

EFFECT OF FOLIAR APPLICATION OF SALICYLIC ACID ON THE RESPONSE OF TOMATO PLANTS TO OXIDATIVE STRESS AND SALINITY

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Abstract

The aim of the study has been to evaluate the effect of an increased salt concentration in a nutrient solution and foliar application of salicylic acid and KMnO_4 (the latter causing oxidative stress) on the yield, fruit quality and nutritional status of tomato plants. Salinity stress was stimulated by elevating the electrical conductivity (EC) of a nutrient solution by a proportional increase in the content of all macro- and micronutrients. In 2009-2010, tomato plants were grown on rockwool, in a heated foil tunnel. The experiment included two sub-blocks with two EC levels (2.5 and 4.5 mS cm^{-1}). Within each sub-block, the following foliar application variants were distinguished: 1. control, without foliar application; 2. salicylic acid (SA); 3. SA/ KMnO_4 . In the SA/ KMnO_4 combination, solutions of these compounds were applied alternately every 7 days. SA was applied in the concentration of 0.01%, while the concentration of KMnO_4 was 0.1%. Foliar treatments were conducted at 7-day intervals from the 3rd cluster flowering stage until ten days before the first harvesting of fruits. Irrespective of the EC of the nutrient solution, foliar application of SA as well as SA/ KMnO_4 had no significant effect on the tomato yield, total acidity and dry matter or soluble sugar content in fruits. Neither did it affect significantly the mineral status of plants except for an increase in the Mn level induced by SA/ KMnO_4 . A significantly higher content of ascorbic acid together with a decreased content of phenolic compounds and free amino acids resulted from the foliar application of SA and SA/ KMnO_4 . Salicylic acid counteracted the oxidative stress caused by KMnO_4 .

Key words: tomato, salicylic acid, KMnO_4 , salinity, yield, fruit quality, mineral content.

WPLYW DOLISTNEJ APLIKACJI KWASU SALICYLOWEGO NA REAKCJĘ ROŚLIN POMIDORA NA STRES OKSYDACYJNY I ZASOLENIA

Abstrakt

Celem badań było określenie wpływu zwiększonej koncentracji soli w pożywce oraz dolistnej aplikacji kwasu salicylowego i KMnO_4 (stymulowanie stresu oksydacyjnego) na plonowanie, jakość owoców oraz stan odżywienia roślin pomidora w składniki mineralne. W badaniach stymulowanie stresu zasolenia osiągano przez zwiększenie przewodności elektrycznej (EC) pożywki, w wyniku proporcjonalnego zwiększenia zawartości wszystkich makro- i mikroskładników pokarmowych. Rośliny pomidora uprawiano na wełnie mineralnej w ogrzewanym tunelu foliowym, w latach 2009-2010. Badania obejmowały dwa podbloki ze zróżnicowanym poziomem EC pożywki (EC 2.5 i 4.5 mS cm^{-1}). W każdym podbloku wyróżniono kombinacje z dolistną aplikacją roślin: 1) kontrola – bez dolistnej aplikacji, 2) kwas salicylowy (SA), 3) SA/ KMnO_4 . SA i KMnO_4 były aplikowane przemienne, co 7 dni. SA aplikowano w stężeniu 0,01%, a KMnO_4 – 0,1%. Zabiegi opryskiwania roślin roztworami wykonywano co 7 dni od momentu kwitnienia trzeciego grona, a zakończono na 10 dni przed pierwszym zbiorem owoców. Bez względu na EC pożywki dolistna aplikacja roślin SA oraz SA/ KMnO_4 nie miała istotnego wpływu na plonowanie, kwasowość owoców oraz zawartość suchej masy i cukrów rozpuszczalnych w owocach oraz na stan odżywienia liści roślin w makro- i mikroskładniki, z wyjątkiem zwiększenia zawartości Mn w przypadku przemiennej aplikacji SA/ KMnO_4 . Wykazano zwiększenie zawartości kwasu askorbinowego w owocach pomidora oraz zmniejszenie zawartości związków fenolowych i sumy wolnych aminokwasów wskutek dolistnego zastosowania SA oraz SA/ KMnO_4 . Kwas salicylowy przeciwdziałał stresowi oksydacyjnemu stymulowanemu przez KMnO_4 .

Słowa kluczowe: pomidor, kwas salicylowy, KMnO_4 , zasolenie, plonowanie, jakość owoców, stan odżywienia roślin.

INTRODUCTION

The influence of osmotic stress on plants in both *in vitro* and *in vivo* conditions is relatively well documented (MARSCHNER 1995). Nevertheless, most scientific papers describe response of plants to salinity (salinity of growth media, soils and substrates) caused by application of high doses of a particular salt, i.e. NaCl (ARFAN et al. 2007, STEVENS et al. 2006, TARI et al. 2002). With just a few exceptions, sodium chloride is not a typical mineral fertilizer. Thus, in intensive crop production (including protected soilless cultivation), salinity of soil or substrate is not caused by excessive concentrations of Na^+ and Cl^- ions alone, but by a much wider spectrum of cations and anions introduced with mineral fertilizers. It is even recommended to use chloride-free and low-sodium fertilizers in protected cultures. In this type of crop production, medium salinity is most commonly caused by excessive concentration of K^+ , NH_4^+ , Na^+ , NO_3^- , SO_4^{2-} and BO_3^{3-} as well as Na^+ and Cl^- , with the latter two being ballast elements in applied fertilizers (AUERSWALD et al. 1999, JAROSZ et al. 2011). Currently, soilless or hydroponic culture, a specific method of crop production, predominates in greenhouses

and foil tunnels (GAJC-WOLSKA et al. 2008). In this type of plant production, the plant shoot weight to substrate volume ratio is unfavorable. In agricultural practice, an increase in the salt concentration in substrates used for hydroponic cultivation results from the presence of all cations and anions introduced with a nutrient solution into the root environment (AUERSWALD et al. 1999, GRATTAN, GRIEVE 1999).

Endogenous salicylic acid (SA) is said to act like a growth regulator. It functions as an indirect signal stimulating many physiological, biochemical and molecular processes and therefore it affects the plant growth and development (KLESSIG, MALAMY 1994, MALAMY et al. 1990). Numerous studies have documented the influence of endo- and exogenous SA on the content of photosynthetic pigments in leaves (YILDIRIM et al. 2008), on plant photosynthesis (FARIDUDDIN et al. 2003) and on nitrogen metabolism owing to SA producing a positive impact on the activity of nitrate reductase (FARIDUDDIN et al. 2003, MIGUEL et al. 2002, JAIN, SRIVASTAVA 1981), on the synthesis of secondary plant metabolites and on antioxidant activity (ERASLAN et al. 2007) or the improved plant tolerance to heavy metals (GUO et al. 2009, POPOVA et al. 2008, GUO et al. 2007, METWALLY et al. 2003). In the context of the present study, the following findings seem particularly interesting: the beneficial effect of SA on plant adaptation (resistance, increased tolerance) to stress factors including heat (LIU et al. 2006, SHI et al. 2006, LARKINDALE, HUANG 2005), low temperature (KANG et al. 2003), fungal or bacterial infection (LEE et al. 1995) and excessive salinity of soil or nutrient solutions, i.e. osmotic stress (ARFAN et al. 2007, SAWADA et al. 2006, STEVENS et al. 2006, TARI et al. 2002). Studies on tomato cultivation have revealed that exogenous application of SA into a nutrient solution (TARI et al. 2002), soil (STEVENS et al. 2006) or sprayed over leaves (HE, ZHU 2008) improved the plant's tolerance to osmotic stress caused by high concentration of NaCl. However, there are few reports on the influence of exogenous salicylic acid on plants exposed to salinity stress induced by an elevated concentration of other than ions Na^+ and Cl in the rhizosphere.

Potassium permanganate (KMnO_4) is a strong oxidant, which is commonly used in medicine owing to its antifungal and antibacterial properties. It is also applied in chemical reactions as an oxidizing agent. Relatively little is known about its influence on plants. The information provided by MOLLENHAUER (1959) and KNOTH (1981) indicates that application of KMnO_4 can be recommended when preparing microscope slides from plant samples. Noteworthy is the study conducted by CHEN and YE (2005) on use of KMnO_4 for removal of *Chodatella* sp. algae from water. Results presented by these authors indicate that the response of *Chodatella* sp. to KMnO_4 included a release of extracellular organic matter and promotion of algal cell aggregation, which was additionally stimulated by increased water hardness. Foliar application of KMnO_4 could be of some importance for disinfection or control of fungal spores or bacteria on plant leaves and shoots during cultiva-

tion as a measure to prevent infection. On the other hand, such a treatment may increase the oxidative stress in epidermal cells, particularly in plants grown in a protected culture, whose leaves develop a thinner cuticular layer.

The available literature lacks information on the effect or possible use of exogenous salicylic acid to stimulate the plant's resistance or mitigate the negative symptoms of oxidative stress caused by KMnO_4 in plant cultivation. In practice, this type of plant stress can be caused by numerous agrochemicals (including chemical plant protection products), which may have oxidative properties. It is worth emphasizing that in large-scale protected cultivation, the co-occurrence of both stress factors – salinity and oxidative stress – is possible. No studies documenting the impact of SA application on plants exposed to both types of stresses have been presented so far.

The aim of this study has been to evaluate the effect of an elevated EC in a nutrient solution and of foliar application of salicylic acid (SA) and KMnO_4 (the latter stimulating oxidative stress) on yield, fruit quality and nutritional status of tomato plants. The idea has been to induce salinity stress by proportionally increasing the content of all macro- and micronutrients in the nutrient solution.

MATERIAL AND METHODS

The study, which was conducted in 2009-2010, consisted of trials on cv. Admiro F_1 tomato (*Lycopersicon esculentum* Mill.). Tomato plants were cultivated in a foil tunnel, on slabs filled with Grotop Master Dry rockwool (Grodan®). An open system plant fertigation was applied. The plants were divided into two sub-blocks, each treated with a nutrient solution characterized by a different EC: 2.5 and 4.5 mS cm^{-1} . Within each sub-block, the following foliar application variants were distinguished:

- 1) control – plants without foliar spraying,
- 2) plants sprayed with 0.01% solution of salicylic acid,
- 3) plants sprayed alternately with 0.01% salicylic acid or 0.1% KMnO_4 .

The plants were sprayed at 7-day intervals from the 3rd cluster flowering stage until ten days before the first fruit harvest. In total, the plants were sprayed four times (four treatments every seven days). In combination 3, the first plant spraying included salicylic acid, while the treatment conducted seven days later consisted of a solution of potassium permanganate. Spraying was performed so as to cover all plants, including flowers, with the solution (approximately 2000 dm^3 of solution ha^{-1}). In order to improve the efficiency of the treatment, Superam 10 AL (Danmar) was added to the working solution as an adjuvant.

Plant fertigation with nutrient solutions of two different EC values (2.5 or 4.5 mS cm⁻¹) started at the 2nd cluster flowering. Until then, plants from both sub-blocks had been fertigated with a nutrient solution of the same EC value. During the vegetative growth period, the pH of the nutrient solution was adjusted to 5.5 in all the variants.

The higher EC, i.e. 4.5 mS cm⁻¹, was obtained by a proportional increase of the concentration of all mineral nutrients in relation to their levels in the nutrient solution with EC equal 2.5 mS cm⁻¹. The composition of a medium with EC 2.5 and 4.5 mS cm⁻¹ was as follows (in mg dm⁻³): N 200 and 310, P 50 and 78, K 300 and 470, Mg 60 and 94, Ca 210 and 330, Fe 1.80 and 2.8, Mn 0.60 and 0.94, Zn 0.33 and 0.51, B 0.33 and 0.51, Cu 0.05 and 0.08, Mo 0.05 and 0.08, respectively. In the subsequent plant growth stages, N:K ratios were modified as recommended. Under intensive light exposure and high temperature, automatic compensation of the solution's EC was initiated in both series so as to obtain the maximum value of 0.5 mS cm⁻¹. Single and two-component fertilizers were used for making the nutrient solution. Microelements were introduced in the form of Superba Mikromix and Tenso (Yara) as well as ammonium molybdate.

Each sub-block consisted of 108 plants, 36 of which represented a study variant (12 plants × 3 replications). The crop density was 2.5 plants per m². The plants were pruned to a single stem and the topping was conducted above the 5th cluster.

The nutritional status of tomato plants was assessed according to the content of N, P, K, Ca, Mg, S as well as Fe, B, Cu, Mn, Mo and Zn in leaves. The determinations were made on the fifth (from the top) fully developed leaf harvested when fruits from the 3rd cluster were 3-4 cm in diameter. The nitrogen content was analyzed using Kjeldahl method, while the other macro- and micronutrients were determined using ICP-OES after mineralization in nitric acid. The dry matter content was assessed by drying leaf samples at 105°C.

Ripe fruits from all clusters were harvested and classified as marketable and non-marketable yield. Marketable yield included fully colored fruits, without visible deformations and above 35 mm in diameter.

The chemical composition was analyzed on ripe fully coloured fruits, similar in size, harvested from the 3rd cluster. For each replication of every variant, eight fruits were chosen, washed in distilled water, dried and homogenized. The following assessments were made on the fruit pulp: the content of dry matter by drying at 70°C, ascorbic acid using Tillmans method and total acidity by titration method. Spectrophotometric methods were applied to determine the levels of soluble sugars (with antrone), phenolic compounds (with Folin-Ciocalteu reagent) and total free amino acids (with ninhydrine).

As the results of all determinations in both years of the study were comparable, statistical analysis was conducted on mean values. The results

were verified using a two-way analysis of variance with the following factors: foliar application and nutrient solution EC. Statistical significance of differences between means was analyzed using Duncan's test at $P < 0.05$.

All the results are presented in Tables 1-4 as means from 2009-2010.

RESULTS

Tomato yield (both total and marketable) was significantly affected only by the EC of a nutrient solution. In contrast, no significant effect of foliar application or its interaction with the EC values on tomato yield was found (Table 1). The higher EC of a nutrient solution (4.5 mS cm^{-1}) reduced total and marketable yield by 1.04 and 1.05 kg m^{-2} , respectively, compared to tomato plants cultivated on a medium with the EC equal 2.5 mS cm^{-1} .

Table 1

Effect of foliar application and EC of nutrient solution on yield of tomato
(mean for years 2009-2010)

| EC (mS cm^{-1}) | Foliar application | Yield of tomato fruits (kg m^{-2}) | |
|-------------------------------|---------------------|-----------------------------------------------|-------------------|
| | | total | marketable |
| 2.5 | control | 9.07 | 9.05 |
| | SA | 8.68 | 8.68 |
| | SA/ KMnO_4 | 8.97 | 8.95 |
| 4.5 | control | 7.99 | 7.96 |
| | SA | 7.72 | 7.71 |
| | SA/ KMnO_4 | 7.90 | 7.87 |
| Mean for: | | | |
| EC | 2.5 | 8.91 ^b | 8.89 ^b |
| | 4.5 | 7.87 ^a | 7.84 ^a |
| foliar application | control | 8.54 | 8.51 |
| | SA | 8.20 | 8.19 |
| | SA/ KMnO_4 | 8.43 | 8.41 |
| Test <i>F</i> for: | EC | <i>x</i> | <i>x</i> |
| | foliar application | n.s. | n.s. |
| | interaction | n.s. | n.s. |

x – significant differences at $P < 0.05$; n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P < 0.05$.

Among all the tested tomato fruit quality parameters, that is dry matter, ascorbic acid, total acidity, soluble sugars, phenolic compounds and free amino acids (Table 2 – means for EC), the higher EC resulted in the production of tomato fruits with an increased content of dry matter and phenolic

Table 2

Effect of foliar application and EC of nutrient solution on fruit quality of tomato fruits
(mean for years 2009-2010)

| EC (mS cm ⁻¹) | Foliar application | Dry matter (%) | Ascorbic acid (mg 100 g ⁻¹ f.w.) | Acidity | Sugars | Phenolic compounds | Amino acids |
|------------------------------|-----------------------|----------------------|------------------------------------------------------|------------------------------|--------|-------------------------------|--------------------|
| | | | | (g 100 g ⁻¹ f.w.) | | (mg 100 g ⁻¹ f.w.) | |
| 2.5 | control | 6.06 | 10.08 ^a | 0.369 | 3.16 | 35.25 ^b | 87.41 |
| | SA | 6.11 | 12.50 ^c | 0.352 | 2.60 | 29.46 ^a | 69.00 |
| | SA/KMnO ₄ | 6.09 | 11.81 ^{bc} | 0.357 | 2.78 | 29.87 ^a | 71.37 |
| 4.5 | control | 6.53 | 10.58 ^a | 0.386 | 3.02 | 35.03 ^b | 82.48 |
| | SA | 6.21 | 11.67 ^b | 0.341 | 3.12 | 34.38 ^b | 70.49 |
| | SA/KMnO ₄ | 6.30 | 12.43 ^{bc} | 0.344 | 3.01 | 35.12 ^b | 73.05 |
| Mean for: EC | 2.5 | 6.09 ^a | 11.46 | 0.359 | 2.85 | 31.52 ^a | 75.93 |
| | 4.5 | 6.35 ^b | 11.56 | 0.357 | 3.05 | 34.84 ^b | 75.34 |
| foliar application | control | 6.29 | 10.33 ^a | 0.377 | 3.09 | 35.14 ^b | 84.94 ^b |
| | SA | 6.16 | 12.08 ^b | 0.347 | 2.86 | 31.92 ^a | 69.74 ^a |
| | SA/KMnO ₄ | 6.20 | 12.12 ^b | 0.351 | 2.89 | 32.49 ^a | 72.21 ^a |
| Test <i>F</i> for: | EC | x | n.s. | n.s. | n.s. | x | n.s. |
| | foliar application | n.s. | x | n.s. | n.s. | x | x |
| | interaction | n.s. | x | n.s. | n.s. | x | n.s. |

x – significant differences at $P < 0.05$, n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P < 0.05$.

compounds. Foliar spraying with SA and alternate application of SA/ KMnO₄ (Table 2 – means for foliar application) were similar in that they caused an increased ascorbic acid accumulation and reduced amounts of phenolic compounds and free amino acids in tomato fruits versus the control (plants without foliar treatment). Noteworthy is also the fact that the interaction between foliar application and nutrient solution's EC generated a statistically significant effect only on two tested fruit quality parameters: the level of ascorbic acid and phenolic compounds. When comparing the impact of foliar spraying with SA and SA/KMnO₄ independently for both sub-blocks (with the lower and higher EC value), a decrease in the ascorbic acid level was found only in plants treated with SA and grown in the solution of EC 4.5 mS cm⁻¹ versus the plants cultivated in a nutrient solution with the lower EC. In both sub-blocks, fruits of plants treated with SA contained significantly more ascorbic acid than the control ones. Moreover, a significant decrease in the content of phenolic compounds was found in fruits of plants sprayed with SA and SA/KMnO₄ in the sub-block with EC 2.5 mS cm⁻¹ when compared to the control as well as all to the combinations with plants cultivated in a nutrient solution of the higher salinity (EC 4.5 mS cm⁻¹).

No significant influence of EC, foliar application of SA or SA/KMnO₄ or interaction between EC and foliar treatments on the dry matter content in tomato leaves was found (Table 3). Taking into consideration the analyzed macro- and micronutrients, a significant increase in N, K, Cu and Mo and a decrease in the Ca content were noted in leaves of plants cultivated in a nutrient solution with the higher EC (Tables 3 and 4 – means for EC value). Among all the mineral nutrients determined in tomato leaves, foliar treatment had a significant effect only on Mn (Table 4, means for foliar application). When compared to the control and SA application, the foliar SA/KMnO₄ treatment contributed to an increased accumulation of manganese in leaves. The correlation between the EC value of a nutrient solution and foliar application was statistically significant only for the S and Zn content in leaves (Tables 3 and 4). Foliar application of SA led to a significant but relatively small increase in the sulphur level in leaves of plants grown on a nutrient solution with EC 4.5 mS cm⁻¹ compared to plants from the sub-block with the lower EC value. Regarding Zn, the foliar application of SA/KMnO₄ in the sub-block with the higher EC value significantly decreased the content of this element in tomato leaves in comparison to plants grown on a nutrient solution with EC 2.5 mS cm⁻¹. In the remaining combinations with foliar application, no significant differences in the content of S and Zn were found between plants from both sub-blocks.

Table 3

Effect of foliar application and EC of nutrient solution on the content of macronutrients in tomato leaves (mean for years 2009-2010)

| EC (mS cm ⁻¹) | Foliar application | Dry matter (%) | N | P | K | Ca | Mg | S |
|------------------------------|-----------------------|----------------------|-------------------|-------|-------------------|-------------------|-------|----------------------|
| | | | (% d.w.) | | | | | |
| 2.5 | control | 12.47 | 3.53 | 0.799 | 4.16 | 2.35 | 0.559 | 1.041 ^a |
| | SA | 12.08 | 3.51 | 0.788 | 4.06 | 2.46 | 0.572 | 0.987 ^{ba} |
| | SA/KMnO ₄ | 12.93 | 3.49 | 0.767 | 4.02 | 2.44 | 0.574 | 1.006 ^{abc} |
| 4.5 | control | 12.65 | 3.76 | 0.837 | 4.37 | 1.96 | 0.529 | 0.948 ^b |
| | SA | 12.69 | 3.76 | 0.886 | 4.43 | 2.27 | 0.574 | 1.070 ^c |
| | SA/KMnO ₄ | 12.60 | 3.80 | 0.781 | 4.46 | 2.14 | 0.514 | 1.079 ^c |
| Mean for: EC | 2.5 | 12.50 | 3.51 ^a | 0.784 | 4.08 ^a | 2.42 ^b | 0.568 | 1.011 |
| | 4.5 | 12.65 | 3.77 ^b | 0.834 | 4.42 ^b | 2.13 ^a | 0.539 | 1.032 |
| foliar application | control | 12.56 | 3.64 | 0.818 | 4.26 | 2.16 | 0.544 | 0.994 |
| | SA | 12.39 | 3.63 | 0.837 | 4.24 | 2.37 | 0.573 | 1.028 |
| | SA/KMnO ₄ | 12.77 | 3.64 | 0.774 | 4.24 | 2.29 | 0.544 | 1.042 |
| Test <i>F</i> for: | EC | n.s. | <i>x</i> | n.s. | <i>x</i> | <i>x</i> | n.s. | n.s. |
| | foliar application | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| | interaction | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | <i>x</i> |

x – significant differences at $P < 0.05$, n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P < 0.05$.

Table 4

Effect of foliar application and EC of nutrient solution on the content of micronutrients in tomato leaves (mean for years 2009-2010)

| EC (mS cm ⁻¹) | Foliar application | Fe | B | Cu | Mn | Mo | Zn |
|------------------------------|-----------------------|----------------------------|-------|-------------------|--------------------|-------------------|---------------------|
| | | (mg kg ⁻¹ d.w.) | | | | | |
| 2.5 | control | 106.16 | 35.33 | 8.59 | 40.64 | 2.16 | 21.06 ^{ab} |
| | SA | 101.06 | 33.69 | 7.68 | 40.29 | 1.92 | 19.75 ^a |
| | SA/KMnO ₄ | 105.15 | 33.99 | 7.98 | 55.78 | 1.99 | 23.44 ^b |
| 4.5 | control | 97.57 | 30.95 | 8.54 | 42.00 | 2.14 | 21.60 ^{ab} |
| | SA | 103.16 | 34.04 | 9.15 | 47.64 | 2.32 | 21.80 ^{ab} |
| | SA/KMnO ₄ | 94.72 | 31.40 | 8.50 | 58.19 | 2.23 | 20.72 ^a |
| Mean for: | | | | | | | |
| EC | 2.5 | 104.12 | 34.34 | 8.08 ^a | 45.58 | 2.02 ^a | 21.42 |
| | 4.5 | 98.48 | 32.69 | 8.73 ^b | 49.28 | 2.23 ^b | 21.37 |
| foliar application | control | 101.87 | 33.14 | 8.57 | 41.34 ^a | 2.15 | 21.33 |
| | SA | 102.11 | 33.87 | 8.41 | 43.97 ^a | 2.12 | 20.77 |
| | SA/KMnO ₄ | 99.93 | 32.69 | 8.24 | 56.98 ^b | 2.11 | 22.08 |
| Test <i>F</i> for: | EC | n.s. | n.s. | <i>x</i> | n.s. | <i>x</i> | n.s. |
| | foliar application | n.s. | n.s. | n.s. | <i>x</i> | n.s. | n.s. |
| | interaction | n.s. | n.s. | n.s. | n.s. | n.s. | <i>x</i> |

x – significant differences at $P < 0.05$, n.s. – non-significant difference;
Means within columns, marked with different letters differ at $P < 0.05$.

DISCUSSION

Salinity stress – effect of the EC of a nutrient solution on plants

Tomatoes belong to plant species moderately tolerant to salinity stress. The yield reduction among plants cultivated on a nutrient solution of EC 4.5 mS cm⁻¹ compared to plants treated with a nutrient solution of EC 2.5 mS cm⁻¹ was most probably due to salt stress. The effect of salinity on plant growth and yield is relatively well documented in numerous reports. Well-known are the effects caused by increased values of osmotic pressure within the rhizosphere (soil solution, nutrient solution in hydroponics) on plants, manifested by growth inhibition, blue-green colour of leaves or a slender phenotype. These changes are caused by the increased biosynthesis of ABA (inhibitor of plant growth and development) under salt stress conditions. ABA plays an important role in enhancing plants' tolerance to salinity (MARSCHNER 1995). Improved tolerance to salt stress is obtained by the higher expression of genes responsible for the biosynthesis of osmoprotectants and stress proteins. This effect, however, is achieved at substantial energetic expense, which eventually contributes to the growth retardation

and reduction of plant yield. Under stress conditions (irrespective of the etiology of stress), phenolic compounds are more intensively synthesized in plants (WU et al. 2005, ALI, ABBAS 2003, RIVERO et al. 2001). In the present study, increased levels of phenolic compounds were determined in fruits of plants cultivated on a nutrient solution with EC 4.5 mS cm⁻¹. Another cause of the higher accumulation of phenolic compounds in these plants could have been the improved nitrogen status of plants, which led to the increased activity of enzymes responsible for its biosynthesis (MATSUYAMA, DIMOND 1973).

One of the physiological responses of plants to osmotic stress is an increased demand for potassium, which is explained by the participation of K⁺ ions in osmoregulation (MARSCHNER 1995). In the research conducted by WEST and TAYLOR (1980), a higher uptake of mineral nutrients (Na and Cl) was observed in tomato plants subjected to NaCl-induced salinity stress and the effect was positively correlated to an increase in the temperature and moisture of the substrate. In the present study, changes in the content of all mineral nutrients analyzed in leaves of plants cultivated under the higher EC value included increased concentrations of K, N, Cu and Mo and a reduced level of Ca. These results demonstrate clearly that when the salinity stress is induced by a proportional increase in the concentration of all macro- and micronutrients in a nutrient solution, plants can selectively regulate the uptake of particular mineral nutrients. The reduced accumulation of Ca in plants cultivated on a nutrient solution of EC 4.5 mS cm⁻¹ (when compared to EC 2.5 mS cm⁻¹) could have resulted from the well-known antagonism between K⁺ and Ca²⁺ (during its uptake by plant roots), but also from the fact that these two cations participate in the regulation of water relations albeit producing opposite effects. With an increased demand for K⁺ by plants grown at 4.5 mS cm⁻¹ EC, a reduction in the Ca²⁺ uptake was observed. On the other hand, the improved nitrogen status found in these plants could have been caused by the synergistic action of K⁺ on the NO₃⁻ uptake. As a result, the demand for Mo was higher as well. This element is a cofactor for nitrate reductase, an enzyme responsible for the reduction of nitrate ions (CAMPBELL 1999). It is worth mentioning than effects induced by salinity stress could have included an impaired uptake of calcium, in extreme cases leading to its deficiency (MARSCHNER 1995). In our study, a reduction in the total yield was observed, with no relationship between a lower calcium status of plants and the incidence of blossom-end rot in tomato fruits. The difference between total yield and marketable yield was created by harvesting smaller tomato fruits. It should be underlined that the aforementioned interactions between the influence of a nutrient solution's salinity level on the mineral nutrition of plants had no significant effect on the dry matter content in leaves. Nonetheless, the dry matter content was found to have increased in fruits of plants subjected to salt stress.

Influence of SA and KMnO_4 foliar application and its interaction with the EC of a nutrient solution

There is a wealth of research results indicating that exogenous salicylic acid increases plant tolerance/resistance to salinity (HE, ZHU 2008, ARFAN et al. 2007, SAWADA et al. 2006, STEVENS et al. 2006, TARI et al. 2002). It is therefore puzzling that foliar application of salicylic acid in a relatively high dose of 0.01% had no effect on yield and mineral nutrition (with the exception of sulphur) of plants exposed to salt stress compared to plants grown on a nutrient solution with the lower value of EC. One possible explanation is that our experiment was conducted in a foil tunnel. In protected culture, droplets of working solution dry on the leaf surface very rapidly. The absorption of compounds applied with the working solution depends strongly on how long the leaves remain moist. This process may also be resumed after rehydration of the dry deposit (formed after water evaporation) on the leaf surface by its humidification with water vapour. The level of rehydration depends on air humidity as well as the POD (Point of Deliquescence) of particular compounds applied through foliar spraying (SCHÖNHERR 2002). When a mixture of salts (organic compounds) is sprayed, a change in the ionic strength of the solution occurs depending on its concentration. As a consequence, the value of the POD, which is difficult to estimate experimentally, also changes. It should be mentioned that the POD is not temperature-dependent (KOLTHOFF et al. 1969). Generally, organic compounds used commonly for foliar applications are characterized by high values of the POD (SCHÖNHERR 2002), a characteristic responsible for the low level of rehydration of the dry deposit formed on the plant's surface.

It can be assumed that due to the rapid drying of working solution droplets, absorption of compounds applied foliarly (salicylic acid and KMnO_4) was hampered. Additionally, in our study, the rehydration of the dry deposit formed on leaves was hindered by a relatively low air humidity in the foil tunnel (as well as the limited dew deposition on plants). Visual observations revealed small violet spots (from evaporated KMnO_4 solution) on leaves of plants treated alternately with SA/ KMnO_4 . Moreover, it can be suspected that most of the applied SA (in both tested combinations) could remain on the leaf surface (dry deposit) as the absorption of organic compounds is generally slower than that of dissociated cations and anions when applied foliarly (SCHÖNHERR 2002). This can also explain why SA, a compound which theoretically protects plants from potential yield loss caused by a higher EC of a nutrient solution, did not produce any effect. It is worth mentioning that SALEHI et al. (2011), who examined tomatoes grown in a subtropical climate in pots irrigated with saline water (0, 4, 8 and 12 dS m^{-1}), reported that foliar application of SA (in concentrations of 0, 10^{-6} , 10^{-4} and 10^{-2} mol) did not significantly affect fresh and dry weight of shoots or the number of flowers per plant. However, these authors suggest further studies including lower SA doses. In this context, the study by MADY (2009) seems particularly

relevant. It was conducted on a field plantation of tomatoes, where foliar application of 50 and 100 mg SA dm⁻³ contributed to an increase in the number of branches and leaves per plant, leaf area per plant, dry weight of leaves as well as the concentration of photosynthetic pigments, N, P, K, Fe, Zn, Mn, total carbohydrates and crude protein in leaves.

The results of our study indirectly indicate that both compounds applied through foliar spraying were to some extent absorbed by plants, but the rate of their absorption was insufficient to significantly affect the fruit yield or mineral nutrition of plant leaves. The exceptions included an increase in the Mn content in leaves of plants treated alternately with SA/KMnO₄ and an improved sulphur status in leaves owing to a foliar application of SA in the sub-block with the higher EC level. Salicylic acid plays a role of an indirect signal triggering numerous biochemical processes in plants (MALAMY et al. 1990, KLESSIG, MALAMY 1994). The induction of plants' resistance to salinity is related to the stimulated synthesis of protective proteins, and because sulphur amino acids are required in this process, the plants' demand for sulphur increases.

The applied compounds were found to have produced a relatively small influence on the quality of tomato fruits. However, the results on ascorbic acid and phenolic compounds in fruits are interesting. Plant spraying with solutions of SA and SA/KMnO₄ increased the content of ascorbic acid in fruits from both sub-blocks when compared to the control. It seems that SA may have stimulated the synthesis of ascorbic acid while reducing the phenolic content, particularly in the sub-block with the lower EC value. The results obtained in the sub-block with the lower EC indicate that SA effectively counteracted the oxidative stress caused by foliar application of KMnO₄. What was intriguing was the comparable levels of ascorbic acid noted in fruits of plants from both sub-blocks and treated with SA/KMnO₄. It seems that salt stress (induced by application of a nutrient solution with the higher EC value) did not weaken the cultivated plants, hence the temporary oxidative stress (caused by foliar application of KMnO₄) had no significant effect on their growth and development. It is possible that plants exposed to a constant stress of the high EC value managed to adapt to unfavorable conditions. Frequently, the plant's adaptation or "immunization" to one stress factor contributes to its increased tolerance/higher sensitivity threshold to other stressors. This is indirectly confirmed by our study, where an increase in the content of phenolic compounds occurred in plants treated with the foliar application of SA and SA/KMnO₄ in the sub-block with EC 4.5 mS cm⁻¹ compared to plants from analogous combinations in the other sub-block. The effect of a higher synthesis of phenolic compounds in response to salinity stress was observed previously (WU et al. 2005, ALL, ABBAS 2003). Nevertheless, it is surprising why the spraying of tomato plants with SA and SA/KMnO₄ reduced the level of phenolic compounds in the plants treated with the lower EC value. SA is one of the phenolic compounds

present in plants and an application of its exogenous forms, at least theoretically, could increase the content of phenolics and other secondary metabolites as its derivatives. The influence of exogenous salicylic acid on the accumulation of phenolic compounds and ascorbic acid in plants is most likely dependent on the SA concentration and method of application (also with respect to simultaneous introduction of other compounds), cultivation conditions and species-specific variation. In the experiment conducted by SMOLEŇ and SADY (2012), a double application of SA in the concentration of 10 mg dm^{-3} as well as a simultaneous application of urea+Mo+BA+sucrose+SA contributed to a comparable decrease in the accumulation of ascorbic acid, with no effect on the phenolic content in radish roots in comparison to the control. In carrot cultivation, an application of $0.5 \text{ mmol SA kg}^{-1}$ of soil resulted in a higher accumulation of carotenoids and anthocyanins in storage roots (ERASLAN et al. 2007).

CONCLUSIONS

1. Plant cultivation on a nutrient solution of a higher electrical conductivity (EC) value caused a significant reduction of tomato fruit yield accompanied by an increased content of N, K, Cu and Mo and a decrease in the Ca level in leaves. The higher EC value of a nutrient solution increased the accumulation of dry matter and phenolic compounds in tomato fruits.

2. Foliar application of salicylic acid and salicylic acid/ KMnO_4 had no significant influence on the fruit yield, total acidity and content of soluble sugars in fruits or the nutritional status of leaves with respect to macro- and micronutrients, with the exception of an increased Mn content in plants treated alternately with salicylic acid and KMnO_4 .

3. An increased content of ascorbic acid and reduced accumulation of phenolic compounds and free amino acids were noted in tomato fruits following the foliar application of salicylic acid and salicylic acid/ KMnO_4 .

4. Results of the determination of ascorbic acid and phenolic compounds in tomato fruits from the sub-block with the lower EC value indicate that salicylic acid effectively counteracted the oxidative stress stimulated by a temporary foliar application of KMnO_4 . No such relationship was found in the sub-block with 4.5 mS cm^{-1} , most probably due to the plant's adaptation (resistance) to the main stress factor, i.e. salinity.

5. No influence of exogenous salicylic acid applied foliarly was found such as the prevention of fruit yield reduction caused by the high EC level (salt stress).

6. A significant interaction between foliar application of the compounds and the EC level was observed for the content of ascorbic acid and phenolic compounds in fruits as well as the S and Zn accumulation in leaves.

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