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THE INFLUENCE OF FOLIAR FERTILIZATION WITH HUMIC ACIDS - BASED PRODUCTS ON THE QUALITY OF TOMATO FRUITS

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Abstract

The use of natural biostimulators on crops is an innovative approach, friendly to the environment and with beneficial effects on the quality of production. In this sense, the objective of this study was to evaluate the application of the Lignohumate biostimulator to the tomato crop on fruit quality, depending on the fertilization dose. This is a bio-fertilizer, consisting of a complex of potassium salts of humic and fulvic acids, supplemented with microelements. The biological material was represented by the 'Giraffe F1' tomato hybrid. The specifics of the variants were: V1 (Mt) - unfertilized, V2 - fertilized with 1 g/10 L water, V3 - fertilized with 1.5 g/10 L water, V4 - fertilized with 2.0 g/10 L water. The monitored quality parameters were: total soluble substance, titratable acidity, reducing carbohydrates, ascorbic acid, total carotene, total lycopene and total polyphenols. The recorded data showed that all quality parameters had significant values compared to the control in the variants fertilized with Lignohumate 1.5 g/10 L water and 2.0 g/10 L water.

Key words: *humic substances, Lignohumate, Lycopersicon esculentum, quality.*

INTRODUCTION

A manner in which the use of fertilizers can be reduced without affecting the nutritional balance of plants is the use of biostimulants and natural extracts. Foliar application is widely used as a method to supplement the deficiency of nutrients that are difficult to decompose in the soil. This practice is used both for the application of macro and micronutrients and humic fertilizers, favouring the assimilation of nutrients by plants and increasing the yield and quality of crops (Iancu et al., 2019; Tejada et al., 2018).

Du Jardin (2015) stated that humic and fulvic acids and are part of the humic compounds in the soil, with the addition of humins and originating from soil organic matter decomposed by plants, animals and microorganisms. A series of fertilizers containing mainly humic or fulvic acids are considered organic fertilizers, which have an effect on plant growth and crop yield by improving nutrient absorption.

Humic substances, humic and fulvic acids are major components (65-70%) in organic matter from soil, determining plant growth, by increasing cell membrane permeability, intensifying respiration, photosynthesis, oxygen and phosphorus absorption, ensuring the growth of root cells (Shen et al., 2020; Abou & Husein, 2016). Abd El-Razek et al. (2020) stated that the application of humic acids directly to the soil improves soil structure and fertility, water retention capacity, increases microbial activity and cation exchange capacity.

Fulvic acid chelates and binds dozens of minerals into a bioavailable form used by the cells. These trace minerals serve as catalysts for vitamins inside the cell, fulvic acid being one of the most efficient transporters of vitamins into the cell (Abou & Husein, 2016).

Other authors have demonstrated that humic substances stimulate root growth and absorption of nutrients at vegetable crops (El-Nemr et al., 2012; Soare et al., 2018). In addition, these crops are less sensitive to stress conditions (drought, extreme temperatures, excessive moisture in the rhizosphere, insufficient light and salinity) due to the high production of antioxidant compounds (Itelima et al., 2018). Unlu et al. (2011), through the foliar application of humic acids, to a cucumber crop, observed an increase in fruit and implicitly in crop yield. Soare et al. (2020) indicated that foliar application of Humusoil, a humic fertilizer product, had favourable effects on the genotypes of groundnuts cultivated on sandy soil from South of Romania. In addition, Matei et al. (2020) in their study showed that the level of the registered productions increased with the increase of the nutrient doses to grain sorghum comparative with unfertilized variants. According to other authors, the application of humic acids influenced the accumulation of carbohydrates and improved the colour and nutritional value of plum fruits (Lu et al., 2023), tomato (Dinu et al., 2013) and pepper (Apostol et al., 2022). In other studies, polyphenol extracts from seeds of *Vitis vinifera* were used along with humic acids, and the germination of tomato seeds was found (Dinu et al., 2014). Soare et al. (2017) found that humic acid applied to a cabbage crop significantly increased plant height, stem diameter, yield and quality.

Tomatoes (*Lycopersicon esculentum*) belong to the *Solanaceae* family and are among the most consumed vegetables in the world. The area cultivated with tomatoes, worldwide, was 4,917,735 ha in 2022, and the production reached 186.1 million tons. Currently, China is the largest tomato producing country in the world. In Romania, the area cultivated with tomatoes was 17,170 ha at the level of 2022, and the production was 298,920 tons (FAOSTAT, 2024). Tomato fruits contain high levels of antioxidants such as vitamin C, polyphenols, carotenoids and minerals, which are needed daily for a healthy diet. The interest in consuming high-quality fresh fruit is continuously increasing (Bădulescu et al., 2020). The levels of bioactive compounds are highly variable and can be influenced by variety, cropping system, harvesting stage and storage period (Dinu et al., 2017) and nutrient doses.

This study aimed to evaluate the application of the Lignohumate biostimulator organic to the tomato crop on fruit quality, depending on the fertilization dose.

MATERIALS AND METHODS

The researches were established in the experimental field of the Faculty of Horticulture in Craiova (Romania), in 2022, to evaluate the influence of the foliar application of the organic biostimulator Lignohumate on the quality of tomato fruits, depending on the fertilization dose. Lignohumate is a mixture of potassium salts of humic and fulvic acids, supplemented with trace elements. The product was applied in 3 stages, 10 days between treatments and in three different doses. The specifics of the variants were: V1 (Control) - unfertilized, V2 - fertilized with Lignohumate 1 g/10 L water, V3 - fertilized with Lignohumate 1.5 g/10 L water, V4 - fertilized with Lignohumate 2.0 g/10 L water.

The biological material was represented by the 'Giraffe F1' tomato hybrid. The culture was established by seedlings produced in a warm greenhouse, of 55 days. Planting was done in the last decade of April, at a distance of 0.9 m between rows and 0.3 m between plants. The crop, at planting, was fertilized with the organic fertilizer Orgevit 4-2.5-2.3 in a dose of 1.5 t/ha. In order to carry out the quality analyses of the fruits, they were harvested by variants and repetitions and prepared according to the standards.

Analytical Methods

The total soluble substance (TSS) content was determined using an Optical Digital Handheld Refractometer Dr301-95 set at t = 20^0C.

The determination of titratable acidity (TA)

From a sample of 5-10 g of tomatoes homogenated with a vertical blender Braun MR 404 Plus for 1 minute, 1-2 mL were taken which were diluted in 10 mL of distilled water and titrated with 0.1 N sodium hydroxide in the presence of phenolphthalein.

The acidity calculation is made using the formula:

AT (%) = V x N x 100/m

V - volume of NaOH solution used for titration, (mL); m - sample weight (g); N -normality of NaOH solution.

Reducing sugars (%) were extracted in distilled water (1: 50 w/v) and assayed colorimetric with 3,5-dinitrosalicylic acid.

The determination of ascorbic acid

A sample of 10 g of tomatoes, previously ground with quartz sand has been transferred into a 100 mL balloon by using a solution of 2% hydrochloric acid. It has been stirred and after sedimenting it has been filtered into a dry glass. A 10 mL aliquot has been passed into a Berzelius glass, to which 30 mL of distilled water, 5 mL of 1% potassium iodate and 1 mL solution of starch have been added. It has been then titrated with potassium iodate N/250 and stirred until becoming bluish.

The calculation of ascorbic acid concentration is made by using the equation:

Ascorbic acid mg/100 g FW = 352 x V.f/m,

where: 352 = 3.52 x 100; 3.52 mg acid ascorbic which corresponds to 1 mL potassium iodate N/250 used for titration and 100 = reporting per 100 g FW; V - volume solution used for titration (mL); f - the factor of the potassium iodate N/250; m - sample weight (g).

The total phenolics content (TPC) was determined colorimetric at 765 nm by the Folin-Ciocalteu method. Gallic acid was used to plot standard curve and the results were expressed as mg of gallic acid equivalents (GAE)/ 100 g FW.

Lycopene and β-carotene were extracted in 2:1:1 hexane: methanol:acetone. The non-polar layer was collected and spectrophotometrically analyzed (Evolution 600 UV-Vis; Thermo Scientific, UK).

The contents of lycopene and β-carotene were calculated according to the following equations: lycopene mg/100 mL = $-0.0458 \times A663 + 0.204 \times A645 + 0.372 \times A505 - 0.0806 \times A453$;

β-carotene mg/100 mL = $0.216 \times A663 - 1.220 \times A645 - 0.304 \times A505 + 0.452 \times A453$.

The results were expressed in mg/100 g FW.

Statistical Analysis. The data obtained were analysed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), followed by the Dunnett's test and 95% Confidence.

RESULTS AND DISCUSSIONS

In tomatoes, taste is the main factor influencing consumer preferences. This largely depends on the content of total soluble substances, reducing carbohydrates and organic acids which are often used as important indicators for fruit flavour evaluation (Zhang et al., 2023). Fruits with a high dry matter concentration have better taste, higher processing yield, better transportability and keeping quality during storage. In this study, the total soluble substance (TSS) recorded variations from 3.3 to 5.2°Brix, and the total acidity from 0.347% to 0.454%, the highest values being recorded in the variant treated with Lignohumate with 2 g/10 L. The results in the present study are in accordance with the studies carried out by Sun et al. (2022) who found that as the application dose of humic acids increases in cherry tomato plants, the TSS content in the fruits also increases. Molla et al. (2012) in their study on the effects of applying biofertilizers on tomatoes showed an increase in sugar content (5.11 mg/100 g) through the combined use of household waste composted with *T. harzianum* T22. Some authors noted that applying humic acids to the soil at a dose of 1.5 g/L significantly improved the content of total soluble substances, total acidity and total sugar content in tomato fruits, 'Tessera' cultivar (Alenazi & Khandake, 2024).

Regarding the reducing sugar content, it varied from 2.58% (V1) to 3.14% (V4), and in this case it was found that in the variants treated with the Lignohumate biostimulator, a higher amount of reducing sugars was accumulated, but insignificant values were recorded between variants, from a statistical point of view ($P < 0.05$) (Table 1).

Table 1. The effect of applying the Lignohumate biostimulator on total soluble solids, titratable acidity and reducing sugar

Variant	TSS (°Brix)	Total acidity (%)	Reducing sugar (%)
V1 (Ct)- unfertilized	3.3 A	0.347 A	2.58A
V2- fertilized with 1 g/10 L of water	3.7 A	0.381 A	2.73A
V3- fertilized with 1.5 g/10 L of water	4.8*	0.429*	2.80A
V4- fertilized with 2 g/10 L water	5.2*	0.454*	3.14A

Means not labelled with the letter A are significantly different from the control level mean, P≤5.

In the present study, the foliar application of humic acids indicated positive effects on the ascorbic acid content, which varied from 16.20 (mg/100 g FW) (V1) to 22.40 mg/100 FW (V4). The highest values were recorded in the fruits harvested from the variants treated with Lignohumate, while the tomato fruits harvested from the control variant had the lowest values (Table 2). The total content of ascorbic acid increased significantly with the increase in the dose of Lignohumate (P≤5), the highest value being recorded at the dose of 2 g/10 L water. The results are in agreement with those reported by other authors as well. Alenazi and Khandake (2024), demonstrated that the foliar application of humic acids (HA) improves the content of vitamin C in the fruits harvested from the treated variants, higher values being recorded at the dose of 2 g/L of HA.

Table 2. The effect of applying the Lignogumate biostimulator on the biochemical composition

Variant	Ascorbic acid (mg/100 g FW)	Total lycopene (mg/100 g FW)	Total carotene (mg/100 g FW)	Total phenolics content (mg GAE/100 g FW)
V1 (Ct) - unfertilized	16.20A	6.40A	1.21A	24.53A
V2 - fertilized with 1 g/10 L of water	18.22*	6.82A	1.44A	26.81*
V3 - fertilized with 1.5 g/10 L of water	18.75*	7.24A	1.83*	29.42*
V4 - fertilized with 2 g/10 L water	22.40*	8.51*	1.76*	28.19*

Means not labelled with the letter A are significantly different from the control level mean, P≤5.

In previous studies carried out by the authors of this paper, in different melon hybrids, it was found that the higher the concentration of Lignohumate, the better the accumulation of nutrients (Dinu et al., 2019; Soare et al., 2018). According to He et al. (2022), the application of humic/fulvic acids to the lemon crop determined the increase in the content of vitamin C, total sugar and total soluble substance, in the treated variants, compared to the control variant ($p < 0.05$).

Regarding the total lycopene content, values were recorded between 6.40 and 8.51 mg/100g FW (at V1 and V4 respectively), finding that the application of the biostimulator based on humic and fulvic acids induces an increase in the total lycopene content in all variants compared to the control variant. These results suggest that the application of humic acids can contribute to enhancement of the efficiency in the absorption of major nutrients, especially based on K from the soil, and which improves the quality of tomato fruits.

The total carotenes in tomato fruits varied from 1.21 mg/100 g FW in the unfertilized variant to 1.83 mg/100 g FW in the variant fertilized with 1.5 g/10 L water (Table 2). It is also found that in the case of this component, variable accumulations are recorded, being influenced by the applied dose with Lignohumate based on potassium humate.

Regarding the content of total polyphenols, it varies from 24.53 mg GAE/100 g FW to 29.42 mg GAE/100 g FW. These values indicate a higher accumulation in all the treated variants compared to the control variant. This fertilizer induced positive effects on the quality of tomato fruits. He et al. (2022), reported a higher content of total polyphenols in the peel, pulp and seeds of lemons, in variants treated with humic acid fertilizers compared to the control variants, at three different harvest periods ($p < 0.05$).

CONCLUSIONS

The results of the research showed that the use of the natural biostimulator Lignohumate, based on potassium salts of huic and fulvic acids, applied in three different doses to the tomato crop induced positive effects on the quality of the tomato fruits. In particular, in the variants treated with Lignohumate, the content of total soluble substance, total acidity and reducing sugar increased, the values being significant compared to the control (Ct.) ($p < 0.05$), highlighting in particular the variant fertilized with a dose of 2 g/ 10 L of water. The accumulation of ascorbic acid, total carotene, total lycopene and total positive effects on the quality of the tomato fruits. Regarding the accumulation of ascorbic acid, total carotenes, total lycopene and total polyphenols was more evident both at variants with 1.5 g/10 L and at 2 g/10 L.

It can be stated that fertilization with Lignohumate is a viable alternative to organic culture for obtaining higher quality yields compared to the unfertilized, at cultures in the greenhouse where large amounts of chemical fertilizers are applied that can cause pollution of the entire trophic chain (soil, underground water, plants).

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