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## Research Article

# Effect of High Salicylate Concentrations on Growth and Levels of Aromatic Compounds in Six Plant Species

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### Abstract

**Background and Objective:** Salicylic acid, a natural phenolic compound synthesized in plants through the shikimic acid pathway. In the search for a cheap nature product with high and selective effects on weeds, this study was conducted to evaluate the phytotoxicity of salicylates at high doses on plant growth and determine the changes that induced in the levels of endogenous aromatic compounds in the tissues of six plant species. **Materials and Methods:** Salicylic acid and acetylsalicylate at 25, 50 and 100 mM were foliar sprayed on 5-weeks-old of three summer crops, maize, sunflower and cotton and three summer weeds, purslane, jew's mallow and barnyard grass. Analyses were performed in triplicate. Analysis of variance and comparison of treatment means with LSD at 5% level were performed using Stat Graphics Plus Version 5.1. **Results:** Salicylate treatments caused severe injury effects on all examined plants, after 7 days from treatments. Acetyl salicylate treatments tended to cause higher injury effects on target species than salicylate acid treatments. However, after 35 days of treatments, the growth criteria of treated plants did not differ significantly from those of control plants. All treatments tended to increase the levels of endogenous salicylic acid, phenylalanine and tyrosine and decreased tryptophan contents. **Conclusion:** Salicylates tended to induce great changes in the levels of endogenous salicylic acid and aromatic amino acids, however, these compounds did not produce any permanent phytotoxic effects on tested plants.

**Key words:** Salicylic acid, acetyl salicylate, weeds, endogenous aromatic amino acids, phytotoxicity, sunflower, cotton, maize

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Weeds have the largest negative impact on crop productivity among various pests<sup>1</sup>. Herbicides tended to be used in far larger volumes than the other agricultural pesticides applied in the world<sup>2</sup>. There is a growing need for new natural herbicides with safer toxicological and environmental profiles. This need was driven by both the loss of herbicides due to safety issues and the rapidly increasing evolution of resistant weeds to herbicides<sup>3</sup>. Phenolic compounds, natural products, constituted an important class of plant secondary metabolites. Many investigators studied the phytotoxicity potential of phenolic compounds and found that many of these compounds at very high rates were very effective against weeds<sup>4-8</sup>.

Salicylic Acid (SA), a natural phenolic compound derived in plants from the shikimic acid pathway, to which  $\geq 30\%$  of photo-synthetically fixed carbon was directed<sup>9</sup>. Chorismate, the end product of the shikimic pathway is the precursor of the three aromatic amino acids: phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). Such three aromatic amino acids were considered the precursors to a wide variety of secondary plant, especially SA, condensed tannins, anthocyanin, vitamin E, indole-3-acetic acid, flavones, isoflavones, phenylpropanoids and coumarins, which are essential for plant growth and development<sup>10</sup>. SA can be synthesized from Phe and Tyr through cinnamic acid, an intermediate in the shikimic acid pathway. The decarboxylation of cinnamic acid may generate benzoic acid, which may then undergo hydroxylation at the C-2 position forming SA<sup>11</sup>. SA is known as a phytohormone which contributes to the regulation of the growth and development processes, like seed germination, photosynthesis, respiration, flowering and senescence<sup>12</sup>. Moreover, SA is considered as a potent signaling molecule in the activation of the plant defense responses against biotic and abiotic stress factors<sup>13-15</sup>. Acetyl salicylate (AcSA) is a commercially manufactured form of SA and in aqueous solution, it hydrolyzed completely to SA<sup>16</sup>. Many studies were conducted to evaluate the phytotoxic effect of high doses of SA and AcSA on seed germination that were previously conducted<sup>17,18</sup>. Although, the literature about the phytotoxicity effect of high doses of SA on plant growth was limited. The effect of foliar application of SA may be returned to its direct effect or connected with the accumulation of endogenous SA or its aromatic precursors in plant tissues. This study aimed to investigate the effect of high doses of SA and AcSA on growth and the changes induced in the levels of endogenous SA and the three aromatic amino acids in the tissues of six plant species.

## MATERIALS AND METHODS

**Study area:** This study was conducted to study the phytotoxic effect of SA and AcSA at high doses on three weed species and three crops species grown under controlled conditions in the experimental greenhouse station ( $25 \pm 3^\circ\text{C}$ , 12 hrs photoperiod) of National Research Centre, Giza, Egypt, during the summer season of 2018.

**Plant materials and pot experiment:** Seeds of maize (*Zea mays* cv. Giza 2), sunflower (*Helianthus annuus* cv. Eroflor) and cotton (*Gossypium barbadense* cv. Giza 9-2) were obtained from Agricultural Research Center, Egypt. Whereas, mature and uniform seeds of purslane (*Portulaca oleracea*), jew's mallow (*Corchorus olitorius*) and barnyard grass (*Echinochloa crus galli*) were collected from weeds grown in the experimental station of National Research Centre, Egypt. The 30 cm pots were filled with 4 kg soil (sand: peat moss, 3:1, w/w) and 20 seeds of each plant were sown per photon the 7th April 2018. All pots watered daily and after 2 weeks, they were flushed with half-strength Hoagland solution. Different concentrations of SA or AcSA were prepared using 0.8mM of sodium hydroxide and this solution used as control. When plants were 5 weeks old, they sprayed with SA or AcSA at 0, 25, 50, 100 mM (w/v) concentrations using an Epoca sprayer (Italy). The solution was sprayed evenly over the entire surface of the plant, including the adaxial and abaxial surface of leaves. For each plant type, 28 pots were maintained (i.e., 7 concentrations  $\times$  4 replicates) in a completely randomized design. Seven days after treatments the plants were examined for visible injury levels and the percent of chlorotic and necrotic areas recorded. Thirty-five days after treatments, plant growth parameters were recorded in terms of plant height (cm), fresh weight of plant (g) and dry weight of plant (g).

**Extraction and determination of salicylic acid and aromatic amino acids:** One day after treatments, vegetative plant samples were collected, fast cleaned with distilled water to remove the SA or AcSA residues, oven-dried at  $50^\circ\text{C}$ , ground in a mortar. The extraction of aromatic compounds was performed according to Matallo *et al.*<sup>19</sup>. One hundred milligrams of plant powder was placed in a centrifuge tube with 15 mL capacity and 10 mL of acidified water (pH 2.5) was added in each sample, which was subjected to an ultrasonic bath with an ultrasonic frequency of 50/60 Hz for 30 min. Subsequently, the samples were subjected to centrifugation at 4,000 g for 10 min at  $20^\circ\text{C}$ . The supernatant was collected and filtered in a nylon filter  $45 \mu\text{m}$ . Quantification of Phe, Tyr, Trp and SA were performed by High-Performance Liquid

Chromatography (HPLC), LC-10 AD, Shimadzu, Japan. Aromatic compounds were analyzed using a Luna RP-C18 (2) column (250×4.6 mm i.d, 5 µm, Phenomenex). The mobile phase consisted of 5 mM of ammonium acetate in methanol (72:28) at a flow rate<sup>20</sup> of 0.5 mL/min. The detecting wavelength was 230 nm. Standard of Phe, Tyr, Trp and SA acids were purchased from Sigma Aldrich. For each compound, the calibration curve was carried with the concentrations of the standards (0.01-0.1 mg mL<sup>-1</sup>) that covered the range of levels of the compounds found in the plant tissues.

**Statistical analysis:** Analyses were performed in triplicate. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Stat Graphics Plus Version 5.1.

## RESULTS AND DISCUSSION

**HPLC analysis of aromatic compounds:** Endogenous SA, AcSA, Phe, Tyr and Trp compounds were analyzed by using Shimadzu HPLC under previously mentioned conditions. The retention time in minutes of each compound in the chromatographic column was 6.08 for Tyr, 8.85 for AcSA, 9.32 for Phe, 11.59 for Trp and 11.98 for SA (Fig. 1).

**Injury effects of salicylate treatments after 7 days after a treatment:** The AcSA considered as a commercial source of SA, whereas the AcSA can be hydrolyzed to SA in aqueous solutions<sup>16</sup>. In this study, the phytotoxic effects of SA and AcSA at 25, 50 and 100 mM on the growth of three tested crops as well as on three tested weeds after 7 days after exposure were evaluated. As shown in Table 1, high doses of SA and AcSA caused significant injury effects on the growth of tested plants at 7 days after treatments. Such injury effects depended on sources of SA, applied concentrations and tested plants. The most injury effects exhibited the three examined crops were produced by application of AcSA at 50 and 100 mM, in most cases. Among of these crops, sunflowers had the greatest phytotoxic effects, especially at a concentration of 50 mM for AcSA (25±5%) as well as at a concentration of 100 mM for either SA (25±5%) or AcSA (35±10%). However, the three tested weeds tended to exhibit low sensitivity as affected by SA and AcSA treatments. Foliar spray of SA at 25-100 mM and AcSA at 25 mM did not produce any significant injury effect on purslane. As for jew's mallow, the injury effect was observed only when SA or AcSA applied at high concentration (100 mM) (10±1 and 20±5%, respectively). Moreover, a relatively high injury effect was observed when sprayed barnyard grass with SA at 100 mM (20±4%) and AcSA at all tested concentrations

Table 1: Injury effects (% of control) of SA acid and AcSA treatments, 7 days after treatments

Treatment	Maize	Sunflower	Cotton	Purslane	Jew's mallow	Barnyard grass
Control	0.0±0 <sup>c</sup>	0.0±0 <sup>c</sup>	0.0±0 <sup>c</sup>	0.0±0 <sup>c</sup>	0.0±0 <sup>c</sup>	0.0±0 <sup>c</sup>
25 mM SA	5.0±2 <sup>b</sup>	3.0±1 <sup>c</sup>	3.0±1 <sup>bc</sup>	0.0±0 <sup>c</sup>	0.0±0 <sup>c</sup>	2.0±1 <sup>c</sup>
50 mM SA	5.0±1 <sup>b</sup>	4.0±1 <sup>c</sup>	5.0±2 <sup>b</sup>	0.0±0 <sup>c</sup>	5.0±2 <sup>c</sup>	3.0±1 <sup>c</sup>
100 mM SA	6.0±2 <sup>b</sup>	25.0±5 <sup>a</sup>	5.0±1 <sup>b</sup>	5.0±1 <sup>c</sup>	10.0±1 <sup>b</sup>	20.0±4 <sup>a</sup>
25 mM AcSA	3.0±1 <sup>b</sup>	10.0±1 <sup>b</sup>	2.0±1 <sup>bc</sup>	4.0±1 <sup>c</sup>	1.0±1 <sup>c</sup>	10.0±3 <sup>b</sup>
50 mM AcSA	10.0±2 <sup>a</sup>	25.0±5 <sup>a</sup>	16.0±2 <sup>a</sup>	11.0±3 <sup>b</sup>	5.0±2 <sup>c</sup>	15.0±3 <sup>a</sup>
100 mM AcSA	12.0±1 <sup>a</sup>	35.0±10 <sup>a</sup>	18.0±4 <sup>a</sup>	12.0±3 <sup>b</sup>	20.0±5 <sup>a</sup>	18.0±4 <sup>a</sup>

The values in the table are Mean±SE, Data within a column followed the same letter are not significantly different at the level 5%

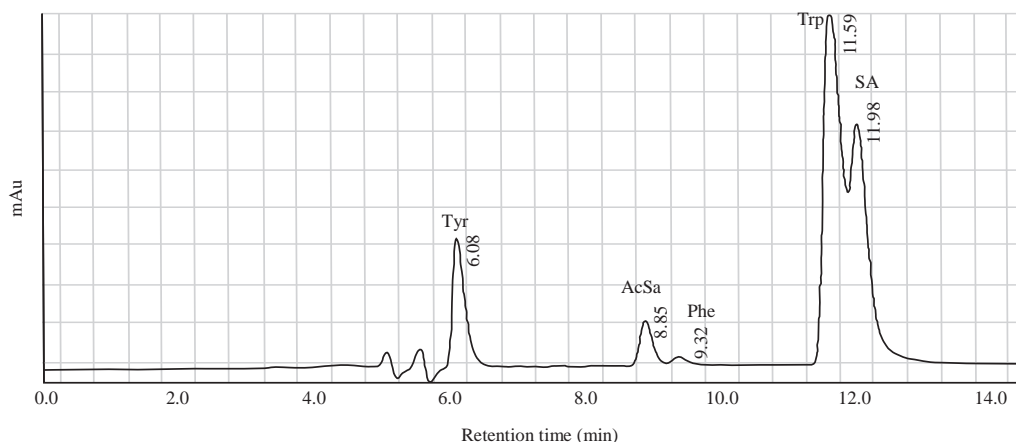


Fig. 1: HPLC chromatogram of Phe, Tyr, Trp, SA and AcSA standards, 10 µL of mg/150 mL

Table 2: Effect of SA and AcSA on growth criteria of different plant species, 35 days after treatments

Treatment	Maize	Sunflower	Cotton	Purslane	Jew's mallow	Barnyard grass
<b>Plant height (cm)</b>						
Control	148.0±3	112.0±2	96.0±4	39.0±5	98.0±5	64.0±6
25 mM SA	156.0±6	109.0±6	98.0±4	41.0±1	100.0±4	67.0±3
50 mM SA	157.0±6	104.0±4	96.0±4	39.0±1	97.0±6	63.0±3
100 mM SA	155.0±2	105.0±9	98.0±4	40.0±1	99.0±2	68.0±4
25 mM AcSA	158.0±2	114.0±9	106.0±4	42.0±7	89.0±5	68.0±3
50 mM AcSA	153.0±2	105.0±5	107.0±4	38.0±2	94.0±4	69.0±5
100 mM AcSA	156.0±4	107.0±3	98.0±12	37.0±1	94.0±6	64.0±3
LSD <sub>5%</sub>	NS	NS	NS	NS	NS	NS
<b>Plant fresh weight (g plant<sup>-1</sup>)</b>						
Control	147.0±3	96.0±4	48.7±2.9	38.1±5.4	21.3±1.4	66.5±2.2
25 mM SA	148.0±3	101.0±2	48.5±2.1	41.0±1.0	22.9±3.4	67.0±10.0
50 mM SA	145.0±6	103.0±5	48.9±4.0	39.0±1.0	20.5±3.3	65.7±0.6
100 mM SA	145.0±7	94.0±7	47.9±2.9	39.7±0.6	22.4±2.8	66.3±4.3
25 mM AcSA	149.0±9	102.0±4	51.2±1.8	41.0±3.6	20.2±1.6	68.3±5.3
50 mM AcSA	147.0±6	101.0±6	49.2±2.2	37.0±2.0	20.0±1.7	69.0±5.0
100 mM AcSA	137.0±8	97.0±4	47.1±3.1	37.3±10.1	20.4±5.4	65.3±0.6
LSD <sub>5%</sub>	NS	NS	NS	NS	NS	NS
<b>Plant dry weight (g plant<sup>-1</sup>)</b>						
Control	17.7±0.4	13.1±1.0	11.5±0.7	2.98±0.42	4.09±0.27	12.5±0.4
25 mM SA	17.7±0.5	13.8±0.3	11.5±0.5	3.21±0.07	4.13±0.34	12.6±1.9
50 mM SA	17.4±0.8	14.1±0.7	11.6±0.9	3.06±0.08	3.94±0.63	12.3±0.3
100 mM SA	17.4±0.7	12.9±0.9	11.4±0.7	3.11±0.05	4.09±0.19	12.5±0.3
25 mM AcSA	17.9±0.3	14.0±0.6	12.2±0.4	3.22±0.28	3.87±0.30	12.9±1.1
50 mM AcSA	17.7±0.7	13.9±0.9	11.7±0.5	2.90±0.20	3.84±0.33	13.0±0.9
100 mM AcSA	15.6±2.4	13.2±0.6	11.2±0.7	2.93±0.79	3.91±1.0	12.3±0.1
LSD <sub>5%</sub>	NS	NS	NS	NS	NS	NS

The values in the table are Mean ± SE, Data within a column followed the same letter are not significantly different at the level 5%

ranged between 10±3 and 18±4%. The phytotoxic effect of high doses of SA and AcSA on plant growth was previously mentioned by many investigators<sup>17,21</sup>. SA one of the phenolic acids and the exogenous application of phenolic compounds had been reported to alter plant growth by affecting different physiological processes such as leaf area expansion, chlorophyll synthesis, photosynthesis rate, leaf water potential and dry matter production<sup>8,22,23</sup>. The severe injury effects produced by SA and AcSA at high concentrations are in the line of those obtained results Gao-Feng *et al.*<sup>24</sup>. They found that the plant height of barnyard grass varied in their response to different SA doses (250, 500 and 1000 mg L<sup>-1</sup>) and the significant reduction was observed only at high concentration. Moreover, variations in the response of different plant species to SA and AcSA treatments are in agreement with the results obtained previously<sup>18</sup>. The inconsistent effects of SA were related to the fact that SA itself might induce the oxidative stress in the treated cells<sup>25</sup>, thus the effect depended on the applied SA concentrations<sup>26</sup>. Generally, AcSA treatments tended to cause higher negative effects on target plants than SA treatments. Since, among tested plants, the phytotoxic effect of applied SA at a high dose ranged between 6-15% corresponded with 12-35% for a high dose of AcSA. These differences are closed to our findings of the high capability of

different plant species to accumulate more SA in their tissues in the case of AcSA treatments as compared with those accumulated under SA treatments. In this concern, Ozpinar *et al.*<sup>18</sup> showed that the presence of carboxyl or hydroxyl or methoxyl groups in phenolic compounds induced different inhibitor effects on American ginseng.

#### Salicylate effects on growth parameters of tested plants, after 35 days from treatment:

After 35 days from treatments, SA and AcSA treatments did not produce any significant effects on plant height, fresh weight and dry weight of different plant species (Table 2). Since the temporary phytotoxic effects of SA and AcSA treatments that observed after 7 days of treatments were disappeared after 35 days. Thus, it is reasonable to suppose that SA and AcSA could not play a dominant role in controlling the growth of cultivated plants and weeds. These findings are in line with those obtained by Gao-Feng *et al.*<sup>24</sup>, who found that the inhibition rate in plant height and root length of barnyard grass was the greatest at 48 hrs after the application of exogenous SA and then gradually reduced.

**Changes in endogenous salicylic acid:** Data in Table 3 presented the concentration of endogenous SA in the leaves

Table 3: Effect of SA and AcSA treatments in the concentration of endogenous SA ( $\mu\text{mol kg}^{-1}$ ) in leaves of different plant species, one day after treatments

Treatment	Maize	Sunflower	Cotton	Purslane	Jew's mallow	Barnyard grass
Control	2.28 <sup>f</sup>	11.75 <sup>e</sup>	5.07 <sup>e</sup>	3.41 <sup>e</sup>	3.15 <sup>e</sup>	6.03 <sup>e</sup>
25 mM SA	12.07 <sup>e</sup>	36.79 <sup>d</sup>	14.20 <sup>d</sup>	13.91 <sup>d</sup>	7.31 <sup>d</sup>	17.54 <sup>c</sup>
50 mM SA	21.92 <sup>d</sup>	39.78 <sup>d</sup>	16.59 <sup>d</sup>	19.13 <sup>d</sup>	19.66 <sup>c</sup>	22.62 <sup>c</sup>
100 mM SA	47.35 <sup>c</sup>	70.41 <sup>bc</sup>	71.23 <sup>b</sup>	99.64 <sup>a</sup>	62.03 <sup>b</sup>	38.22 <sup>b</sup>
25 mM AcSA	23.09 <sup>d</sup>	64.12 <sup>c</sup>	29.13 <sup>c</sup>	20.94 <sup>d</sup>	20.32 <sup>c</sup>	12.40 <sup>d</sup>
50 mM AcSA	60.28 <sup>b</sup>	80.34 <sup>b</sup>	67.97 <sup>b</sup>	31.01 <sup>c</sup>	59.73 <sup>b</sup>	29.88 <sup>b</sup>
100 mM AcSA	104.62 <sup>a</sup>	116.59 <sup>a</sup>	99.57 <sup>a</sup>	47.10 <sup>b</sup>	121.44 <sup>a</sup>	94.26 <sup>a</sup>

Mean of four samples, Data within a column followed the same letter are not significantly different at the level 5%

Table 4: Changes in the concentrations of aromatic amino acids in leaves of different plant species, one day after treatments

Treatment	Maize	Sunflower	Cotton	Purslane	Jew's mallow	Barnyard grass
<b>Endogenous Phe (<math>\mu\text{mol kg}^{-1}</math>)</b>						
Control	18.73 <sup>a</sup>	8.56 <sup>c</sup>	2.94 <sup>b</sup>	4.87 <sup>a</sup>	8.13 <sup>a</sup>	ND
25 mM SA	18.67 <sup>a</sup>	8.51 <sup>c</sup>	2.90 <sup>b</sup>	4.76 <sup>a</sup>	8.06 <sup>a</sup>	ND
50 mM SA	18.33 <sup>a</sup>	8.55 <sup>c</sup>	3.19 <sup>ab</sup>	4.74 <sup>a</sup>	8.32 <sup>a</sup>	ND
100 mM SA	18.49 <sup>a</sup>	10.12 <sup>b</sup>	3.24 <sup>ab</sup>	4.68 <sup>a</sup>	8.39 <sup>a</sup>	ND
25 mM AcSA	19.42 <sup>a</sup>	13.44 <sup>a</sup>	2.90 <sup>b</sup>	4.79 <sup>a</sup>	8.28 <sup>a</sup>	ND
50 mM AcSA	19.64 <sup>a</sup>	11.44 <sup>ab</sup>	3.36 <sup>a</sup>	4.84 <sup>a</sup>	8.44 <sup>a</sup>	ND
100 mM AcSA	18.16 <sup>a</sup>	8.85 <sup>c</sup>	3.17 <sup>ab</sup>	4.65 <sup>a</sup>	7.95 <sup>a</sup>	ND
<b>Endogenous Tyr (<math>\mu\text{mol kg}^{-1}</math>)</b>						
Control	48.76 <sup>a</sup>	50.83 <sup>d</sup>	67.82 <sup>b</sup>	12.90 <sup>a</sup>	9.52 <sup>a</sup>	28.34 <sup>a</sup>
25 mM SA	47.67 <sup>a</sup>	78.98 <sup>c</sup>	67.18 <sup>b</sup>	13.23 <sup>a</sup>	9.34 <sup>a</sup>	28.56 <sup>a</sup>
50 mM SA	50.40 <sup>a</sup>	88.43 <sup>bc</sup>	67.92 <sup>b</sup>	12.71 <sup>a</sup>	9.81 <sup>a</sup>	34.36 <sup>a</sup>
100 mM SA	47.43 <sup>a</sup>	103.51 <sup>a</sup>	69.23 <sup>b</sup>	12.92 <sup>a</sup>	9.70 <sup>a</sup>	29.11 <sup>a</sup>
25 mM AcSA	51.80 <sup>a</sup>	104.91 <sup>a</sup>	91.97 <sup>a</sup>	13.90 <sup>a</sup>	9.25 <sup>a</sup>	29.89 <sup>a</sup>
50 mM AcSA	51.54 <sup>a</sup>	104.53 <sup>a</sup>	92.00 <sup>a</sup>	12.97 <sup>a</sup>	9.72 <sup>a</sup>	31.77 <sup>a</sup>
100 mM AcSA	51.92 <sup>a</sup>	96.72 <sup>ab</sup>	72.43 <sup>b</sup>	12.76 <sup>a</sup>	8.91 <sup>a</sup>	29.17 <sup>a</sup>
<b>Endogenous Trp (<math>\mu\text{mol kg}^{-1}</math>)</b>						
Control	0.62 <sup>a</sup>	0.34 <sup>a</sup>	1.03 <sup>a</sup>	1.39 <sup>a</sup>	0.88 <sup>a</sup>	0.80 <sup>a</sup>
25 mM SA	0.53 <sup>a</sup>	0.35 <sup>a</sup>	0.96 <sup>a</sup>	1.29 <sup>b</sup>	0.83 <sup>a</sup>	0.78 <sup>a</sup>
50 mM SA	0.62 <sup>a</sup>	0.33 <sup>a</sup>	0.96 <sup>a</sup>	0.98 <sup>c</sup>	0.86 <sup>a</sup>	0.78 <sup>a</sup>
100 mM SA	0.28 <sup>b</sup>	0.32 <sup>a</sup>	0.49 <sup>b</sup>	0.47 <sup>d</sup>	0.49 <sup>b</sup>	0.76 <sup>a</sup>
25 mM AcSA	0.52 <sup>a</sup>	0.34 <sup>a</sup>	1.00 <sup>a</sup>	1.07 <sup>bc</sup>	0.59 <sup>b</sup>	0.76 <sup>a</sup>
50 mM AcSA	0.55 <sup>a</sup>	0.13 <sup>b</sup>	0.77 <sup>a</sup>	1.06 <sup>bc</sup>	0.57 <sup>b</sup>	0.78 <sup>a</sup>
100 mM AcSA	0.47 <sup>a</sup>	0.13 <sup>b</sup>	0.76 <sup>a</sup>	0.48 <sup>d</sup>	0.14 <sup>c</sup>	0.75 <sup>a</sup>

Mean of four samples, Data within a column followed the same letter are not significantly different at the level 5%

of non-treated plants (control) and SA and AcSA treated plants, one day after treatments. As for control plants, the endogenous SA of six plant species ranged between 2.28  $\mu\text{mol kg}^{-1}$  (maize) to 11.75  $\mu\text{mol kg}^{-1}$  (sunflower). Whereas, foliar spray of either SA or AcSA resulted in a magnitude increase in the level of endogenous SA. Concentrations of SA in leaves tissues of examined plants depended on plant species, applied concentration and source of salicylate. A gradual increase in the endogenous SA as the exogenous concentration of either SA or AcSA increased. Foliar spray of SA at low concentration (25 mM) produced leaves of different plant species with 2.3-5.3 times of SA that in control plants. Whereas, application SA at high concentration (100 mM) produced plants with 6.0-29.2 times of endogenous SA, as compared with corresponded control plants. These results are in agreement with the previously obtained results<sup>15</sup>. It can be noticed that AcSA treatments tended to increase the endogenous level of SA more than the

increase produced by SA treatments (Table 3). The endogenous SA in the leaves of all tested species was possessed a great increase due to foliar spray of AcSA at all examined doses. At a low dose of AcSA (25mM), endogenous SA constituted between 2.1-10.1 times that in control plants, corresponding with 9.9-45.9 times for the highest dose (100 mM). These differences may be reflecting the difference in the chemical structure of two SA sources that lead to increase in the capability of tested plants to absorb and accumulate more SA from AcSA solutions than from SA solutions<sup>18</sup>. In addition, these findings are in harmony with our above-mentioned results about the superiority injury effects of AcSA treatments when compared to SA effects after 7 days from treatments.

**Changes in endogenous aromatic amino acids:** Level of endogenous Phe, Tyr and Trp in the leaves of six plant species was determined by using HPLC, data represented Table 4. The

concentration of Phe in the leaves of control plants ranged between 2.94  $\mu\text{mol kg}^{-1}$  (cotton) to 18.73  $\mu\text{mol kg}^{-1}$  (maize). As shown in Table 4, Phe did not identify in the leaves of the barnyard grass plant. While, Tyr in control plants was presented in the range 9.52  $\mu\text{mol kg}^{-1}$  (jew's mallow) and 67.82  $\mu\text{mol kg}^{-1}$  (cotton). Tested species varied in their Phe and Tyr contents as affected by exogenous application of SA and AcSA, when determined after one day from treatments. Phe and Tyr contents varied in their response to SA and AcSA treatments between tested plant species (Table 4). These treatments did not produce any significant effect on the level of Phe in maize or purslane or jew's mallow or barnyard grass. However, SA and AcSA treatments tended to increase the level of Phe in the leaves of sunflower and cotton, when compared with corresponded control plants. A maximum increase in the level of Phe of sunflower was observed when applied AcSA at 25 mM and 50 mM as well by SA at 100 mM. With less extent, an increase in the level of Phe in cotton was observed due to application SA or AcSA at 50 mM and 100 mM. As for Tyr, the greatest effect was recorded for AcSA at 25 and 50 mM, which increased Tyr in the sunflower and cotton. Since, SA synthesized from shikimic acid through different pathways, one of them through the activity of phenylalanine ammonia-lyase enzyme (PAL) on either Phe or Tyr<sup>27</sup>. Hence, in some plants, Phe and Tyr serve as indirect precursors of SA via PAL activity. Therefore, the great accumulation of endogenous SA due to SA and AcSA treatments could probably inhibit PAL activity and lead to accumulating more Phe and Tyr as in the case of sunflower and cotton plants<sup>28</sup>. This suggestion might explain the enhancement effect of SA and AcSA treatments on Phe and Tyr contents in leaves of sunflower and cotton. Variations in the response of endogenous Phe and Tyr to salicylate treatments between tested plants may be attributed to the differences in their SA metabolic pathways<sup>27</sup>.

The concentration of Trp in the leaves of control plants varied greatly between six plant species and ranged from 0.34  $\mu\text{mol kg}^{-1}$  (sunflower) to 1.39  $\mu\text{mol kg}^{-1}$  (purslane) (Table 4). SA and AcSA treatments tended to reduce endogenous Trp in the tested plants, but the reduction in the case of barnyard grass did not reach the level of significance. As for maize, cotton, purslane and jew's mallow, all SA and AcSA doses tended to suppress endogenous Trp contents, after one day of treatments. The reduction was more severe when four plant species were treated with 100 mM of either SA or AcSA. These two treatments suppressed endogenous Trp in maize, cotton, purslane and in Jew's mallow. In the case of sunflower, the significant reduction effect on Trp content was obtained by the application of AcSA at 50 or 100 mM. The literature about the effect of exogenous application of SA AcSA on endogenous Trp was limited, but the reduction effect

on Trp content may be due to the indirect effect of accumulation of endogenous SA under SA and AcSA treatments.

## CONCLUSION

Foliar application of salicylic acid and acetylsalicylate at high doses did not produce any permanent effects on the growth of tested plants; however, these compounds tended to induce great changes in the levels of endogenous salicylic acid and aromatic amino acids.

## SIGNIFICANCE STATEMENT

This study discovered that the exogenous application of high doses of salicylic acid or acetylsalicylate compounds caused great changes in endogenous salicylic acid and aromatic amino acids. Although, the three tested weeds and the three tested crops did not exhibit any permanent phytotoxic effects due to SA or AcSA treatments. This study helps the researchers to know that salicylic acid as a phenolic acid inappropriate for further herbicide development.

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