DETERMINATION OF ANTIOXIDANT ACTIVITY IN PEPPER FRUITS FERTILIZED WITH NATURAL BIOSTIMULATORS FORMED FROM SPORES AND MICHELIA OF TRICHODERMA ATROVIRIDE NON-GENETICALLY MODIFIED AND FULVIC ACID

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ABSTRACT

The aim of this study was to determine the role of fertigation with Trichoderma atroviride and fulvic acid at 6 varieties and a hybrid of long pepper (Capsicum annuum L.) var. longum.For this purpose, determinations were made on the content of total polyphenols, ascorbic acid and antioxidant activity of peppers that have reached physiological maturity.

The determinations were made by the Folin-Cicâlteu method, by titration and the DPPH method. The results obtained in the samples fertigated with Trichoderma atroviride are superior to the samplesfoliarly fertilized with fulvic acid. Although there is a difference between them, we believe that it is appropriate to use both biostimulators in pepper culture.

INTRODUCTION

Proper practices for sustainable management of agricultural soils, the use of biofertilizers is a common method in the agro-horticultural system. Through this process, a stimulation of the natural processes that take place in the soil with a direct effect on the assimilation of nutrients is obtained with the increase of their efficiency and the obtaining of stable multiannual productions.

The study aimed to evaluate the physiological response to fertilization of pepper plants with natural biostimulators formed by spores and mycelium of Trichoderma atroviride non-genetically modified and fulvic acid, from 6 varieties and a hybrid of long pepper (Capsicum annuum L.) var. longum (Bogdan, Lung de Işalniţa, Lung românesc, Doljan, Cosmin, Fermier, Kaprima F1). The culture took place in a cold solarium in Șimnicu de Sus locality, Dolj county (southwest of Romania) (44 ° 24′23 ″ N 23 ° 48′09 ″ E) In a particularly authorized farm.

Trichoderma atroviride is a fungus that promotes the strengthening of the root system, the absorption of nutrients and the increase of the efficiency of fertilizers. Contributes to the formation of healthier, stronger, and more resistant plants. It has a phytostimulating action and contributes to soil fertility and vitality. It has a high level of adaptability to soil and environmental conditions [1].

Fulvic acid is described by Schnitzer, M. (1977) [2] as part of the humic structure of the soil created in extremely small quantities by the action of millions of beneficial microorganisms, which work to degrade plant matter in a soil environment with adequate oxygen. It has a low molecular weight [3] and is very biologically active.Due to its low molecular weight, it has the property and ability to easily bind minerals and elements in its molecular structure, causing them to dissolve and become mobilized fulvic complexes. From humic deposits it usually carries 60 or more minerals and trace elements dissolved in its molecular complexes.They are then in natural form ideal for being absorbed and interacting with living cells [4]. The roots and cells of plants easily absorb large amounts of fulvic acid and maintain it in their structure [3]. It is easily complexed with minerals and metals, making them available for plant roots and easily absorbable through cell walls. Fulvic acids also dissolve and transport vitamins, coenzymes, auxins, hormones and natural antibiotics that are generally found throughout the soil, making them available. Catalyzes enzymatic reactions having a close association with enzymes [5; 6].

Fulvic acid increases the process of assimilation of nutrients in the roots by transporting them quickly to the shoots of plants. It relieves oxygen deficiency and increases the vital activity of cells. It changes the pattern of carbohydrate metabolism, resulting in an accumulation of soluble sugars that increase the osmotic pressure inside the cell wall and allows plants to resist wilting. Improves growth and can stimulate the immune system.

MATERIAL AND METHOD

Standard laboratory grade chemicals/reagents including, Folin–Ciocalteau reagent, sodium carbonate, pure water, 2% hydrochloric acid;potassium iodate, potassium iodide, starch, gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH).

Obtaining the extract

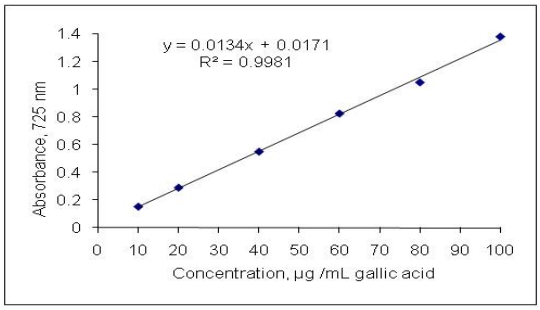
To compare the antioxidant activity of fruits fertilized with Trichoderma atroviride and fulvic acid, the fruits of the 7 types of peppers reached physiological maturity were selected.

The fruits were washed and cut into pieces and then placed in a kitchen blender.

Weigh 1-25 g of fruit into a 100 mL vial over which 40 mL of 50% methanol is added and mix. Leave to stand for 60 minutes at ambient temperature and then centrifuge at 15,000 rpm for 15 minutes. Filter and transfer the obtained supernatant to a 100 mL volumetric flask. 40 mL of 70% acetone is added to the residue obtained and homogenized. Leave to stand for 60 minutes and then centrifuge for 15 minutes at 15,000 rpm. Filter and supernatant is placed in a 100 mL flask containing the methanolic extract. Make up to 100 mL with distilled water.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) values of the capsicum annuum extracts were measured using the Folin-Ciocalteu reagent. The reaction mixture contained 100 µL of antioxidant extract and solvent, 500 µL of the Folin-Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL of pure water. After 2 h of reaction at ambient temperature, absorbance was read at 725 nm and used to calculate the TPC, using Gallic acid as the standard. Triplicate measurements were taken and the results were expressed as mg Gallic acid equivalents (GAE)/g of fresh sample, using the following equation based on the calibration curve (Fig.1): $y = 0.0134x + 0.0171$, [7].



Gallic acid calibration curve.

Determination of ascorbic acid by iodatometric method

An amount of 5-10 g of each pepper sample, previously ground with quartz sand, is passed into a 100 mL volumetric flask using a 2% hydrochloric acid solution. Stir. After settling, filter into a dry glass. Take a 10 mL aliquot of the filtrate, place in a Berzelius beaker, add 30 mL of distilled water, 5 mL of 1% potassium iodide and 1 mL of starch solution. Titrate with potassium iodate N / 250, stirring until a bluish coloration appears. The determination of scurvy acid content is done using equation [8]:

Ascorbicacid, mg% = 352. n.f / G

where:

n - mLused for titration;

f - the factor of the potassium iodate solution N / 250;

G - sampleweight in grams.

Evaluation of DPPH radical scavenging activity

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers [9].

The antioxidant effect is proportional to the disappearance of DPPH• in test samples. Monitoring DPPH• with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH• shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant.

The measurement of DPPH radical scavenging activity was carried out according to the method of Barros et al. (2007) [10].

0.1mL extract (sample) was mixed with 3.9 mL 0.06 mM DPPH solution and the absorbance on the spectrophotometer was read minute by minute until stabilized.

RESULTS AND DISCUSSIONS

From the obtained data are distinguished values that signify the role played by the two biofertilizers on the content of total polyphenols, Table 1, ascorbic acid, Table 2 and antioxidant activity, Table 3.

Sample name	Polyphenols, mEq GAE/g fresh sample		
	Witness	*Trichoderma atroviride*	Fulvic acid
BOGDAN	83.0	104.1	86.7
LUNG DE IȘALNIȚA	76.5	83.2	78.7
LUNG ROMÂNESC	82.1	99.0	85.4
DOLJAN	56.1	60.0	63.0
COSMIN	98.0	101.2	99.3
FERMIER	111.0	124.2	118.7
KAPRIMA F1	113.1	129.0	122.4

Table 1. Total polyphenol content of pepper samples

Sample name	Ascorbic acid, mg/100 g fresh sample		
	Witness	*Trichoderma atroviride*	Fulvic acid
BOGDAN	109.2	115.0	96.3
LUNG DE IȘALNIȚA	76.7	85.0	88.5
LUNG ROMÂNESC	78.7	90.3	87.9
DOLJAN	78.7	80.2	83.0
COSMIN	115.7	119.5	121.2
FERMIER	108.2	134.0	145.1
KAPRIMA F1	101.9	114.2	130.0

Table 2. Ascorbic acid content of pepper samples

Sample name	DPPH radical scavenging, %		
	Witness	*Trichoderma atroviride*	Fulvic acid
BOGDAN	27.6	52	40.0
LUNG DE IȘALNIȚA	25.5	49.2	38.0
LUNG ROMÂNESC	27.3	51	40.2
DOLJAN	18.6	40,2	34.2
COSMIN	32.6	51	57.0
FERMIER	37.0	62.4	60.0
KAPRIMA F1	37.7	59.7	63.0

Table 3. Antioxidant activity of pepper samples

In a biological system, an antioxidant can be defined as any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate [11].

In nature there are many plants with antioxidant content. Vegetables are an important class with a high content of antioxidants. The most common antioxidants are polyphenols and ascorbic acid. Molecular structures, in particular the number and positions of hydroxyl groups and the nature of substitutions on aromatic rings, give phenolic compounds the ability to inactivate free radicals [12].

Ascorbic acid is a natural organic compound with antioxidant properties, which is found in both animals and plants. It functions as a redox buffer that can reduce and therefore neutralize reactive oxygen species [13]. It is involved in a series of processes that take place in living cells. Ascorbic acid plays a complex and important role in the human body, protecting biologically active compounds from oxidative degradation. The iodometric method allows the determination of ascorbic acid in foods with different complexities.

The experimental data highlight the role played by the two fertilizers in the content of total polyphenols, ascorbic acid and the antioxidant activity of the pepper fruits studied.Thus, in the samples fertilized with Trichoderma atroviride, the content in polyphenols varies depending on the variety and presents superior values compared to the control sample and the samples foliar fertilized samples with fulvic acid.

The values of the total polyphenol content in the control samples are between 56.1 mEq GAE / g in the Doljan sample and 113.1 mEq GAE / g in the Kaprima F1 sample. The ascorbic acid content has a minimum value of 76.7 mg / 100g in the Lung de Işalniţa test and a maximum of 115.0 in the Cosmin test and the antioxidant activity has values between 18.6% in the Doljan test and 37.7% in the Kaprima F1 test.

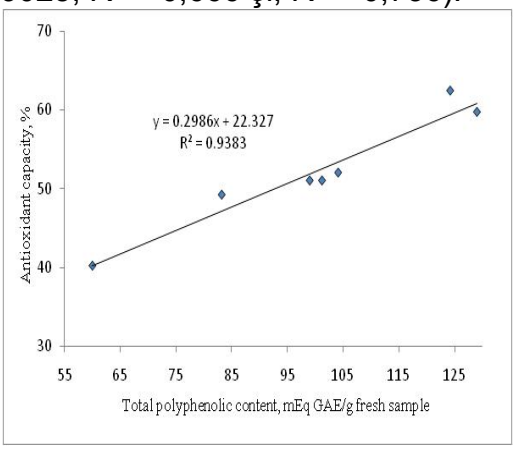
Trichoderma atroviride fertigation samples show values of total polyphenol content ranging from 60.0 mEq GAE / g in the Doljan sample to 129.0 mEq GAE / g in the Kaprima F1 sample. The ascorbic acid content has a minimum value of 80.2 mg / 100g in the Doljan sample and a maximum of 134.0 in the Cosmin sample and the antioxidant activity is between 40.2% in the Doljan sample and 62.4% in the Fermier sample.

Foliar fertilized samples with fulvic acid have values of the total polyphenol content between 63.0 mEq GAE / g in the Doljan sample and 122.4 mEq GA / g in the Kaprima F1 sample.The ascorbic acid content has the minimum value of 83.0 in the Doljan test and the maximum of 145.1 in the Fermier test and the value of antioxidant activities between 38.0% in the test: Lung de Işalniţa 63.0% in the Kaprima F1 test.

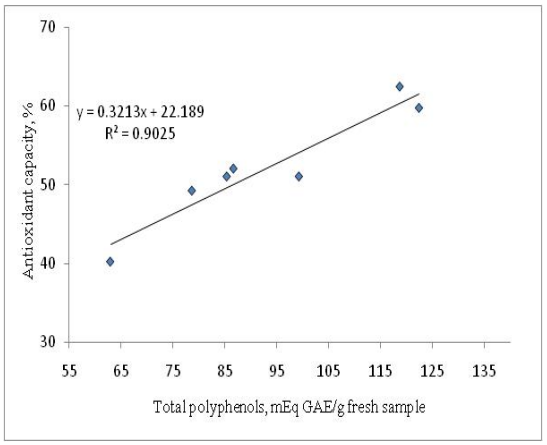
The corroboration of the experimental data highlights the role played by the two fertilizers in the changes of the biochemical composition in the culture of the 7 varieties of peppers. The stronger influence of fertigation with Trichoderma atroviride is noticeable.

The antioxidant activity of pepper can be attributed to the total content of polyphenols and the content of ascorbic acid and, therefore, it is important to analyze the correlation between the antioxidant activity and these bioactive compounds of pepper.

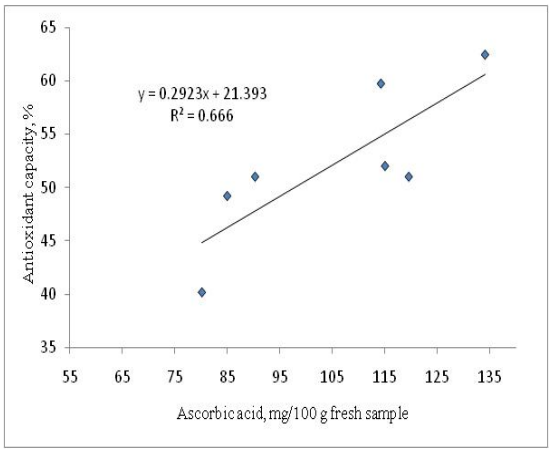
A regression analysis was used to correlate the results of the total content of polyphenols and ascorbic acid with the antioxidant activity of the cultivated pepper samples (Fig. 1, 2,3). For all samples a significant correlation was obtained (R^2 = 0,9383, R^2 = 0,9025, R^2 = 0,666 şi, R^2 = 0,736).



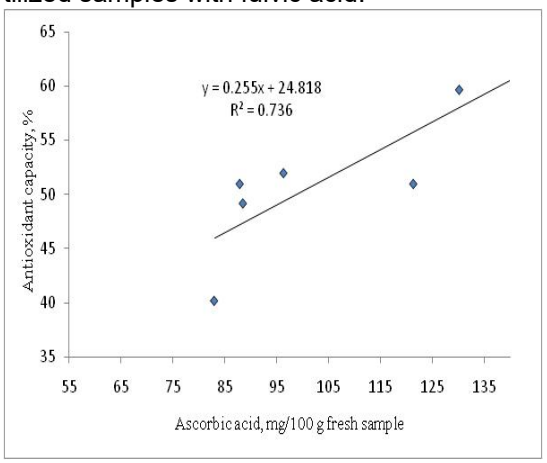
Linear regression between total polyphenol content and antioxidant capacity in samples fertilized with Trichoderma atroviride.



Linear regression between total polyphenol content and antioxidant capacity in foliar fertilized samples with fulvic acid.



Linear regression between ascorbic acid content and antioxidant capacity in samples fertilized with Trichoderma atroviride.



Linear regression between ascorbic acid content and antioxidant capacity in foliar acid fertilized samples.

There is a better correlation between the total polyphenol content and the DPPH value (R^2 = 0,9383, R^2 = 0,9025) of the samples fertilized with Trichoderma atroviride and fulvic acid compared to the correlation between ascorbic acid and DPPH (R^2 = 0,666 şi, R^2 = 0,736). This confirms that antioxidant activity is mediated mainly by polyphenols.

The correlation between total polyphenol content and antioxidant activity has been widely studied in various foods such as fruits and vegetables [14 - 20].

CONCLUSIONS

1. The efficiency of the fertilizer is highlighted by the values obtained from the samples fertilized with the two biostimulators.
2. The values obtained by fertilizing the pepper with Trichoderma atroviride are higher than those obtained by foliar fertilization with fulvic acid.
3. Although there is a difference between the results obtained with the two biofertilizers, we believe that it is appropriate to use them in pepper culture.

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