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# THE EFFECT OF APPLYING EXOGENOUS SALICYLIC ACID ON APHID INFECTION AND ITS INFLUENCE ON HISTO-PHYSIOLOGICAL TRAITS AND THERMAL IMAGING OF CANOLA

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**ABSTRACT.** Insect damage in canola adversely affects its productivity and quality and is considered one of the most important degrading factors in Egypt. The effect of foliar application of salicylic acid (SA) on aphid populations, growth and yield of canola (*Brassica napus*, L.) cv. serw 4 was the major goal of this study. Two experiments were conducted at the farm of Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, during 2014 and 2015 seasons, to achieve this target. Each experiment included four levels of SA (0, 50, 100, 200 mg $l^{-1}$). The experimental results revealed that SA, at low concentration (50 mg $l^{-1}$), was an effective treatment for reduction the number of aphid populations and colony depth on the main inflorescence, contributed with reducing the thickness of secretory tissue of flower pedicel. The level of 50 mg $l^{-1}$ of SA-treated canola had the highest number of stomata $cm^{-2}$, along with the lowest width of both stoma and its aperture. Thickness of xylem tissue and the number of xylem vessels $bundle^{-1}$ in leaf midrib, reducing sugars and free amino acids was increased at 50 mg $l^{-1}$ SA, but free phenolics content did not affected significantly. Under controlled conditions, changes in temperature of infected leaves allowed the discrimination between healthy and infected areas in thermo-image, even before visible symptoms of aphid infestation appeared. The detection of modifications in plants or canopies, associated with low insect severity in the early stages of infestation, was crucial for the targeted, site-specific or on demand application of integrated aphid control. Canola, which was treated with 50 mg $l^{-1}$ of SA, gave 30.5 and 27.9 kg of oil $ha^{-1}$ over the control. It was concluded that

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spraying of SA at 50 mg $l^{-1}$ was an effective elicitor to diminish the aphid numbers on canola inflorescence and improve its yield.

**Keywords:** oil crop; aphid populations; biochemical; infrared thermal image; histology.

## INTRODUCTION

Canola (*Brassica napus* L. var. *oleifera*) is one of the most important oil crop (Miri, 2007) and, at present, is the third largest source of crop oil all over the world (Kandil and Gad, 2012). In Egypt, canola has a bright future to contribute in reducing oil deficiency gap between production and consumption of edible oil; particularly it could be successfully grown during winter season in newly reclaimed land outside the old one of Nile valley to get around the competition with other crops occupied the old cultivated area (Sharaan *et al*., 2002).

Canola crops are most susceptible to damage by a wide range of pests from seedling establishment to seed development. There are several pests, which attack canola crop in Egypt, but aphids are the most serious of all the insect pests attack canola (Mahmoud and Shebl 2014). Cabbage aphid, *Brevicoryne brassicae*, is known to be the most abundant and destructive species of *Aphididae* on canola crop during the flowering and capsule-forming period (Sayed and Teilep 2013; Mahmoud and Shebl 2014). Because aphids have the capability of reproducing very quickly, they can injure canola plants during a very small window of time. Several investigators have proposed the use of elicitors of plant resistance as a mean of controlling pests in agriculture (Thaler *et al*., 1999; Boughton *et al*., 2006). This new control approach is gaining in topicality, because pests and diseases are serious constraints in efforts to increase productivity per hectare.

Salicylic acid (SA), an endogenous elicitor and plant growth regulator, has been found to generate a wide range of metabolic and physiological responses in plants, thereby affecting their growth and development, such as ion uptake, transport and membrane permeability (Raskin, 1992). In addition, SA is an important signaling molecule in systemic acquired resistance, thus induces plant tolerance to various abiotic and biotic stresses (Horvath *et al*., 2007; Kamel *et al*., 2016).

Enhancement synthesis and accumulation of secondary metabolites as SA in plants are part of pest defense mechanism (Schneider *et al*., 1996). The high rate of pupal mortality of cotton bollworm (*Helicoverpa armigera*) was correlated with high concentration of SA under laboratory conditions (Sivamani *et al*., 1992). Exogenous application of jasmonic acid or SA analog induces systemic defenses, that reduce potato aphid (*Macrosiphum euphorbiae*) population growth on tomato (Goggin, 2005).

Thermal imaging is a technique, which converts the radiation emitted

by an object into temperature data, without establishing contact with the object. Application of thermal images was used in detection of bruises in fruits and vegetables (Varith *et al.,* 2003) and detection of *Cryptolestes ferrugineus* (a *Coleoptera* beetle) infestation inside stored-wheat kernels (Manickavasagan *et al.,* 2007).

The objective of the current study is to investigate the efficacy of SA elicitor for managing *Brevicoryne brassicae* L. on canola plants under field conditions, and their impact on canola yield. Also, to demonstrate the histo-physiological defense mechanism of canola to this pest under SA application. In addition to, using the infrared thermo-imaging technique, as a new tool for detection aphid's infestation.

## MATERIALS AND METHODS

**Experimental layout**

Two field experiments were conducted at the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt (30º 58′ N latitude, 32º 23′ E longitude and 13 m above sea level), during 2013/14 and 2014/15 seasons. Canola (*Brassica napus*, L.) cv. Serw 4 was sown on 21 Oct. 2013 and on 22 Oct. 2014, the most suitable time to become naturally infested with aphids. Plants were thinned to one plant per ridge; 20 cm between hills. Recommended cultural and agronomic practices were adopted from sowing to harvest. No insecticide was sprayed in and around the experimental fields. The experimental area was divided into four treatment areas, including the control. Each treatment included four replicates. The experiment was distributed in a randomized complete block design.

**Salicylic acid treatments**

Canola plots were sprayed with salicylic acid (SA) at the rate of 0, 50, 100 and 200 mg $l^{-1}$. Plants were sprayed after 45 days of sowing by one week interval for four times using hydraulic sprayer. Control plots were applied only with distilled water.

**Growth parameters and yield components**

Total dry weight (g $plant^{-1}$) was determined after drying at 70°C up to constant weight for 72 h. Plant water content (%) was calculated as: (fresh weight - dry weight)/ (fresh weight)*100. Seed oil content (%) was determined by using the Soxhelt continuous extraction apparatus, with petroleum ether (60-80°C) as an organic solvent, for a period of 12 hours, according to AOAC (1990); oil yield $ha^{-1}$ was calculated by multiplying seed oil content (%) by seed yield $ha^{-1}$. At maturity, after 150 days from sowing in both seasons, seed yield (g $plant^{-1}$) were estimated from plants of the two middle ridges (the 3rd and 4th ridges) in each plot, then seed yield (kg $ha^{-1}$) was calculated.

**Aphid assessment**

The experiment was left for natural infestation; data were recorded at weekly interval, from first occurrence of pest at the fifteenth week of sowing till crop harvest. Aphid counts were recorded from each of the 50 randomly plants, on 10 cm of the main inflorescence, in each replicate. A scoring protocol was used to measure both the incidence of cabbage aphid in the crop (percentage of affected inflorescence) and the level of infestation

(depth of colony in centimeters). The rating system was as follows, with scores averaged as a measure over the whole treatments: 0 = nil present, 1 = 1.0 cm colony, 2 = 2.5 cm colony, 3 = 5.0 cm colony, 4 = 5.0-7.5 cm colony and 5 = 10 cm colony or more.

**Determination of some organic chemical components**

All biochemical and histological investigations were examined on the 3rd leaf and flower after 95 days from sowing. Ethanolic extract (96% EOH) of leaf was prepared according to Abdel-Rahman *et al.* (1975) to determine the content of reducing sugars, free phenolics and free amino acids. Reducing sugars determined with alkaline copper and arsenomolybdate reagents spectrophotometricaly at 540 nm, according to Moore (1974). Free phenolics were determined spectrophotometricaly at 650 nm with Folin-Ciocâlteu reagent, according to William *et al*. (1965). Free amino acids estimated using the method of Rosen (1957) with ninhydrin reagent spectrophotometricaly at 570 nm. All spectrophotometric measurements were conducted in 3rd leaf using UV/VIS spectrometer, T80, PG instrument Ltd, USA.

**Histological studies**

The 3rd leaf and flower pedicel specimens from second season plants were fixed in Formalin acetic acid, dehydrated in ethyl alcohol series, embedded in Paraffin wax, sectioned to thickness of 15 µm, double stained with Safranin and Light green, cleared in Xylene and mounted in Canada balsam, according to Willey (1971). All measurements were calculated by eyepiece micrometer.

**Number and dimensions of stomata**

Epidermal strip of the 3rd leaf was made, then number, length and width of stomata by eyepiece micrometer were determined (Willey, 1971).

**Thermal image acquisition**

Thermal images of the plots were taken with infrared thermal cameraTi-32 (Fluke Thermography, USA), equipped with a 640 x 480 pixel microbolometer sensor. Thermal and visual image can be presented simultaneously as full thermal image, or as a picture in picture image in various blend modes. the temperature measurements range starts at -20°C and goes up to + 600°C sensitive in the spectral range of 7.5-13 µm. The canopy height was 1 m, images were analyzed in Ti-32 Pro software (Infrared Solutions). Emissivity for measurements of inflorescences and plant canopies was set at 0.97, while transmission correction was 85%. For more accuracy, the span of auto adjusted thermal image is automatically set, in addition to level of the displayed as an important camera feature, in order to detect maximum and minimum temperature of the entire display (Wilcox and Makowski, 2014).

**Statistical analysis**

The experiment was conducted in a randomized complete block design with four replicates. Data were statistically analyzed using the appropriate analysis of variance, according to Steel *et al*. (1997). Data obtained in the present study was subjected to an analysis of variance (ANOVA) with the honestly significant difference value, calculated as Tukey's statistic at $p \leq 0.05$ (SAS Institute, 2004).

## RESULTS

### Effect of salicylic acid on plant growth and yield

Over the two successive seasons, salicylic acid (SA) at 50 mg $l^{-1}$ gave the highest significant values of plant shoot and dry weight (g) as well as its water content (%), compared to control and other SA concentrations. In this respect, the shoot fresh weight was increased by 228 and 74%. The shoot dry weight was increased by 117 and 90%. The shoot water content was increased by 18.6 and 15.6%, compared to untreated canola, in 2014 and 2015 seasons, respectively (*Table 1*).

**Table 1 - Effect of salicylic acid application on some growth parameters of canola after 95 days of sowing during 2014 and 2015 seasons**

| SA (mg $l^{-1}$) conc. | Shoot fresh weight (g) plant$^{-1}$ | | Shoot dry weight (g) plant$^{-1}$ | | | Water content (%) |
|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| 0.0 | 114.0c | 202.0b | 34.0b | 36.4b | 67.98b | 71.00b |
| 50 | 375.0a | 352.0a | 74.0a | 69.2a | 80.64a | 82.12a |
| 100 | 206.0bc | 253.0ab | 64.0a | 51.8b | 76.64ab | 79.94ab |
| 200 | 231.0b | 235.0b | 56.0ab | 38.0b | 73.96ab | 81.36ab |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

**Table 2 - Effect of salicylic acid application on seed and oil yield of canola after 150 days of sowing during 2014 and 2015 seasons**

| SA (mg $l^{-1}$) conc. | Seed yield (g) plant$^{-1}$ | | Seed yield (kg ha$^{-1}$) | | Oil content % | | Oil yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| 0.0 | 30.3d | 29.1c | 1949.2d | 1869.9c | 38.8c | 39.6b | 74.0c | 74.0c |
| 50 | 40.4a | 38.2a | 2598.3a | 2454.7a | 40.2bc | 41.5a | 104.5a | 101.9a |
| 100 | 37.3b | 36.3a | 2399.0b | 2334.8a | 41.4ab | 41.4a | 99.3a | 96.6ab |
| 200 | 34.1c | 33.3b | 2193.4c | 2142.0b | 42.0a | 42.9a | 92.1b | 91.9b |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

Canola which was treated with 50 mg $l^{-1}$ of SA produced the highest seed yield (g plant$^{-1}$ and kg ha$^{-1}$), over the two successive seasons and under 100 mg $l^{-1}$ of SA, in the second season (*Table 2*). In this respect, foliar applying 50 mg $l^{-1}$ of SA increased the seed yield by 33.3 and 31.2% over the control, in 2014 and 2015 seasons, respectively. All levels of SA under study significantly increased the % of oil content in canola, compared to control (*Table 2*). Plants of canola, that was treated with 200 mg $l^{-1}$ of SA, contained the maximum significant % of oil content only in the second season. Although the % of oil content decreased in 50 mg $l^{-1}$ treated

canola, but increment of seed yield $ha^{-1}$ led to increase the oil yield $ha^{-1}$, compared to other SA concentrations. The oil yield increased by 30.5 and 27.9 kg $ha^{-1}$ in plants, which was treated with 50 mg $l^{-1}$ of SA, compared to control ones during the cultivated seasons, respectively.

## Effect of salicylic acid on number of aphids

All SA concentrations significantly ($p \leq 0.001$) reduced the mean number of aphids 10 $cm^{-1}$ of plant inflorescence, during the 15th to 21th week of investigation (full blooming and capsule-forming periods) in 2013/14 and 2014/15 seasons, compared to untreated plants (*Tabs. 3* and *4*). At the end week of investigation, SA at 50 mg $l^{-1}$ reduced the number of aphids 10 $cm^{-1}$ of plant inflorescence by five times, in the first season, and by four times, in the second season, in comparison to control.

**Table 3 - Impact of salicylic acid elicitor (SA) on the mean number of cabbage aphid, *Brevicoryne brassicae,* on canola throughout the growing season of 2013/14**

| SA (mg $l^{-1}$) conc. | Mean number of aphids/ 10 cm of inflorescence | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15th week | 16th week | 17th week | 18th week | 19th week | 20th week | 21th week |
| 0.0 | 7.2 a | 12.5 a | 30.5 a | 40.0 a | 83.0 a | 172.0 a | 213.0 a |
| 50 | 1 cd | 2.0 c | 6.4 cd | 9.1 bc | 13.0 c | 30.5 bc | 41.9 d |
| 100 | 2.4 c | 2.5 c | 8.6 c | 16.5 b | 33.0 b | 52.5 b | 98.0 c |
| 200 | 4.5 b | 4.9 b | 14.8 b | 21.5 b | 47.0 b | 69.5 b | 153.0 b |
| $p \leq$ | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

**Table 4 - Impact of salicylic acid elicitor (SA) on the mean number of cabbage aphid, *Brevicoryne brassicae,* on canola throughout the growing season of 2014/15**

| SA (mg $l^{-1}$) conc. | Mean number of aphids/ 10 cm of inflorescence | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15th week | 16th week | 17th week | 18th week | 19th week | 20th week | 21th week |
| 0.0 | 10.0 a | 18.3 a | 45.0 a | 53.0 a | 157.0 a | 269.0 a | 353.0 a |
| 50 | 3.4 c | 4.5 c | 10.7 d | 14.9 bc | 36.5 d | 58.0 d | 86.0 c |
| 100 | 7.4 b | 7.7 b | 24.8 c | 25.0 b | 83.0 c | 119.0 c | 200.0 b |
| 200 | 6.5 b | 8.6 b | 32.0 b | 33.5 b | 120.0 b | 191.0 b | 256.0 a |
| $p \leq$ | 0.0008 | 0.0004 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

Canola plots, particularly their edges, were severely impacted by cabbage aphids both in incidence (e.g., 80 or 90% of affected inflorescence), and level of infestation (e.g., colonies in excess 10 cm in depth). *Figs. 1* and *2* showed that SA application at the low rate of 50 mg $l^{-1}$ caused reduction in infestation percentage (inflorescence affected in both seasons percentage). The infestation percentages were 10 and 15%, in 2013/14 and 2014/15, respectively. Also, data showed that application of SA at the low rate was more efficient on reduction of depth (0.7 and 0.5 cm), in comparison with the middle rate 100 mg $l^{-1}$ (1.3 and 0.9 cm) and the high rate of SA 200 mg $l^{-1}$ (2.0 and 0.5 cm), in 2013/14 and 2014/15, respectively.

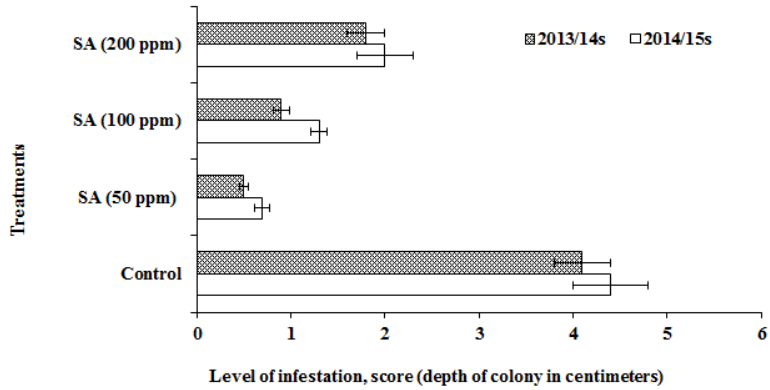


**Figure 1 - Effects of SA on level of aphid infestation score (depth of colony in centimeters) on canola, during the 2013/14 and 2014/15 seasons**

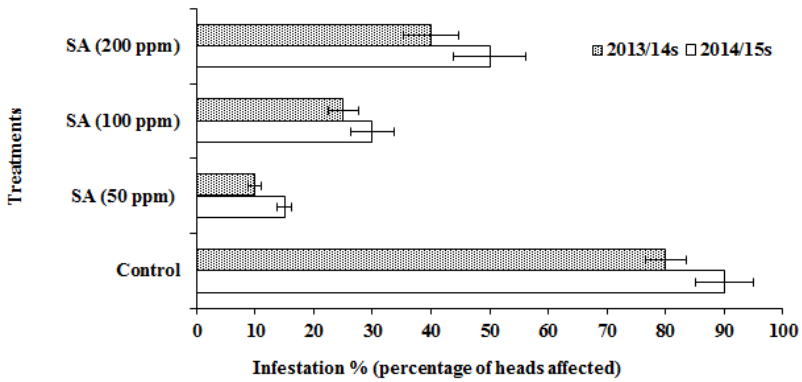


**Figure 2 - Effects of SA on aphid infestation % (percentage of inflorescence affected) on canola during the 2013/14 and 2014/15 seasons**

**Effect of salicylic acid on biochemical components in leaves and flowers**

Content of reducing sugars in leaves and flowers was enhanced by 115 and 70%, respectively, in 50 mg $l^{-1}$ of SA-treated canola, compared to untreated one (*Table 5*). In general, content of free phenolics was non-significantly increased under all SA concentrations in leaves and flowers, in comparison to untreated plants. Content of free amino acids was significantly increased in leaves and flowers under all SA concentrations, compared to control plants. Leaves of canola, treated with 50 mg $l^{-1}$ of SA, synthesized the maximum content of free amino acids (3.39 mg $g^{-1}$ FW) with 113% increment, compared to untreated plants. Flowers sprayed with100 mg $l^{-1}$ of SA accumulated 2.62 mg $g^{-1}$ FW with 167% increment, in comparison to control plants.

**Table 5 - Effect of salicylic acid application on some chemical components of 3rd leaf and flower of canola after 95 days of sowing, during 2014/15 season**

| SA (mg $l^{-1}$) conc. | Reducing sugars, mg $g^{-1}$ FW | | Free phenolics, mg $g^{-1}$ FW | | Amino acids, mg $g^{-1}$ FW | |
|---|---|---|---|---|---|---|
| | Leaf | flower | Leaf | flower | Leaf | flower |
| 0.0 | 20.4b | 23.2b | 1.75a | 2.83a | 1.59b | 0.98b |
| 50 | 43.9a | 39.6a | 1.93a | 2.92a | 3.39a | 1.39a |
| 100 | 20.7b | 22.4b | 2.28a | 2.84a | 2.76a | 2.62a |
| 200 | 19.6b | 30.8ab | 2.23a | 2.98a | 1.32b | 2.32a |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

**Effect of SA on some histological characters of leaf and its stomata**

In general, SA application had modified some characters of leaf tissues, as shown in *Tabs. 6, 7* and *Figs. 3, 4*. The maximum thickness of mesophyll and phloem tissues was found in both 100 mg $l^{-1}$ SA-treated plants and untreated ones. Maximum thickness of xylem tissue (235 µm) was observed in 50 mg $l^{-1}$ SA-treated canola with 37.9 % increment, in comparison to control plants. Six vascular bundles were detected in leaves treated with 200 mg $l^{-1}$ of SA, in compared to four vascular bundles in untreated plants. Maximum numbers of xylem vessels were observed in both 50 and 100 mg $l^{-1}$ of SA.

Length of stoma was gradually increased with increment of SA concentrations under investigation, without significant differences among them. However, the maximum value of stoma width was observed in 100 mg $l^{-1}$ of SA-treated leaf and untreated one. The minimum width of stoma (38.3 µm) was detected in 50 mg $l^{-1}$ of SA. Both high concentrations of SA (100 and 200 mg $l^{-1}$) had reduced the length of soma aperture, in comparison to control and 50 mg $l^{-1}$ of SA treatment. All SA concentrations

gradually decreased the width of stoma aperture, in comparison to untreated canola. The maximum number of stomata (3300 stoma $cm^{-2}$) was found in 50 mg $l^{-1}$ of SA treatment with 35.6% of increment, compared to untreated leaf. The highest concentration of SA (200 mg $l^{-1}$) gave the minimum values of both length and width of stoma, as well as the number of stomata $cm^{-2}$.

**Table 6 - Effect of salicylic acid application on some histological parameters of 3rd leaf after 95 days of sowing, during 2014/15 season**

| SA (mg $l^{-1}$) conc. | Thickness (µm) of | | | Number of | |
|---|---|---|---|---|---|
| | mesophyll tissue | xylem tissue | phloem tissue | vascular bundles | xylem vessels |
| 0.0 | 606.7 a | 170.3 c | 135.0 a | 4 b | 71 b |
| 50 | 572.0 b | 235.0 a | 105.0 b | 4 b | 93 a |
| 100 | 633.3 a | 206.7 b | 135.0 a | 3 b | 98 a |
| 200 | 540.0 b | 210.0 b | 110.0 b | 6 a | 77 b |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

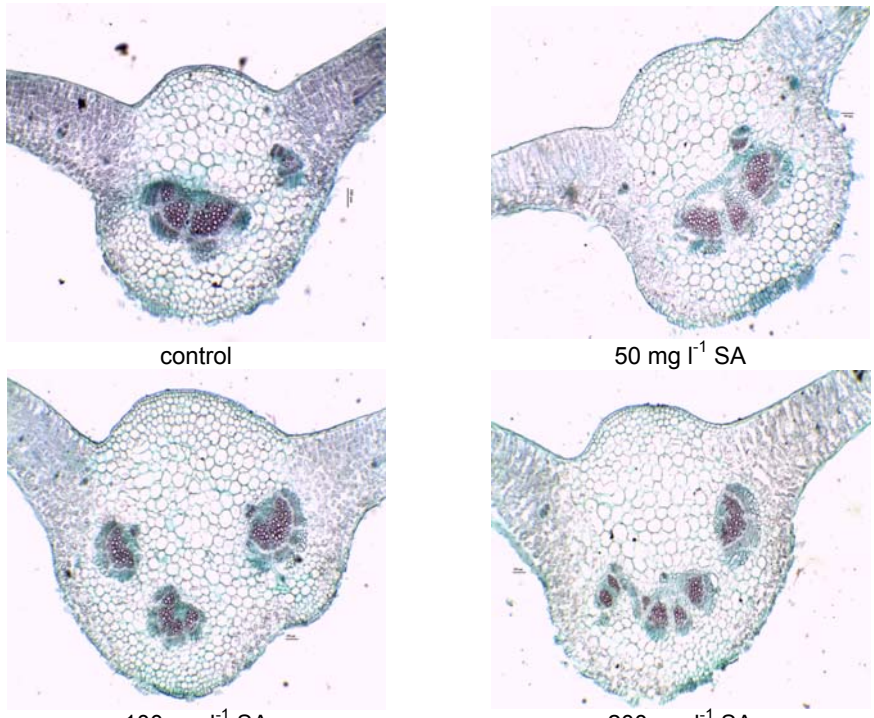


**Figure 3 - Transverse section on median ribs or midribs of 3rd leaf of canola under different concentrations of SA applications after 95 days of sowing**

**Table 7- Effect of salicylic acid application on stomatal characteristics after 95 days of sowing, during 2014/15 season**

| SA (mg $l^{-1}$) conc. | Characteristics of stoma (µm) | | | | Number of stomata ($cm^{-2}$) |
|---|---|---|---|---|---|
| | Length | Width | Aperture length | Aperture width | |
| 0.0 | 51.7 b | 53.3 a | 30.7 a | 11.7 a | 2433.0 b |
| 50 | 58.3 a | 38.3 c | 31.7 a | 7.7 b | 3300.0 a |
| 100 | 61.7 a | 53.3 a | 24.0 b | 6.0 bc | 2533.0 b |
| 200 | 63.3 a | 45.0 b | 21.7 b | 4.7 c | 2133.0 c |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

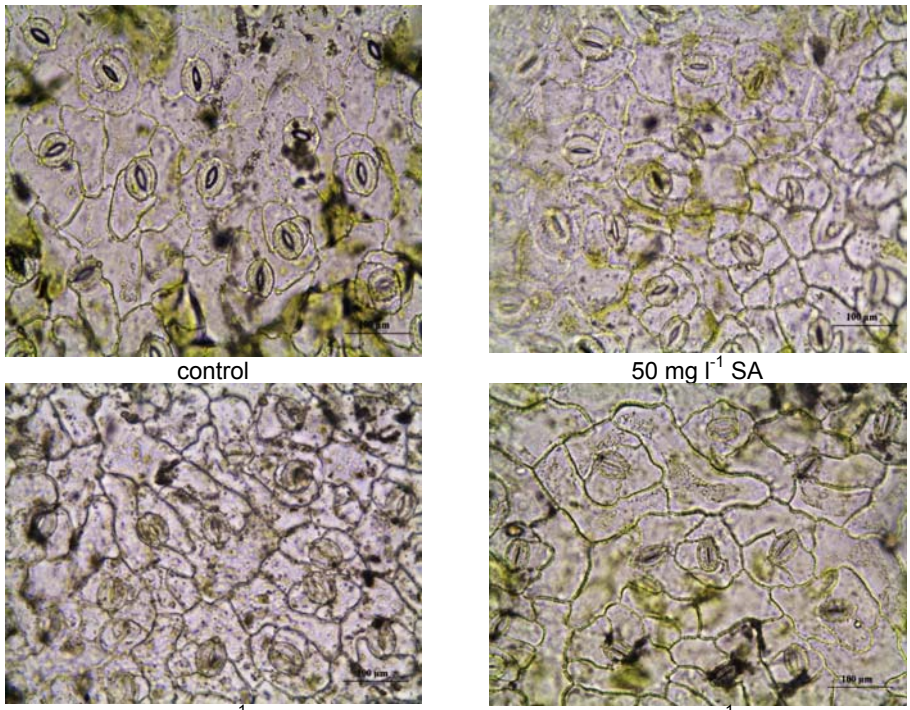


**Figure 4 - Stomatal dimensions of canola after 95 days of sowing under different concentrations of SA**

## Effect of SA on some histological characters of flower pedicel

Maximum thickness of epidermis, xylem and phloem tissues and number of xylem vessels $bundle^{-1}$ was recorded in 50 mg $l^{-1}$ of SA treatment, in compared to untreated or the other both high concentrations of SA (*Table 8* and *Fig. 5*). The previous parameters were increased by 45.4, 10.3, 37.1 and 44.4%, respectively, in comparison to control plants. All SA concentrations decreased the thickness of secretory tissue, in

compared to control. Both high concentrations (100 and 200 mg $l^{-1}$) of SA gave the maximum number of vascular bundles (8 and 9), but there were only six bundles in control and 50 mg $l^{-1}$ of SA treatment.

**Table 8 - Effect of salicylic acid application on histology of flower pedicel after 95 days of sowing, during 2014/15 season**

| SA (mg $l^{-1}$) conc. | Thickness (µm ) of | | | | Number of | |
|---|---|---|---|---|---|---|
| | epidermis | secretory tissue | xylem tissue | phloem tissue | vascular bundles | xylem vessels/ bundle |
| 0.0 | 27.3 b | 242.3 a | 77.3 b | 54.7 c | 6 b | 9 b |
| 50 | 39.7 a | 189.0 c | 85.3 a | 75.0 a | 6 b | 13 a |
| 100 | 27.7 b | 155.0 d | 78.0 b | 65.7 b | 8 a | 9 b |
| 200 | 29.0 b | 205.0 b | 65.7 c | 55.0 c | 9 a | 9 b |

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; $p \leq 0.05$).

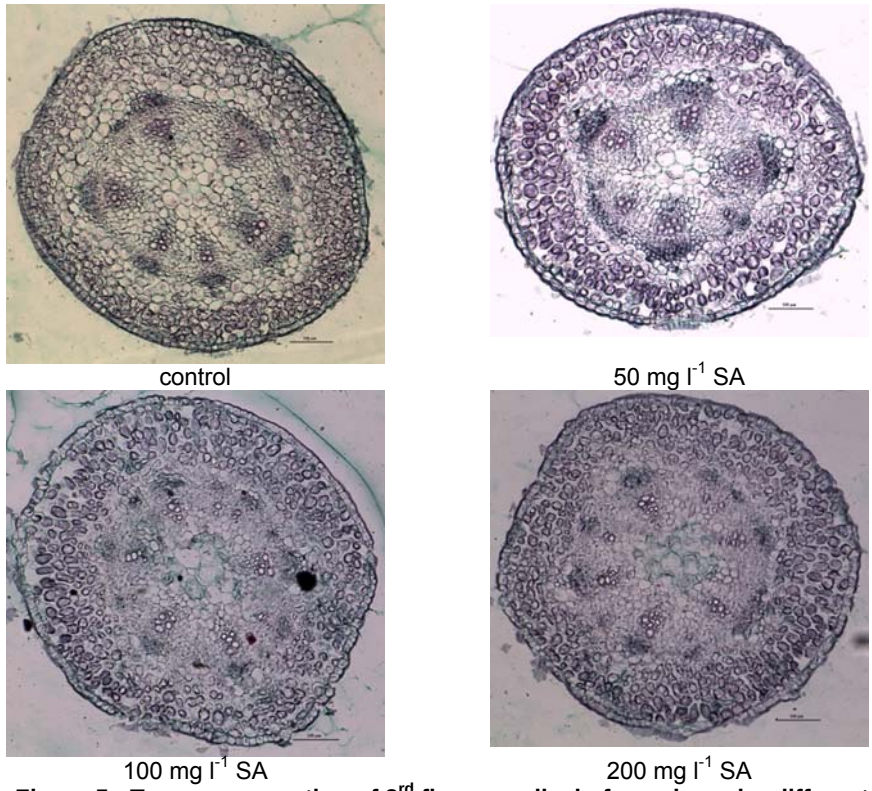


**Figure 5 - Transverse section of 3rd flower pedicel of canola under different concentrations of SA after 95 days of sowing**

**Infrared thermal imaging**

Thermal cameras provide the opportunity to evaluate the temperature variations in crop canopies as affected by insects. *Fig. 6* depicts a close-distance image of canola, containing different plant parts, such as stalk, leaf, and flowers under various SA treatments. According to this thermal image, the majority of canola plants in 50 mg $l^{-1}$ SA treatment had a temperature range of 27.3-30.3˚C, while the range was 25.2-26.9˚C and 24-26.2˚C for 100 mg $l^{-1}$ SA and 200 mg $l^{-1}$ SA treatments, respectively. The image acquisition occurred for healthy and infected plants (*Fig. 7*) demonstrated that plants infected with aphids had a higher temperature than healthy plants. Where, the average temperature over all means of SA treatments were 22.6˚C for healthy plants and 25.8°C for infected plants (*Table 9*).

The thermal images *Fig. 6* demonstrated that the temperature of the healthy inflorescence of canola at 20 pixels was 22.1±0.27°C, whereas temperature aphid-infected ones was 22.4±0.35°C (*Fig. 7*). The maximum and minimum temperatures of healthy inflorescence were 22.5±0.32°C and 21.6±0.22°C, respectively. The maximum and minimum temperatures of infected ones were 22.8±0.35°C and 22.0±0.24°C, respectively. However, the maximum temperature (22.5°C) of inflorescence was detected thermally in both 50 and 100 mg $l^{-1}$ of SA treatment (*Fig. 6*). The minimum values of inflorescence temperature (20.2 °C) were recorded in untreated one. SA treatment at 50 and 100 mg $l^{-1}$ increased the temperature of the main inflorescence by 2.3°C over the control plants.

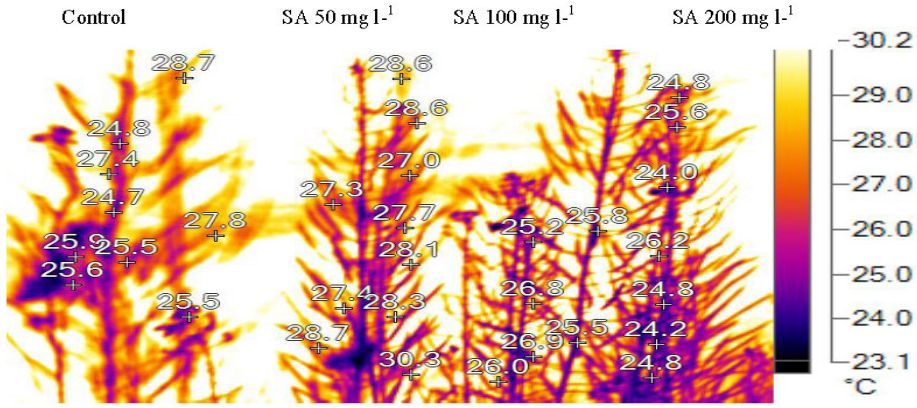


**Figure 6 - Thermal images of canola plant treated with salicylic acid after 140 days of sowing**

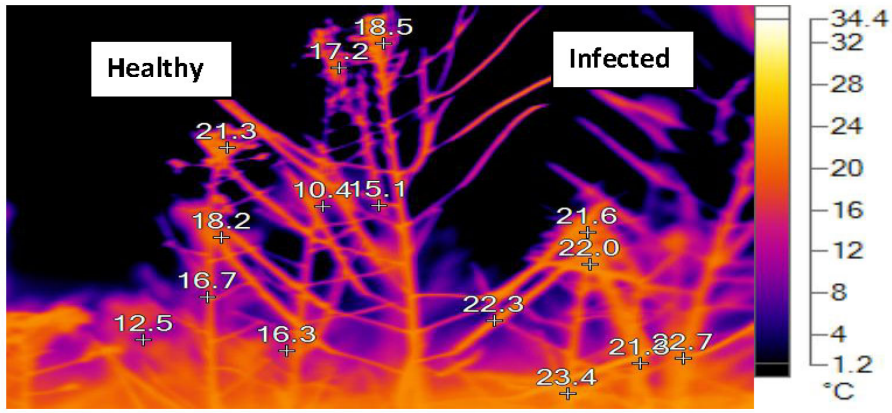


**Figure 7 - Thermal image of canola plant contains the aphid infested plant (left) and the healthy plant (right) after 140 days of sowing**

**Table 9 - Average thermal leaf canopy temperature for canola plants treated with various salicylic acid levels**

| SA treatments (mg $l^{-1}$) | Leaf canopy temperature | |
|---|---|---|
| | Healthy | Infected |
| 0 | 18.2 | 20.5 |
| 50 | 24.4 | 27.2 |
| 100 | 23.8 | 24.9 |
| 200 | 24.0 | 25.8 |
| Mean | 22.6 | 24.6 |
| LSD | 0.27 | 0.98 |

## DISCUSSION

Salicylic acid (SA) is a phenolic phytohormone in plants with roles in plant growth and development, photosynthesis, transpiration, ion uptake and transport (Hayat *et al.,* 2005). Results showed that it induces specific changes in leaf anatomy, thickness of epidermis, xylem, phloem tissues and number of xylem vessels bundle structure, as shown in SA-treated leaves and flower pedicel of canola, compared to untreated one (*Figs. 3* and *5*). SA is involved in endogenous signaling, mediating in plant defense against pathogens. It plays a role in the resistance to aphids by inducing the production of pathogenesis-related proteins (Thaler *et al*., 1999; Thiruvengadam *et al*., 2015). It is involved in the systemic acquired resistance (Raskin, 1992). Aphids can disrupt cuticular and/or stomatal regulation of transpiration and influence plant water relations (Taiz and Zeiger, 2006). Recording the maximum number of stomata $cm^{-2}$ with minimum aperture width of stoma in 50 mg $l^{-1}$ SA-treated leaf resulted in diminish the transpire surface area of leaves (*Table 7* and

*Fig. 4*). Uzunova and Popova (2000) found that exogenous application of SA reduced width of the adaxial and abaxial epidermis, as well as mesophyll tissue subsequently reduced the transpiration rate in barley (*Hordeum vulgare*). Also, transpiration rate was significantly decreased in SA-treated (1 and 10 mM) leaves of common been (*Phaseolus vulgaris*) (Larque-Saavedra, 1978). However, increment the thickness of xylem tissue and number of xylem vessels in midrib of SA-treated leaves, as shown in *Table 7* and *Fig. 3*, demonstrated the functional role of SA in xylem development and, subsequently, enhancement the water and nutrient transportation in plant. Humphreys and Chapple (2002) showed that SA, as a simple phenolic compound, participates in lignin biosynthesis, constituting important compound of xylem vessels walls.

In our results, a clear proof that SA can close stomata is reported here. SA could be used to mitigate the harmful effects of aphid infestation in canola plants. Infestation of inflorescence by cabbage aphid tended to decrease the canola yield and quality (Sayed and Teilep, 2013). Aphids suck the sap from the plant. All treatments appeared to show a yield response against the untreated control plots. High densities may prevent pod set or even kill plants. Aphids can also reduce the accumulation and movement of assimilates (Taiz and Zeiger, 2006).

The results had shown the positive effect of the eco-friendly compound, salicylic acid (SA), at low concentration, in decreasing the number of aphid on inflorescence and enhancement growth and productivity of canola. Also, the response of canola depend on SA concentration as previously mentioned (Metwally *et al*., 2003), whereas low concentration of SA had a positive effect on plant growth and yield, but the high concentration had a negative effect. This might be due to that SA overdose could lead to salicylate intoxication (Raskin, 1992). Aphids can be found on canola from emergence to flowering. Because aphids have the capability of reproducing very quickly, they can injure canola plants during a very small window of time. There is a variety of phenotyping methodologies, that can be very helpful to screen resistance under controlled conditions. Increasing of shoot water content as a result of SA application led to enhance the shoot fresh weight of canola. Fresh weight of other crops as pepper (*Capsicum annuum*) (Elwan and Elhamahmy, 2009), pea (*Pisum sativum*) (Elhamahmy and Hossein, 2011) were also positive affected by SA application at low concentration ($10^{-6}$M). Also, SA promotes plant growth through another mechanisms, such as stimulate plant cell proliferation and expansion (Hayat *et al.,* 2005), altered auxin: cytokinin balance (Shakirova *et al.,* 2003), induced source-sink mediated invertase (Elwan and Elhamahmy,

2009), increased photosynthesis rate (Shi *et al.,* 2006), enhanced the absorption and transportation of nutrient (Harper and Balke, 1981) or, indirectly, regulates both local disease resistance mechanisms, including host cell death, defense gene expression and systemic acquired resistance (Schneider *et al.,* 1996).

In contrary, reduction of growth parameters in canola, under high concentration of SA may due to closure of stomata subsequently, reduced the photosynthetic electron transport, leading to reduction of photosynthetic rate, as previously showed by Mateo *et al.* (2006). Also, the high concentration of SA reduced chlorophyll content in leaves of both cowpea (*Vigna unguiculata*) and wheat (*Triticum aestivum*) (Rao *et al*., 1997; Chandra and Bhatt, 1998). However, the negative effect of SA may due to its oxidative stress on cell, through induction the activity of superoxide dismutase, responsible for formation of $H_2O_2$ and inhibit the activity of catalase and peroxidase, responsible for its degradation (Horvath *et al*., 2007).

Although, application of SA at all concentrations decreased the numbers of aphids on inflorescence of canola, but phenolics content not changed in leaves and flowers. Phenolic derivative may participate in lignin synthesis, therefore thickness of epidermis, xylem and phloem, as well as number of xylem vessels in flower pedicel was increased under SA application (*Fig. 5*). Other phenolics serve as allelopathic or phytoalexin compounds participate in defense activation network (Raskin, 1992). Metabolites of phloem sap represented the main feeding diet to cabbage aphid, due to its specific feeding apparatus (Cole, 1996).

Due to the protective role of SA on plant enzymes (Raskin, 1992), SA-treated seeds contain the highest percent of oil content (*Table 2*). Increment of free amino acids content in all SA-treated concentrations may due to up regulate the activity of nitrate reductase, as previously demonstrated in spinach (*Spinacea oleracea*) and parsley (*Petroselinum hortense*) (Elwan and El-Hamahmy, 2015). Amino acids, as tryptophan, is a precursor of auxin. Auxin promote plant growth through increase the amount of water content, as mentioned in tobacco (*Nicotiana tabacum*) transformed with auxin biosynthesis genes, which had higher water content than the non-transformed ones (Sitbon *et al.,* 1992).

SA application led to increase the reducing sugars and amino acids contents in leaves and flowers. These findings were agreed with Kiddle *et al*. (1994) showed that SA induced the accumulation of glucosinolates in leaves of oilseed rape, which contribute to the plant's defense against microorganisms and pests. Glucosinolates glucosides are natural secondary metabolites toxic to aphids, synthesized in all *Brassica* species from glucose and amino acids precursors (Cole, 1996). In contrary, SA significantly increased individual phenols, total polyphenols contents

and enhanced biological activity in Chinese cabbage (*Brassica rapa* spp. *Pekinensis*) plants (Thiruvengadam *et al*., 2015).

Traditional methods of measuring growth have been time-consuming and have often involved the destructive harvest of plants. Recent imaging technologies to estimate biomass and growth parameters have gone some way to alleviate these issues. Thermography can be useful in monitoring plant aphids development based on the local temperature changes resulting from either plant defense mechanisms or insect impact. As a result, the recorded temperature of SA-treated inflorescence was increased by 2.3°C over the untreated inflorescence. SA inhibit the mitochondrial electron transport or induce the alternative respiratory pathway (Norman, 2004), which led to determine the production of the energy biomolecules, ATP, therefore, the energy dispersed as heat (Kapulnik *et al*., 1992). These findings demonstrated the functional role of SA as a key signal in regulating thermogenesis and disease resistance (Raskin, 1992). Temperature raised in infected inflorescence may due to the respiration of aphids populations, compared to the free-aphids ones (*Fig. 6*). All SA concentrations also raised the temperature of inflorescence, subsequently decrease the infestation of aphids (*Figs. 6* and *7*). Using of high-resolution infrared camera showed an increment by 0.5 to 1.0$^{o}$C of temperature of SA-treated leaf surface of tobacco (Van Straeten *et al*., 1995). Temperature may increase where the disease closes stomatal closure, as a result of decreased xylem water flow (Taiz and Zeiger, 2006).

However, decreasing the number of aphids was correlated with reducing the thickness of secretory tissue of flower pedicel (*Fig. 5*). Horvath *et al*. (2007) contributed the high efficiency of SA in activation of plant tolerant to biotic and abiotic stressors due to the generation of reactive oxygen species and alters the activities of antioxidant enzymes led to maintain cellular redox homeostasis. Indirect effect of SA through its effect on other phytohormones as jasmonic acid, ethylene, and auxin. Generally, combined thermal imaging and histological studies under laboratory conditions showed that the accumulation of salicylic acid (a hormone produced in plant defense against infections) in response to aphids infestation in canola leaves was paralleled by stomatal closure. However, if the infection has only a minor effect on transpiration, detection using thermography may not be possible, particularly in field conditions.

## CONCLUSION

Low level of salicylic acid (SA) reduced the number of aphid on canola inflorescence. SA induced thermogenesis in canola inflorescence. SA modified the number and dimensions of leaf

stomata, as well as the histological constituent of leaf. SA increased the content of feed-aphid metabolites (reducing sugars and amino acids) and did not affect the level of phenolics in leaves or flowers of canola. SA reduced the thickness of secretory tissue of flower pedicel. SA enhanced the growth, yield and oil content in canola. There is a variety of phenotyping methodologies, that can be very helpful to screen aphids resistance under controlled conditions. However, in order to be applicable for breeders in their large scale screenings, assessments should be fast, cheap and sufficiently reliable. Therefore, infrared thermal imaging was used as an innovative techniques for detecting aphid's infestation and can aid in accurately quantifying the damage caused by insect pests.

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