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STUDIES REGARDING THE INFLUENCE OF EXOGENOUS SALICYLIC ACID TREATMENT ON SOME BIOACTIVE COMPOUNDS OF TWO VARIETIES OF CHERRY TOMATOES

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Abstract

Salicylic acid belongs to a diverse group of plant phenolics. It is a natural signaling molecule involved in the regulation of different physiological and biochemical processes. The way in which exogemous salicylic acid acts depends on several factors, such as the plant species, the environmental conditions (light, temperature etc.) and the concentration of salicylic acid solutions. Tomatoes (Lycopersicum esculentum) are an important dietary source of micronutrients and antioxidants. The beneficial properties appear to be related to the lycopene and carotene content and the antioxidant content particularly ascorbic acid, polyphenols, total flavonoids, which may play a role in inhibiting reactions mediated by reactive oxygen species.

In the present research the influence of exogenously applied salicylic acid on the content of some bioactive compounds was analyzed. Two different concentrations of salicylic acid solution (50 and 100 ppm) were applied on the leaves of two variety of cherry tomato (Idyll and Red Pear). The treatments were applied at 21, 30 and 45 days after the seedlings were planted. The results obtained indicated that 100 ppm concentration of SA was most effective in the foliar treatment in terms of content in bioactive compounds.

Key words: tomatoes, salicylic acid, bioactive compounds

INTRODUCTION

Salicylic acid (SA) is a phenolic compound and natural constituent of plants (Raskin, 1992). SA was recognized as an endogenous regulator in plants after it was discovered that it is involved in the regulation of different physiological and biochemical processes, including membrane permeability (Barkosky, Einhellig, 1993), ion uptake, enzyme activities, photosynthesis (Hayat et al., 2005), growth and development of plants (Hayat and Ahmad, 2007), and may function as a plant growth regulator (Arberg, 1981).

Quiroz-Figueroa et al., in 2001 suggested that it is possible that these phenolic compounds act as a signal which triggers the differentiation processes. An explanation would be the possibility that due to the chelating properties of these compounds, some inhibitors present in the embryogenic cultures are inactivated.

The effects of exogenously applied SA on plant physiological processes under optimal environmental conditions are controversial. Several studies suggest that SA may have a positive effect on germination or plant growth in various plant species. However, SA may also act as a stress factor, having a negative influence on various physiological processes. The

way in which it acts depends greatly on several factors, such as the plant species, the environmental conditions (light, temperature etc.) and the concentration. Exogenous SA may also alleviate the damaging effects of various stress factors, and this protection may also manifest through a higher photosynthetic capacity (Janda et al., 2014).

One of the most studied functions of SA is associated with its involvement in plant resistance response to different pathogen attacks (Enyedi et al., 1992; Durner et al., 1997).

Application of SA to plants has been shown a variety of biological responses. Enzyme activities such as amylase and nitrate reductase were increased by SA application (Sharma et al., 1986; Chen et al., 1993).

Many researchers studied the influence of exogenous applied SA to improve productivity of some vegetables. To analyse the potential benefits of SA to enhance food production, an extended series of experiments were conducted with different plant species of interest for food production, in different agroecosystems, while taking into account climatic conditions of soil, temperature, humidity and the quality of water for irrigation (Martin-Mex et al., 2013).

Tomatoes (*Lycopersicum esculentum*) are an important dietary source of micronutrients and antioxidants (Agarwa, Rao, 2000).

The role of dietary antioxidants, including vitamin C, vitamin E, carotenoids, lycopene and polyphenolic compounds in disease prevention, has received much attention in recent years (Hallivel et al., 1995; Feris, 1994). It is also an important source of minerals.

The research performed by Ardelean (2013), underlined the additional and correctional character of the foliar fertilizers on the production and quality of the fruits, by the continuation of the period of vegetation, of the maturity and reaping.

Exogenous application of SA increases plant productivity, acting on the stress physiology (Larqué-Saavedra, 1978) in the root system (Gutiérrez-Coronado et al., 1998).

The aim of this study was to analyze the influence of exogenously applied SA solutions (50 and 100 ppm) applied on the leaves of two variety of cherry tomato seedlings (Idyll and Red Pear), on the content of some bioactive compounds (lycopene, β carotene, total phenolic, flavonoid, vitamine C), in harvested fruit.

MATERIAL AND METHOD

The experiments were performed in 2016-2017, at the Laboratory of Secondary Metabolits in Food Industry from the Faculty for Environmental Protection, University of Oradea.

For this study, the analysed materials are represented by two varieties of cherry tomatoes (*Solanum lycopersicum)*, Idyll and Red Pear variety. The seedlings were purchased from the market and planted in plastic flowerpots. Three seedlings were planted from each variety in 3x3 different pots.

Treatment was made with different concentrations of SA solution: 50 ppm (0,005%), 100 ppm (0,01%) and the control sample, which was only water. The solutions were applied by spraying on the leaves.

The first treatment was applied at 21 days after the seedlings were planted, followed by another two applications after 30 and 45 days. The volume applied was 30 ml /plant.

Harvested fruits were frozen at -80ºC in individual plastic bags from each plant according to their variety and type of treatment. After all the fruits were harvested, the samples were homogenized separately and analysed.

The analysed parameters were: lycopene and β-carotene content, total polyphenolic compounds, total flavonoids content, vitamin C, antioxidant activity by FRAP assay.

Lycopene and βcarotene content determination

Lycopene extraction from tomatoes was realized following the method of Sharma and Le Maquer, 1996. One gram of the homogenized samples and 25 ml of hexane:ethanol:acetone, (2:1:1) (v/v) mixture were placed on the rotary mixer for 30 min., adding 10 ml distilled water and continued agitation for another 2 min. The solution was left to separate into distinct polar and non-polar layers. The absorbance was measured at 450 and 502 nm, using hexane as a blank and a Shimadzu–UV-mini–1240 spectrophotometer.

Lycopene and βcarotene concentration were calculated using Misbaudeen et al., 2012 formulas. All determinations were made in triplicates.

$$[\beta\text{caroten}]\ \mu g/g = \frac{(A450 \times \varepsilon_{\underline{lycopene}}502/\ \varepsilon_{\underline{lycopene}}450) - A502}{(\varepsilon_{\underline{lycopene}}502/\ \varepsilon_{\underline{lycopene}}450) \times \varepsilon_{carotene}450 - \varepsilon_{carotene}502}$$

$$[\text{Lycopen}]\ \mu g/g = \{A450 - \varepsilon_{carotene}450 \times [\beta\text{caroten}]\}/\ \varepsilon_{\underline{lycopene}}502$$

Were:

$\varepsilon_{carotene}\,450 = 1.39 \times 10^5$ L/mol/cm

$\varepsilon_{carotene}\,502 = 2{,}63 \times 10^5$ L/mol/cm

$\varepsilon_{\underline{lycopene}}\,450 = 1{,}16 \times 10^5$ L/mol/cm

$\varepsilon_{\underline{lycopene}}\,502 = 1{,}72 \times 10^5$ L/mol/cm

βcaroten mg/kg = (1,483 x A 450 – A 502)/1,798x 105

lycopene mg/kg = A 502/1.72 x 105

Extract preparation for polyphenolic compounds determination and for FRAP-assay

For each sample an alcoholic extraction was made: 1:1 with 50% ethanol solution, and after 30 minutes they were filtered. Ethanol extracts were diluted then 1/10 with ethanol solution (50%) (Moigrădean et al., 2007).

Total Phenolic content

The total phenolic (TP) content was determined by using the Folin-Ciocâlteu colorimetric method developed by Singleton and Rossi (1965). A diluted extract (0.5 ml) or phenolic standard was mixed with 2.5 ml Folin-Ciocâlteau reagent and after 5 minutes, 2.0 mL sodium carbonate (7.5%) was added. The absorption was read after 2 h at 20°C, at 750 nm. For the preparation of calibration, curve aqueous gallic acid solution was used as the standard and expressed as mg of gallic acid equivalent (GAE)/100g fresh weight (Gergen, 2004).

Total Flavonoid compounds content

The total Flavonoid compounds content (FC) was measured with $AlCl_3$ colorimetric assay (Atanassova et al., 2011). The absorbance was measured at 510nm. As a standard we used catechin.

Ascorbic acid (Vitamin C)

Ascorbic acid was extracted using xylen as solvent and determined using spectrophotometric determination (Ranganna, 1986).

5g of each sample were homogenized in 3% metaphosphoric acid, making up the volume to 20 ml. This solution was filtered. Then acetate buffer (pH 4), 2,6 dichlorophenol indophenol solution and xylene were added in rapid succession. After phase separation, the xylene phase was extracted, and the absorbance was measured at 520 nm. For the preparation of calibration curve ascorbic acid solution in 3% H_3PO_3 (0.1mg ml^{-1}) was used. The ascorbic acid content was expressed in mg ascorbic acid/ 100 g sample.

Frap assay

The reducing capacity of samples was assayed with the original method of Benzie and Strain 1996. For calibration curve we used Trolox (0–1000µM).

To 200µl of extract/standard solution, a volume of 1800 µl of freshly prepared FRAP reagent was added. The mixture was shaken and thermostated for 10 minutes at 37°C. After cooling, absorbances have been read at 593 nm, against the reagent blank. The results were expressed as µM of Trolox equivalent (TE) from calibration curve that corresponds to the sample concentration.

RESULTS AND DISCUSSION

The results obtained after performing analysis for studying the influence of SA applied exogenously on the content of some bioactive compounds, tested in two different concentrations and applied on two varieties of cherry tomato seedlings, Idyll and Red Pear, were inserted in table 1 and 2.

Table 1

Mean values for some tested bioactive compounds on Idyll variety

Parameters for Idyll variety		**Treatment**		
		Control C1	**SA 50 ppm (P1a)**	**SA 100 ppm (P1b)**
		mean±sd	**mean±sd**	**mean±sd**
Carotenoid pigments	Lycopene mg/kg FW	37.24±1.1	45.57±0.61 ***	85.70±0.79 ***
	β-carotene mg/kg FW	23.25±1.27	27.25±0.76 *	52.86±0.37 ***
Phenolic copmpounds	Total polyphenols mgGAE/100g FW	73.88±0.2	87.65±0.3 ***	84.10±0.21 ***
	Flavonoids mgQE/100g FW	27.77±0.2	23.69±0.25 ***	19.05±0.4 ***
Vitamin C mg/100 FW		21.34±0.5	18.45±0.29 ***	19.23±0.27 **
Antioxidant activity µM TROLOX equivalent		212.31±0.6	234.52±0.74 ***	298.45±0.69 ***

p>0.05= non-significant; p<0.05=* significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot

Table 2

Mean values for some tested bioactive compounds on Red Pear variety

Parameters for Red Pear variety		**Treatement**		
		Control C2	**SA 50 ppm (P2a)**	**SA 100 ppm (P2b)**
Carotenoid pigments	Lycopene mg/kg FW	53.05±0.98	54.61±1.23 n.s.	97.93±1.32 ***
	β-carotene mg/kg FW	21.31±0.21	26.55±0.34 ***	36.63±0.54 ***
Phenolic copmpounds	Total polyphenols mgGAE/100g FW	65.74±1.01	83.24±1.13 ***	80.59±1.35 ***
	Flavonoids mgQE/100g FW	22.25±0.23	18.48±0.35 ***	14.09±0.22 ***
Vitamin C mg/100 FW		29.15±0.11	22.94±0.05 ***	24.52±0.1 ***
Antioxidant activity µm TROLOX equivalent		204.52±1.15	226.70±1.2 ***	286.22±1.31 ***

p>0.05= non-significant; p<0.05=* significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot.

Lycopen and βcarotene content – increased significantly after SA treatments in comparison with the control lot. The highest increases were recorded for Idyll variety treated with a 100 ppm SA solution. In this case the lycopene content increased with 130.12% and the carotene content with 127.35% compared to the control lot. For the Red pear variety these values increased with 84.6% and 71.9% compared to the control lot (Table 1, Fig.1).

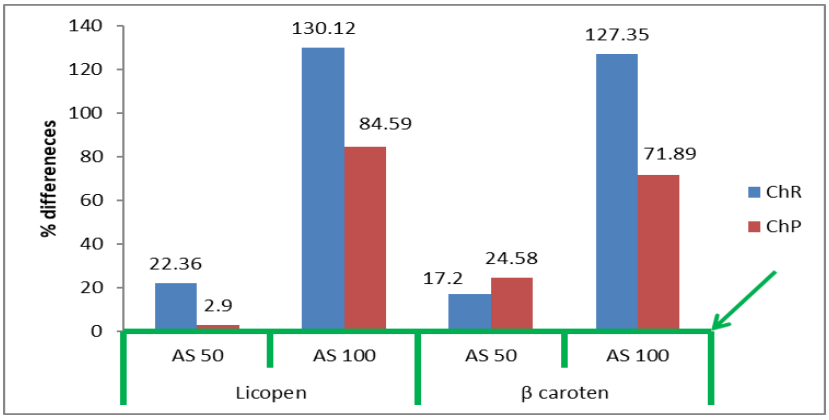

Fig. 1. Graphical representation of the percentage differences for lycopene and βcarotene between the control lot and the lots treated with SA

Total Phenolic content - Evaluating our results for the determination of TP content we can observe that the total polyphenolic content (TP) increases were higher in the Red Pear variety treated with a 50ppm SA solution (Table 2, Fig. 2).

Total Flavonoid compounds and vitamin C content were negatively influenced by the treatment with SA, the highest decrease was registered for Red Pear variety, in case of 100ppm SA solution (Table 2, Fig. 2).

Antioxidant activity presents a very significant increase for both varieties and both concentrations of SA solution. The highest values were registered in case of Idyll variety treated with 100ppm SA solution, 298.45 µM Trolox in comparison with control lot 212.31 µM Trolox (Table 1 and Table 2).

There are numerous studies related to the effect of SA on tomatoes but differ from one another, in terms of tomato varieties, the SA application, the stage of development of the plant in which the treatment was applied, but also the SA concentrations used.

Kowalska and Smolen (2013) obtained a decrease for TP content and an increase in vitamin C content in case of foliar treatment of tomato leaves with 0.01% (100ppm) SA solution.

Kumar et al., 2018, explore the effect of exogenous SA treatment on the post harvest life and quality of tomato fruit. The results obtained in their research indicated that the exogenous application of SA is an effective approach in enhancing the shelf life of the fruits.

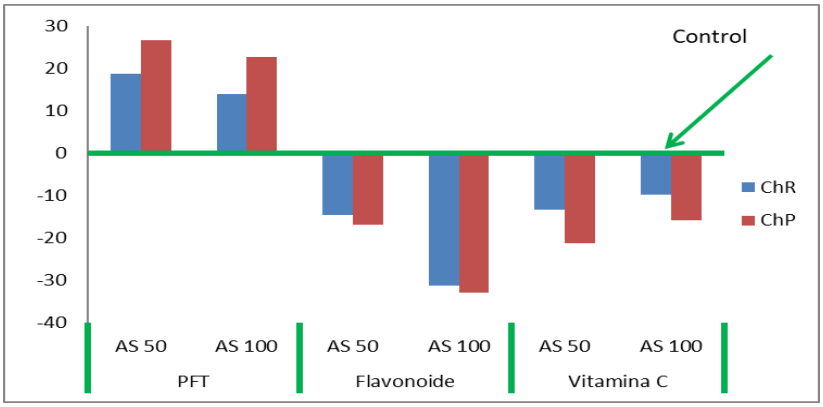

Fig. 2. Graphical representation of percentage differences for polyphenols, flavonoids and vitamin C between the control lot and the lots treated with SA

CONCLUSIONS

After analyzing the obtained results, the following conclusions can be drawn:

1. The foliar application of SA increases the content of lycopene, βcaroten, total phenolic content and antioxidant activity, in the harvested fruit, but decreased the content of total flavonoid and vitamin C.
2. The preferred concentration for SA solution was 100ppm.
3. In order to obtain more precise result, a various number of repetitions on a larger batch of tomatoes are necessary.
4. The treatments will be based on the analysed parameters and varieties. They can not be generalized for all parameters and varieties, a fact that was also specified in the literature.

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