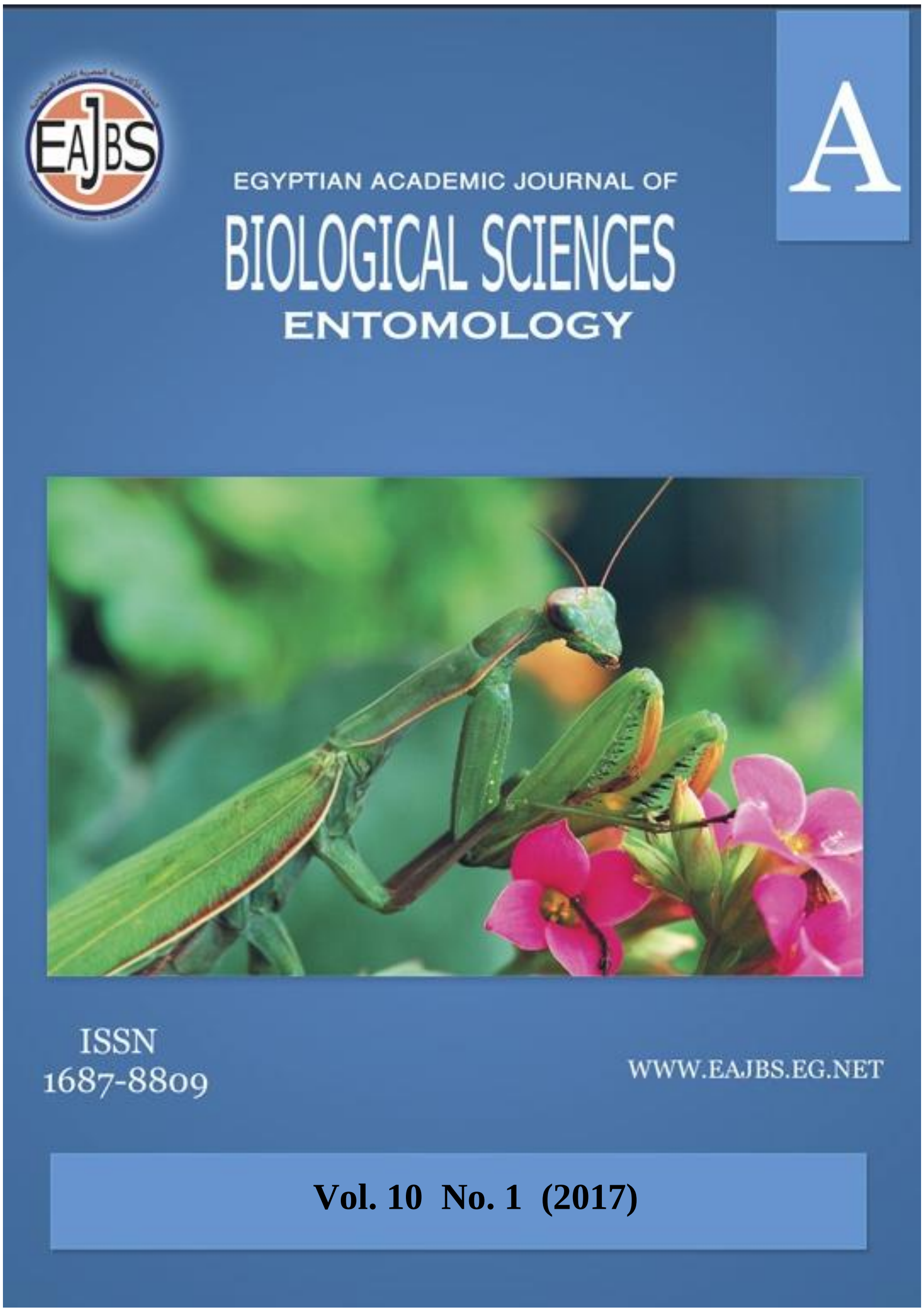

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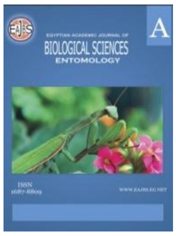

Laboratory Evaluation of the Effect of Insecticides on Non-target Organisms: 1- The Predatory Green Lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)

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ABSTRACT

A study purposed to evaluate the acute residual toxicity of six insecticides (different groups); Dursban and Malathion (OP), Chess (Selective feeding broker), Spintor (bioinsecticide), Biogard: (*Bacillus thuringiensis*) (bacteria), and Biover: (fungus) on immature stages of the predator, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) was carried out under laboratory conditions. Lethal and sub-lethal effects of the tested insecticides (4 concentrations each) on the development of the eggs (3 ages) and the 2nd instar larvae of the predator were assayed. The eggs were treated using the dipping method, while the larvae were treated using the residual film contact method. LC_{50}, LC_{90}, LC_{99} and the sub-lethal concentration (LC_{20}) for each insecticide were estimated. Hatchability values, percentages of the treated eggs as well as percentages of larvae were calculated. Pupation and adult emergence percentages were estimated and compared with those of the untreated check to study the residual effect on the treated larvae, using LC_{20} of each insecticide. The dose-mortality responses of the eggs were compared in terms of differences in slopes and LC_{50} values. Data showed that the 3-day old eggs were more tolerant to the tested insecticides than the newly ones. The mean mortality percentages of treated larvae were: 100, 100, 55, 50, and 20% for Dursban, Malathion, Biogard, Chess and Spintor, respectively. According to the classification of (IOBC/WPRS), Dursban and Malathion were classified as harmful insecticides, Biogard and Chess as moderately harmful, and Spintor as harmless insecticde. *C. carnea* larvae showed a relative tolerance to the insecticides tested as they pupated and developed successfully to the adult stage. The highest larval mortality (24%) was recorded for Dursban, while the lowest one (18%) was for Spintor compared with (10%) for the control. Statistical analysis of accumulated larval mortality indicated that there were insignificant differences among the mortality percentages in any of the studied insecticides and also between the treated larvae with each insecticide and the control. Spintor was the most suitable insecticide recommended to be used in IPM programs as it is safe for different stages of *C. carnea*.

INTRODUCTION

Insecticides are used to control pests either by killing the target ones or by preventing them from engaging in behaviors or alter their life cycle. Insecticides are classified based on their structure and mode of action. 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.

Existence of naturally occurring biological control agents is one reason that many plant-feeding insects do not ordinarily become economic pests. Problems associated with reliance on chemical control include 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 (Lugojja *et al.*, 2001). 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 in the laboratory for tolerance to one or more common used insecticide is very helpful. Use of selective insecticides is an important strategy for pest control and they should have no adverse effects on beneficial organisms (Hassan, 1989 and Nasreen *et al.*, 2007).

The green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is one of most common predatory species worldwide. It has a great role in reducing several pest populations in field crops and vegetables (Dean and Sterling, 1992). An ideal insecticide should be toxic to pests, but not to predators and parasitoids (Ishaaya and Cassida. 1981). Some insecticides showed less toxicity to biocontrol agents. Nasreen *et al.* (2005) found that Chlophyrifos recorded the lowest LC_{50} values when 2^{nd} instar of *C. carnea* was treated with it. As well, Spinosad was slightly toxic to *C. carnea* (Elzen *et al.*, 1998).

The present study was purposed to screen out some of the recommended insecticides that are used in IPM programs and to evaluate their acute and sub-lethal residual toxicity to the immature stages of *C. carnea* under laboratory conditions.

MATERIALS AND METHODS

Toxicity of some of the commonly recommended insecticides against economic pests in the Egyptian fields, represent different chemical groups, was evaluated against eggs (1, 2, and 3-day old) and 2^{nd} instar larvae of the green lacewing, *C. carnea*. Experiments were carried out under the laboratory conditions of 27±2°C and 70±5% RH. Insecticides stock solutions were prepared in distilled water by serial dilutions, while that of the control were prepared with distilled water only.

Selected insecticides

The selected insecticides and their field rates of application were:

1- Dursban (OP) H 48% EC, with a rate of application of 250 cm/100 L.
2- Malathion (OP) 57% EC, with a rate of application of 500 cm/100 L,
3- Chess (Selective feeding broker) 50 % WG, with a rate of application of 20 gm/100 L,
4- Spintor (bioinsecticide derived from an actinomycete bacterium species, *Saccharopolyspora spinosa* (Mertz and Yao, 1990), that displays the efficacy 24%, with a rate of application of 12.5 cm/100 L,
5- Biogard: (Bioside) (*Bacillus thuringensis*) with a rate of application of 125 gm/100 L,
6- Biover: bioside group (fungus) with rate of application of 250 gm/ml

Eggs and larvae of *Chrysoperla carnea*

Processed egg cards and 2^{nd} instar larvae of *C. carnea* were obtained from the *Chrysoperla* Mass-production Unit at the Faculty of Agriculture, Cairo University, Giza, Egypt. A total of 300 eggs was used as follows: 5 eggs/ concentration (x 4 concentrations) / insecticide (x 5 insecticides) / replicate (x 3 replicates) and 15 eggs (x 3 replicates) for control. A total of 300 2nd instar larval individuals was used as follows: 5 larvae/ concentration (x 4 concentrations) / insecticide (x 5 insecticides) / replicate (x 3 replicates) and 15 larvae (x 3 replicates) for control.

Bioassay

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

Treatment of *C. carnea* eggs

Three ages of *C. carnea* eggs were tested. The eggs of each age were received on a black paper sheet that divided into strips, each had 15 eggs. The strips were dipped in different dilutions/ insecticide or in water (control) for 10 seconds. The treated strips were placed on paper tissues to dry, based on the methodology described by Medina *et al.* (2001). Then the eggs were placed individually in glass tubes (2x7 cm) to avoid cannibalism after hatching. Treated eggs were kept in labeled groups, each contained the name of the insecticide and the concentration. Eggs were observed daily until hatching. The average number of the hatched eggs/ treatment was calculated as percentage of the total treated eggs.

Treatment of *C. carnea* 2nd instar larvae

Toxicity of insecticides to the 2nd instar larvae was assessed, using the residual contact method. An amount of 0.5 ml of each insecticide dilution was added to each glass Petri dish and allowed to dry at the ambient temperature. Fifteen individuals in three groups (five each) were used for each concentration and the control as well. The treated larvae were transferred individually to glass tubes (2x7 cm) and provided with aphid nymphs as food and as well was the control larvae (Sabry and El-Sayed, 2011). The mortality percentage of the treated larvae was assessed. Larvae were considered dead when they no longer moved or twitched when being touched 2-3 times with a fine brush (Nasreen *et al.*, 2007).

Experiments

Preliminary preparations of each of the tested insecticides started with the recommended field rates of applications. The rates were prepared as concentrations in water dilutions. Eggs and/or 2nd instar larvae were treated and mortality percentage was estimated to determine the concentration range of each insecticide. This range was used as a guide for determining the lethal concentrations, LC_{50}, $LC_{90,}$ LC_{99}, and the sub-lethal concentration (LC_{20}) for each insecticide. To estimate the lethal and sub-lethal concentrations of the studied insecticides against the eggs and 2nd instar larvae of *C. carnea*, 4 concentrations within the concentration range for each insecticide were prepared and both eggs and larvae were treated. The hatching percentage of the treated eggs and mortality percentage of larvae were estimated. To study the residual effect of the treated larvae, using LC_{20} of each insecticide, the larvae were separated individually and provided with aphids as food, and inspected daily until pupation. As well, the pupae until adult emergence. The pupation and the adult emergence % were estimated and compared with those of the controls.

According to the classification of the (IOBC/WPRS) working group (Hassan, 1997), harmless insecticide causes < than 50% mortality, slightly harmful causes 50-79% mortality, moderately harmful causes 80-89% mortality and harmful causes > than 90% mortality. 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, if the slope (b) is < than 1, then the susceptibility of the population to the insecticide is heterogeneous. To compare the toxicity of the tested insecticides, Toxicity Index (T.I.) and Relative Potency levels (R.P.) were calculated based on the LC_{50} values (Sun, 1950), as follows:

$$\text{T. I.} = \left(\frac{\text{LC50 of the most toxic compound}}{\text{LC50 of the tested compound}}\right) \times 100$$

$$\text{R. P.L.} = \left(\frac{\text{LC50 of the least toxic compound}}{\text{LC50 of the tested compound}}\right)$$

Statistical analysis

All experiments were conducted in a completely randomized design, with the 63 treatments for eggs + 3 for control, and 21 treatments for larvae + one for control. The test was not considered valid, if there was more than 12% mortality in the control (Hassan, 1989). Percent mortality was corrected through Abbot's formula (Abbott, 1925) when necessary. The lethal concentrations and sub-lethal concentration (LC_{20}) were deduced by extrapolation from the regression line obtained by probit analysis (Finney, 1971). Significance among mortality percentage was determined by F-value.

RESULTS AND DISCUSSION

Lethal effect on *Chrysoperla carnea* immature stages

Toxicity to the eggs

All insecticides had lethal effects on eggs of *C. carnea* at the 3 different ages (1, 2, and 3-days). LC_{50} values varied according to the insecticide and the age of the egg. Data for Dursban and Chess confirmed that 3-day old eggs were more tolerant to the tested insecticides than the newly ones which were compatible with those reported by El-Arnaouty and Badawy, (1999). Referring to the data summarized in Table (1), the LC_{50} values of all the tested insecticides against the 3 ages of eggs were < than (100%), LC_{50} values as percentages estimate of the recommended field rates ranged from 0.2 to 12% of the recommended field rates. The dose-mortality responses of the eggs were compared in terms of differences in slopes and LC_{50} values (Table 2). Toxicity of the insecticides to the eggs was also compared in terms of Toxicity Index (T.I.) and Relative Potency levels (R.P.L.) based on the LC_{50} values (Table 3). As indicated in the table, Dursban (OP) showed the highest T.I. value among the other insecticides. The results were in agreement with those reported by Aida Ayubi *et al.* (2013).

Table 1: LC_{50} values (ppm) and slope for the studied insecticides against different ages of *C. carnea* eggs

Insecticide	1- day old eggs		2-day old eggs		3-day old eggs		Recommended field rate (ppm)
	LC_{50}	Slope	LC_{50}	Slope	LC_{50}	Slope	
Dursban	10.7	0.81	5.0	0.79	25.12	0.69	2500
Malathion	269	0.75	55.0	0.87	42	0.84	5000
Chess	24	1.45	8.71	1.33	11.48	1.44	200
Biovar**	26.7	2.3	23.44	2.25	7.94	2.55	2500
Spintor	7.76	0.48	5.0	0.62	4.73	0.72	125

** 1 ppm equivalent to 32 conidia/ml.

Table 2: Estimated LC_{50} values as percentages of the recommended field rates of the studied insecticides against different ages of *C. carnea* eggs

Insecticide	1-Day	2-Days	3-Days
Dursban	0.4	0.2	1.0
Malathion	5.4	1.1	0.84
Chess	12	4.36	5.74
Biovar	1.07	0.94	0.33
Spintor	6.2	4	3.78

Table 3: Toxicity of the recommended insecticides to different ages of *C. carnea* eggs

Insecticide	1- day old eggs				2-day old eggs				3-day old eggs			
	LC_{50}	slope	T.I.*	R. P.L.** (ppm)	LC_{50}	slope	T. I*.	R.P.L.** (ppm)	LC_{50}	slope	T. I*.	R.P.L.** (ppm)
Dursban	10.7	0.81	72.52	25.14	5	0.79	100	11	25.12	0.69	18.83	1.67
Malathion	269	0.75	2.88	1	55	0.87	9.09	1	42	0.84	11.26	1
Chess	24	1.45	32.33	11.20	8.71	1.33	57.40	6.31	11.48	1.44	41.20	3.66
Biovar***	26.7	2.3	29.06	10.07	23.44	2.25	21.33	2.35	7.94	2.55	62.22	5.29
Spintor	7.76	0.48	100	34.66	5	0.62	100	11	4.73	0.72	100	8.88

***** T. I. = toxicity index calculated on the basis of LC_{50} values (Sun, 1950)

** R. P.L. = relative potency levels based on the LC_{50} value

* ** 1 ppm equivalent to 32 conidia/ml.

Toxicity to 2nd instar larvae

Dursban and Malathion were highly toxic to the larvae, followed by Biogard, then Chess, and Spintor. The corresponding mean mortality percentages were: 100, 100, 55, 50, and 20% for Dursban, Malathion, Biogard, Chess and Spintor, respectively (Table 4). According to the classification of (IOBC/WPRS), Dursban and Malathion could be classified as harmful insecticides, Biogard and Chess as moderately harmful, and Spintor as harmless insecticide. These results agree with Williams *et al.* (2003), Varghese and Beevi (2004) and Cisneros *et al.* (2006) who reported that Spintor was less toxic to the 2nd instar larvae of *C. carnea* and is a safe insecticide for predators. The LC_{50} values of the studied insecticides to the 2nd instar larvae was estimated. Its values of Dursban, Malathion, Chess, Spintor, and Biogard were: 2.98, 92, 171, 286, and 1136 ppm, respectively (Table 5). They were much < than the recommended field rates, except Spintor which its LC_{50} was higher than the recommended field rate. Referring to the data of the slope in Table (5), the highest slope value (b = 3.66) was found by Spintor and the last one (b = 1.43) was by Chess. It could be concluded that the susceptibility of *C. carnea* larvae to Spintor was homogeneous and relatively homogeneous to Chess, while no heterogeneous susceptibility was found.

Table 4: Mortality % and toxicity classification of the 2nd instar larvae of *C. carnea* treated with field rates of five recommended insecticides

Insecticide	Mortality %	Toxicity
Biogard	55 ± 3.3 (51 – 62)	Moderately harmful (Class 3) 51 ≤ Mortality ≤ 75%
Chess	50 ± 2.9 (44 – 55)	Slightly harmful (Class 2), 25 ≤ Mortality ≤ 50%
Dursban	100 ± 0.0 (100 – 100)	Harmful (Class 4) Mortality > 75%
Malathion	100 ± 0.0 (100 – 100)	Harmful (Class 4) Mortality > 75%
Spintor	20 ± 3.2 (16 – 25)	Harmless (class 1) Mortality <25%
Control	9.75 ± 0.2 (8 – 11)	====

Table 5: Toxicity index (T. I.) and relative potency levels (R.P.L.) of the studied insecticides against the 2nd instar larvae of *C. carnea*

Insecticide	Slope and homogeneity	T. I.*	R. P.L.**	Concentrations (ppm) (95% CL)			
				LC_{50}	LC_{90}	LC_{99}	LC_{20}
Dursban	1.54	1.00	318.21	2.98	20	97	1.17
Malathion	2.0	3.24	12.35	92	403	1348	35
Chess	1.43	1.74	6.64	171	1338	7234	44
Spintor	3.66 (homogeneous)	1.04	3.97	286	640	1239	169
Biogard	1.46	0.26	1	1136	8557	44828	262

*T. I. = toxicity index values calculated on the basis of LC_{50} values
** R. P.L. = relative potency levels based on the LC_{50} values

Latent effect

Second instar larvae of *C. carnea* were treated with the LC_{20} values of each of the tested insecticides. The values of the accumulated larval mortality, pupation and development to adult percentage resulted from the 2nd instar larval treatment were presented in Table (6). *C. carnea* larvae showed a relative tolerance to the insecticides tested, they pupated and completed successfully to the adult stage. Referring to the data in Table (6), it could be concluded that the highest larval mortality (24%) was recorded for Dursban, while the lowest (18%) was for Spintor compared with (10%) for the control. Pupation % ranged from 64 to 75%, while it was (86%) in the control. Larvae treated with LC_{20} of all insecticides succeeded to complete their development to adults with a developmental % ranged from (71.6%) for larvae treated with Chess to (78%) for that treated with Spintor, while it recorded (92.2%) for the control. Obtained data showed that the pupation rate decreased with the increase of insecticide concentration. Slight effect of insecticides was recorded at the adult emergence rate in the treatments with lower concentrations. Results were in agreement with Badawy and El Arnaouty, (1999) who reported that 1st and 2nd instar larvae of *C. carnea* were most susceptible to OP and Carbamates chemical groups. Also, with Duffie *et al.* (1998) who mentioned that OP classes were the most toxic group causing dramatic reductions in predator numbers. Statistical analysis of accumulated larval mortality indicated that there were insignificant differences among the mortality % in any of the studied insecticides and also between the treated larvae with each insecticide and the control, since F-value ranged from 1.025 to 2.487, as the tabulated F-value was 5.929 (Table 6). Also, the differences among treatments or between each treatment and control were insignificant for the larvae developed to pupae or the pupae developed for adults.

Table 6: Accumulated larval mortality, pupation, and development to adult percentages of *C. carnea* 2nd instar larvae treated with LC_{20} of the studied insecticides

Insecticide	Accumulated larval mortality %	Pupation %	Development to adult %
Dursban	24 ± 1.85 (20 – 29)	70 ± 1.4 (68 – 72)	72 ± 1.96 (69 – 75)
Malathion	23 ± 1.9 (18 – 28)	70 ± 1.6 (67 – 73)	71.7 ± 1.94 (68 – 75)
Chess	22 ± 2.72 (20 – 25)	64 ± 2.0 (61 – 67)	71.6 ± 1.99 (68 – 76)
Spintor	18 ± 1.39 (16 – 24)	75 ± 2.36 (72 – 78)	78 ± 1.41 (76 – 80)
Biogard	20 ± 2.36 (17 – 22)	73 ± 1.41 (71 – 75)	75 ± 3.29 (70 – 80)
Control	10 ± 1.3 (8 – 12)	86 ± 2.24 (83 – 89)	92.4 ± 1.84 (90 – 96)

In conclusion, all insecticides had lethal effects on eggs of *C. carnea* at the 3 different ages, while the larvae showed a relative tolerance to some of the insecticides tested, as they pupated and developed successfully to the adult stage. The susceptibility of *C. carnea* larvae to Spintor was homogeneous and relatively homogeneous with Chess, while no heterogeneous susceptibility was recorded.

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ARABIC SUMMERY

تقييم معملى لتأثير المبيدات الحشرية على الكائنات غير المستهدفة: مفترس أسد المن الأخضر
Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae)

منى عبد الحميد شعيب ـ عبد العزيز أبو العلا خضر ـ أحمد حسين الهنيدى
معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الجيزة – مصر.

أجريت دراسة تحت الظروف المعملية لتقييم السمية المتبقية الحادة لستة من المبيدات الحشرية التابعة لمجموعات مختلفة: Dursban و Malathion (المجموعة الفسفورية العضوية)، chess (وسيط التغذية الانتقائية)، Spintor (مبيد حيوى) ، Biogard: (مبيد بكتيرى)، وBiover: (مبيد فطرى) على الأطوار غير الكاملة لمفترس أسد المن الأخضر، *Chrysoperla carnea* (Stephens): Neuroptera: Chrysopidae). تمت دراسة الأثرالمتبقى المميت وتحت المميت للمبيدات المختبرة (٤ تركيزات لكل مبيد) على كل من طور البيضة (٣ أعمار) ويرقات العمر الثانى للمفترس. تم معاملة البيض باستخدام طريقة الغمس، بينما تم معاملة اليرقات باستخدام طريقة الملامسة. تم حساب كل من نسبة التطفل ونسبة الخروج والنسبة الجنسية فى حالة البيض المعامل لكل عمر من الأعمار الثلاث لكل مبيد من المبيدات على حده. أيضا تم حساب نسبة الموت والنسبة الجنسية لليرقات المعاملة لكل مبيد على حدة. ولدراسة الأثر المتبقى على اليرقات المعاملة تم حساب LC_{50}، LC_{90}، LC_{99} وكذلك التركيز تحت المميت (LC_{20}) عن طريق تقدير نسبة الموت لكل مبيد حشرى. أيضا تم معاملة اليرقات بالتركيز (LC_{20}) لكل مبيد حشري، وتم تقييم النسبة المئوية لكل من التحول الى طور العذراء وخروج الأفراد الكاملة ومقارنتها باليرقات غير المعاملة. أوضحت النتائج المتحصل عليها بعد معاملة البيض بالمبيدات أن البيض ذو عمر ثلاث أيام كان اكثر تحملا للمبيدات عن البيض الحديث. أظهرت النتائج وفقا لتصنيف (IOBC / WPRS)، عند معاملة اليرقات بالمبيدات أن Dursban وMalathion من المبيدات الحشرية الضارة، أما Biogard وChess من المبيدات متوسطة الضرر، بينما تم تصنيف Spintor من المبيدات غير الضارة. وعند معاملة اليرقات بالتركيز (LC_{20}) أبدت اليرقات استجابة نسبية للمبيدات المختبرة حيث تم التحول بنجاح الى طور العذراء ثم خروج الأفراد الكاملة. وسجلت أعلى نسبة موت لليرقات (٢٤٪) فى حالة المعاملة بمبيد Dursban، في حين كان أقل نسبة موت لليرقات (١٨٪) فى حالة المعاملة بمبيد Spintor مقارنة مع (١٠٪) لليرقات غير المعاملة. وأشارت نتائج التحليل الإحصائي لوجود فروق غير معنوية بين نسبة الموت التراكمية لليرقات المعاملة في أي من المبيدات الحشرية محل الدراسة. كما توصى الدراسة بأن المبيد Spintor أكثر ملائمة لاستخدامه بأمان ضمن برامج المكافحة المتكاملة للآفات حيث أنه آمن على الأطوار المختلفة للمفترس *C. carnea*.