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EFFECT OF BIOSTIMULANT TREATMENT ON THE ANTIOXIDANT ACTIVITY IN TOMATO FRUIT

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Abstract

One of the most widely grown and consumed vegetable, tomato (Lycopersicon esculentum Mill.) is valuable both for their rich taste and for their nutritional value and antioxidant properties. Vitamin C, carotenoids (lycopene, β-carotene) and polyphenols are the main bioactive compounds present in tomato, their regular consumption being correlated with a low risk of various cancers and some cardiovascular diseases. Biostimulants products represent nowadays a sustainable alternative to the chemical fertilizers used for improving crop yields and quality. A product obtained from fish gelatin and collagen hydrolysate was physicochemically characterized and used for foliar application on tomato crop in the fruiting stage. The objective of this study was to assess the effect of the biostimulant treatment on antioxidant activity of tomatoes. The tomatoes were analyzed regarding carotenoids, ascorbic acid, phenolic compounds content and antioxidant activity. The treated tomatoes presented higher content by 26.64% for lycopene, by 12.8% for ascorbic acid and by 17.25% for total phenolics. These positive effects can be attributed to the supplementary amino acids amount provided by the biostimulator product.

***Key words**: antioxidant activity, ascorbic acid, fish gelatin, lycopene, phenolic compounds.*

INTRODUCTION

Recent research has reported protective effects on human health of a diet rich in vegetables and fruits, among which tomatoes occupy an important place. One of the most widely grown and consumed vegetable, tomato (*Lycopersicon esculentum* Mill.) is valuable both for their rich taste and for their nutritional value and antioxidant properties. The main bioactive compounds present in tomato are antioxidants such as carotenoids (lycopene, zeaxanthin, β-carotene), vitamin C and polyphenols which are involved in inhibition of the harmful effects of free radicals, preventing oxidative damage in DNA and proteins. The health-beneficial properties of tomato have been proven by epidemiological evidence that suggests their role in reducing serum levels of oxidative stress biomarkers. Research performed by Abete et al. (2013) demonstrated that daily consumption of 160 g of tomato sauce rich in lycopene produces a decrease in oxidized LDL cholesterol levels. Consequently, a diet rich in tomato was correlated with a low risk of various cancers and some cardiovascular diseases (Collins et al., 2022).

The nutritional content of the tomato fruit depends on the cultivation conditions and on the growth environment that must ensure the maximum potential accumulation of the bioactive compounds under organic farming conditions. Since the EU Council Directive 91/676/EEC called for a significant reduction in the amount of nitrogen-containing fertilizers used in agriculture and horticulture, environmentally friendly agricultural techniques to improve crop yields and quality were adopted. As an alternative to chemical fertilizers, biostimulants began to be used on a large scale to promote plant growth, so that the market size for biostimulants is expected to reach USD 4.14 billion by 2025 (Madende & Hayes, 2020). Although they do not supply nutrients directly to plants, the biostimulants can facilitate the nutrients achievement by supporting metabolic processes in plants improving the general health and vitality of plants.

Hydrolysates of food by-products such as animal tissue (Cristiano & De Lucia, 2021; Luta et al., 2024) or fish waste (Chalamaiah et al., 2012; Madende & Hayes, 2020) have been used as biostimulants provided by animal sources. Fish processing produce large quantities of waste (head, skin, bones, tail, viscera) representing a source of environmental pollution if improperly disposed. Only about 30% of the 91 million tons of fish harvested every year is transformed into fishmeal (Madende & Hayes, 2020). But fish by-products have potential applications as plant biostimulants. The residues rich in collagen and gelatin are processed by chemical or enzymatic hydrolysis and converted into protein hydrolysates containing free amino acids, short-chain peptides and proteins as active agents that positively influence some physiological processes, including photosynthesis, assimilation and translocation of nutrient in plants, and also accumulation of valuable compounds (Malécange et al., 2023). Various studies have shown that fish hydrolysates can improve nutrient utilization by plants, achieving beneficial effects on root and leaf growth, inducing flowering, improving fruit set, and also reducing fruit drop (Chalamaiah et al., 2012; Yakhin et al., 2017; Xu & Mou, 2017).

The present study was focused on testing a product obtained from fish gelatin and collagen hydrolysate with inorganic potassium added. This protein gel was physico-chemically characterized and used for foliar application on tomato in the fruiting stage. The aim was to assess the effect of the tomato plants treatment with the biostimulant product on the antioxidant compounds content and on the total antioxidant capacity of tomato fruit compared to the plants grown in conventional cultivation.

MATERIALS AND METHODS

Obtaining of biostimulant gel (protein hydrolysate)

A new product representing a protein gel made from fish gelatin and collagen hydrolyzate enriched with macroelements (K) with a ratio of NPK 1:1:3 was obtained and tested for biostimulant properties by application on tomato plants during the fruiting stage.

Fish gelatine was obtained from fish scales treated for 1 h with 0.1 M NaOH to remove non-collagen proteins, then demineralization using 1 M HCl (1.5 hours) have been made. The demineralized fish scales were subjected to a hot water extraction to produce gelatin, which was then filtered in several stages to obtain a clear gelatin solution. The biostimulant product was obtained by mixing equal parts of fish gelatine with collagen hydrolysate and inorganic potassium. The collagen hydrolysate was obtained from the residues of bovine hide after extraction of gelatine, which were dispersed in water in a ratio solid to liquid 1:2 at pH 8 and 50°C. Then a hydrolysis with Protamex (edopeptidases) added in a ratio of 1.6 to residue weight was made for two hours. The potassium was added in a ratio of 3:1 to total nitrogen. The obtained gel was analyzed regarding certain chemical characteristics (Table 1). Determination of the dry substance, total ash, total nitrogen and protein content, pH, bloom test, and viscosity were made using standards in force.

Table 1. Physico-chemical characteristics of protein gel

Characteristics	Analytical methods	Parameters value
Dry substance (%)	SR EN ISO 4684:2006	15.18
Total ash (%)	SR EN ISO 4047:2002	2.7
Total nitrogen (%)	SR EN ISO 5397:1996	1.20
Protein content (%)	SR EN ISO 5397:1996	6.74
pH	STAS 8619/3:1990	7.02
Bloom test (g)	European Pharmacopoeia. Gel strength	54
Viscozity (mPa*s)	European Pharmacopoeia. Viscosity.	1.75

Biological material

The experiment was conducted in the experimental greenhouse of the University of Agronomic Sciences and Veterinary Medicine from Bucharest in 2023 on pink tomato crop from BPK 16021 hybrid (*Lycopersicon esculentum* Mill.), provided by Marcoser SRL (Matca, Galati, Romania). The experimental variants were: V1 - untreated plants (control) and V2 - plants which received treatment with fish gelatin based gel. The substrate used for cultivation was Traysubstrate, a well structured mixture of blond peat and black peat, with addition of macro and microelements and a pH of 5.5-6.5. Three successive foliar treatments with dilluted solution of protein gel (10% v/v) were applied, once every seven days, between May 22 and June 5, 2023. No treatment with other fertilizers was administered except for biostimulant applications with tested protein gel. The tomato harvests started on June 26, 2023. Random tomato samples were collected from each variant and were analyzed in laboratory regarding bioactive compounds.

Biochemical analysis of the tomato fruit

Tomato fruit harvested at the red-ripening stage were subjected to biochemical analyses. The extractions were made according to the protocol provided by the analysis method used.

Carotenoids (lycopene and *β-carotene)* were determined according to the protocol described by Anthon & Barrett (2007). An amount of blended tomatoes was homogenized with a triple mixture of hexane: ethanol: acetone (2:1:1), then sonication and shaking were applied so that two phases were separated. A sample was collected from the upper phase and the absorbance was measured at two wavelength (503 and 444 nm). Using the extinction coefficient lycopene and β-carotene contents has been calculated. Results were expressed as mg/100 g FW.

Vitamin C content was determined using titration with potassium iodate solution by volumetric method described by Elgailani et al. (2017). A 2% solutrion of oxalic acid was used for the extraction of vitamin C with the purpose to prevent the oxidation. The extracts were treated with KI solution, hydrochloric acid and starch solution, then were subjected to titration with iodate KIO_3 until the a blue color appears indicating the end of the reaction. Results were expressed as mg/100 g FW.

Total polyphenols content was performed using the modified Folin-Ciocalteu method as Singleton et al. (1999) described. The determination is based on the reduction of the Folin-Ciocalteu reagent, obtaining a blue colored compound, followed by measuring the absorbance at 750 nm. The results were expressed as gallic acid equivalents (mg GAE/g FW).

Total antioxidant activity was measured according to the method adapted by Brand-Williams et al. (1995) after Blois (1958), using the stable free radical DPPH. 100 μM solution of DPPH in methanol were mixed with different concentrations of a tomato extract in 80% aqueous methanol, then were maintained 30 min in a dark place at room temperature. After that the absorbance (A) was measured at 515 nm. The percentage of the radical scavenging activity (RSA) was calculated as follows:

$$\% RSA = (1-[A_{sample}/A_{control\ t=0}])/100$$

A DPPH solution in 80% methanol was used as control. The linear regression curve of the sample extracts (mg/mL) against the percentage of the radical scavenging activity was made and used for calculating EC_{50} for each sample. The EC_{50} parameter is defined as the concentration of sample which is required to scavenge 50% of DPPH free radicals.

Statistical analysis was performed with the one-way Analysis of Variance (ANOVA) using Microsoft Excel Office 2019 for Windows. Comparisons of means were calculated using the Duncan's test at the 5% significance level ($p < 0.05$). All measurements were carried out in five replicates, and results are presented as means ± standard deviation (S.D.). In charts, values marked with different letters show significant differences ($p < 0.05$).

RESULTS AND DISCUSSIONS

Effect of the biostimulant treatment on the antioxidant compounds of the tomato

Biostimulants act by inducing a physiological response in the treated plants, influencing metabolic processes of plants and consequently, their growth and development. The current research aimed to assess the response of the tomato crop to the application of tested protein gel regarding the antioxidants content of the fruit.

Vitamin C is the most commonly found vitamin in tomato fruit. Considered as a major natural antioxidant, vitamin C is involved in limiting the damaging effects of free radicals therefore it is supposed to help prevent or delay the development of certain diseases caused by oxidative stress. Besides that, vitamin C stimulates the immune system and improves the absorption of iron and calcium (Bianchi et al., 2023).

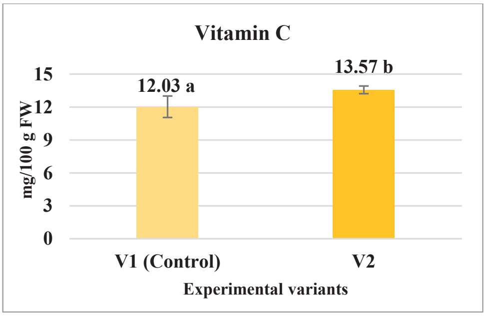

Figure 1. Vitamin C content in the analyzed tomato samples

The application of the biostimulant protein gel resulted in increased vitamin C content in tomato fruit. Significant differences were noted between the control and the treated variant (Figure 1), which recorded a 12.8% higher vitamin C content than the untreated plants.

Also previous research performed by Luta et al. (2024) found that the biostimulant treatment of tomato plant with a bovine gelatine based gel stimulated the accumulation of vitamin C, which was 1.29 times higher compared to the untreated plants. These results are in according to Tallarita et al. (2021), which reported an increasing of vitamin C in tomato fruit when applied 9 times the biostimulant treatment with an enzymatic protein hydrolysate. On the contrary, evaluating the effects of three biostimulants (protein hydrolysate, plant and seaweed extract) on the nutritional quality of greenhouse tomato, Colla et al. (2017) noted no influence of the treatment on the total ascorbic acid.

Lycopene and ***β-carotene*** are the main carotenoids detected in tomato fruit, representing almost 75% of tomato carotenoids (Hernández-Herrera et al., 2022). Lycopene and other carotenoids are suggested to protect against carcinogenesis through antioxidant mechanisms. Thorough studies demonstrated by in vitro experiments that both lycopene and β-carotene manifest anticancer activities, being able to induce apoptosis in cancer cells and to inhibit their proliferation (Jayappriyan et al., 2013; Arathi et al., 2016).

The analysis of the obtained results highlighted a 26.64% increase in the lycopene content from 7.17±0.2 mg/100 g FW in the untreated tomato fruit to 9.09±0.11 mg/100 g FW in the variant treated with fish gelatin based gel (Figure 2).

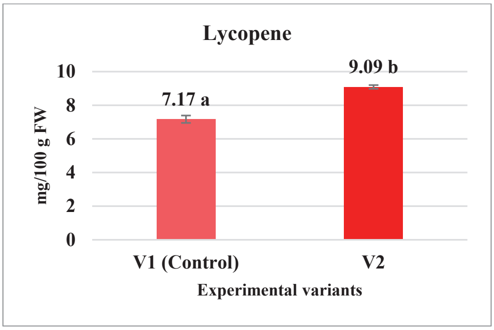

Figure 2. Lycopene content in the analyzed tomato samples

And the β-carotene synthesis in the tomato increased by 20.37% under the same biostimulant treatment (Figure 3).

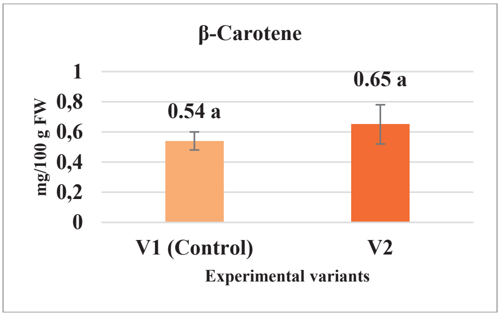

Figure 3. β-carotene content in the analyzed tomato samples

Tallarita et al. (2021) noted also that lycopene content recorded a quantitative improvement (from 11.05 to 14.13 mg/g FW) after repeated application of the tested protein hydrolysate on the tomato crop.

An increased by 125% accumulation of lycopene has been reported by Colla et al. (2017) under the foliar application of a commercial legume-derived protein hydrolysate. Consequently, a positive correlation between lycopene and potassium concentration was suggested, reporting that biostimulant application appeared to stimulated lycopene accumulation through the increase in mineral absorption.

Polyphenols are bioactive compounds with major role in free radical scavenging activity and in plant protection against stress.

Moreover, polyphenols are involved in plant development and growth (Sun et al., 2024) and also a correlation between total phenols of plants and antifungal activity has been reported (Mohamed et al., 2017).

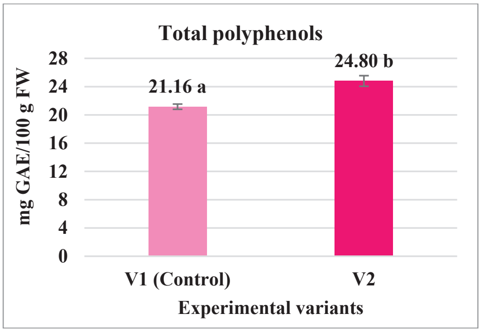

Figure 4. Total polyphenols content in the analyzed tomato samples

Figure 4 shows a stimulating effect for the total polyphenols biosynthesis under the treatment with the tested fish gelatin based gel. The obtained values were between 21.16±0.37 and 24.80±0.75 mg GAE/100 g FW, indicating a 17.25% increase in the average content of total polyphenols in the treated tomato compared to the untreated control.

Also previous studies reported that using of biostimulants enhanced the polyphenol content in tomato (Ali et al., 2021), broccoli (Kałuzewicz et al., 2017), and zucchini (Abd-Elkader et al., 2022).

Effect of the biostimulant treatment on the antioxidant activity of the tomato

Various studies have reported that biostimulants facilitate plant resilience, mainly by increasing antioxidant activity in the plants under unfavorable environmental conditions (Colla et al., 2015; Gonzalez-Morales et al., 2021; Malécange et al., 2023). The antioxidant capacity in plants is correlated with the content of compounds with antioxidant potential, such as carotenoids, flavonoids, vitamin C, polyphenols. Furthermore, a linear correlation of the increase in polyphenol content with antioxidant capacity is reported by previous research (Asadi et al., 2022; Balan et al., 2023). Samples of tomato collected from the experimental variants has been screened for a potential radical scavenging activity, and the EC50 values were calculated. Significant differences between the analyzed variants were noted (Figure 5).

The result indicated that variant V2 (tomato treated with protein gel) recorded the highest antioxidant activity (91.21±0.81 mg/mL expressed as the EC50 value), as expected due to the higher content in total polyphenols compared to the untreated variant, which required a higher concentration (121.39±0.80 mg/mL) to scavenge 50% of DPPH free radicals.

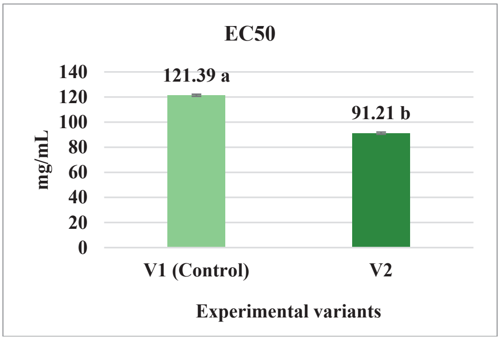

Figure 5. EC_{50} values of DPPH scavenging activities in the analyzed tomato samples

Overall, the treatment with biostimulant protein gel positively impacted the tomato plants growth and an improved fruit quality has been obtained.

These beneficial effects can be attributed to the supplementary amino acids amount provided by the new biostimulant product. Positive effects of the foliar application of amino acids and biostimulants based on amino acids on quality parameters have been reported also as a result of research performed on *Foeniculum vulgare* Mill. (Elsayed et al., 2022), *Achillea* *millefolium* L. (Shafie et al., 2021), *Nigella sativa* L. (Ayyat et al., 2021), *Ocimum basilicum* L. (Aghaye et al., 2019). Amino acids are molecules essential in metabolite synthesis promoting cell growth and plant development (Sun et al., 2024). The free amino acids present in the tested fish gelatin based gel represent a support for the development of tomato plants, helping the rapid biosynthesis of different types of proteins. Important for plant is not only the presence of these free amino acids, but also their accessibility for use in biosynthesis processes (Rentsch et al., 2007) therefore providing ready-for-uptake amino acids represents an advantage for plants, especially during critical periods of plant development such as the flowering or the fruiting stage (Bulgari et al., 2015).

CONCLUSIONS

Biochemical determinations performed in this study revealed beneficial effects of the treatments with biostimulant fish gelatin based gel on the tomato fruit quality.

Applications of the tested protein gel resulted in a good accumulation of the antioxidant compounds and a high radical scavenging activity of the tomato fruit, increasing their potential in preventing oxidative stress.

The tested new biostimulant could be considered better alternative for chemical fertilizers in obtaining vegetable with valuable nutritional and health-related properties.

This research also encourages the reuse of waste products supporting the circular economy in favor of a healthier environment.

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