

Efficiency of the foliar application of salicylic acid to reduce the infestation with citrus leaf miner

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Abstract

This work was performed for studying the effectiveness of the foliar application of salicylic acid along with the bio-insecticide Abamectin (as standard) on the infestation with citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in three species of citrus trees, summer orange, lemon (adalia) and mandarin (morkite) in Giza Governorate, Egypt during seasons, 2021 and 2022. Two foliar sprays for each compound were applied in each season. Salicylic acid was applied by a concentration 1 kg /20L water, Abamectin was applied by 3ml/20L water and tensotec was added by 40ml/20L water as emulsifier, the previous concentration and low concentration, 0.5kg of salicylic acid /20L water (half of the used concentration) was also used in the second spray in each season. The obtained results clear that, the mean number of mines, larvae, pupae and eggs of citrus leaf miner in orange, lemon and mandarin trees scored very highly decrement after treatment with the two tested concentrations of salicylic acid and the standard compared with pre-treatment and the control. The highest percent reduction of mines 85.7 % was recorded in mandarin at the highest concentration of S. acid, followed by 85.6% with the low concentration compared to 88.6 % with Abamectin while lemon recorded the highest percent reduction of larvae 91.98% with the low concentration of S. acid compared to 81.7% with Abamectin. Mandarin scored the highest reduction percentage of pupae 81.08% with the low concentration of S. acid comparing to 82.2 % for Abamectin while lemon recorded the highest reduction percentage of eggs 78.5% with the same concentration. No significance was found in the percent reduction for mines and eggs between high and low concentration of S. acid and the standard among the three citrus species. Also there were no found significant differences in percent reduction of pupae obtained in case of lemon and mandarin either with the two concentrations of S. acid or Abamectin. The spray 2 was more efficient than the spray 1 in reducing the mean number and increasing the percent reduction of the tested CLM aspects in the three tested citrus species. In case of orange and lemon, mean number of parasites associated with this pest after treatment with the two concentrations of S. acid was higher than that obtained after treatment with Abamectin. In this study salicylic acid may, enhance the citrus trees to resist the infestation with the citrus leaf miner, may cause death of CLM larvae or may repelled the adult leaf miner. In the same time S. acid did not negatively affect on mean number of its parasites and can be incorporated for controlling of this dangerous pest.

Keywords: Salicylic acid, citrus leaf miner, percent reduction

Introduction

The citrus leaf miner (CLM) is very serious and destructive herbivorous pest of citrus and many other species related to the same family (Abbas *et al.*, 2013) [1]. It is a lepidopteran insect, causes great lose in the production annually. The pest laid their individual eggs on the lower surface of young leaves (Knapp *et al.*, 1995) [7]. After hatching of eggs, larvae emerge and enter the leaves, causing great damage to them and new flushes during feeding, this makes mines and wounds. The damaged spaces turns into colortic patch and this results in bacterial infection which lead to hazards in citrus trees such as, sunscald, leaf drop and premature fruit drop. These adversely affect on the growth and delay maturity in trees leading to reduction in the yield (Graham *et al.*, 2004) [4]. To increase qualitative and quantitative

yield, it is essential to control this serious pest and overcome its damage. Because of toxicological problems and environmental and health hazards of chemical control in addition to high costs (Vianna *et al.*, 2009) [13], there is a need for developing alternative methods. Induce or strengthening the plant defense is one of these methods. Salicylic acid, is a vital component of the plant responsible for pathogen and disease resistance (Maleck and Dietrich, 1999) [8]. Key roles are played by salicylic acid and jasmonic acid in regulating plant defenses against the herbivore (Peng *et al.*, 2004) [9]. Therefore this study was done to clarify under field condition efficiency of the foliar applications of salicylic acid on the infestation of (CLM) in three types of citrus trees: lemon (adalia) summer orange and mandarin (morkite).



Citrus Leaf Miner (CLM) *P. citrella*

Materials and methods

This present study was carried out on different species of citrus trees(5 years old), summer orange, lemon (adalia) and mandarin (morkite) in Giza Governorate, Egypt, in two successive seasons , 2021and 2022, to clarify effectiveness of the foliar application of salicylic acid along with the bio-insecticide Abamectin for reducing the infestation by leaf miner in citrus. Salicylic acid was applied by a concentration 1kg /20L water, Abamectin was applied by 3ml/20L water and tensotec was added by40ml/20L water as emulsifier, the spray solution was applied to the different citrus trees by using motor sprayer (20 Liter in capacity) in early morning and completely cover spray form up to down for the treated trees was done, untreated row of the trees was left between every two treatments. Two sprays (spray 1 and spray2) of each compound were carried out for each season. In this experiment six trees (6 replicates) of each tested citrus species were used for each treatment along with six non treated control (sprayed with water only). The second spray was carried out after 14 day from the first one, in this spray the six replicates of each citrus species used with salicylic acid were divided into two groups (3 trees for each group), the first group was treated with the same concentration of salicylic acid applied in the spray 1and the second group was treated with lower concentration, of salicylic acid 0.5kg /20Lwater (half of the used concentration) and Tensotec was added also as emulsifier by the same used concentration. A randomize specimens were taken from each tested citrus tree; three branches (15leaves) were taken from each replicate and the control after7 days from each spray. The specimens were put in plastic bag and examined in the laboratory under the binocular and some live CLM aspects: number of mines, larvae, pupae, eggs and parasites per branch were recorded.

Statically analysis

Percent reduction were estimated according to Henderson and Tilton (1955) ^[5] %Reduction= $[1-(Ta \times Cb / Tb \times Ca)] \times 100$
 Ta= Number of live (mines, larvae, pupae or eggs) in the treatment after spraying. Cb= Number of live (mines, larvae, pupae or eggs) in the control before spraying. Tb= Number of live (mines, larvae, pupae or eggs) in the treatment before

spraying. Ca= Number of live (mines, larvae, pupae or eggs) in the control after spraying. Data was analyzed as factorial, using Proc GLM in SAS (Anonymous. 2003) ^[3]. Means were compared by Duncan multiple range test (P= 0.05 level) in the same program.

Results and discussion

Results of the foliar application of salicylic acid and the bioinsecticide, Abamectin on the three tested citrus types of trees were illustrated in Tables (1-4). Results obtained and tabulated in Table (1) show that the mean number of mines in orange, lemon and mandarin trees is very highly decreased after treatment with the two concentrations of salicylic acid and Abamectin compared with pre-treatment and untreated control. After treatment with S. acid the highest percent reduction in number of mines 85.7 % was recorded in mandarin at the highest concentration followed by 85 .6% with the low concentration compared to 88.6 % with Abamectin. In case of orange, the highest percent reduction 82.43% was scored at the highest concentration and 82.15% was obtained with the low one compared to 88.15% with Abamectin. Lemon recorded 82.73% percent reduction of mines with the low concentration and 78.5%with the highest one compared to 80.25 % with Abamectin. The data in the table also show that no found significant differences in the reduction percentage of mines between high and low concentration of S. acid and Abamectin among the three citrus species.

Data obtained and tabulated in Table (1) show also that the mean number of larvae of citrus leaf miner in the all tested types of citrus trees highly reduced after treatment with the two tested concentrations of S. acid and Abamectin . Lemon recorded the highest percent reduction 91.98% with the low concentration 0.5kg/L and 80.53% with high concentration compared to 81.7 % with Abamectin, there is no significant difference between the high concentration and Abamectin while there is a significant difference between them and the low concentration. No significant differences found in the percent reduction of larvae obtained in both of orange and mandarin after treatment with the two tested concentrations of S. acid and Abamectin.

Table 1: influence of the foliar treatment of orange, lemon and mandarin with salicylic acid and Abamectin on mean number and percent reduction of mines (per twig) and larvae (per twig) of (CLM) during season 2021 and 2022

The treatments	Mean number and % reduction of mines					
	Orange		Lemon		Mandarin	
	Mean	% reduction	Mean	% reduction	Mean	% reduction
Pre-treatment	23.8	--	18.8	---	18	---
Control	26.25	--	23.2	---	23.2	---
S. acid (1kg/20L)	4	82.15 a	3.8	78.50 a	2.4	85.7 a
S. acid (0.51kg/20L)	3.8	82.43 a	3.0	82.73 a	1.8	85.6 a
Abamectin	2.75	88.15 a	3.7	80.25 a	2	88.6 a
F	--	5.33	--	0.73	--	0.57
P	--	0.012	--	0.49	--	0.57
Mean number and % reduction of larvae						
Pre-treatment	11	--	12.2	--	8	--
Control	13.75	--	12.7	--	10	--
S. acid (1kg/20L)	2.33	84.36 a	2.2	80.53 b	1.25	82.73 a
S. acid (0.51kg/20L)	1.8	85.90 a	1.0	91.98 a	1.25	86.20 a
Abamectin	1.75	89.14 a	2.25	81.7 b	1.2	83.00 a
F	--	2.19	--	2.84	--	0.10
P	--	0.13	--	0.77	--	0.90

Effects of the foliar application of *S. acid* on the mean number and percent reduction of pupae of citrus leaf miner are shown in Table (2), the data clear that the mean number of pupae highly decreased in the three citrus types compared to pre-treatment and control, mandarin recorded the lowest mean number 0.5 and the highest reduction percentage of pupae 81.08 % at the low concentration of *S. acid* comparing to 0.7 and 82.2 %, respectively with Abamectin. While Abamectin scored 90.48% percent reduction with orange, *S. acid* recorded 80.12% at the high concentration. There is no found significance in percent reduction of pupae obtained in lemon and mandarin either with the two concentrations of *S. acid* or Abamectin.

Results obtained and tabulated in Table (2) show also that a highly reduction in mean number of eggs per twig after treatment with the two concentrations of *S. acid* and the bio-insecticide. Regarding to the percent reduction, lemon recorded the highest percent reduction 78.5 % at the low concentration of *S. acid* compared to Abamectin 74.72 %. Lemon and mandarin recorded equal percents 74.45 % at the high concentration of *S. acid* while orange recorded 68.69 %. There was no significant differences found in percent reduction of eggs obtained at the two different concentrations of *S. acid* and Abamectin among the three citrus species.

Table 2: influence of the foliar treatment of orange, lemon and mandarin with salicylic acid and Abamectin on mean number and percent reduction of pupae (per twig) and eggs (per twig) of (CLM) during two successive seasons 2021 and 2022

The treatments	Mean number and % reduction of Pupae					
	Orange		Lemon		Mandarin	
	Mean	% reduction	Mean	% reduction	Mean	% reduction
Pre-treatment	9	--	8.3	--	8.3	--
Control	10.25	--	9.25	--	9.0	--
<i>S. acid</i> (1kg/20L)	1.5	80.12 ab	1.5	79.5 a	1.1	79.5 a
<i>S. acid</i> (0.51kg/20L)	1.83	76.78 b	1.8	74.88 a	0.5	81.08 a
Abamectin	0.83	90.48 a	1.3	82.2 a	0.7	82.2 a
F	--	2.90	--	0.53	--	0.09
P	--	0.074	--	0.59	--	0.91
	Mean number and % reduction of Eggs					
Pre-treatment	44.3	--	42.2	--	28.8	--
Control	43	--	40.0	--	28.5	--
<i>S. acid</i> (1kg/20L)	13.8	68.69 a	10.75	74.45 a	9.5	74.45 a
<i>S. acid</i> (0.51kg/20L)	12.5	71.5 a	9.0	78.5 a	7.2	69.30 a
Abamectin	13.9	68.6 0 a	10.6	74.72 a	11	74.72 a
F	--	0.37	--	0.98	--	1.93
P	--	0.69	--	0.39	--	0.17

For the influence of the two sprays on mean number and percent reduction of the tested CLM aspects. Data obtained and tabulated in Table (3) show that the spray 2 was more efficient than spray1 and a high significant difference found in mean number and percent reduction for mines and larvae between the two sprays among the three tested citrus

species. While there was no found significant difference between the two sprays on the percent reduction of pupae, a very highly significant difference was found between spray1 and spray2 in the percent reduction for eggs in orange, lemon and mandarin.

Table 3: effect of the spray (1) and the spray (2) of the foliar application of orange, lemon and mandarin with salicylic acid and Abamectin on mean number and percent reduction (per twig) for some aspects of (CLM)

CLM aspects	Spray	Mean number and percent reduction					
		Orange		Lemon		M Mandarin	
		Mean	% reduction	Mean	% reduction	Mean	% reduction
Mines	Spray1	12.2	80.34 b	10.9	72.39 b	9.2	81.98 b
	Spray 2	8.1	87.45 a	7.2	85.1 a	6.4	90.08 a
	F	--	13.38	-	23.54	--	11.4
	P	--	0.001	-	< .0001	--	0.002
Larvae	Spray1	7.1	81.16 b	6.0	73.47 b	4.3	75.23 b
	Spray 2	3.4	90.19 a	3.4	89.83 a	2.6	89.07 a
	F	--	10.84	--	13.85	--	5.53
	P	--	0.003	--	0.0001	--	0.027
Pupae	Spray1	4.6	82.61 a	4.2	76.32 a	3.8	76.32 a
	Spray 2	3.8	84.25 a	3.2	81.87 a	2.7	83.93 a
	F	--	1.01	--	2.44	--	2.06
	P	--	0.32	--	0.13	--	0.16
Eggs	Spray1	26.16	56.22 b	22.4	64.01 b	18.2	64.01b
	Spray 2	15.4	77.89 a	12.9	82.95 a	10.1	79.88 a
	F	--	70.52	--	71.21	--	76.70
	P	--	< .0001	--	< .0001	--	< .0001

For the effect of the two seasons (1 and 2) on mean number and percent reduction of the studied CLM aspects. Results obtained and tabulated in Table (4) show that the mean number of the different aspects tested was higher in first season than the second one and the percent reduction was higher in the first season than the second. Orange and mandarin recorded nearly equal percent reduction of mines 89.05 and 89.5 %, respectively in the first season which is higher than that recorded in lemon 85.5 %. While a highly significant difference was found in percent reduction for mines between the two seasons in orange and lemon, there was no found significance between the two seasons in mandarin. The results of the table also show a highly significant difference in the percent reduction of larvae between the two seasons in orange while no significant

difference found in lemon and mandarin. Orange recorded the highest percent reduction of larvae 92.14 % in the first season while lemon and mandarin recorded nearly equal percent reduction 85.35 and 85.16 %, respectively. Orange also recorded the highest percent reduction of pupae 92.31 % in the first season and there was a high significant difference in the percent reduction between the two seasons. There was a significant difference in the percent reduction of pupae in the two seasons in lemon and mandarin. Lemon scored the highest percent reduction of eggs 77.66 % in the first season followed by mandarin 75.24 % in the same season. There was no significant difference found between the two seasons in the percent reduction of eggs for the three tested citrus species.

Table 4: Effect of the two seasons 2021 (season 1) and 2022 (season 2) of the foliar application of orange, lemon and mandarin with salicylic acid and Abamectin on mean number and percent reduction of different aspects of citrus leaf miner

CLM aspects	Season	Mean number and percent reduction/ twig					
		Orange		Lemon		Mandarin	
		Mean	% reduction	Mean	% reduction	Mean	% reduction
Mines	Season1	12.3	89.05 a	10.6	85.25 a	9.45	89.5 a
	Season2	9.8	80.16 b	8.6	74.84 b	7.42	84.18 a
	F	--	14.29	--	16.38	--	3.79
	P	--	0.0009	--	0.0004	--	0.06
Larvae	Season1	5.7	92.14 a	5.8	85.35 a	4.1	85.16 a
	Season2	5.2	81.03 b	4.4	81.23 a	3.4	81.90 a
	F	--	13.36	--	1.26	--	0.31
	P	--	0.0012	--	0.27	--	0.58
Pupae	Season1	4.8	92.31 a	4.4	86.32 a	4.1	87.37 a
	Season 2	4.3	74.88 b	3.6	72.97 b	3.0	74.40 b
	F	--	13.30	--	6.66	--	5.29
	P	--	0.0012	--	0.016	--	0.03
Eggs	Season1	23.30	69.45 a	19.5	77.66 a	15.9	75.24 a
	Season2	20.57	68.99 a	18.5	73.08 a	13.6	71.82 a
	F	--	0.03	--	4.17	--	2.5
	P	--	0.86	--	0.05	--	0.13

The obtained results proved that S. acid suppressed the mean number and reduction percentage of different aspects of citrus leaf miner infesting the three different types of citrus trees, so S. acid may increase the tolerance of the leaves to resist the infestation of CLM. The obtained results are supported by (Peng *et al.*, 2004) [9] who proved that salicylic acid was involved in defense responses of tomato plant to the herbivore. Also, Ali *et al.*, (2010) [2] reported that, using S. acid under field conditions at 0.02% concentration was effective against *Bemisia tabaci*. The obtained results may also due to many reasons :- the first reason after spraying S. acid on citrus trees, it was exposed to the sun light and became powder, the surface of the citrus leaves as a result became slightly rough and this resulted in two effects firstly caused injuries in larvae cuticle which led to dryness and death of the larvae in the first larval stage, secondly the rough surface of citrus leaves may also have repellence effect as it became difficult for the adult insects to lay eggs because of preference of the smooth surface for laying eggs. The second reason :- After the exposure of S. acid to the sun, citrus leaves gained the silver colour and this may cause disturbance and repellence of the adult citrus leaf miner to lay eggs (repellence effect) and this consequently may lead to decrement of the infestation, this

agree with (Rodríguez-Álvarez *et al.*, 2015) [10] who reported that, when tomato plants treated with S. acid, volatile terpenes increased and this repelled *Bemisia tabaci*. Comparing the percent reduction of CLM aspects in the three different species of citrus trees regardless the type of treatment, the spray or the season, data from the statistical analysis are presented in Fig. (1) And clear that, mandarin recorded the highest percent reduction in mines 86.84% followed by orange 84.6 % and there is a highly significant difference between lemon and both of orange and mandarin, $p=0.0002$. Orange recorded the highest percent reduction in both of larvae 86.58% and pupae 83.59% and there is no found significant difference between the three citrus species $p=0.48$ and $p=0.51$ for larvae and pupae, respectively. No significant difference found between lemon and mandarin in the percent reduction of eggs while there is a high significant between them and orange, $p=0.0005$. The obtained results concluded that, there was a difference in the percent reduction between the three citrus types, these results may be attributed to the variations in the nature for each citrus tree species, this agree with Jacas *et al.* (1997) [6] who said that, the flushing patterns of the trees affected on the susceptibility in each citrus species.

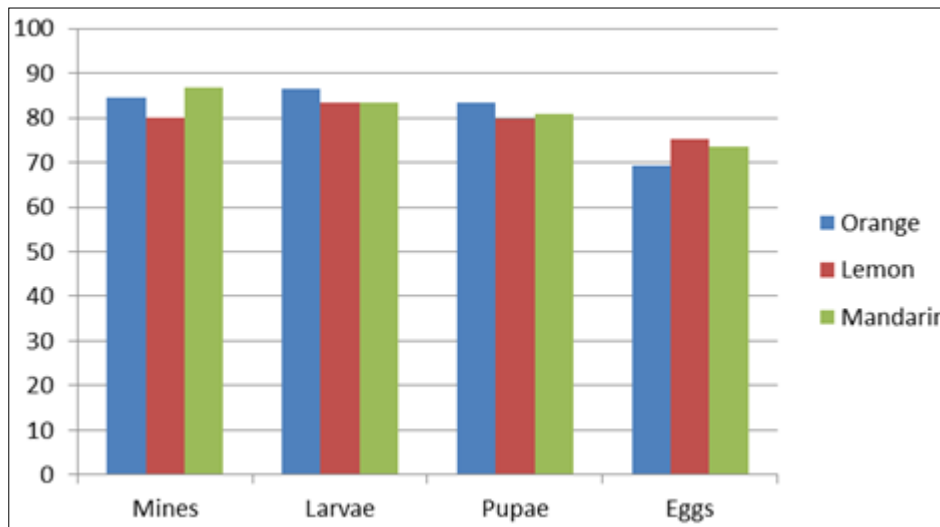


Fig 1: Percent reduction of some aspects of CLM in orange, lemon and mandarin after treatment with salicylic acid and Abamectin in two successive seasons 2021 and 2022

The present study concern also on the effect of the tested compounds on the number of parasites of CLM. The obtained results show that all parasites found associated with late larval instars of citrus leaf miner, this agree with Vercher *et al.*, 2005^[11] who reported that all species of *P. citrella* parasites developed mostly from third and fourth instars. Obtained results and tabulated in Table (5) and clear that in case of orange and lemon, mean number of parasites after treatment with the two concentrations of S. acid was higher than that obtained after treatment with Abamectin and there was a highly significant difference but a non

significant difference found in case of mandarin. The obtained data of this table also cleared that, the mean number of the parasites associated with CLM was higher in season 2022 than season 2021 in the three tested citrus types and there was a significant difference between them. Results in the table also showed that, no significant difference found between the influences of the spray 1 and spray 2 on the mean number of the parasites in the three tested citrus types. It can be concluded that, foliar spray with S. acid did not negatively affect on the mean number of the parasites associated with CLM in the three tested citrus types.

Table 5 : Influence of the foliar treatment of orange, lemon and mandarin with salicylic acid and Abamectin on mean number of parasites (per twig) accompanied with (CLM) during two successive seasons 2021 and 2022

The treatment	Mean number		
	Orange	Lemon	Mandarin
Pre-treatment	10.33 a	10 a	9.83 a
Control	9.50 a	10 a	10.25 a
S.acid(1kg/20L)	2.58 cb	3.67 b	2 b
S.acid(0.51kg/20L)	3 b	2.58 cb	0.83 b
Abamectin	0.92 c	1 c	0.92 b
F	54.37	48.59	80.23
P	< .0001	< .0001	< .0001
The season			
Season 2021	4.25 b	4.21 b	5.17 a
Season 2022	5.58 a	6.0 a	4.08 b
The spray			
Spray1	4.94 a	4.72 a	4.67 a
Spray2	3.54 a	4.17 a	3.29 a

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