 mg 100 g^{-1} fruit, and the yield of 14.23 to 19.01 t ha^{-1} (Kadri Bozokalfa et al., 2009).

Conclusion

In new technologies of pepper cultivation in an integral and/or organic cultivation system in a protected area (greenhouse), where abiotic factors are only partially controlled, the year as an investigated factor had an influence only in some measurement components of yield and vitamin C content). The reason is that in some years the average temperature in the greenhouse was higher and combined with high relative humidity. Generally, the application of EM and *Trichoderma spp*. had a positive effect on most of the tested properties, and the effect was particularly pronounced in terms of yield. Considering everything that has been said, it is recommended that in an integral/organic system of growing pepper producers, above all in a protected area, the studied technology of growing, because the achieved yields are higher, of good quality, and the priority is health-safe food, as well as a fresh program - permanent supply markets, products for consumption, as well as for further processing. Microbiological preparations also had a positive effect on the fact that there were no diseases and pests on the pepper crop.

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Conflict of interests. No potential conflict of interest was reported by the authors.

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