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The Impact of Faba Bean Seeds Soaking in Salicylic Acid, Acetyl-Salicylic Acid and Methyl Salicylate on Inducing Plant Resistance against the Cowpea Aphid, *Aphis craccivora* Koch.

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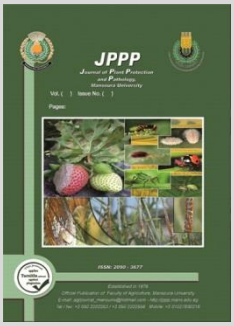

ABSTRACT

The present work was conducted in the laboratory of Plant protection, Shandweel Agricultural Research Station, Sohag Governorate to investigate the effect of priming of faba bean seeds with three concentrations, 50, 100 and 200ppm for each substances of salicylic acid, acetyl salicylic acid and methyl salicylate on survival, duration and wing formation of the cowpea aphid, *Aphis craccivora* Koch., also, their repellent effect was determined. Results obtained showed that the highest effect was recorded in salicylic acid at 200ppm (86.96%) after 24 hours, however, no repellent effects were recorded from acetyl-salicylic acid. Priming faba bean seeds with salicylic acid and methyl salicylate elongated the nymphal stage, decreased their survival and increased wing formation compared to control. The effects of the three substances were found to be concentrated dependant. The use of salicylic acid and methyl salicylate as resistance inducers hold great promise for application for *A. craccivora* control as eco-friendly method.

Keywords: salicylic acid, methyl salicylate, acetyl-salicylic acid, broad bean, *Aphis craccivora*, priming, repellent, survival and wing formation.

INTRODUCTION

Faba bean (*Vicia faba* L.) is considered one of the most important legume vegetables in Egypt, consumed as a fresh vegetable in the local market or to be exported. Faba bean is generally used as a vegetable, green or dried, fresh or canned human food. *A. craccivora* is a polyphagous insect pest, but it shows preference to those of Leguminoseae (Singh and Singh, 2017). It considered as one of the most dangerous insect pest attacking faba bean in Egypt (Mousa and Metwally, 2014). The induced resistance was defined by Karban and Myers (1989) as changes in plants following damage or stress can increase the resistance to further herbivore attack by reducing the preference or effect of herbivores. The application of plants by salicylic acid and methyl salicylate can stimulate herbivore damage (Chamberlain *et al.*, 2000 and Tang *et al.*, 2015). Primed seeds in compounds such as salicylic acid and methyl salicylate display faster, stronger and activations of the various cellular defense responses that are induced following the attack by insects or in response to abiotic stress (Sarwar *et al.*, 2008 and Engelberth *et al.*, 2011). Moreover, recent studies have shown that using priming strategy to enhance plant resistance was suggested by many researchers (El-Khawas, 2012; Worrall *et al.*, 2012; Smart *et al.*, 2013 and Sharma *et al.*, 2015). The objectives of this study were to test the repellent effect of salicylic acid, methyl salicylate and acetyl salicylic acid on *Aphis craccivora*, and whether the priming of faba bean seeds with the previous substances affected the performance of newly emerged nymphs of *Aphis craccivora*.

MATERIALS AND METHODS

These experiments were conducted in the laboratory of Plant protection, Shandweel Agricultural Research Station, Sohag Governorate. All laboratory studies were conducted under room conditions where the ambient temperature ranged between 11 and 25 ◦C, and RH varied from 44% to 60%.

1. – The cowpea aphid culture:

Aphis craccivora was reared in the Laboratory of Plant protection at Agricultural Research Station of Shandaweel, Sohag Governorate. Aphids were obtained from cowpea cultivated at the Farm of Agricultural Research Station of Shandaweel. Faba bean seeds were sown in plastic pots containing 2 soil : 1 sand ratio. Pots were, then, kept in a screen cage (1×1×1m) covered with anti-aphid screen, and kept under laboratory conditions. The collected aphids were placed on the growing seedlings. This constituted the stock culture. The artificial infestations by aphids were successively repeated on new uninfested faba bean seedlings.

2. - Chemicals and plants:

The salicylic acid and methyl salicylate were obtained from (El-Nasr Co. for Intermediate Chemicals, Egypt (NCIC)) and Acetyl salicylic acid from (Future Pharmaceutical industries Company, Egypt. Seeds of *Vicia faba* (variety weam) were obtained from The Agricultural Research Center at Giza. Three concentrations were used for each material, 50, 100 and 200 ppm. Salicylic acid, acetyl salicylic acid and methyl salicylate were solved in little drops of ethanol then dispersed in the distilled water to the required concentration.

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3. - Seed Priming:

The seeds were soaked for 3 hours in solutions of salicylic acid, methyl salicylate and acetyl salicylic acid at different concentrations, 50, 100 and 200ppm for each substance, meanwhile, distilled water was used for control. Before preparing all solutions, salicylic acid, acetyl salicylic acid and methyl salicylate were dissolved in ethanol. After that, seeds washed thoroughly with distilled water to prevent the effects on seedlings. Then ten seeds were sown in plastic pots of 30 cm in diameter and 55 cm high containing soil taken from the field. The plants were left to grow under natural environmental conditions. All experiments started when the plants were three weeks old.

4. – The repellent effect:

To evaluate whether the treatments affected the response of *A. craccivora* behavior (repellent effect), the free choice test was used. One faba bean leaflet from each treatment was put on petri dish (15 cm diameter with wetted filter paper) against untreated one. A number of 20 aphid adults were released in the centre of each petri dish. After 1 and 24 hours, the numbers of aphid settled on treated and untreated leaflets were counted. This experiment was arranged in a complete randomized design with five replicates. The data were expressed as percentage of repulsion (PR) using the following formula:

$$PR\ (\%) = (Nc\text{-}50) \times 2$$

Where,

Nc = the percentage of aphids found in the control leaflets. Then the average values were categorized according to the scale of McDonald *et al.* (1970) as following: 0: >0.01-0.1%, I: 0.1-20%, II: 20.1-40%, III: 40.1-60%, IV: 60.1-80% and V: 80.1-100%.

5. – The effect of seed priming on nymphal duration, survival and wing formation of *A. craccivora*:

After 25 days from seeds sowing, one faba bean leaflets were collected from each treatment and placed in a Petri-dish (1 cm height × 9 cm diameter) which contained a wetted filter paper. After that, 10 aphid adults were transferred from the stock culture to each Petri-dish using hair- camel brush. The leaflets were inspected after 6 hours, and 20 new born nymphs were left, after that the aphid adults were removed. The nymphs were observed daily and the number of dead was recorded, at last, the number of nymphs reached mature stage were counted. The nymphal duration was calculated. The percent of alate and apterious adults were recorded. This experiment was arranged in a complete randomized design with ten replicates

6. - Statistical analysis:

Statistical analysis was conducted by using one – way analysis of variance. 'F' test used to evaluate the differences significance between treatments. The Duncan's Multiple Range Test at P = 5% was used to separate the means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSIONS

1. - Repellent effect:

The data in Tables (1) and (2) show the repellent effect of priming faba bean seeds at three concentrations of salicylic acid, acetyl salicylic acid and methyl salicylate on the acceptance of *A. craccivora* adults after 1 and 24 hours, respectively.

Table 1. Repellent effect of salicylic acid, acetyl salicylic acid and methyl salicylate against *A. craccivora* on faba bean after 1 hour.

Treatment	Concentration (ppm)	Non responded %	Responded%		Repellent %	Class
			Treat	Cont.		
Salicylic acid	50	17.00	20.00	63.00	51.81 b	I I I
	100	15.00	12.00	73.00	70.11 a	IV
	200	13.00	13.00	74.00	71.76 a	IV
Acetyl-salicylic acid	50	31.00	31.00	38.00	10.14 d	I
	100	28.00	32.00	40.00	11.11 d	I
	200	27.00	30.00	43.00	17.81 d	I
Methyl salicylate	50	30.00	22.00	48.00	37.14 c	I I
	100	20.00	23.00	57.00	42.52 bc	I I I
	200	15.00	19.00	66.00	55.29 b	I I I

Means in each column followed by the same letter are not significantly different at the 5% level according to Duncan ,s Multiple Range Test.

Table 2. Repellent effect of salicylic acid, acetyl salicylic acid and methyl salicylate against *A. craccivora* on faba bean after 24 hours.

Treatment	Concentration (ppm)	Non responded %	Responded%		Repellent %	Class
			Treat	Cont.		
Salicylic acid	50	14.00	19.00	67.00	55.81 bc	III
	100	8.00	12.00	80.00	73.91 ab	IV
	200	8.00	6.00	86.00	86.96 a	V
Acetyl salicylic acid	50	27.00	30.00	43.00	17.81 e	I
	100	26.00	28.00	46.00	24.32 e	II
	200	23.00	27.00	50.00	29.87 de	II
Methyl salicylate	50	14.00	24.00	62.00	44.19 cd	III
	100	7.00	22.00	71.00	52.69 c	III
	200	6.00	19.00	75.00	59.57 bc	III

Means in each column followed by the same letter are not significantly different at the 5% level according to Duncan ,s Multiple Range Test.

Data in Table (1) revealed that all salicylic acid concentrations proved a repellent effect (>50%) against *A. craccivora* adults, also, methyl salicylate at 200ppm, however, no repellent effects were recorded from acetyl salicylic acid after one hour. The highest repellent percent was recorded on salicylic acid at 200ppm (71.76%), followed insignificantly by salicylic acid at 100ppm (70.11%). Also, salicylic acid at 50ppm and methyl salicylate at 200ppm showed repellent percent of 51.81% and 55.29%, respectively. While, the acetyl salicylic acid

did not exceed 17.81%. The repellency class of salicylic acid concentration levels varied between III to IV, however, the repellency class of methyl salicylate concentration levels varied between II to III.

After 24 hours, it is clear that the acetyl salicylic acid had a weak effect on aphid behavior, the repellent effect percents of acetyl salicylic acid at 50ppm, 100ppm and 200ppm were 17.81%, 24.32% and 29.87%, respectively, and their repellency class varied between I to II (Table 2). However, the highest effect was recorded in salicylic acid at 200ppm (86.96%), also salicylic acid at 50ppm and 100ppm gave 55.81% and 73.91% repellent, respectively, salicylic acid repellency class varied between III to V. On the other hand, only two concentrations of methyl salicylate at 200ppm and 100ppm showed a repellent effect with 59.57% and 52.69%, respectively, and its repellency class was III.

For the three substances, the repellent activity increased with increasing of concentration. It is clear that the aphid behavior affected by time, the repellent percents of all treatments increased with time. Also, the number of non-responded aphids decreased with time and by increasing of concentration. In previous reports, methyl salicylate was reported as aphid repellent (Chamberlain *et al.*, 2000; Glinwood and Pettersson, 2000; Glinwood *et al.*, 2007; Blande *et al.*, 2010 and Tang *et al.*, 2015). Smith and Boyko (2007) suggested that the use of salicylic acid and its methyl conjugate to stimulate the expression of defense response genes which lead to the development of direct chemical defenses against aphids. Sambangi and Usha Rani. (2016) found that increased levels phenolic compounds as salicylic acid in guar plant can be explained as a mechanism of defense that acts as a barrier to aphid feeding. On the other hand, no repellent effect was found for acetyl salicylic acid by Karatolos and Hatcher (2009) as a number of aphids recorded on plants.

2. - Nymphal stage:

The data in Figure (1) showed that priming faba bean seeds with salicylic acid at the three concentrations and acetyl salicylic acid at 100ppm and 200ppm increased the duration of aphid nymphal stage comparing to control, however, no effects were recorded in case of MeSA. The longest nymphal duration was found in salicylic acid at 200ppm (6.56 days), followed insignificantly by salicylic acid at 100ppm (6.54 days), salicylic acid at 50ppm (6.43 days) and acetyl salicylic acid at 200ppm (6.45 days). The shortest nymphal duration was found in control (5.81 days). The present results are in agreement with those of Mallinger *et al.* (2011) who reported that methyl salicylate treatment had no effect on mean generation time of soybean aphid.

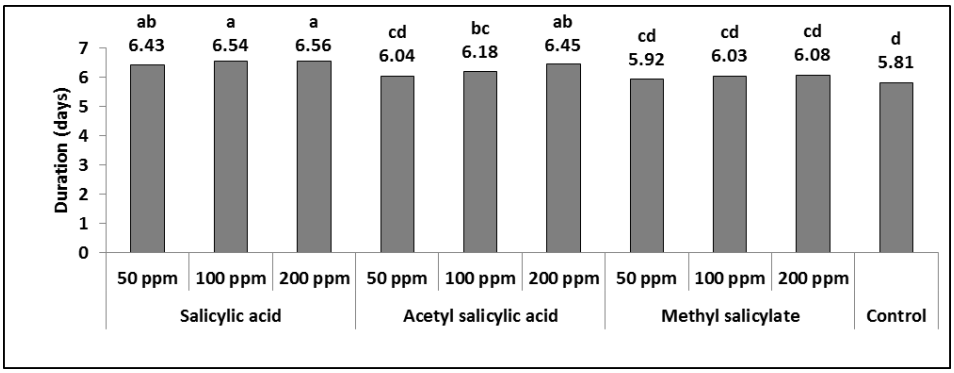

Figure 1. Effect of salicylic acid, acetyl salicylic acid and methyl salicylate different concentrations on nymphal duration of *Aphis craccivora* on faba bean. Different letters next to each bar indicate significant differences at p<0.05 by Duncan' s Multiple Range Test.

Nymphal survival:

The percent of nymphs reached to mature stage differed significantly by treatment (Figure 2). Acetyl salicylic acid at 50ppm and 100ppm had no significant effect on the percent of nymphs reached to adulthood, however, the rest treatments decreased the mean number of nymphs reached to mature stage. The lowest percent of matures was recorded in salicylic acid at 200ppm (33.50%) by insignificant difference with salicylic acid at 100ppm (37.00%). The highest percent of matures was recorded in the control (83.00%).

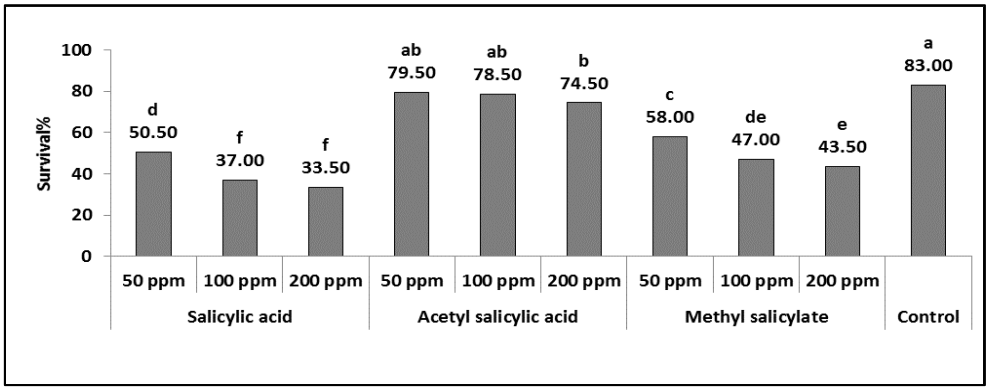

Figure 2. Effect of salicylic acid, acetyl salicylic acid and methyl salicylate different concentrations on nymphal survival of *Aphis craccivora* on faba bean. Different letters next to each bar indicate significant differences at p<0.05 by Duncan' s Multiple Range Test.

In previous studies, Karatolos and Hatcher (2009) reported that direct application of acetyl salicylic acid increased *Myzus persicae* mortality, however, its foliar application did not induce resistance against aphids. Sobhy *et al.* (2015) found that tomato plant treatment with salicylic acid mimic reduced larval survival rate of *Spodoptera littoralis* (Boisduval) compared with the control treatment. Also, Lu *et al.* (2020) found that methyl salicylate was effective on thrips.

Wing formation

The priming of faba bean with salicylic acid and methyl salicylate increased significantly the wing formation on aphid comparing to control and decreased the apterious viviparous one. However, no effects were found in acetyl salicylic acid treatments. The highest alate individuals percent recorded in salicylic acid at 200ppm (22.14%) followed insignificantly by methyl salicylate at 200ppm (19.04%), salicylic acid at 100ppm (18.96%) and methyl salicylate at 100ppm (17.26%), however, the lowest was recorded at control (1.21%) with insignificant differences with acetyl salicylic acid treatments (Figure 3).

For the percent of apterious adults, no effects were found in case of acetyl salicylic acid, however, salicylic acid and methyl salicylate decreased significantly the percentage of apterious adults compared to control. The lowest apterious individuals percent was recorded in salicylic acid at 200ppm (77.86%) followed insignificantly by salicylic acid at 100ppm (81.04%), methyl salicylate at 200ppm (80.96%) and methyl salicylate at 100ppm (82.74%), however, the highest one was recorded in control (98.79%) with insignificant differences with acetyl salicylic acid treatments (Figure 4). The previous results mean that the priming faba bean seeds with salicylic acid or methyl salicylate make plants unfavorable to *A. craccivora*.

In previous studies, environmental conditions, aphid density and host plant quality may influence wing production (Müller *et al.*, 2001 and Braendle *et al.*, 2006). In the same line with this study, Shi *et al.* (2016) suggested that the wingless aphids became winged aphids when the plant defense of salicylic acid signaling pathway was induced.

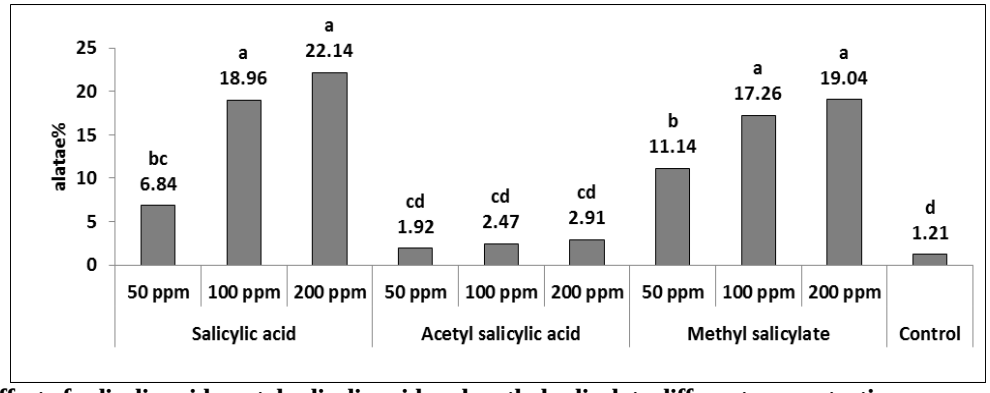

Figure 3. Effect of salicylic acid, acetyl salicylic acid and methyl salicylate different concentrations on percent of alatae of *A. craccivora* on faba bean. Different letters next to each bar indicate significant differences at p<0.05 by Duncan's Multiple Range Test.

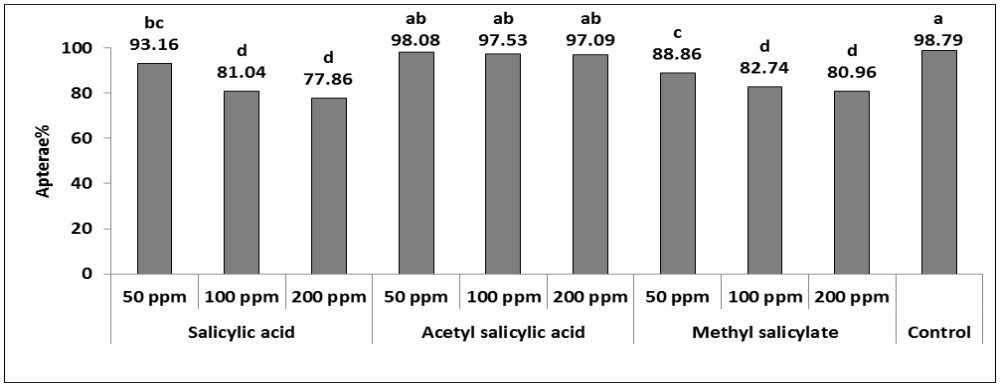

Figure 4. Effect of salicylic acid, acetyl salicylic acid and methyl salicylate different concentrations on percent of apterae of *A. craccivora* on faba bean. Different letters next to each bar indicate significant differences at p<0.05 by Duncan's Multiple Range Test.

From the foregoing results, it could be concluded that the effectiveness of salicylic acid and methyl salicylate as plant resistance inducers for reducing faba bean infestation with *A. craccivora*. The seed treatment by salicylic acid and methyl salicylate elongated nymphal duration and decreased their survival, beside a strong repellent effect. The effects of the three substances were found to be concentrated dependant. The use of salicylic acid and methyl salicylate as resistance inducers hold great promise for application against *A. craccivora* control as eco-friendly method.

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تأثير غمر بذور الفول الرومي في محاليل حمض السالسيلك وحمض الأسيتايل سالسيلك والميثايل سالسيلات علي تحفيز المقاومة النباتية لمن البقول.

عصمت أحمد السليماني

معهد بحوث وقاية النباتات – مركز البحوث الزراعية

أجريت هذه الدراسة في معمل وقاية النباتات بمحطة البحوث الزراعية بجزيرة شندويل – محافظة سوهاج لبحث تأثير نقع بذور الفول الرومي في ثلاث تركيزات (50, 100 و200 جزء في المليون) من حمض السالسيلك, الميثايل سالسيلات وحمض الأسيتايل سالسيك علي معدل البقاء ومدة التطور وتكوين الأجنحة لحشرة من البقول, كذلك تم تقدير التأثير الطارد لهذه المركبات. أوضحت النتائج أنه لا يوجد أي تأثير طارد لحمض الأسيتايل سالسيك, بينما سجل أعلي تأثير طارد في معاملة حمض السالسيك بتركيز 200 جزء في المليون (86.78%).أظهرت النتائج أن غمر بذور الفول الرومي في محاليل حمض الساليسيلك والميثايل سالسيلات يزيد من مدة طور الحورية للحشرة ويقلل من معدل بقاء الحوريات ويزيد من نسبة تكوين الأفراد المجنحة مقارنة بالكنترول. وجد أن تأثير المواد الثلاثة يزيد بزيادة التركيز. استخدام حمض الساليسيلك والميثايل سالسيلات كمحفزات للمقاومة يمكن أن يكون أسلوب مبشر وصديق للبيئة لمكافحة من البقول.