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# Article

## Significance of foliar sprayed salicylic acid in kidney bean resistance against *Tetranychus urticae* (Trombidiformes: Tetranychidae) attack

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**ABSTRACT**
The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is capable of dramatically decreasing growth and yield of bean plants (*Phaseolus vulgaris* L.) in Egypt. Aiming to offer an alternative method to be used for its control, we evaluated the effect of salicylic acid (SA) on induced resistance in bean seedlings against spider mite attack. Possible defense responses that were involved were also elucidated. The 9 and 18-day results proved that foliar application of SA at 50 and 100 mg $l^{-1}$ has a clear influence on containing mite populations, with higher efficiency observed at the higher concentrations. Consistent with the incidence of induced resistance and defense reactions, the remarkable increase in peroxidase (POD), polyphenol oxidase (PPO) activities and phenolic and flavonoid contents was detected in SA and/or the infestation bean treatments. In contrast, catalase (CAT) activity showed a different trend, as it was significantly decreased in the leaves subjected to individual infestation. The highest levels of all tested enzymes and compounds were noticed after 18 days at 100 mg $l^{-1}$ SA combined with the infestation treatments. In addition, increased mite population density led to a reduction in chlorophyll content, but SA was able to partly revert that loss in a concentration and time–dependent manner with 100 mg $l^{-1}$ concentration being more effective at 18 days following application. Together, these results indicate that SA treatments at the proper concentration and time could potentiate the resistance in bean plants against *T. urticae*.

**KEY WORDS:** Chlorophyll; defense; foliar treatment; *Phaseolus vulgaris* L., phytohormone; two-spotted spider mite.

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## INTRODUCTION

The red spider mite, *Tetranychus urticae* Koch belonging to web-spinning mites, is a pest having a wide distribution all over the world. It attacks a broad range of host plants and is well adapted to greenhouses and open-field conditions in temperate, tropical and subtropical areas (Chaudhri and Akbar 1985; Ahmadi *et al*. 2007). *Tetranychus urticae* causes plant damage by sucking out the mesophyll cell contents using its piercing mouth parts (Warabieda *et al*. 1997), resulting in a decline in the photosynthetic ability of the damaged cells. Therefore, continued feeding can cause complete defoliation and yield reduction in many economic crops including bean plants (Farouk and Osman 2009).

The excessive use of synthetic pesticides against these pests, combined with their high proliferative potential, leads to resistance even after just few applications (Stumpf and Nauen 2001).

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Furthermore, in order to produce safer crops in a less polluted environment, a need has emerged to develop alternative mite control methods that meet the modern criteria of the integrated crop protection. Such practices include host plant resistance. Plants have two broad categories of defense responses, one of them is preformed or constitutive and the other is induced. Preformed defenses are mechanisms which are naturally existent in plants with individually varying levels. Examples of these are morphological traits and biochemical components which can affect resistance of host plant to mites (Wilson 1994; Sadras and Wilson 1997). On the other hand, when under stress from biotic or abiotic factors, plants are able to protect themselves by mounting an arsenal of inducible defense mechanisms ranging from the release of biochemical compounds to longer-term structural changes. The damage caused by mite feeding can play a role in plant-inducible defenses. Such mechanisms may allow the plant to respond to further attacks launched by the same or another herbivore more strongly and rapidly compared to plants that have not been previously exposed to infestation (Conrath *et al*. 2006). The induced defenses are often regulated through various signaling pathways controlled mainly by plant phytohormones such as jasmonic acid (JA) and salicylic acid (SA), and these tend to be mediated according to the type of damage.

SA is a natural phenolic product of phenylproponoid metabolism (Lee *et al*. 1995). It has an essential role in regulating many aspects of physiological and biochemical processes such as plant growth, photosynthesis, nutrient uptake, plant thermogenesis and chlorophyll synthesis (Raskin 1992; O'Donnell *et al*. 2001). Additionally, SA has been reported to stimulate systemic acquired resistance (SAR) in plants under stress (Ryals *et al*. 1996). Exogenous SA treatment has also been shown to promote natural inducible plant defenses against many pathogens and herbivores (Tierranegra-Garcia *et al*. 2011; Neerja *et al*. 2013). However, little is known about its effects on plant resistance against mites. The activation of resistance by chemical applications that mimic natural induction may therefore give a simple way to enhance the level of plant protection against pests with minimal influence on plant health and productivity. Therefore, the present study was conducted to evaluate the effectiveness of foliar SA applications in containing *T. urticae* infestation on bean plants. Additionally, the effects of SA, infestation and a combination of both were evaluated on some compounds that are implicated in plant defenses against stressors.

## MATERIALS AND METHODS

### *Plants*

Seeds of common kidney bean *Phaseolus vulgaris* L. were wrapped in wet absorbent dark tissue paper in small boxes at 25 ℃ for approximately 72 h to enhance seed germination. The germinated seeds were planted individually in plastic pots (500 ml) containing soil and vermicompost in a 3:1 proportion. The pots were watered as needed and maintained inside a climate room (24 ± 2 ℃; 70 ± 10% R.H. and L10: D14 hours) at the Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University. Three-week-old seedlings were used for the experimentations.

### *Mites*

The laboratory strain of *T. urticae* used in the present study was originally collected from infested cucumber plants (*Cucumis sativus* L.). A culture of mites was maintained on kidney bean seedlings inside cages in an isolated compartment of the climate room. Two experiments were carried out as a part of the present study.

### *Impact of SA in kidney bean resistance to* T. urticae

Kidney bean pots were divided into two groups (assessments I and II) based on treatment duration. Seedlings of both groups were sprayed by hand-sprayer with aqueous solutions of SA (SA was obtained from Merck Co.) at concentrations of 50 and 100 mg $l^{-1}$. These concentrations were selected due to their ability to induce resistance to some herbivores such as *T. urticae* and pathogens

such as *Rhizoctonia solani* in several plant species (Farouk and Osman 2009; Neerja *et al*. 2013). Trial solutions were foliar-sprayed uniformly on the two leaf blades until the runoff point. Control plants were sprayed with distilled water only. After the leaf surface had dried, approximately 100 adult females of *T. urticae* were transferred to each plant. Afterwards, the treated pots in each treatment group were separately screened with plastic bags to avoid mite escape. The pots were set inside the climatic room and laid out in a completely randomized design. At 9 (assessment I) and 18 (assessment II) days after the application of the spray treatment and artificial infestation, the final populations of spider mites (eggs, juveniles and adults) on each plant in the first and second groups were estimated on the whole seedling using a binocular microscope (Fisher Scientific). Each treatment comprised three plants with three replications.

*Impact of SA and/or infestation on defense induction*

Along with the infested treatments used in the above experiment, non-infested treatments were also maintained for this experiment by applying the same procedural and experimental design used above. The background levels of some defense-related chemical attributes in kidney beans were compared with the potential of mite infestation, SA foliar application and a combination of both to indicate whether altered levels of these attributes were attained.

Nine leaf samples from each treatment were randomly collected at 9 and 18 days post the initial spraying and infestation date. The leaves were collected and transferred to the laboratory under cold conditions for different biochemical assays. Other pests were not noticed in the climate room during the execution of both experiments.

*Enzyme activity assay*

For assessment of catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO), 5 g of fresh leaf samples from each treatment, including controls, were homogenized with 50 ml of Phosphate buffer pH 6.0 (0.05 M) containing 0.05 g polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 419.25 g for 15 min. at 4 ℃. The supernatant was then assayed for enzymatic activity (Colowick and Kaplan 1955).

*Determination of catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO)*

The titrimetric method illustrated by Chance and Maehly (1955) was used to assess the catalase activity. 1 ml of the enzyme extract was added to 5 ml of 300 µM phosphate buffer (pH 7.4) containing 100 µM $H_2O_2$ then incubated for 1 min. at 25 ℃. The reaction was stopped by adding 10 ml of 2% $H_2SO_4$. The residual $H_2O_2$ was titrated with 0.01 N $KMnO_4$ until a faint pink color persisted for at least 15s.

Peroxidase activity was quantified by blending 1 ml of the enzyme extract with 5 ml of 300 µM of phosphate buffer (pH 7.4), 5 µM of pyrogallol and 50 µM of $H_2O_2$. The reaction was resumed for 5 min at 25 ℃. The enzyme activity was halted by adding 0.5 ml of 5% $H_2SO_4$. After centrifugation at 3000 g for 15 min., the amount of purpurogallin produced was deduced by measuring the absorbance at 420 nm (Chance and Maehly 1955).

Polyphenol oxidase activity was measured following the methodology of Kar and Mishra (1976) with some modifications. 1 ml of the enzyme extract was added to 2.5 ml acetate buffer pH 5.6 (0.01M) and 0.5 ml pyrogallol. The absorbance of the purpurogallin formed was detected at 420 nm.

***Secondary metabolites assay***

*Determination of total phenols and flavonoids*

Phenolic content was evaluated according to the method of Singleton *et al*. (1999). Plant samples were dried at 70 ℃ then finely ground. To 1 g of the dried powder, 5 ml of 80% methanol was added to form a homogenate followed by filtration. 2.5 ml of 0.2 N Folin-Ciocalteu reagent was mixed with 0.5 ml of the homogenate for 5 min. followed by the addition of 2 ml of $Na_2CO_3$ 7.5%. The solution

was incubated for 2 h at room temperature and the absorbance was assessed at 760 nm. The results were calculated in mg of gallic acid equivalents (GAE)/g of dry weight.

Flavonoid content was determined as described by Wang *et al*. (2011). 0.5 ml of methanol extract solution, prepared as above, was mixed with 5 ml of 30% ethanol and 0.75 ml of sodium nitrate 5% for 5 min followed by the addition of 0.75 ml of aluminum nitrate 10%. 5 ml of sodium hydroxide 1 M was added to stop the reaction. The absorbance was recorded at 510 nm. The flavonoid content was estimated as mg of rutin equivalent per g of sample.

*Chlorophyll assay*

Leaf greenness of the kidney bean plants was measured using SPAD-502 chlorophyll meter (Konica Minolta, Japan). A total of 30 leaves from each treatment were sampled for chlorophyll measurement. Four SPAD recordings (two on each side of the midrib) were averaged for each leaf.

*Statistical analysis*

Data about final spider mite densities at different treatments and data concerning biochemical assays were subjected to analysis of variance (ANOVA). Mite density was transformed using square root. Means were then separated by Tukey's test at $P \leq 0.05$ in the event of a significant treatment effect, using Statistics Analysis System (SAS Institute, Cary, USA).

## RESULTS

*Mite population growth*

Application of SA on kidney bean (*Phaseolus vulgaris*) affected the survival of *T. urticae*. The impact of SA application on spider mite densities indicated a significant reduction in both the final number of each life stage and the total population either at 50 or 100 mg $l^{-1}$ SA whether assessed at 9 or 18 days post-treatment when compared to control except adults. When they were counted after 9 days of both applications, no significant difference was found compared to control (Fig. 1A–D). There was no pronounced difference observed in the number of eggs and immatures on kidney beans when sprayed with SA at 50 mg $l^{-1}$ as compared to 100 mg $l^{-1}$ at both assessments (Fig.1 A, B). Whereas by the end of assessment II, the number of adults and total population notably decreased at 100 mg $l^{-1}$ SA as compared to 50 mg $l^{-1}$ (Fig. 1C, D). Generally, the total population of *T. urticae* on kidney beans was reduced by 78% and 84% following the application of 50 and 100 mg $l^{-1}$ SA, respectively at assessment I compared to control. Nine days later, the total population was further reduced reaching 92% and 98%, respectively (Fig. 1D).

*Activity of antioxidant enzymes*

Data presented in Table 1 showed that when leaves of *P. vulgaris* were subjected to either SA foliar spray (50 and 100 mg $l^{-1}$) or infestation by *T. urticae* or a combination of both, there was a significant increase in POD and PPO activities at assessment I and II compared to their respective controls (sprayed with water only). The application rate of SA used individually or in combination treatments had a statistically significant effect on POD and PPO activities at both assessments. Within 18 days, the two tested concentrations of SA combined with *T. urticae* resulted in the highest activities of both enzymes, where the activity was increased at 50 and 100 mg $l^{-1}$ SA by 5.4 and 9.5 folds, respectively for POD and by 1.9 and 3.1 for PPO compared with the control. In contrast, kidney bean leaves subjected to individual infestation showed a significant decline in the CAT activity at both assessments as compared to control. However, CAT activity significantly increased in comparison to control both when SA was applied alone at 100 mg $l^{-1}$ and in combination with infestation at 50 and 100 mg $l^{-1}$, being higher at assessment II as compared to assessment I.

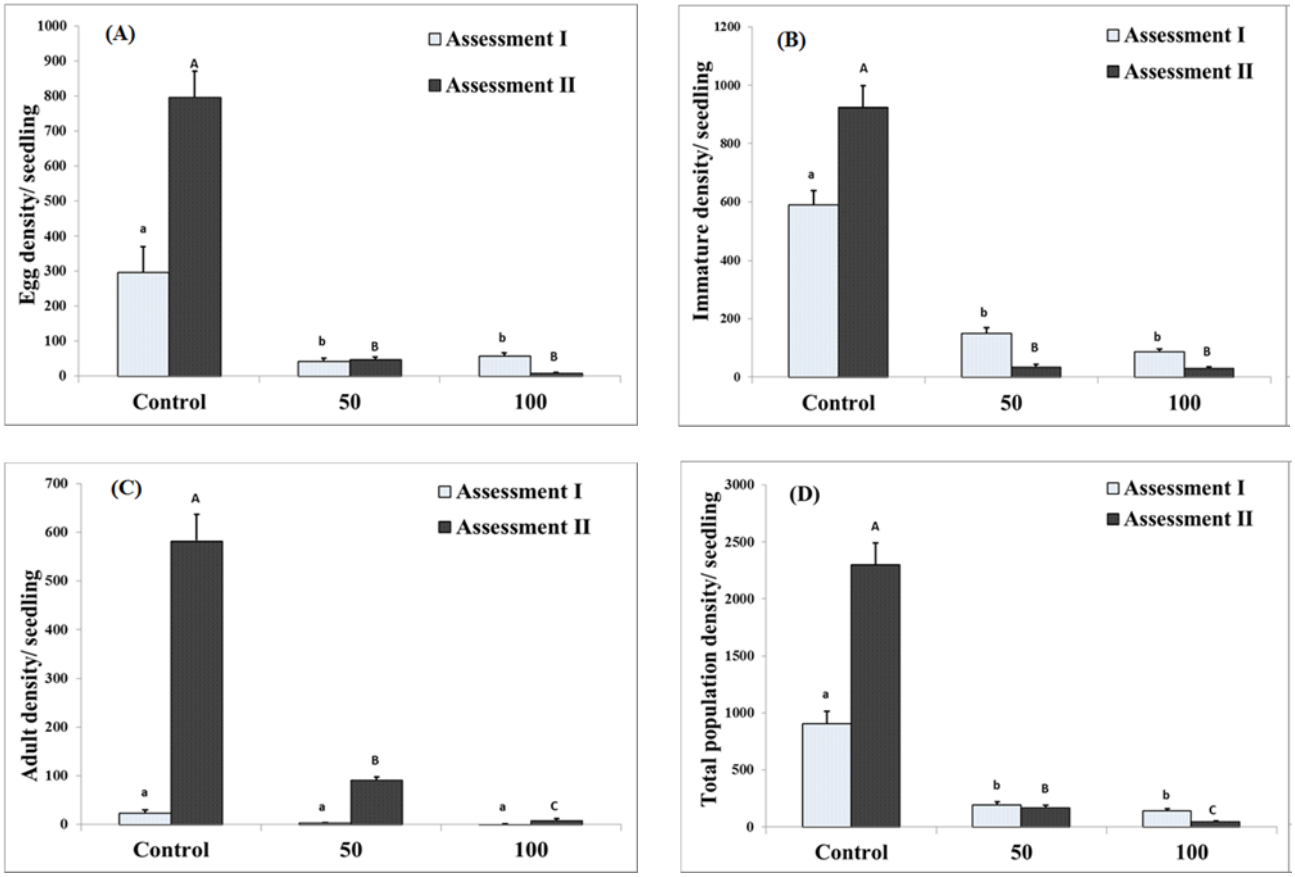


**Figure 1.** Density (mean ± SE) of different stages – A. Eggs; B. Immatures; C. Adults; D. Total population of *T. urticae* at 9 (assessment I) and 18 (assessment II) days after foliar application of salicylic acid (SA) and mite infestation on kidney beans *P. vulgaris*. Within each assessment, means followed by the same letter are not significantly different (Lower case letters for assessment I, upper case letters for assessment II) by Tukey's test ($P < 0.05$).

**Table 1.** Effects of foliar spray of salicylic acid (SA) and/or infestation with *T. urticae* (TU) on catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) activities in kidney beans *P .vulgaris* at 9 (assessment I) and 18 (assessment II) days post-treatment.

| Treatment | CAT (U $g^{-1}$) | | POD (U $g^{-1}$) | | PPO (U $g^{-1}$) | |
|---|---|---|---|---|---|---|
| | Assessment I | Assessment II | Assessment I | Assessment II | Assessment I | Assessment II |
| **Water** | 57.60 ± 6.33$^{d}$ | 58.80 ± 2.57$^{d}$ | 36.26 ± 2.75$^{f}$ | 45.86 ± 1.04$^{f}$ | 37.93 ± 1.17$^{d}$ | 101.33 ± 2.75$^{e}$ |
| **TU** | 42.00 ± 3.32$^{e}$ | 46.20 ± 2.57$^{e}$ | 72.53 ± 1.08$^{e}$ | 186.26 ± 3.94$^{e}$ | 42.38 ± 0.64$^{c}$ | 138.46 ± 0.67$^{d}$ |
| **SA (50 mg $l^{-1}$)** | 58.64 ± 0.92$^{d}$ | 56.02 ± 0.87$^{d}$ | 82.63 ± 0.41$^{d}$ | 201.12 ± 0.32$^{d}$ | 41.14 ± 0.74$^{c}$ | 138.58 ± 0.71$^{d}$ |
| **SA (100 mg $l^{-1}$)** | 68.21 ± 1.20$^{c}$ | 72.28 ± 0.61$^{c}$ | 89.25 ± 1.34$^{c}$ | 221.44 ± 0.52$^{c}$ | 49.06 ± 0.42$^{b}$ | 152.04 ± 0.35$^{c}$ |
| **SA (50 mg $l^{-1}$) +TU** | 117.60 ± 7.71$^{b}$ | 191.10 ± 5.14$^{b}$ | 106.33 ± 2.44$^{b}$ | 247.73 ± 4.01$^{b}$ | 50.86 ± 0.75$^{b}$ | 197.46 ± 1.82$^{b}$ |
| **SA (100 mg $l^{-1}$) +TU** | 189.00 ± 6.64$^{a}$ | 218.40 ± 8.40$^{a}$ | 244.53 ± 8.72$^{a}$ | 436.40 ± 1.98$^{a}$ | 64.86 ± 0.47$^{a}$ | 307.87 ± 2.58$^{a}$ |

Data represent mean ± SE of three replicates. Different letters in the same column correspond to significantly different values for $P < 0.05$ (Tukey's test).

### *Accumulation of secondary metabolites*

Effects of SA foliar spray and/or infestation on total phenolic and flavonoid content in kidney bean leaves are shown in Table 2. SA was effective in elevating the phenolic and flavonoid contents

in a concentration and time-dependent manner. The application of SA resulted in a significant increase in the total phenolic and flavonoid content compared to the control both in the infested and non-infested leaves at 9 and 18 days post-treatment. In addition, total phenols and flavonoids were significantly increased with individual infestation by *T. urticae* as compared to the control for both assessments. For all treatments, the phenol and flavonoid contents were found to be higher at assessment II as compared to assessment I as they reached the maximum values when SA was applied at 100 mg $l^{-1}$ either alone or in combination with infestation treatments.

**Table 2.** Effects of foliar spray of salicylic acid (SA) and/or infestation with *T. urticae* (TU) on total phenols and flavonoids in kidney beans *P. vulgaris* at 9 (assessment I) and 18 (assessment II) days post-treatment.

| Treatment | Total phenols (mg $g^{-1}$) | | Total flavonoids (mg $g^{-1}$) | |
|---|---|---|---|---|
| | Assessment I | Assessment II | Assessment I | Assessment II |
| **Water** | $13.60 \pm 0.32^{e}$ | $14.95 \pm 0.48^{e}$ | $2.57 \pm 0.14^{d}$ | $2.91 \pm 0.15^{d}$ |
| **TU** | $17.55 \pm 0.90^{d}$ | $21.41 \pm 1.11^{d}$ | $3.47 \pm 0.17^{c}$ | $4.24 \pm 0.15^{c}$ |
| **SA (50 mg $l^{-1}$)** | $34.52 \pm 0.41^{ab}$ | $41.18 \pm 0.68^{b}$ | $5.84 \pm 0.41^{ab}$ | $7.18 \pm 1.21^{a}$ |
| **SA (100 mg $l^{-1}$)** | $36.34 \pm 0.46^{a}$ | $44.32 \pm 0.63^{a}$ | $6.18 \pm 0.31^{a}$ | $7.41 \pm 0.91^{a}$ |
| **SA (50 mg $l^{-1}$) +TU** | $26.50 \pm 0.89^{c}$ | $32.34 \pm 1.08^{c}$ | $5.14 \pm 0.15^{b}$ | $5.38 \pm 0.28^{b}$ |
| **SA (100 mg $l^{-1}$) +TU** | $33.34 \pm 1.04^{b}$ | $40.68 \pm 1.21^{b}$ | $6.13 \pm 0.24^{a}$ | $7.33 \pm 0.38^{a}$ |

Data represent mean ± SE of three replicates. Different letters in the same column correspond to significantly different values for $P < 0.05$ (Tukey's test).

**Table 3.** Effects of foliar spray of salicylic acid (SA) and/or infestation with *T. urticae* (TU) on total chlorophyll in kidney beans *P. vulgaris* at 9 (assessment I) and 18 (assessment II) days post-treatment.

| Treatment | Assessment I | | Assessment II | |
|---|---|---|---|---|
| | SPAD value* | RC** | SPAD value* | RC** |
| **Water** | $35.38 \pm 0.17^{a}$ | 1.0 | $35.98 \pm 0.19^{a}$ | 1.0 |
| **TU** | $20.22 \pm 0.40^{b}$ | 0.6 | $10.04 \pm 0.38^{d}$ | 0.3 |
| **SA (50 mg $l^{-1}$)** | $35.48 \pm 0.23^{a}$ | 1.0 | $35.78 \pm 0.81^{a}$ | 1.0 |
| **SA (100 mg $l^{-1}$)** | $34.48 \pm 0.45^{a}$ | 1.0 | $34.72 \pm 0.63^{a}$ | 1.0 |
| **SA (50 mg $l^{-1}$) +TU** | $20.14 \pm 0.31^{b}$ | 0.6 | $26.72 \pm 0.72^{b}$ | 0.7 |
| **SA (100 mg $l^{-1}$) +TU** | $20.44 \pm 0.40^{b}$ | 0.6 | $28.78 \pm 0.53^{c}$ | 0.8 |

Data represent mean ± SE of three replicates. Different letters in the same column correspond to significantly different values for $P < 0.05$ (Tukey's test).
* A numerical value which is indicated in Minolta units following the manufacturer of the Minolta SPAD 502 meter.
** Relative Content, chlorophyll content values are expressed relative to water treatment.

### *Chlorophyll content*

In kidney bean leaves, chlorophyll levels were not significantly affected by any of the SA concentrations evaluated, either those assessed after 9 or 18 days of application relative to the control (Table 3). Conversely, at assessment I, chlorophyll levels were markedly declined after infestation with *T. urticae* alone or in treatments combined with SA at both concentrations tested compared to the control without any significance between treatments. The chlorophyll level maintained at these treatments was of 0.6 relative to the water control. Thereafter, at assessment II, although the total chlorophyll level was sharply reduced by the infestation alone reaching 0.3 relative to the control, SA combined with the infestation was capable of partly reverting that loss in a concentration-dependent manner where 100 mg $l^{-1}$ SA was more effective. At this SA concentration, the chlorophyll level was closest to that of the non-infested leaves (0.8) (Table 3).

## DISCUSSION

This study investigated the application of SA as a prospective defense compound or signal for synthesis of other related secondary substances in kidney bean spider mite infestation. Our results confirmed that exogenous application of 50 or 100 mg $l^{-1}$ SA on *Phaseolus vulgaris* significantly reduced the survival and the offspring production of *T. urticae*. These results are consistent with the observations of Favaro *et al*. (2019), who reported that SA at 50 mg $l^{-1}$ was effective in reducing oviposition and adult survival of *T. urticae* on strawberry cultivars in leaf disc bioassay. In accordance, Miyazaki *et al*. (2014) revealed that SA and its methyl derivative, methyl salicylate (MeSA), when applied externally to cotton (*Gossypium arboretum* L.) at 1 mM did not affect the numbers of eggs and adult females of *T. urticae* compared to the control after 12 days of application. Conversely, they mentioned that application of SA and MeSA on cotton (*G. hirsutum* L.) with the same rate mentioned above significantly reduced the number of eggs by 44 and 33%, respectively relative to the control. However, this is a smaller reduction compared with the one estimated for the eggs in our study (≈ 86% and 80 % reduction at 50 and 100 mg $l^{-1}$ , respectively) at assessment I. Moreover, the reduction in our second assessment increased to 94% and 98%, in respect.

It seems that the effectiveness of SA in reducing the mite performance is not the same for all plants. Kareem *et al*. (2017) noticed that the efficacy of SA in mitigating the adverse effects of stress differs according to the type of plant species and the physiological conditions of the plant. In addition, our obtained data showed that the increase of contact time between SA-treated plants and *T. urticae* caused higher reduction in its population size. This may be due to the increase in the levels of defense-related enzymes and compounds, which in turn led to an increase in their activity at 18 days following treatment compared to 9 days (Tables 1, 2) which could have played a part in providing more plant adaptation against mite infestation.

Plant defense mechanisms are initiated rapidly in response to pest attack. One of the first defense responses in plant cells is the rapid rise in concentration of reactive oxygen species (ROS) possessing high level of chemical reactivity in a phenomenon known as “oxidative burst”. ROS inherently exist in plant cells at low concentrations and have a pivotal role in their functioning. Cell wall and membrane are the central locations for the synthesis of ROS in plants. The formation of ROS within the cell wall and their subsequent extracellular release seems to be intended, permitting their direct harmful effect on pest cells (Kulbat 2016) and sometimes engaging in the infestation reduction (Santamaria *et al*. 2018). In contrast, the high levels of ROS were found to be detrimental to plant cells as they cause degradation of cell components, resulting in disruption of their metabolic function and finally leading to cell death (Lu and Finkel 2008).

The application of plant activators or elicitors, as they have been nominated, results in the elicitation of a series of plant defense responses such as the induction of a range of enzymatic and non-enzymatic antioxidant compounds (Neerja *et al*. 2013). The activation of ROS scavenging enzymes, including CAT, POD and PPO, is the most established channel in ameliorating the adverse effects of ROS synthesis during the plant-herbivore interactions (Jiang *et al*. 1994; Mittler 2002; Constabel and Barbehem 2008).

Catalase, the tetrameric heme protein, is ubiquitous in aerobic organisms and is present mainly in the cytosol, mitochondria and peroxisomes (Krych *et al*. 2014). The induction of CAT may prevent the accumulation of toxic levels of ROS, especially hydrogen peroxide ($H_2O_2$), formed under pest stress (Mittler 2002). Additionally, CAT is implicated in the plant cell wall resistance and acts as a signal for the defense gene induction (Chen *et al*. 1993). Our results showed that SA at the higher concentration (100 mg $l^{-1}$) was effective in eliciting the CAT activity in the bean leaves. These are in harmony with the findings of Kolupaev *et al*. (2011) where they noticed an increase in the CAT activity in millet plantlets when treated with SA.

Among the proteins induced through plant defense which perform a key role in various physiological responses are POD and PPO (Almagro *et al*. 2009). Plant peroxidases are glycoprotein

in nature and are present in vacuoles and cell walls (Passardi *et al*. 2005). They are engaged in cell wall rigidification, metabolism of ROS and lignin formation (Almagro *et al*. 2009). Polyphenol oxidases, on the other hand, are copper-containing enzymes that engage in oxidation of phenolic compounds to their quinones, which may subsequently alkylate dietary protein during herbivore feeding (Constabel and Barbehem 2008). Chandra *et al*. (2007) observed that when cowpea plants were treated with SA, the activity of POD and PPO increased, and that was considered as a marker of resistance. Elevation in POD and PPO activity levels during incompatible plant ± herbivore/elicitor interactions is well authenticated and, in some cases, POD and PPO have been associated with the inhibition of herbivore growth (Felton *et al*. 1989; Taggar *et al*. 2012). In consistence with these studies, our results revealed that SA remains effective in promoting the enzyme activities.

Importantly, as spider mite is inevitably associated with cellular damage, it is relevant that wounding is known to enhance the activities of oxidative enzymes (Hiraga *et al*. 2000). Our results revealed that the infestation of kidney bean leaves with *T. urticae* caused an increase of enzyme activities, except in catalase enzyme, as compared to their respective non-infested ones. Similar results were noticed by Trevisan *et al*. (2003) for POD in hop plant, Liang *et al*. (2017) for PPO in Cassava cultivar and Steinite and Ievinsh (2002) for CAT in strawberry plants. Inactivation of catalase by infestation and its reactivation by the application of SA alone or in combination with infestation may depend on the concentration of $H_2O_2$ present (Durner and Klessig 1996).

Moreover, out of the secondary metabolites that deserve attention in plant defense are phenols and flavonoids. It has been found that SA prompts the generation of $H_2O_2$, which catalyzes the activity of phenylalanine ammonia-lyase that is, in turn, accountable for the synthesis of a phenyl propane unit, which is a constituent of phenolic and flavonoid compounds (Deenamo *et al.* 2018). Phenolic compounds take part in defense responses induced by wounding stress and have the ability to quench free radical reactions (Kulbat 2016). Whereas, flavonoids stabilize plant cell membranes by limiting their fluidity, which in turn reduces the diffusion of free radicals (Arora *et al*. 2000). In the present study, the increase in phenolic and flavonoid contents was observed after elicitor treatment and in the resistance reaction. Increased phenolic and flavonoid contents in response to SA foliar spray has been also reported in cucumber (Preciado-Rangel *et al*. 2019). As indicated from other investigations, phenolic and flavonoid compounds were found to be highly overaccumulated in leaves of strawberry and citrus, respectively following herbivory by *T. urticae* (Agut *et al*. 2014; Golan *et al*. 2017). In addition, the expression of a key gene, chalcone synthase (CHs), in the biosynthesis of flavonoids was also enhanced following infestation (Agut *et al*. 2014).

Chlorophyll, a magnesium-tetrapyrrol pigment, has a vital role in photosynthesis as it plays a major role in the process of light energy harvesting and leading electron transfer (Liu and Guo 2013). One of the main visible symptoms of plant injury related to spider mite infestation is leaf discoloration as the leaf becomes reddish or silvery due to chlorophyll degradation. The degradation of chlorophyll is probably due to the mechanical damage done to the chloroplasts during mite feeding (Landeros *et al*. 2004). In strawberry plants, Kielkiewicz (1981) noticed a general distortion of the structure of the chloroplasts in cells close to those damaged by feeding of *T. urticae*. Our data indicated that in bean leaves damaged by *T. urticae*, chlorophyll levels declined very drastically and the decline was correlated with the population density and the feeding period duration. Earlier studies have documented that a decrease in the chlorophyll content coincided with an increase in the population size and the duration of the feeding period of the spider mites (De Angelis *et al*. 1983; Landeros *et al*. 2004). Furthermore, our findings revealed that non-infested bean plants that were treated with the two concentrations of SA conserved the same chlorophyll content compared to the control. In addition, our results clarified that when SA was provided exogenously at a proper dose (100 mg $l^{-1}$) and time (for 18 days), it largely eliminated the negative effects of infestation on chlorophyll content in kidney bean seedlings. This might be related to the induction of plant defense mechanisms leading to mite death, thus limiting the damage caused by the infestation. Additionally, SA was able to

enhance potassium and nitrogen leaf contents (data not shown) which may increase the number of chloroplasts per cell and, consequently, chlorophyll synthesis (Possingham 1980).

Stimulation of the host plant own defense responses by pre-treatment with elicitors is considered to be a modern alternative approach for mite management and crop protection. Since there is a clear correlation between the capacity of the elicitor to stimulate plant resistance and induce defense-related enzymes and secondary metabolites in kidney bean plants, it is concluded that SA could contribute to resistance against *T. urticae*. However, field trials are still required to confirm its effectiveness in reducing *T. urticae* population under natural conditions.

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# اهمیت پاشش برگی اسید سالیسیلیک در مقاومت لوبیا قرمز به حملهٔ *Tetranychus urticae* (Trombidiformes: Tetranychidae)

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**چکیده**

کنهٔ تارتن دو لکه‌ای، *Tetranychus urticae* Koch (Acari: Tetranychidae)، می‌تواند به مقدار زیادی موجب کاهش رشد و محصول‌دهی گیاه لوبیا (*Phaseolus vulgaris* L.) در مصر شود. برای کمک به یافت روشی جایگزین برای استفاده در مهار این آفت، تأثیر اسید سالیسیلیک بر مقاومت القایی نشاهای لوبیا به حملهٔ کنهٔ تارتن بررسی شد. پاسخ‌های دفاعی احتمالی که ایجاد شدند نیز توضیح داده شدند. نتایج ۹ و ۱۸ روزه ثابت کرد که پاشش برگی اسید سالیسیلیک به میزان ۵۰ و ۱۰۰ میلی‌گرم در لیتر اثر مشخصی بر جمعیت‌های کنه با تأثیر بیشتر در غلظت‌های بیشتر داشت. مطابق با بروز مقاومت القایی و واکنش‌های دفاعی، افزایش زیادی در فعالیت‌های پراکسیداز (POD)، پلی فنول اکسیداز (PPO) و محتویات فنولیک و فلاونوئید در اسید سالیسیلیک و/یا تیمارهای لوبیا آلوده تشخیص داده شد. در مقابل، فعالیت کاتالاز (CAT) روند متفاوتی را نشان داد، زیرا به میزان زیادی در برگ‌های آلوده به فرد کاهش می‌یابد. بالاترین سطح کل آنزیم‌ها و ترکیبات مورد آزمایش پس از ۱۸ روز در ۱۰۰ میلی گرم در لیتر اسید سالیسیلیک همراه با تیمارهای آلوده دیده شد. همچنین، افزایش انبوهی جمعیت کنه منجر به کاهش میزان سبزینه شد، اما اسید سالیسیلیک توانست تا حدودی آن خسارت را به صورت غلظت و وابسته به زمان در غلظت ۱۰۰ میلی گرم در لیتر جبران کند که بازهٔ ۱۸ روز پس از پاشش موثرتر بود. در کل، این نتایج نشان می‌دهد که تیمارهای اسید سالیسیلیک در غلظت و زمان مناسب می‌توانند مقاومت گیاهان لوبیا را در برابر *T. urticae* تقویت کنند.

**واژگان کلیدی:** سبزینه؛ دفاع؛ پاشش برگی؛ لوبیا؛ هرمون برگی؛ کنهٔ تارتن دو لکه‌ای.

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