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EFFECT OF SOME INSECTICIDES ON PARASITOID, *APHELINUS MALI* HALD (HYMENOPTERA: APHELINIDAE) OF THE WOOLLY APPLE APHID *ERIOSOMA LANIGERUM* HAUSMANN

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ABSTRACT

The toxicity of six insecticides (Deltamethrin, Chlorpyrifos, Acetamiprid, Diflubenzuron, Lufenuron and Fenoxycarb) was investigated on *Aphelinus mali* Hald. Infests the woolly apple aphid *Eriosoma lanigerum* Hausmann using three laboratory test methods. The first test was the initial toxicity, by exposing the adult parasitoids to fresh-dry insecticides film applied on glass plates. The second test was the exposure of adults to insecticide film applied on leaves of apple. The third test was carried out to evaluate the toxicity of insecticides on parasitoid within the aphid mummy (pupa stage). The results of the first test indicated that the Chlorpyrifos was highly toxic to the adult of *A. mali* causing 100% mortality. Diflubenzuron was harmless causing 27.16% mortality. The other four insecticides were classified as slightly to moderately harmful. The second test showed that the Chlorpyrifos also was highly toxic to the adult of *A. mali* causing 100% mortality; the rest (Deltamethrin, Acetamiprid, Diflubenzuron, Fenoxycarb and Lufenuron) showed less toxicity on *A. mali,* causing 94.81, 75.32, 18.18, 37.66 and 3.90% mortality, respectively. In the third test, the tested insecticides showed low toxicity to pupa of *A. mali* within host mummies. Diflubenzuron, Lufenuron and Fenoxycarb were harmless, and adults

emergence was 19.83, 23.96 and 29.75%, respectively. Deltamethrin, Chlorpyrifos and Acetamiprid were slightly harmful, and adults emerged at 46.67, 48.67 and 52.67%, respectively.

Keywords: Insecticides, *Aphelinus mali*, *Eriosoma lanigerum*, Woolly Apple Aphid, Parasitoid, Toxicity

INTRODUCTION

Beneficial arthropods in IPM program were become widespread and widely recognized **[1]**. *Aphelinus mali* (Haldeman) (Hymenoptera: Aphelinidae) is a specific endoparasite of the woolly apple aphid (WAA) *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae) that introduced as a biological control agent from its native home, North America, into apple growing regions throughout the world and is considered to be the most important parasitoid of WAA **[2]**. It was introduced into Syria from France by Abou Al-Naser in 1933, and was observed to be highly effective at the end of the season. The importance of natural enemies plays an important role concerning apple aphid populations in Syria **[3]**. **[4]** found that the life parasitoid stages (egg, larva and pupa) inside its host need 3, 10- 12, 6-7 days to develop, respectively. WAA continues to be an acute problem in orchards when insecticides used, where in South Africa was found that insecticides minimize the activity of *A. mali* and increased WAA over the economic threshold **[5]**. The routine use of broad-spectrum organo-phosphorous insecticide (OP) sprays are to control major pests, such as the codling moth; provide significant restraint of aphid and parasitoid populations, and minimize effectiveness of biological control **[6]**. Unfortunately, most insecticides are toxic to *A. mali* [**7]**; consequently chemicals should be chosen selectively.

The main objective of this study was to determine the effects of six insecticides on *A. mali* by using three laboratory methods.

MATERIALS AND METHODS

The experiments were carried out in 2008, Faculty of Agriculture, Damascus, Syria, using three laboratory tested methods.

1. Residual Film Method on Glass

This is a laboratory initial toxicity test at the highest recommended concentration field dosages **[8]** of a product to expose parasitoids adults to fresh-dry insecticides film applied on glass plates.

2. Residual Film Method on Leaves of Apple

Expose parasitoid adults to insecticide film applied on plant leaves under controlled laboratory conditions (25±2°C, 75±20% RH, and a photoperiod of 16:8 (L:D) hr**)**.

The two methods were used mainly to determine toxicity of insecticides on the adults of *A. mali*.

3. Expose of Pupal Stage of the Parasitoid

This method was used to evaluate the toxicity of insecticides on parasitoid within the aphid mummies (pupa stage).

The three testes were carried out in laboratory, Studies and Researches Center of Biological Control, and laboratory of Pesticides, Faculty of Agriculture, Damascus University, under laboratory conditions (25±2°C, 75±20% RH, and a photoperiod of 16:8 (L:D) hr**)**.

Insecticides and Dosages

Six insecticides listed below in **Table 1** were used, which are the most commonly insecticides applied in apple orchards in Syria (Directorate of Plant Protection, Personal communication). The application rates used in the experiments were based on the highest recommended field dosages (FD) **[8]**.

Table 1: List of Insecticides Used

Common name	Trade name	Formulation	% of a.i.	Group	Rate of application ml or g/100 L
Deltamethrin	**Decis**	**EC**	**50**	**Pyrethroides**	**25**
Chlorpyrifos	**Lentrek 4**	**EC**	**48**	**Organophosphate**	**150**
Acetamiprid	**Zenith**	**SP**	**20**	**Neonicotinoid**	**50**
Diflubenzuron	**Dimilin**	**WP**	**25**	**Benzoylurea**	**60**
Lufenuron	**Match**	**EC**	**50**	**Benoylurea**	**100**
Fenoxycarb	**Insegar**	**WP**	**250**	**Carbamate**	**40**

Adult Parasitoids

Adults of *A. mali* were collected from apple tree branches at the Center of Agricultural Scientific Research, Sweida'a - Governorate Southern Syria. WAA and its parasitoid are exist naturally in the orchards. The chosen branches of apple trees were cut, kept in to cooler box and transferred into the laboratory.

The branches put in plastic cans, covered with white-smooth cloth material, tightened at the top to next day. The newly emerged adults of *A. mali* were collected into small vials using an aspirator.

Initial Toxicity Test on Parasitoid Adults (Susceptible Life Stage)

The initial toxicity was tested by exposing the adult parasitoids to a fresh dry insecticide film applied on glass plates. Separate glass petri dishes (9 cm diameter) were sprayed with 2 ml of an aqueous suspension of each insecticide using a hand sprayer. Five replicates were sprayed for each tested insecticides. Controls were sprayed with distillated water. Two hours left to dry. Twenty adults of *A. mali* were transferred to each petri plate that held at 25±2°C, 75±20% RH, and a photoperiod of 16:8 (L:D) hr for 24 hours. Adults were classified as dead when they were no longer standing as were described by **[9]**. Results were corrected for mortality in water using Abbott's formula **[10]**. All data were subjected to analysis of variance (ANOVA) and means were compared by using LSD procedures. The SPSS statistical analysis program was used. Insecticides were classified using IOBC classification into four categories according to the corrected mortality percentage: 1, harmless(< 30%); 2, slightly harmful (30-79%); 3, moderately harmful (80- 99%); 4, harmful (> 99%) **[11]**.

Expose of Parasitoid Adults to Insecticide Film Applied on Plant Leaves

This test was carried out by exposing the adult parasitoids to insecticide film applied on apple leaves. A solution of each insecticide sprayed on an apple tree (4 years old). The control was sprayed with distillated water. Three hours after applying insecticides, 16 leaves were selected from the tree. Five replicates were used with each treatment. The base of the petri dish was covered by apple leaves (5x1 cm). Twenty newly emerged adults of *A. mali* were transferred to each petri dish. The dishes were then held as in initial toxicity section. After 24 hr the numbers of dead parasitoid were counted to determine rate of mortality with each tested insecticides. The mortalities were corrected and analyzed as in initial toxicity.

Parasitoid within the aphid (pupa stage): A few (3 to 4) branches of four apple trees (4 years old) were covered at the end of April by white-smooth cloth material, tightened at the base. The branches previously were naturally infested by *E. lanigerum*. In the mid of June the *E. lanigerum* colonies were infested by *A.*

mali adults that stayed 24 hr on colonies. *A. mali* at the end of this period were gently brushed off with a camel hair brush. The branches were covered again tightly. After 17 days, the mummies of WAA were appeared; infested branches were collected and removed to the laboratory. Mummies were collected using a camel hair brush into a petri dish. 30 mummies were concentrated in the middle of a filter paper placed into the lid of a petri dish (9 cm) and exposed to insecticide by pipetting 0.5 ml of the insecticide solution directly onto mummies and left to dry (3 hr). The control group was exposed to distillated water with five replicates. The effect of the insecticide was evaluated by counting the number of *A. mali* adults that emerged. Results were corrected and analyzed as in initial toxicity.

RESULTS AND DISCUSSION

Initial Toxicity Test on Parasitoid Adults (Susceptible Life Stage)

The results of the initial toxicity on glass surfaces were illustrated in the **(Table 2)** using the highest recommended concentration of the preparation, these results showed that the insecticides differed in their toxicity. The insecticide **C**hlorpyrifos (Lentrek 4®) was harmful to *A. mali* causing 100% mortality. This falls into the class 4 category according to the guidelines of IOBC (11), which is consistent with another study in New Zealand **[9]**. Deltamethrin (Decis®) and Acetamiprid (Zenith®) were classified as moderately harmful and they each caused 98.77% mortality. The insect growth regulators (Diflubenzuron and Fenoxycarb) and the insect growth inhibitor (Lufenuron) were less effective on the adult parasitoids. Diflubenzuron (Dimilin®) caused 27.16% mortality and was classified as harmless (class 1) according to IOBC guidelines. Lufenuron (Match®) and fenoxycarb (Insegar®) were classified as slightly harmful, with mortality of 30.86 and 37.04%, respectively. No significant differences was observed between the means of alive adults 11.20 and 9.20, categorized in class 2 according to IOBC. These chemicals (insect growth regulator and insect growth inhibitor) can be considered to be highly selective and thus safe to bees, and to other predatory and parasitic insects **[12]**.

Expose of Parasitoid Adults to Insecticide Film Applied on Plant Leaves

The preparations that were harmful and moderately harmful in the initial toxicity test (Deltamethrin and Chlorpyrifos) were classified as harmful and moderately harmful (class 3 and 4) respectively according to

IOBC guidelines in the test of insecticides applied on apple leaves, and without significant differences between the mean of alive adults 0.80 and 0.00, correspondingly, Chlorpyrifos and Deltamethrin are broad-spectrum insecticides **[13]**. These two insecticides, especially Chlorpyrifos, were highly toxic to adult *A. mali* which can limit the biological control of WAA **(Table 3)**. Most of the other insecticides were less toxic to the adult parasitoids when applied on plant leaves than in the initial toxicity test. Acetamiprid (Zenith®) falls into class 2 as slightly harmful (75.32%) mortality (class 3 in the initial toxicity test), and Lufenuron (Match®) is classified class 2 category according to IOBC with a percent of mortality 3.90%, (9) found that Lufenuron caused no or little toxicity.

Lufenuron (insecticide) belongs to insect growth inhibitor, which can be considered to be highly selective to predatory and parasitic insects **[12]**. The results of mean of alive adults indicating that most of the tested compounds showed less toxicity on *A. mali* when applied on plant leaves than on glass plates. The mean of Deltamethrin, Acetamiprid, Diflubenzuron and Lufenuron were 0.80, 3.80, 12.60, 14.80, respectively when compared with the results of initial toxicity of 0.20, 0.20, 11.80, 11.20, respectively.

Parasitoid within the Aphid (pupa stage)

The results showed that the insecticides were less toxicity on pupa of *A. mali* within host mummies **(Table 4)**. Diflubenzuron, Fenoxycarb and Lufenuron were harmless and adults emerged at 64.67, 61.33 and 56.67%, respectively. Deltamethrin, Chlorpyrifos and Acetamiprid were slightly harmful and resulted of adults emerged at 46.67, 48.67 and 52.67%, respectively. Our result is similar to the result of Vogt and Ternes **[14]**, where they found that two insecticides of neonicotinoid did not affect the protected stage of the parasitoid in woolly apple aphid mummy, which were directly sprayed. This means that mummy protects the pupa stage from insecticides effect, which indicates that pupa stage is less susceptible to the insecticides than adult stage.

Table 2: The effect of Insecticides in the Initial Toxicity Test on Adult Parasitoids *A. mali.*

Treatment	Number of adults tested	Number of alive adults (Mean ± SD)	Percent corrected mortality	Class*
Control	100	16.20 ± 1.924 a	-	-
Deltamethrin	100	0.20 ± 0.447 d	98.77	3
Chlorpyrifos	100	0.00 ± 0.000 d	100.00	4
Acetamiprid	100	0.20 ± 0.447 d	98.77	3
Diflubenzuron	100	11.80 ± 2.775 b	27.16	1
Lufenuron	100	11.20 ± 2.588 bc	30.86	2
Fenoxycarb	100	9.20 ± 2.683 c	37.04	2

Means followed by the same letter in the column were not significantly different at 0.05; *Evaluation categories, IOBC classification (Boller *et al*., 2005): 1- harmless (<30%) ; 2- slightly harmful (30- 79%)., 3- moderately harmful (80- 99%)., 4- harmful (>99%)

Table 3: Exposure of *A. mali* Adults to Insecticide Film Applied on Apple Leaves

Treatment	Number of Adults tested	Number of alive adults (Mean ± SD)	Percent corrected mortality	Class*
Control	100	15.40 ± 1.949 a	-	-
Deltamethrin	100	0.80 ± 0.837 e	94.81	3
Chlorpyrifos	100	0.00 ± 0.000 e	100	4
Acetamiprid	100	3.80 ± 2.490 d	75.32	2
Diflubenzuron	100	12.60 ± 3.209 b	18.18	1
Lufenuron	100	14.80 ± 1.304 a	3.90	1
Fenoxycarb	100	9.60 ± 2.302 c	37.66	2

Means followed by the same letter in the column were not significantly different at 0.05; *Evaluation categories, IOBC classification (Boller *et al*., 2005): 1- harmless (<30%) ., 2- slightly harmful (30- 79%)., 3- moderately harmful (80- 99%)., 4- harmful (>99%)

Table 4: The effect of Spraying Insecticides on the Pupa of *A. mali* Within Woolly Apple Aphid Mummies

Treatment	Number of mummies tested	Number of adults emerged (Mean ± SD)		% of adults emerged	Percent corrected mortality	% reduction of adults emerged	Class*
Control	150	24.20 ± 2.588	a	80.67	-	-	-
Deltamethrin	150	14.00 ± 5.701	c	46.67	42.15	34.00	2
Chlorpyrifos	150	14.60 ± 4.827	c	48.67	39.67	32.00	2
Acetamiprid	150	15.80 ± 2.280	bc	52.67	34.71	28.00	2
Diflubenzuron	150	19.40 ± 2.302	b	64.67	19.83	16.00	1
Lufenuron	150	18.00 ± 2.345	bc	61.33	23.96	19.33	1
Fenoxycarb	150	17.80 ± 2.280	bc	56.67	29.75	24.00	1

Means followed by the same letter in the column were not significantly different at 0.05.
***Evaluation categories, IOBC classification (Boller *et al.*, 2005): 1- harmless (<30%) ., 2- slightly harmful (30-79%)., 3- moderately harmful (80- 99%)., 4- harmful (>99%).**

CONCLUSION

The results of the three methods in the present investigation for the effect of insecticides on *A. mali* indicates the recognition of a harmless insecticide (Diflubenzuron, Fenoxycarb and Lufenuron) that could be used in IPM and other biological control program to control apple pests. The insecticides with high toxicity should not entirely be excluded and could be used in the spring session when *A. mali* does'nt exists. Other investigation concerning the impact of these insecticides on *A. mali* in field should be carried out.

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