INFLUENCE OF FERTILIZATION WITH *TRICHODERMA ATROVIRIDE* AND FULVIC ACIDS UPON THE NUTRITIVE CONSTITUENTS IN LONG PEPPER FRUITS

Florin-Dan APOSTOL[1], Maria DINU[2], Mihaela Gabriela DUMITRU[3], Liviu Cristian MARACINEANU[2], Ana Maria JOSCEANU[4]*, Nicolae GIUGEA[2]

Association of crop plants with arbuscular mycorrhiza (AM) is the most important biological association able to improve the plants growth and productivity. The study presents the influence of fertilization with AM and fulvic acid on the accumulation of bioactive nutritional compounds in the long peppers fruits grown in the solar system. Six Romanian pepper varieties (Capsicum annuum L.) var. longum and Kaprima F1 hybrid were monitored. Moisture, total soluble substances (TSS), polyphenols (TPC), vitamin C, chlorophyll a and b, and total carotens levels were measured. The Doljan genotype fertilized with AM recorded the highest TPC (27.4 mg GAE/g), while Cosmin accumulated 66.7 mg/100 g vitamin C and 2.3 mg/100 g TC. Although the results highlight the efficacy of fertigation with T. atroviride, the contribution of fulvic acids in fertilization should not be neglected.

Keywords: Capsicum annuum; *Trichoderma atroviride*; fulvic acid; fertilization.

1. Introduction

Pepper (*Capsicum annuum*) is a very important vegetable species from nutritional point of view, being part of the *Solanaceae* family. Pepper fruits are an important source of antioxidants, such as ascorbic acid [1], carotenoids, phenolic acids, and flavonoids [2]. Polyphenols and flavonoids are very important in assessing the nutritional value of pepper fruits, as they protect the human body cells against oxidation caused by free radicals [3]. The yield, the quality of the sweet pepper fruits, as well as the accumulation of phytonutrients are influenced by the genotype [4,5], the culture system [6] and the maturity type of fruits [7].

[1] Ph D student, Doctoral School of Plant and Animal Resources Engineering, Faculty of Horticulture, University of Craiova, Romania, e-mail: dan79apostol@yahoo.com
[2] Department of Horticulture and Food Science, Faculty of Horticulture, University of Craiova, Romania, e-mail: dinumariana@hotmail.com; maracineanulc@yahoo.com; giugeanicolae@gmail.com
[3] Department of Chemistry, Faculty of Sciences, University of Craiova, Romania, e-mail: bitamgs@yahoo.com
[4] Department of Analytical Chemistry and Environmental Engineering, Faculty of Chemical Engineering and Biotechnologies, University POLITEHNICA of Bucharest, Romania, e-mail: a_josceanu@chim.upb.ro
*Corresponding author: e-mail: a_josceanu@chim.upb.ro

Plants associate with other life forms (animals, bacteria, or fungi) to complete their life cycle, to fight against pathogens or to grow in unfavourable environments. Arbuscular mycorrhiza (AM) is the most common symbiotic association of plants with microbes. AM fungi occur in most natural habitats, providing a range of important ecological services, by improving plant nutrition, stress resistance and tolerance, soil structure, and fertility [2]. Since phosphorus is important in the biosynthesis of plant metabolites, its availability plays a crucial role [8]. The positive influence of inoculation with arbuscular mycorrhizal fungi on certain plant metabolites [9,10] indicates that their application could be used as a crop biofortification strategy. In the last decade, numerous studies were carried out that sought to identify the specific effects of arbuscular mycorrhizal fungi (AMF) on phytochemicals of economic interest [11,12]. Remarkably, many classes of metabolites are affected by symbiosis with species of arbuscular mycorrhizal fungi, and the results obtained have been observed and reported in several species from different plant families [13].

Arbuscular mycorrhizae (AM) absorb nutrients such as N, P, K, Ca, S, Cu, and Zn from the soil and translocate them to associated plants. However, the most prominent and consistent nutritional effect of arbuscular mycorrhizae (AM) is in the improved absorption of immobile nutrients, especially P, Cu, and Zn [14]. Fungianism improves the absorption of nutrients by increasing the absorption surfaces of the roots [15]. The fungi hyphae spread in the soil and "infect" the roots of the plants, creating specific structures for the exchange of nutrients. This relationship generally benefits both organisms by supplying carbon to the fungus and by increasing the absorption of nutrients (primarily phosphorus) by the plant. Each mycorrhizal plant and fungus are usually involved in several simultaneous relationships. Cavagnaro and Martin [16] have shown that this network of underground connections can facilitate the nutrients exchange from plant to plant.

Trichoderma mushrooms use is favourable for the growth and development of plants [9], productivity increase [17], and nutrition process improvement by absorbing fertilizers [18], accelerating the decomposition of organic matter in the soil, nutrients release from soil organic matter, and xenobiotic pesticide degradation [19]. Most research involving the isolation and identification of *Trichoderma* species has been undertaken to evaluate their potential in protecting plants from pathogen infection [20-23] or as biological control agents.

They can improve the solubility of soil micronutrients (Fe, Zn, Cu and Mn) and produce metabolites with hormonal activities such as auxins and cytokines [20], essential for plant organs formation and physiological processes regulation (growth, photosynthesis, resistance to pathogens), indole acetic acid (plant growth regulator), gibberellins (active in elongating stems, increasing vegetative mass, stimulating flowering, fruit development, and seed germination), and zeatins, active in cellular growth and division, nutrients transformation, and energy transfer [24]. Hoyos-Carvajal et al. [25] reported that certain strains of *Trichoderma* were able to produce soluble forms of phosphate from phosphate rock. They showed a consistent ability to produce siderophores to convert ferric iron into soluble forms by chelation. Along with the synthesis or stimulation of phytohormone production, most *Trichoderma* strains acidify the environment, secreting organic acids such as gluconic, citric or fumaric acid. These acids result from the metabolism of other carbon sources, mainly glucose, being in turn able to solubilize phosphates, micronutrients, and cations such as Fe, Mn, Mg [26]. Therefore, *Trichoderma* addition to soils, whose cation levels are low, results in biofertilization by solubilization of metal ions and increase in crop productivity. It has been demonstrated that there is an increasing number of leaves per plant, thus increasing the intensity of photosynthesis [27-29].

Another valuable and interesting feature of plant colonization by *Trichoderma* is the improved tolerance to abiotic stress (drought, salinity, heat, cold [30]), accompanied by changes in root architecture, and *p*H increase, providing better absorption of water and nutrients [31]. The most studied species of *Trichoderma* in terms of their stimulation of the growth of several vegetable crops are *T. asperellum*, *T. atroviride*, *T. harzianum*, *T. virens*, and *T. viride* [32].

Fulvic acid contains carboxyl, carbonyl, hydroxyl, phenolate, and quinone functional groups, capable to participate in chelation and ion exchange reactions [33-35]. It is considered a valuable fertilizer for sustainable agriculture [36], having positive effects on many important plant functions (respiration, membrane permeability, cell division, elongation, seed germination and seedling development, chlorophyll synthesis, root growth stimulation, increased nutrient uptake, and resistance to water stress), leading to higher crop yields [37-39]. It plays an important role in the growth of RNA in plant cells, influencing the concentration of enzymes and proteins [33].

The aim of this study was to observe the effect of organic fertilization, with a bio-stimulating product consisting of fulvic acid (branded as ‘Furia’) and another based on mycorrhizal fungi *Tricoderma atroviride,* on the main nutrients of long peppers grown organically. The application of mycorrhizal fungi in pepper culture is a very important technological stage because pepper has a trasant root system, developed on the soil surface, and mycorrhiza increases plant resistance to stressors, especially water stress and disease tolerance.

2. Materials and Methods

2.1. Experimental Protocol and Culture Conditions

The culture was established in a cold solarium, in Șimnicu de Sus locality, Dolj county (southwest of Romania, 44°24′23″ N, 23°48′09″ E). It was organized as a two-factor experiment. Factor A was represented by the pepper varieties and had 7 levels: a_1 – Bogdan, a_2 – Lung de Ișalnița, a_3 – Lung Românesc, a_4 – Doljan,

a_5 – Cosmin, a_6 – Fermier and a_7 – Kaprima F_1. Factor B was represented by the fertilizer and had 3 levels: b_1 – unfertilized, b_2 – fertilized with *Tricoderma atroviride*, and b_3 – fertilized with fulvic acids. The culture was established by seedlings produced in the greenhouse multiplier, according to the species-specific technology. The 70-day-old seedlings were planted on 27-04-2020, in rows spaced 80 cm between strips, 50 cm between rows, and 40 cm between plants per row. The experiment was placed in randomized blocks in three repetitions, each variant with 10 plants per repetition. The number of plants per variant was 30, and 3.84 plants were planted per m^2.

The technology applied was the literature recommended version for organic cultivation of long pepper in the solarium, diseases and pests were fought through specific preventive treatments with certified products for organic farming. The treatments applied during the vegetation were fertirigation with *Tricoderma atroviride*, at 30 days intervals, and with fulvic acids, at 15 days intervals, using 0.25% solutions. The first treatment was applied 3 weeks after planting. The main fertilization, before the establishment of the crop, was carried out with well-decomposed manure (30 t/ha). All hybrids were grown under the same conditions of organic farming. Analyses were performed on long pepper fruits at physiological maturity (when the fruit is red) to achieve the established objectives.

2.2. Chemicals and Reagents

Analytical grade chemicals/reagents, including acetone, Follin–Ciocalteau reagent (2 M solution), gallic acid, hydrochloric acid, methanol, potassium iodate, potassium iodide, sodium carbonate, and starch were purchased from Sigma-Aldrich (Steinheim, Germany), being used as shipped. Demineralized water (18.2 MΩ/cm, Smart2Pure System from TKA, Germany) was used for calibration and dilution purposes.

2.3. Extract of Pepper Fruits

Fruits were selected from the 7 pepper types after having reached physiological maturity. The fruits were washed, cut into pieces, and placed in a kitchen blender. 1 – 25 g fruit mass was weighed, treated with 40 mL of 50 % methanol, and homogenized. The mixture was left to stand for 60 min at ambient temperature, centrifuged for 15 min at 15,000 rpm; the supernatant was transferred into a 100 mL volumetric flask. The residue was mixed with 40 mL of 70 % acetone, left for another 60 min, and centrifuged 15 min at 15,000 rpm. This supernatant was made up to 100 mL with demineralized water.

2.4. Moisture Content

The initial moisture content of *Capsicum annuum* L was determined with a moisture analyser (MX-50, A&D Instruments, UK), equipped with an internal Super Hybrid Sensor for weight. Results were expressed as percentage.

2.5. Total soluble substance – TSS

The total soluble substance (TSS) content was determined with a refractometer DR 301-95 (A. Krüss Optronic, Hamburg, Germany) at 20 °C and results were expressed in °Brix.

2.6. Total polyphenols content - TPC

TPC was measured using the Folin-Ciocalteu method. 800 µL deionised water, 50 µL Folin-Ciocalteu reagent (2 M), and 10 – 50 µL extract were accurately mixed [55]. After 1 min, 100 µL Na_2CO_3, 20% solution, were added, making up to a 1 mL with deionised water. Absorbance was measured with a V550 Jasco spectrophotometer (Japan) at 765 nm after 2 h incubation in the dark. Results were expressed as mg of equivalent gallic acid (GAE) /g extract, using a standard curve designed in the 1 - 25 µg/mL gallic acid domain (y = 0.0062 x + 0.0398; R^2 = 0.9992).

2.7. Ascorbic Acid

5-10 g pepper sample, previously grinded with quartz sand, were transferred into a 100 mL flask using HCl, 2% solution. After mixing and 15 min rest, it was filtered in another dry vessel. A 10 mL aliquot from the filtrate was transferred into a Berzelius beaker, mixed with 30 mL deionized water, 5 mL KI, 1% solution, and 1 mL starch solution. The resulting solution was titrated with KIO_3, 0.25 N solution, under stirring, to bluish. Ascorbic acid content was calculated according to relation (1):

$$\text{Ascorbic acid, mg \%} = \frac{352 \times V \times F}{m} \quad (1)$$

where: V = volume of titration reagent used, in mL;
F = volumetric correction factor for the titration reagent used;
m = pepper mass, in g.

2.8. Chlorophyll a, Chlorophyll b, and Total Carotenoid - TC

Weighed pepper samples were mixed with 50 mL of 95% acetone and homogenized with a Braun MR 404 Plus (Germany) blender for 1 min. The homogenate was filtered and centrifuged in a Hettich Universal 320/320R centrifuge (Sigma Aldrich, Germany), 10 min at 2500 rpm. The supernatant absorbance was measured in 400-700 nm domain with a Varian Cary 50 Uv-vis spectrophotometer (Agilent, USA). As peaks for chlorophyll *a* are at 662 nm, chlorophyll *b* at 646 nm, and total carotens at 470 nm, the corresponding levels were calculated as in [40], results being reported as mg /100 g fresh weight:

$$C_a = 11.75 \times A_{662} - 2.350 \times A_{645} \quad (2)$$

$$C_b = 18.61 \times A_{645} - 3.960 \times A_{662} \quad (3)$$

$$C_{x+c} = 1000 \times A_{470} - 2.270 \times C_a - 81.4 \times \frac{C_b}{227} \quad (4)$$

where: C_a = chlorophyll a,
C_b = chlorophyll b,

C_{x+c} = total carotenoid.

2.9. Statistical analysis

Statistical analysis was carried out with the aid of Microsoft Excell facilities, for determining the main statistical characteristics of the monitored chemical and biological properties.

3. Results and Discussion

Pepper fruits contain a high level of antioxidants, such as vitamin C, polyphenolic acids, flavonoids, and carotenoids (lycopene and β-carotene), inducing a high antioxidant activity. Their levels vary with the genetic factors, culture system, environmental factors, cultivar involved, but also with the fruit harvesting stage [41]. The red colour is an important indicator of ripeness and quality. The green colour of a long pepper fruit is due to the presence of chlorophyll, and the degradation of this pigment occurs during ripening, when synthesis of yellow β-carotene and xanthophylls pigments begins. During this study characteristics such as humidity, total soluble substance (TSS), total polyphenolics content (TPC), ascorbic acid, chlorophyll a, chlorophyll b and total carotens levels were determined for the 7 genotypes of peppers, experimental results being presented in Tables 1 and 2.

There was an increase of 0.6 - 6.4 % in the fruit humidity for all fertilized samples compared to the control variants. The increase was larger after fertirrigation with *T. atroviride*, the Lung Românesc pepper enjoying the highest increase. Humidity increase after treatment with fulvic acid was less, Bogdan and Fermier types experiencing a less than 1 % augmentation.

In terms of humidity, Lung de Işalnița pepper has benefited the most from the use of fertilizers. The humidity in the control groups shows two outliers (Lung de Işalnița and Cosmin). This made the mean and first quartile values equal (Fig. 1a). The lack of symmetry is serious, leading to a skewness of -2.7.

The moisture values for samples treated with *T. atroviride* and fulvic acid are different from those reported by Geng et al. [42], demonstrating a better water absorption induced by the two fertilizers. The values obtained for control samples are in accordance with the value reported by Nitesh et al. [43], their fertilized samples displaying values lower than the results reported in this study.

The TSS content varied in the 6.5 - 8.2 °Bx range, Cosmin, Fermier, and Kaprima F_1 genotypes showing higher values when fertilizers were applied compared to the controls. The outlier of *T. atroviride* group pushed the mean above the median value, more values being found in the Q3-Q4 region (Fig. 1b). More TSS values of control group and fulvic acid fertirrigated samples are found in the Q1-Q2 region.

Table 1

Biochemical properties variation in long pepper fruits

Pepper Genotype	Fertilization procedure		
	Control	*Trichoderma atroviride*	Fulvic acid
Humidity (%)			
Bogdan	90.0 ± 1.0	92.0 ± 0.6	90.5 ± 1.0
Lung de Işalniţa	86.0 ± 6.1	91.5 ± 0.9	90.0 ± 0.7
Lung Românesc	90.1 ± 0.1	92.8 ± 0.7	89.3 ± 1.4
Doljan	89.9 ± 0.3	91.0 ± 1.2	91.1 ± 1.2
Cosmin	86.8 ± 5.7	91.7 ± 0.3	90.8 ± 1.5
Fermier	89.8 ± 0.4	92.0 ± 1.2	90.5 ± 1.4
Kaprima F_1	89.2 ± 0.9	91.5 ± 1.3	90.6 ± 0.6
Total Soluble Solids - TSS (°Bx)			
Bogdan	7.5 ± 0.3	7.0 ± 0.3	8.0 ± 0.3
Lung de Işalniţa	8.0 ± 0.2	6.8 ± 0.2	7.5 ± 0.1
Lung Românesc	7.6 ± 0.2	7.8 ± 0.3	7.5 ± 0.6
Doljan	8.0 ± 0.2	6.5 ± 0.6	6.8 ± 0.5
Cosmin	6.7 ± 0.4	7.0 ± 0.3	8.2 ± 0.4
Fermier	6.7 ± 0.4	7.1 ± 0.3	8.2 ± 0.4
Kaprima F_1	7.4 ± 0.1	8.0 ± 0.8	7.7 ± 0.5
Total polyphenols - TPC (mg GAE/g)			
Bogdan	15.8 ± 0.4	22.5 ± 0.8	16.0 ± 0.4
Lung de Işalniţa	8.5 ± 0.5	8.8 ± 0.9	11.2 ± 0.3
Lung Românesc	22.4 ± 0.7	26.5 ± 0.5	18.5 ± 0.5
Doljan	20.9 ± 1.2	27.4 ± 0.6	24.7 ± 1.2
Cosmin	9.8 ± 0.3	13.7 ± 0.3	13.0 ± 0.1
Fermier	10.0 ± 0.4	9.8 ± 0.2	8.0 ± 0.1
Kaprima F_1	7.7 ± 0.5	9.2 ± 0.3	8.0 ± 0.5

TSS values are in accordance with the 7.63°Bx value reported by Gurpinar and Morgonar [44] or 7.83°Bx [45], recorded after the organic fertilizers application to a pepper crop grown in greenhouses. Nzanza et al. [24] observed an 11% increase in TSS for a tomatoes crop grown in the field and fertilized with *T. harzianum*, compared to the control, and by 9 % when AMF (arbuscular mycorrhizal fungi) was applied. Aminifard [36] reported TSS values in the 9.65 - 10.75 Bx range, after fulvic acid fertilization in a field crop of paprika. This difference can be attributed to fulvic acid having a positive effect on fruit quality.

TPC levels for the control varieties fruits differed by genotype, varying from 7.7 mg GAE/g (Kaprima F1) to 22.4 mg GAE/g (Lung Românesc). Fertilization increased the TPC in most samples, the most significant effects being recorded in Bogdan fertilized with *T. atroviride* (42.4 %). The ranges for the experimental values vary in the order fulvic acid < control < *T. atroviride*.

There is significant distortion from normal distribution of TPC, the largest one being characteristic to the control group (Fig. 1c). All means are larger than medians, the control group showing the largest difference.

Aminifard [36] used five doses of fulvic acid (0 - 250 mg/kg) in a study on peppers and found out that the total polyphenols content increased in the fruits, as all, but Fermier, genotypes monitored in the case of the present study.

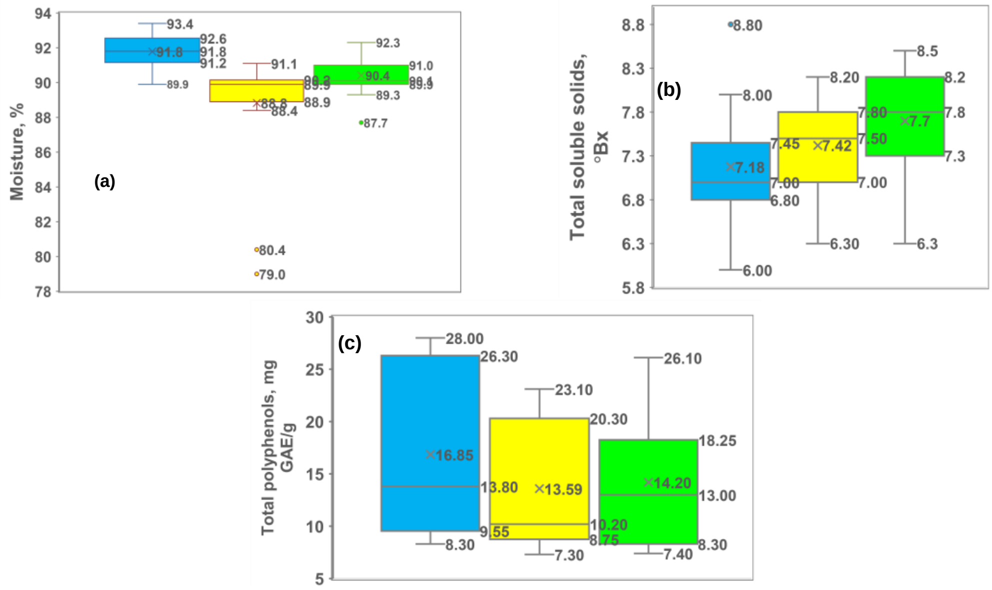


Fig. 1. Boxplot representation of physico-chemical characteristics of the pepper fruits studied with median, maximum, and minimum values and interquartile distances: (a) humidity, (b) total soluble solids, and (c) total polyphenols content.
Tricoderma atroviride and Fulvia fertilized samples, controls.

Following the fertilization treatments, all pepper fruits showed higher ascorbic acid contents than the control samples, the experimentally determined values varying in the 37.6 – 74.5 mg / 100 g range. The increase was more important after fertigation with *Trichoderma atroviride*, a maximum of 38 % being reached in the case of the Fermier variety. The treatment with fulvic acid led to the highest increases in vitamin C for the fruits of the Bogdan variety. These results are in accordance with those reported by Ribes-Moya et al. [46].

Means and medians are almost identical for all three groups, with a rather homogenous dispersion of experimental values in the variation range (**Fig. 2a**). Only *T. atroviride* fertilized group shows a slight skewness, and the *t*-test revealed that it's mean ascorbic acid level is significantly different from that of the control group.

TC levels differed with the genotype and applied fertilizer. The highest concentrations were recorded in the genotypes fertilized with *T. atroviride* (1.7 mg/100 g - 2.3 mg/100 g), Cosmin, Fermier, and Kaprina F1 standing out. Fulvic acids fertilization also increased TC values compared to the controls, but less than *T. atroviride*. Bogdan, Işalnița, Cosmin, Fermier, and Kaprima F_1 responded best to the two fertilizers. The positive action of fulvic acids on the total carotenoids content is also supported by Aminifard et al. [36].

Table 2

Biological parameters for pepper fruits

Pepper Genotype	Fertilization procedure		
	Control	*Trichoderma atroviride*	Fulvic acid
Vitamin C (mg/100 g)			
Bogdan	62.0 ± 3.2	74.5 ± 0.9	72.1 ± 2.1
Lung de Işalnița	35.2 ± 0.4	40.7 ± 0.2	37.6 ± 0.5
Lung Românesc	39.3 ± 0.3	42.1 ± 2.0	40.0 ± 0.2
Doljan	49.3 ± 0.3	58.0 ± 0.5	51.7 ± 1.1
Cosmin	59.4 ± 0.6	66.7 ± 0.6	61.0 ±0.9
Fermier	40.1 ± 0.5	55.3 ± 0.2	44.7 ± 0.2
Kaprima F_1	57.5 ± 0.4	61.0 ± 0.6	58.2 ± 0.4
Total Carotenoids – TC (mg/100 g)			
Bogdan	0.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.2
Lung de Işalnița	1.9 ± 0.1	2.1 ± 0.2	2.0 ± 0.1
Lung Românesc	0.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Doljan	0.9 ± 0.1	1.9 ± 0.1	1.1 ± 0.1
Cosmin	1.3 ± 0.1	2.3 ± 0.2	1.9 ± 0.0
Fermier	1.4 ± 0.1	2.3 ± 0.3	2.1 ± 0.2
Kaprima F_1	1.1 ± 0.1	2.3 ± 0.0	1.9 ± 0.1
Chlorophyll *a* (mg/100 g)			
Bogdan	32.6 ± 0.5	48.7 ± 1.7	40.5 ± 1.0
Lung de Işalnița	50.8 ± 0.4	65.2 ± 0.1	65.0 ± 0.1
Lung Românesc	17.5 ± 0.8	25.0 ± 0.4	20.5 ± 0.5
Doljan	29.0 ± 0.7	34.7 ± 0.7	32.9 ± 0.2
Cosmin	53.7 ± 0.4	61.3 ± 0.4	63.0 ± 0.8
Fermier	66.4 ± 2.7	87.1 ± 0.3	77.0 ± 0.5
Kaprima F_1	70.6 ± 0.6	75.0 ± 0.9	74.8 ± 0.5
Chlorophyll *b* (mg/100 g)			
Bogdan	15.0 ± 0.2	23.2 ± 1.4	18.7 ± 0.2
Lung de Işalnița	27.5 ± 0.9	30.3 ± 0.5	29.7 ± 0.5
Lung Românesc	9.8 ± 0.1	13.0 ± 0.2	11.6 ± 0.4
Doljan	14.6 ± 0.3	16.9 ± 0.1	15.5 ± 0.2
Cosmin	25.4 ± 0.3	38.2 ± 0.3	29.1 ± 0.2
Fermier	29.0 ± 0.2	35.7 ± 4.9	35.9 ± 0.7
Kaprima F_1	27.5 ± 0.3	33.5 ± 0.3	30.8 ± 0.6

While the mode and median for the fulvic acids and *T. atroviride* fertilized groups tend to superimpose, they are distanced by 0.1 units for the control set (Fig. 2b). The two outliers in the fulvic acids fertilized set induce some distortion in the normal distribution and lead to a -1.11 skewness.

The chlorophyll-content dynamics presented obvious changes. All fertilized plants yielded fruits with higher chlorophyll *a* content than control samples. The percentage increase was more significant after *T. atroviride* treatment (6.2 – 49.4 %) than for fulvic acid (5.9 – 28 %). Despite its highest chlorophyll *a* content, Kaprima F_1 hybrid experienced the smallest increase after biofertilization.

Mean and median values are different for all groups (Figs. 2c,d), with no outliers. There are more experimental values in Q1-Q2 region. A *t*-test assuming unequal variances demonstrated that there are significant differences only between the means of the control and *T. atroviride* fertilized samples ($p = 0.026$).

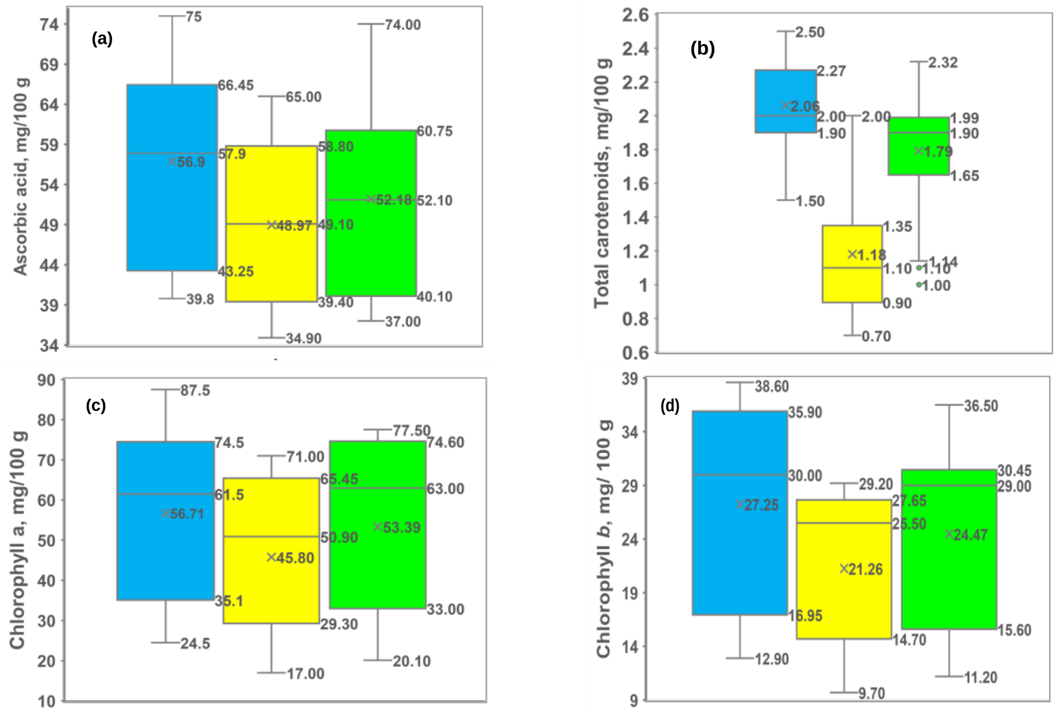


Fig. 2. Boxplot representation of physico-chemical characteristics of the pepper fruits studied with median, maximum, and minimum values and interquartile distances: (a) ascorbic acid; (b) total carotenoids; (c) chlorophyll *a*; (d) chlorophyll *b*. ☐ *Tricoderma atroviride* and ☐ Fulvia fertilized samples, ☐ controls.

The results of this study are like those of Estrada-Luna and Davies [47], demonstrating that application of mycorrhizal fungi causes a larger accumulation of chlorophyll. The effect of inoculation with mycorrhizal fungi determined the increase of SPAD values (relative values of chlorophyll) in peppers, total chlorophyll concentration, and photosynthetic activity, which is a controversial topic [48]. Cekic et al. [49] reported a positive effect, Haghighi and Barzegar [50] found that mycorrhization only improved photosynthetic activity, but not chlorophyll content, while in the works of Kaya et al. [51], Jezdinský et al. [52] and Beltrano et al. [53] no mycorrhization effect was proven. Moreover, Bakr et al. [54] found ambiguous results regarding the chlorophyll content (in SPAD units). Geng et al. [42], studying a cotton crop, found that the application of fertilizers based on fulvic acids significantly improved the SPAD units, thus increasing photosynthesis and fluorescence parameters of chlorophyll in cotton leaves compared to unfertilized samples.

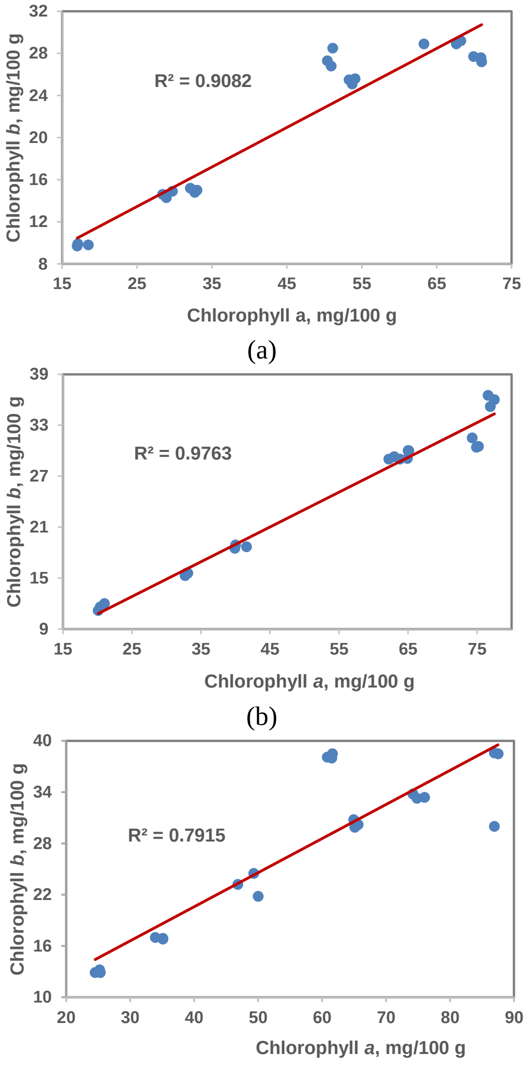


Fig. 3. Linear correlation between chlorophyll *a* and chlorophyll *b* levels in pepper fruits. (a) control; (b) fulvic acid fertirrigation; (c) *Trichoderma atroviride* fertilization.

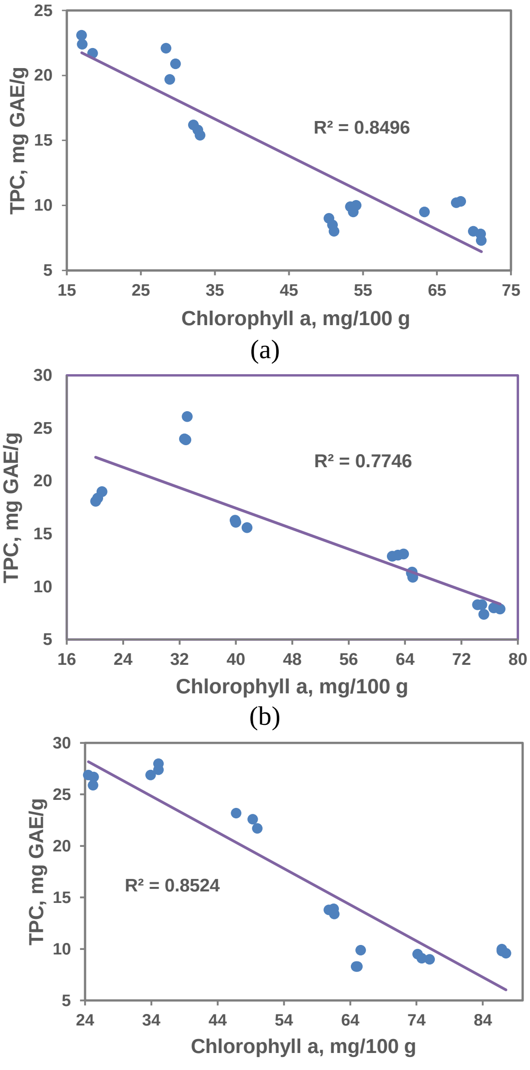
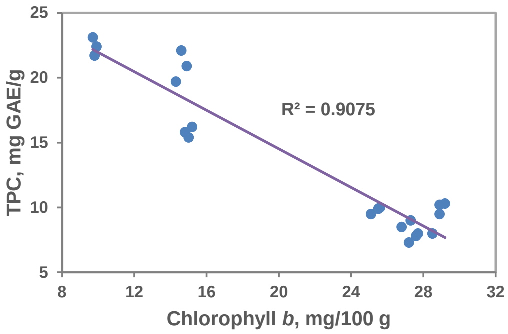


Fig. 4. Linear correlation between chlorophyll *a* and total polyphenols content (TPC) in pepper fruits. (a) control; (b) fulvic acid fertirrigation; (c) *Trichoderma atroviride fertilization*.

Linear regression identified correlations between the investigated properties of all analysed samples, control sets included. A good positive correlation was found between the chlorophyll *a* and chlorophyll *b* contents in all samples (Fig. 3a-c). Chlorophyll *a* varies invers proportionally with TPC, the correlation coefficient ranging from – 0.8801 for the samples fertirrigated with fulvic acid to – 0.9232 when *T. atroviride* was the fertilizer (Fig. 4a-c).



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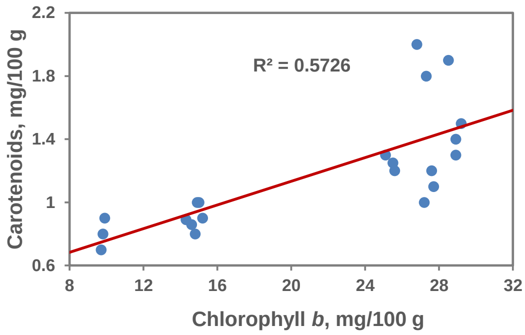

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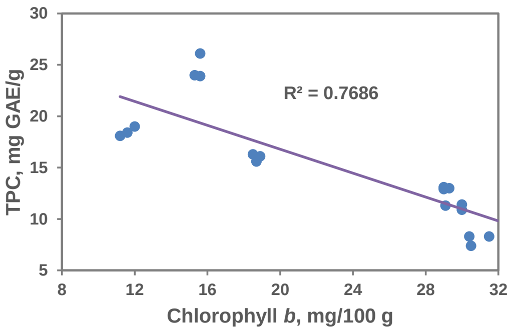
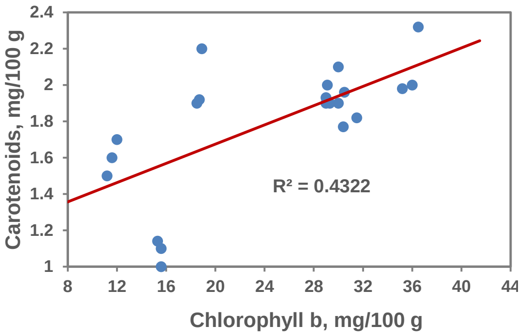
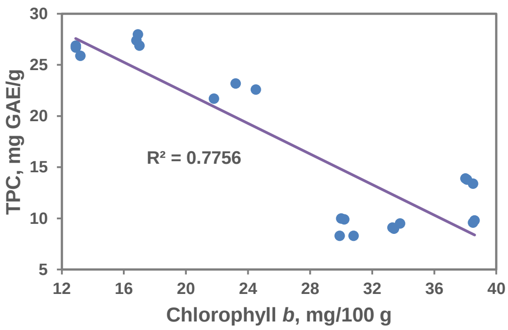


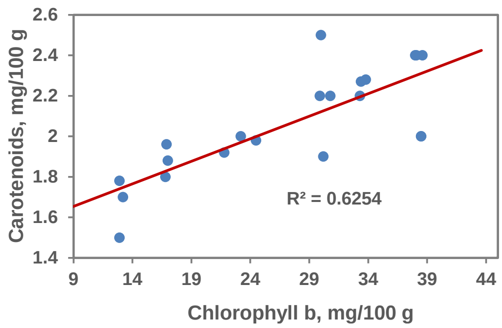
(b)



(b)



(c)



(c)

Fig. 5. Linear correlation between chlorophyll *b* and total polyphenols content (TPC) in pepper fruits. (a) control; (b) fulvic acid fertirrigation; (c) *Trichoderma atroviride* fertilization.

Fig. 6. Linear correlation between chlorophyll *b* and carotenoids content (TPC) in pepper fruits. (a) control; (b) fulvic acid fertirrigation; (c) *Trichoderma atroviride* fertilization.

Even the control showed a comparable dependence, with a correlation coefficient of – 0.8801. It is not surprising that chlorophyll *b* maintained the trend (Fig. 5a-c), with comparable correlation coefficient values (– 0.8767 for fulvic acid, – 0.8807 *T. atroviride* and a larger value for the control set, – 0.9526). Chlorophyll *b* and TC level enjoy positive correlation coefficients, ranging from 0.6578 for fulvic acid fertilizer, 0.7567 in the absence of biofertilization and 0.7908 after treatment with *T. atroviride* (Fig. 6a-c).

5. Conclusions

Although the two biofertilizers improved the composition of the main bioactive parameters of the pepper fruits, obtaining values superior to the control samples, the results varied with the biofertilizer. *Trichoderma atroviride*-fertilized samples presented higher values than fulvic acids biofertilized samples. The varying increase of the bioactive parameters values can be attributed to the biological potential of each sample. Although the results highlight the efficacy of fertirigation with *T. atroviride*, the contribution of fulvic acids in fertilization should not be neglected.

Relatively higher chlorophyll content also demonstrated better nutrient utilization as an effect of mycorrhizal fertilization. Based on the presented results, mycorrhizal treatment can provide an effective and simple method to increase the qualitative yield of peppers grown in cold solar, when producers pay less attention to the plants and suboptimal temperatures occur.

Given the growing consumer demand for healthy products and current policies for ecologically sustainable cropping systems, the use of mycorrhizal mushrooms in pepper cultivation in solarium is a coherent alternative to chemical practices based on chemical inputs and monoculture.

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