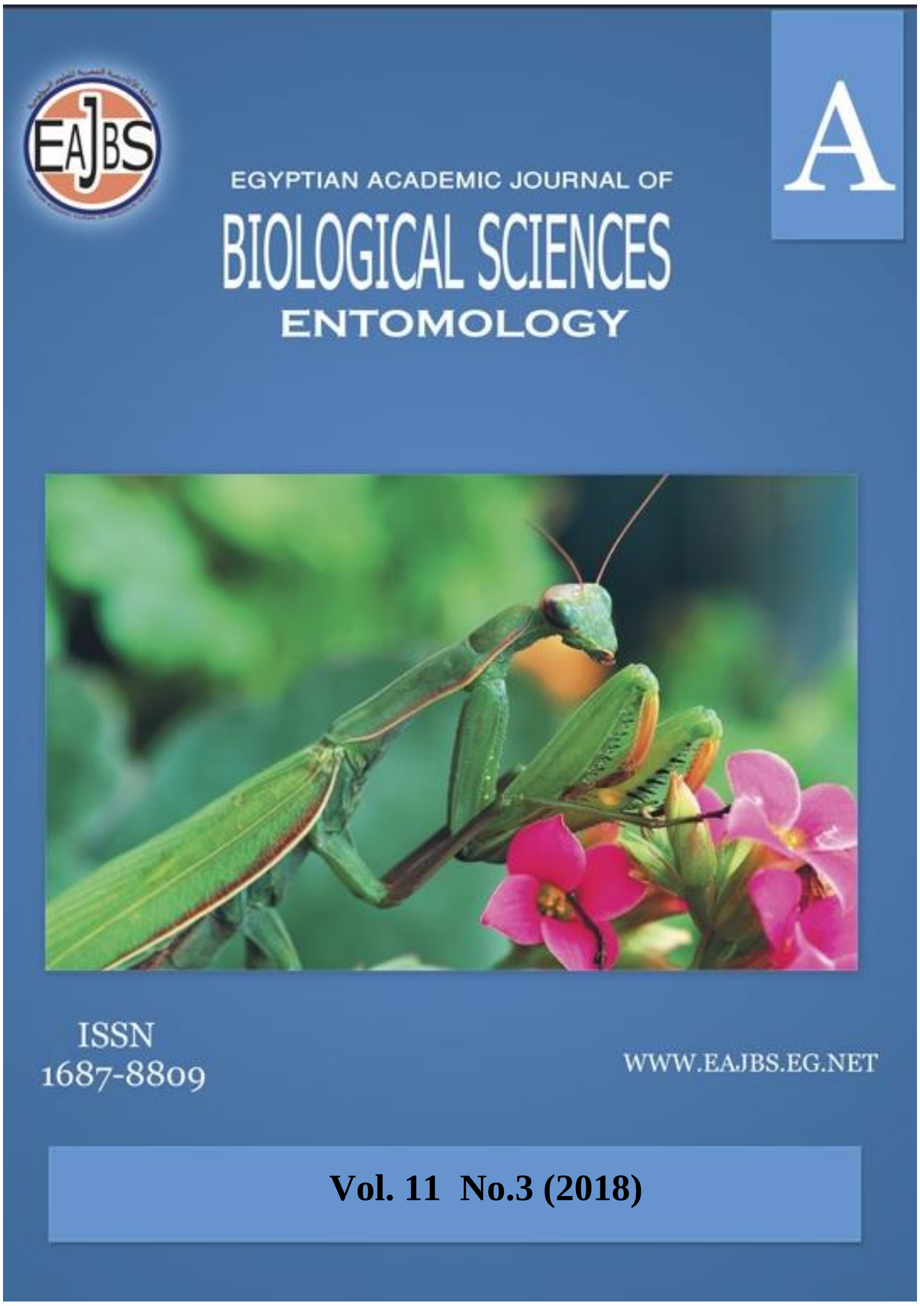

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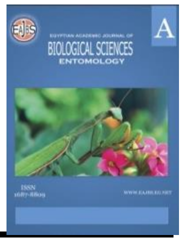

Laboratory Evaluation of the Effect of Insecticides on Non-target Organisms: 2- The egg parasitoid, *Trichogramma evanescens* West. (Hymenoptera: Trichogrammatidae)

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ABSTRACT

The present study aimed to evaluate the effect of different groups of insecticides (*e.g.*, Dipel, Dursban, Biover, Malathion and Spintor) on the immature stages and the parasitism rate of the egg parasitoid, *Trichogramma evanescens* West. (Hymenoptera: Trichogrammatidae) under laboratory conditions. The parasitized eggs of the factitious host, the Mediterranean flour moth, *Anagasta (Ephestia) kuehniella* Zeller were used. Data were recorded in terms of parasitism rate, emergence rate and female percentages. The calculated values of the Slope (b) of the log-dosage-probit mortality curve, by treating the parasitized *A. kuehniella* eggs (ranged from 0.6 to 1.34), indicated that the susceptibility of the population was heterogeneous for all the studied insecticides. Results indicated that each of the tested insecticides showed the different degree of toxicity. Malathion was the most toxic insecticide (Toxicity ratio T.R.: 10-39.7), while the bioinsecticide, Dipel was the least toxic one (Toxicity ratio T.R.:0.24-0.47). The parasitoid developmental stage had a significant effect on the toxicity ratio of all the studied insecticides. The eggs treated 7-day post-treatment showed the highest parasitism rate for all insecticides, (65-94%), followed by 5-day old (52-91%), 3-day old (48-90%), and then 1-day old (43-88%). An inverse relationship between the parasitism rate and the insecticide concentration was found. The emergence rate was affected greatly by both the type of insecticide and the treated immature stage. The highest emergence rate, (14-86%) was recorded for the eggs treated 1-day post-treatment, while parasitized eggs, treated after 7 days at all the tested insecticides recorded the lowest rates of adult emergence of *T. evanescens* (6-49%). The highest emergence rate (42-83%) was found for eggs treated with the fungicide Biover, while the lowest values (7-35%) were recorded for the eggs treated with Spintor compared to a significant high emergence rate for the untreated ones (92%). Sex ratio (female percentage) did not differ significantly between the treated (38-55%) and the untreated parasitized eggs (50-54%).

INTRODUCTION

Insecticides include; bactericides, baits, fungicides, herbicides, insecticides, lures, and repellents. These insecticides control pest organisms by physically, chemically or biologically interfering with their metabolism or normal behavior. Problems associated with reliance on chemical control include the development of insecticide resistance in economic pest species. This encourages an increase in dosage and number of insecticide applications, which magnifies the adverse effects on natural enemies (El-Heneidy *et al.*, 2015). Natural enemies are a key component of Integrated Pest Management (IPM) and they are often recommended as the first line of defense in an IPM program. Biological control agents such as predators and/or parasitoids are usually more sensitive to insecticides than the target pests. Therefore, to improve biological control practices in agricultural IPM system, preliminary selection of natural enemies for tolerance to one or more commonly used insecticide is very helpful. In spite of the important role of the biological control agents in agriculture, chemical control is still indispensable but, the use of non-selective insecticides greatly reduces the beneficial potential of the biological agents, particularly parasitic Hymenoptera that are more susceptible to insecticides than their hosts.

Trichogramma spp. (Hymenoptera Trichogrammatidae) parasitize the eggs of over 400 species belonging to at least seven insect orders (Bao and Chen, 1989). Several species of *Trichogramma* are mass-reared and released annually around the world on an estimated 80 million acres of agricultural crops and forests in 30 countries (Olkowski and Zahang, 1990 and Li, 1994). The harmful effects of insecticides directly and/or indirectly on different *Trichogramma* spp. have been studied by many researchers under laboratory and field conditions (Hewa-Kapuge *et al*., 2003; Desneux *et al*., 2007 and Vianna *et al.*, 2009).

The present study aimed to evaluate the acute and sub lethal residual toxicity of a group of differently recommended insecticides, widely used in Egyptian fields, to the immature stages of *T. evanescens* under laboratory conditions. The results may contribute to the selectivity of such insecticides to conserve the role of parasitoids in Egyptian fields.

MATERIALS AND METHODS

Some of the most commonly recommended insecticides, used in the Egyptian fields, were investigated against the developmental stages of *Trichogramma evanescens* West., using the parasitized eggs of the factitious host, *Anagasta kuehniella* (Zeller) (1, 3, 5, and 7-day) post parasitism. These days corresponded to the developmental stages of *Trichogramma*: 1-day: eggs, 3-days: larvae, 5-days: early pupae, and 7-days: pupae. Experiments were carried out under the laboratory conditions of 27±2°C and 70±5% RH. Insecticides' solutions were prepared in distilled water by serial dilutions, while control was treated with a distilled water.

Selected insecticides:

Five insecticides, represent different chemical groups were tested. Recommended field rates of application were tested.

*Dursban: (OP) H 48 % EC, with a rate of application of 250 cm/100 L of water,

*Malathion: (OP) 57% EC, with a rate of application of 500 cm/100 L of water,

*Spintor: (Entomopathogenic agent derived from an actinomycete bacterium species, *Saccharopoly sporaspinosa* (Mertz and Yao, 1990), with a rate of application of 12.5 cm/100 L of water,

*Dipel: Bioside group (*Bacillus thuringiensis* bacteria), with a rate of application of 125 gm/100 L of water, and
*Biover: Biocide group (fungus), with a rate of application of 250 gm/100 L of water. This rate is equivalent to $80x10^3$ conidia/ml., *i.e.*1 ppm equivalent to 32 conidia/ml.

Eggs of the factitious host, *Anagasta kuehniella* (Zeller):

Processed egg cards of *A. kuehniella* were obtained from the *Chrysoperla* Mass-production Unit at the Faculty of Agriculture, Cairo University, Giza, Egypt. Egg cards were used as follows:-
*Pre-parasitism: egg card (125 eggs)/ concentration (x 4 concentrations)/ insecticide (x 5 insecticides)/ replicate (x 3 replicates) (= 60 treatments) + one egg card (x 3 replicates) for control equal 3 treatments.
*Parasitized egg cards treated (1, 3, 5, and 7-day post parasitism).
A total of 60 treatments for each immature stage x 4 immature stages (= 240 treatments) + (3 treatments for control/ each immature stage) x 4 immature stages = 12 treatments.

Bioassay:

The technique, defined by the (IOBC/WPRS (International Organization of Biological Control - Working Group) for insecticides and beneficial organisms (Hassan, 1994) was used.

Treatment of *T. evanescens*:

a)**Treatment of *A. kuehniella* eggs pre-parasitism**:

A. kuehniella egg cards were dipped into each insecticidal solution for 10 seconds. Treated egg cards were kept on filter papers at room temperature to dry, then processed to *T. evanescens* for parasitism, transferred to clean vials that plugged with muslin, and kept in an incubator at (27±2°C and 70±5% RH). Treated egg cards were checked daily until emergence.

b)**Treatment of immature stages of *T. evanescens*:**

A. kuehniella egg cards were processed to *T. evanescens* for parasitism and then treated either 1, 3, 5, or 7 days post parasitism (Knutson, 1998). After treatment, the cards were kept on filter papers at room temperature until the excess of liquid dried, then transferred to clean vials, plugged with muslin, and kept in an incubator at (27 ± 2°C; 70 ± 5% RH).

According to the average reduction in the survival rate (R), the tested insecticides were grouped into toxicological categories, according to the classification of (IOBC) recommendations: class 1 = harmless (< 30%), class 2 = slightly harmful (30 - 79%), class 3 = moderately harmful (80 - ≤99%) and class 4 = harmful (> 99%) (Van de Veire *et al.*,2002 and Bueno *et al.,* 2008). The average reductions in the beneficial capacity of parasitoids (parasitism and emergence rates) were calculated, using the following equation:

$$\% \text{ Average reduction} = 100 - \left(\frac{\% \text{ general mean of treatment with pesticide}}{\% \text{ general mean of the control treatment}} x100 \right)$$

The average obtained in the control treatment was used as a reference to calculate the reduction in the beneficial capacity of parasitoids.

Experiments:

a) To estimate the lethal concentrations of the studied insecticides against the immature stages of *T. evanescens,* 4 concentrations for each insecticide were prepared and the following biological parameters were estimated: the percentage of parasitization; the percentage of emergence and the sex ratio.
b) The homogeneity or heterogeneity of the selected insecticides was determined according to the value of the slope (b) of the log-dosage-probit mortality curve (LDP).

If the slope (b) is > than 2, then the susceptibility of the population to the insecticide is homogeneous and if it is < than 1, it is heterogeneous.

c) To compare the toxicity of the tested insecticides, the Toxicity Ratio (TR) was calculated based on the LC_{50} values as follows

$$TR = \frac{\text{Recommended field dose of the insecticide}}{\text{LC50 of the tested insecticide}}$$

Statistical analysis:

The treatment was considered valid, when it was more than 10% mortality in the control (Hassan, 1989). Percent mortality was corrected through Abbot's formula (Abbott, 1925) when necessary. The lethal concentrations were deduced by extrapolation from the regression line obtained by probit analysis (Finney, 1971). Significance of mortality percentage was determined by F-value.

RESULTS AND DISCUSSION

The majority of the researchers have reported that the immature stages of the *T. evanescens* are tolerant to insecticides. This high level of tolerance could be made possibe by the chorion of the host eggs. They measured how insecticides are harmful to the egg, larval and pupal stages on the basis of adult emergence (Consoli *et al*., 2001; Hewa-Kapuge *et al.*, 2003; Carvalho *et al*., 2005; Rezende *et al*., 2005; Cristina *et al*., 2006 and Somchoudhury *et al*., 2007).

Lethal Effect of Insecticides on *T. evanescens*:

Pre-Parasitism Host Eggs:

The treated *A. kuehniella* parasitized eggs showed that Malathion was the most toxic insecticide (TR= 208.3), while Dipel was the least toxic one (TR = 0.42) (Table 1). Also. data indicated that the susceptibility of the host eggs to Dipel, Malathion and Spintor was heterogeneous because their Slope (b) values of the log-dosage-probit mortality curve were less than 1, while that for Dursban and Biovair, they were nearly heterogeneous because the (b) values were very close to 1 (1.33 and 1.27).

Treatment of Different Immature Stages of *T. evanescens*:

Results showed varying degrees of toxicity for insecticides tested against different immature stages of *T. evanescens* inside host eggs. The parasitized *A. kuehniella* eggs treated at different developmental immature stages of the parasitoid indicated that the calculated values of the Slope (b) was less than 1 for all the studied insecticides, so the susceptibility of the host was heterogeneous (Table 2). Data also indicated that the immature stage had no effect on the susceptibility of the host to all the studied insecticides. The survival rates of *T. evanescens* development stages on *A. kuehniella* eggs were significantly affected by chemical treatments. Malathion was the most toxic insecticide (TR ranged 10-39.7), according to the developmental stage, while Dipel was harmless to all the developmental stages of the tested *Trichogramma* species (TR ranged (0.24 - 0.47) (Table 3). These results are in agreement with those reported by Shoeb (2005) who found that Protecto (*Bt*) had the least effect. The results suggested that the susceptibility of the developmental stages of *Trichogramma* towards insecticides could differ from one immature stage to another and according to the insecticide type used. Williams *et al.* (2003) recorded Spinosad as a moderately harmful, while *Bt* was proven to be slightly harmful (Burnner *et al*., 2001).

Among all the tested insecticides, the post-treated 7-day eggs showed the highest parasitism rate (65-91%), followed by the 5, 3-day eggs, and lastly the 1-day old (50-88%) that had a little effect on the mortality rate of *Trichogramma*. There was also an inverse relationship between the parasitism rate and the insecticide concentration. The percentage values of the highest mortality rates of the developmental stages ranged

(30-50), (29-52), (27-48), (25-40%) for eggs treated 1, 3, 5 and 7 days post parasitism, respectively, while the eggs treated with Dipel recorded the lowest mortality rate, compared to the significantly low mortality rate (8%) of the untreated eggs (Table 4). Despite, the statistically significant differences among the treatments, only Spintor was categorized as slightly harmful (class 2). The rest of the insecticides to though reduced adult emergence to some extent as compared to the control but were still safe enough to be ranked as harmless (<30% mortality).

For the emergence rate, obtained data revealed that it was affected greatly by both the type of insecticide and the treated immature stage (Table 5). The highest emergence rate (2-83%) was recorded for the eggs treated 1-day post-treatment. Data also showed that the recorded values for the highest emerged adults ranged (69-83), (50-54), (54-66), (42-49%) for the eggs treated with Biover 1, 3, 5 and 7 days post parasitism, respectively. These findings agree with those reported by Costa *et al.* (2014) who reported that all insecticides significantly reduced the emergence of *Trichogramma* wasps, with the lowest emergence observed when they were applied at the pupal stage. The present data reported that the lowest values of emergence were recorded for eggs treated with Spintor (2–35%), compared to a significant (high emergence rate (92%) for the untreated one, which was in agreement with that reported by Shoeb (2010) who found that Spintor gave a very low emergence rate (3-5%). In addition, parasitized eggs treated after 7 days with all tested insecticides recorded significant low rates of adult emergence of *T. evanescens*, compared to those treated 1, 3 and 5 days after parasitism. This was in agreement with that reported by El Sebai and El-Tawil, (2012) who reported that parasitized eggs treated after 8 days (about one day before emergence) with all tested herbicides recorded a significantly low rate of adult emergence of *T. evanescens* compared to those treated 3 and 5 days after parasitism, except for Dipel. Adult emergence rate did not differ significantly at all stages (3, 5 and 7 days), but it was low than the control. Spintor ranked first in reducing adult emergence, while Biover ranked last. Despite, the statistically significant differences among the treatments, only Spintor was categorized as slightly harmful, according to the IOBC ranking. Although, the rest of the insecticides reduced adult emergence rates to some extent as compared to the control but they were still relatively safe to be ranked as harmless.

Referring to the data in the table (6), female percentage (sex ratio) was slightly affected by the different insecticides at all concentrations. These resulted were in agreement with that reported by (Moura *et al.*, 2005 and Braga *et al.*, 2013) who reported that the products did not affect the sex ratio of parasitoids when treated at the egg-larval period and at the pre-pupal and pupal phases. The sex ratio of the parasitoids treated in the egg-larval period (F1 generation) was not affected by any of the evaluated compounds. Moura *et al.* (2005) stated that sprayed imidacloprid and chlorfenapyr/ß-cyfluthrin on *T. pretiosum*had had no effect on the biological characteristics. Female percent ranged (41 to 55%), which was very close to the control (50–55%). So, it could be concluded that the sex ratio in all the tested insecticides did not differ significantly between the treated and untreated experiments. Also, the immature stage had no effect on the female percentage at all the tested insecticides as the sex ratio did not differ from the control (Costa *et al.*, 2014). As well, Suh *et al.* (2000) mentioned that regardless to the developmental stage treated, none of the tested insecticides had a significant effect on the sex ratio or frequency of emerged females.

Several researchers have reported that the immature stages of *T. evanescens* are tolerant to insecticides, this high level of tolerance could be made possible by chorion of the host eggs. The present study revealed that Spintor proved to be slightly harmful to the eggs, larval, and pupal stages on the basis of adult emergence rates. Similar observations were made by Hewa-Kapuge *et al*. (2003) through testing 7 different insecticides. Like other insect species, *T. evanescens* immature stages have long been considered less responsive to insecticides than adults, because the egg chorion serves as a sort of protection to development stages. However, in the present study, the egg chorion protection was not enough in case of Spintor. The results concur with those obtained by Suh *et al*. (2000), Cônsoli *et al*. (2001) and Bueno *et al*. (2008) who found that immature *T. evanescens* stages, exposed to Spintor were characterized by a very high mortality rate.

Table (1): Slope (b) of the log-dosage-probit mortality curve, LC_{50} and Toxicity Ratio (TR) for *Anagasta* eggs treated and then parasitized

Insecticide	Slope B	LC_{50} (ppm)	RFD (ppm)	TR	Homogeneity/Heterogeneity
Biover	1.27	625	2500	4	Nearly Heterogeneous
Dipel	0.6	2400	1000	0.42	Heterogeneous
Dursban	1.33	45	2500	55.6	Nearly Heterogeneous
Malathion	0.62	24	5000	208.3	Heterogeneous
Spintor	1	26	125	4.81	Heterogeneous

Table (2): Slope (b) of the log-dosage-probit mortality curve for the parasitized *Trichogramm*a eggs treated at different immature stages (1-, 3-, 5-, and 7-days postparasitism)

Insecticide	Days post-treatment							
	1		3		5		7	
	Slope b	Hetro or Homo	Slope B	Hetro or Homo	Slope b	Hetro or Homo	Slope b	Hetro or Homo
Biover	1	N Het	1.07	N Het	1.34	N Het	1.1	N Het
Dipel	0.6	Hetro	0.58	Hetro	0.8	Hetro	0.72	Hetro
Dursban	0.859	Hetro	0.46	Hetro	1.0	Hetro	0.727	Hetro
Malathion	0.938	Hetro	0.791	Hetro	0.242	Hetro	0.470	Hetro
Spintor	0.712	Hetro	0.875	Hetro	1.474	30	0.882	Hetro

Table (3): LC_{50} and Toxicity Ratio (TR) for the parasitized *Trichogramm*a eggs treated at different immature stages (1, 3, 5, and 7-days post parasitism)

Insecticide	Days post-treatment							
	1		3		5		7	
	LC_{50}	TR	LC_{50}	TR	LC_{50}	TR	LC_{50}	TR
Biover	502	5.00	457	5.74	610	4.10	714	3.50
Dipel	3847	0.26	4220	0.24	2137	0.47	3980	0.25
Dursban	131	19.10	123	20.32	104	24	241	10.73
Malathion	126	39.7	160	31.25	420	11.9	501	10
Spintor	13	9.61	15	8.33	30	4.17	190	0.67

Table (4): Parasitism rate (%) with *Trichogramma* in parasitized eggs treated at different immature stages (1, 3, 5, and 7-days post parasitism)

Insecticide & concentrations (ppm)		Days post-treatment			
		1	3	5	7
Biover	125	70	71	73	80
	250	61	61	64	75
	375	43	52	57	70
	500	50	48	52	65
Dipel	50	88	86	78	91
	100	86	82	78	88
	200	80	78	75	83
	1000	74	76	70	75
Dursban	5	86	85	90	94
	10	85	84	85	91
	25	81	83	77	90
	40	60	70	62	78
Malathion	10	86	84	66	90
	40	64	65	60	85
	120	53	56	54	78
	500	75	90	90	91
Spintor	2.5	66	70	91	91
	5	53	61	73	91
	7.5	56	59	81	90
	10	67	72	80	90
Control		92	92	92	92

Table (5): Emergence rate (%) with *Trichogramma* in parasitized eggs treated at different immature stages (1, 3, 5, and 7-days post parasitism)

Insecticide & concentrations (ppm)		Days post-treatment			
		1	3	5	7
Biover	125	79	50	54	42
	250	69	54	66	49
	375	83	53	56	48
	500	79	51	57	46
Dipel	50	44	55	44	40
	100	41	64	55	40
	200	70	67	50	29
	1000	74	71	60	34
Dursban	5	86	95	35	18
	10	60	90	55	40
	25	24	90	16	11
	40	40	30	7	6
Malathion	10	51	41	46	42
	40	65	42	39	40
	120	42	33	44	33
	500	40	32	40	36
Spintor	2.5	24	32	35	31
	5	24	23	12	12
	7.5	18	22	21	10
	10	14	7	16	16
Control		92	92	92	92

Table (6): Female rates (sex ratio) of *Trichogramma* emerged from parasitized eggs treated at different immature stages (1, 3, 5, and 7-days post parasitism)

Insecticide & concentrations (ppm)		Days post-treatment			
		1	3	5	7
Biover	125	48	52	44	44
	250	47	51	43	45
	375	48	53	44	45
	500	47	52	42	42
Dipel	50	47	45	46	46
	100	54	51	50	47
	200	44	42	47	48
	1000	49	41	48	47
Dursban	5	54	43	43	42
	10	53	47	47	42
	25	51	42	42	41
	40	53	44	44	44
Malathion	10	44	47	50	44
	40	45	48	49	45
	120	46	49	49	46
	50	43	45	38	47
Spintor	2.5	50	48	60	50
	5	55	44	64	55
	7.5	57	46	58	58
	10	50	52	52	50
Control		52	55	52	50

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RABIC SUMMERY

تقييم معملى لتأثير المبيدات الحشرية على الكائنات غيرالمستهدفة
٢- طفيل البيض *Trichogramma evanescens* West.

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تهدف الدراسة إلى تقييم تأثير ثلاث مجموعات متنوعة من المبيدات الحشرية هى (Dipel, Spintor, Dursban, Biover Malathion) على الأطوارغير الكاملة لطفيل اليض (*Trichogramma evanescens* West.) والذى تم تربيته على بيض العائل البديل (فراشة دقيق البحر الأبيض المتوسط) (*Anagasta (Ephestia) kuehniella* Zeller) وذلك تحت الظروف المعملية. تم تقدير نسبة التطفل ونسبة خروج الحشرات الكاملة والنسبة الجنسية (النسبة المئوية للأناث) للأطوار غير الكاملة للطفيل المعاملة بالمبيدات ومقارنتها بغير المعاملة. أوضحت نتائج قيم الأنحدار (Slope (b للمنحنيات التى درست العلاقة بين الجرعة ونسبة موت أطوار الطفيل المعاملة بالمبيدات، حيث تراوحت بين 0.6 و1.34، مما يعنى أن حساسية الطفيل كانت غير متجانسة لجميع المبيدات المختبرة. كما تم حساب السمية النسبية (Toxicity ratio T.R.) لكل مبيد من المبيدات المستخدمة، حيث أوضحت نتائج الدراسة اختلاف درجة سمية كل مبيد عن الآخر نحو أطوار الطفيل المختلفة. وكان Malathion هو الأكثر سمية (TR: 10-39.7) بينما كانت (0.24-0.47) فى حالة المعاملة بمبيد Dipel وهو يمثل الأقل سمية. أوضحت نتائج الدراسة أيضا تأثر نسبة التطفل تأثرا معنويا بعمرالطفيل المعامل حيث سجل الطفيل المعامل فى عمر ٧ أيام أعلى نسبة تطفل (65-94%)، يليه المعامل فى عمر ٥ أيام (52-91%) ثم المعامل فى عمر٣ أيام (48-90%)، بينما كان أقلها الطفيل المعامل فى عمر يوم واحد (43-88%). كما تأثرت نسب خروج الطفيل تأثرا معنويا بالعمر الذى تم معاملته فيه حيث سجل الطفيل المعامل فى عمر يوما واحدا أعلى نسب خروج (14-86%) فى حين سجل الطفيل المعامل فى عمر ٧ أيام أقل نسب خروج (6-49%). وتأثرت أيضا نسب الخروج بنوع المبيد المستخدم فى المعاملة حيث سجل الطفيل المعامل بالمبيد الفطرى Biover أعلى نسب خروج (42-83%) فى حين سجلت المعاملة بالمبيد Spintor أقل نسبة خروج (7-35%). أظهرت النتائج عدم تأثرالنسبة الجنسية (النسبة المئوية للاناث) معنويا حيث بلغت (38-55%) فى حالة المعاملة و(50-54%) فى حالة عدم المعاملة.