Egypt. Acad. J. biolog. Sci., 3 (1): 1- 9 (2011) F. Toxicology &Pest control
Email: egyptianacademic@yahoo.com ISSN: 2090 - 0791
Received: 21 / 3 / 2011 www.eajbs.eg.net

Assessments the toxic effects of entomopathogenic bacterium, *Bacillus thuringeinsis subsp. kurstaki*, and methomyl insecticide on larval instars of the greater sugarcane borer; *Sesamia cretica* (Lederer)

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ABSTRACT

This study was conducted to evaluate the efficacy of the bio-insecticide *Bacillus thuringiensis* (Dipel 2x® 6.4 % WP) against, 1st and 2nd instar Larvae of *Sesamia cretica* compared with methomyl (Lannate 90 % SP). After 48 hours from treatment, The LC_{50} value of *B. thuringiensis* for 1st instar and 2nd larvae were 0.00526 and 0.7 gm, respectively. While the LC_{50} of methomyl after 24h. for 1st instar and 2nd larvae were 3394 and 5481 ppm, respectively. After treatment of 1st and 2nd instars larvae of *S. cretica* with LC_{50} concentrates of *B. thuringiensis*, the average larval duration of *S. cretica* were (36.53 & 24.69) and (33.78& 20.31) days for treated and untreated, respectively. In addition, the pupation percentage was (47 & 92 %) and (18 & 84 %) for treated and untreated, respectively. The larval mortality percentage was (53 & 8 %) and (82 & 16 %) for treated and untreated, respectively. The pupal weight for (treated & untreated), *S. cretica* were (0.1887 & 0.184 gm) and (0.1842 & 0.1601 gm) when it treated as 1st and 2nd instars larvae, respectively. Furthermore, the pupal duration were (12.14 & 10) and (10.44 & 9.83 days) for (treated & untreated), when *S. cretica* treated as 1st and 2nd instar larvae, respectively. The total adult emergence for 1st instar and 2nd instar larvae were (94 & 100%) and (100 & 100%) for treated and untreated, respectively.

On the other hand results indicated that, after treatment of 1st and 2nd instar larvae of *S. cretica* with LC_{50} concentrates of methomyl, the average larval duration of *S. cretica* were (31.41 & 24.69) and (33.87& 20.31) days for treated and control, respectively. The pupation percentage was (34 & 92 %) and (28& 84 %) for treated and untreated when *S. cretica* treated as 1st and 2nd instars larvae, respectively. The larval mortality percentages were (66 & 8 %) and (72 & 16 %) for treated & untreated, respectively. The pupal weight for treated and control, were (0.1626 & 0.184 gm) and (0.1994 & 0.1601 gm), respectively. The pupal duration were (12.41 & 10) and (10.83 & 9.83) days for treated and untreated, respectively. The total emergence percentage for 1st instar larvae were (100 & 100 %) and (85.7& 100%) for 2nd instar larvae for each (treated & untreated), respectively.

Keywords: *Bacillus thuringeinsis subsp. Kurstaki*, methomyl , the greater sugarcane borer.

INTRODUCTION

The greater sugarcane borer; *Sesamia cretica* is considered the most serious corn bore in Egypt and attacks young maize plants shortly after emergence devours the whorl leaves and may kill the growing points, causing dead hearts. It is also capable of damaging older plants and excavating tunnels into the stems, ears and /or cobs. This pest lays its eggs during March, so its larvae cause complete death of small maize plants in April and May causing drastic yield losses (Mostafa, 1981;

Simeada, 1985; El-Mitwally, 1987 and El-Naggar, 1991). These losses are mainly attributed to the decrease in number of plants per unit area at harvest because of the large number of dead hearts, increase in plant lodging and eardrops and predisposing infested plants to disease organisms.

Over the years, it has been important for humans to control the populations of harmful insects and insecticides used for this purpose in agricultural and horticultural sectors. Synthetic insecticides, owing to their various side effects, have been widely replaced by biological insecticides. *Bacillus thuringiensis* (BT) accounts for 90 % of the bioinsecticide market and it produces insecticidal toxins referred to as delta endotoxins (Chattopadhyay *et al.* (2004). The aim of the present work is to evaluate the toxicological activity and latent effects on some biological aspects of the entomopathogenic bacterium, *Bacillus thuringeinsis subsp. kurstaki*, and chemical insecticide methomyl against 1st and 2nd instar larvae of the greater sugarcane borer; *Sesamia cretica* (Lederer).

MATERIALS AND METHODS

The present study was carried out to study the effect of *Bacillus thuringiensis* and methomyl insecticide against 1st and 2nd instar larvae of the greater sugarcane borer; *Sesamia* cretica (Lederer) under laboratory conditions.

Tested compound

***Bacillus thuringiensis* (Dipel 2X 6.4% WP)**

Bacillus thuringiensis subsp. *Kurstaki,* Dipel 2X 6.4 % WP (each mg contain 32000 international unit according to the analysis certificate).

Methomyl (Lannate 90 SP)

S-methyl N-(methylcarbamoyloxy) thioacetimidate.

Test insect

Infested corn plants with *S. cretica* (larval stage) were taken from the field and transferred to the laboratory. The larvae of *S. cretica* were reared on maize pieces at a controlled conditions in electric incubator at 27˚C ± 2 and relative humidity of 65% ± 10 % R. H., as follows:

1- Obtained larvae kept, individually, in plastic cups containing a layer of moistened sawdust on the bottom (1.5cm thickness). Every two days, larval feces removed as well as dried stems, which replaced by fresh maize stems or young corn ears, always in sufficient amounts. Larvae left under laboratory conditions at 27˚C ± 2 and relative humidity of 65% ± 10 % R. H. until pupation.

2- The obtained pupae were sexed and introduced singly into 1 x 3" glass vials, each provided with a small piece of moistened cotton wool at its bottom, and plugged with cotton wool.

3- Vials were kept under the same laboratory conditions mentioned above until adult emergence.

4- Immediately after emergence, every couple placed in a glass chimney cage fixed on a plastic cup filled with fine sand covered on the top by muslin cloth kept in position by a rubber band. Inside each cage, 4 wax papers were rolled and fixed in the sand to act as an oviposition site for *S. cretica* moths.

5- Pairs replicated in 10 times and each jar covered with pin notched wax paper to provide sufficient ventilation.

6- The daily-obtained egg-masses kept in Petri-dishes containing small pieces of moistened cotton wool, where eggs were counted and left until hatching.

7- Each 10 newly hatched larvae were introduced together into a glass jar containing 10 small pieces (about 10 cm long) of the fresh tender rolled leaves surrounding the growing point of maize plants.

8- Jars tightly covered with muslin and inspected every two days to renew maize leaves.
9- Immediately after the second larval instar, small cutting (about 10 cm long) of fresh maize plant stems or young corn ears were used for feeding. Cuttings were renewed every two days until pupation.

Bioassay studies

Laboratory experiments were conducted to study the toxic effects of *Bacillus thuringiensis* (Dipel 2X) as a biocontrol agent and Methomyl (Lannate) as a carbamate insecticide on 1st, and 2nd instars of *S. cretica* larvae.

For treatment, three equal pieces of tender parts stems of maize plant dipped in the desired solution for about two minutes after which the treated parts were left in shade for about 10 minutes to dry. The experimented larvae kept starved for about 4 hours, before offering the treated food to assure rapid ingestion. Larvae were offered contaminated maize parts for 48 h. when the tested compound is *B.t.* and for 24 when the tested compound is methomyl. The total number of treated larvae / treatment was 50 that divided in 5 replicates of 10 larvae each. The same numbers of larvae considered as a control, these larvae offered tender parts of maize plant stems immersed in distilled water.

Results were presented graphically as log/ probity regression lines and LC_{50} were values calculated by the computer program Sigma Plots for Windows (version2). Furthermore, LC_{50} of tested compound were calculated for both the 1st and 2nd instars larvae. Fresh tender parts of maize plant stems immersed in the LC_{50} of each tested compounds and then left to dry at room temperature before contaminated maize stems as before for each compound. Each treatment comprised 50 larvae and replicated 5 times (larvae/jar).

The same numbers of larval mortality was determined at the end of the larval stage. Corrected mortality counts according to formula (Abbott, 1925), then submitted to probit analysis using Finney (1971). These tests carried out to define the larval mortality Percentage, Larval duration. Pupation Percentage, Pupal weight, Pupal duration, Pupal mortality Percentage, Adult emergence, Sex ratio.

Statistical analysis

Statistical analysis of bioassay results for ANOVA test, the computer.

RESULTS AND DISCUSSION

- Toxicity of *B. thuringiensis* against, 1st and 2nd instars larvae of *S. cretica* under laboratory conditions compared with methomyl.

The toxic action as initial toxic effect of investigated compounds to the 1st and 2nd instars larvae of *S. cretica* were evaluated under laboratory conditions. The main criteria of the toxicity regression lines; LC_{50} and slope value were used as parameter in comparison between different compounds. The toxicants used were *B. thuringiensis* and methomyl.

1- Toxic effect of *B. thuringiensis* against the 1st and 2nd instars larvae of *S. cretica*

Data summarized in Table (1) showed that the toxic effect of *B. thuringiensis* increased by increasing of used concentration, the corrected mortality % were 82.2, 60.0, 53.3, and 24.4 % when the *B. thuringiensis* concentrations were 0.0625, 0.015625, 0.0039 and 0.00097 ppm, respectively. On the other hand, the LC_{50} of *B. thuringiensis* for 1st instar larvae was 0.00526 ppm. The slope for *S. cretica* 1st instar larvae when treated by *B. thuringiensis* was 0.8323. Results also, indicated that the toxic effect of *B.*

thuringiensis increased by increasing the concentrates, the mortality % were 72.92, 62.5, 50, 41.67, 18.75, 14.5, 6.25 and 2 % when the *B. thuringiensis* concentrations were 4, 2, 1, 0.25 0.0625, 0.015625, 0.0039and 0.00097 ppm, respectively. On the other hand, the LC_{50} of *B. thuringiensis* for second instar larvae was 0.7 ppm. The slope for *S. cretica* second instar larvae when treated by *B. thuringiensis* was 0.7048. Harapaz and Wysoki (1984) found that 1% concentration of *B. thuringiensis.* wettable powder (containing 16.000 lU/mg) applied at a rate of 48.000 IU/cm^2, killed 95 % of 4th instar larvae of carob moth, *Ectomyelcis ceratoniae* (Zeller) (Lepidoptera: Pyralidae), after 66 h. Meanwhile, 100% mortality was recorded after 85 h of exposure in a laboratory test. The mortality caused by 0.5 % concentration (24.000 lU/cm^2) was significantly lower and presumably inadequate for practical application against this pest. Yamamoto *et al*. (1990) evaluated the effect of *B. thuringiensis*, cyfluthrin and fenvalerate on the larvae of *Alabama argillacea*. Fenvalerate gave 60 % control and *B. thuringiensis* was the least effective controlling less than 50 % up to 6 days after application. A significant reduction in the pupal population 6 days after application of *B. thuringiensis* indicated some secondary effect on the larvae before pupation. Salama *et al.* (1992) studied the potency-enhancing effect of ultra-sonication of Dipel 2X (R) suspension *(B. thuringiensis* var. *kurstaki)* followed by combination with non-toxic chemical additives or exposure to dynamic magnetic fields against *Spodoptera littoralis*.

Table 1: Toxicity of different concentrations of *B. thuringiensis* against, 1st and 2nd instars larvae of *S. cretica*.

Larvae instars	Concentrate (ppm)	Mortality %		LC_{50}	Slope
		Responded	Corrected		
1st	0.0625	84	82.2	0.00526	0.8323
	0.015625	64	60		
	0.0039	58	53.3		
	0.0097	32	24.4		
	Control	10			
2nd	4.00	74	72.92	0.7	0.7048
	2.00	64	62.5		
	1.00	52	50		
	0.25	44	41.67		
	0.0625	2	18.75		
	0.015625	18	14.5		
	0.0039	10	6.25		
	0.0097	6	2		
	Control	4			

2- Toxic effect of methomyl against the 1st and 2nd instars larvae of *S. cretica*

Results in Table (2) show the toxic effect of methomyl increased by increasing of used concentration, the corrected mortality percentage were 91.56, 73.33, 42.22, 26.67, 22.22and 2.22 % methomyl concentrations were 14062.5, 7031.25, 3515.6, 1757.8, 878.9 and 439.5 ppm, respectively. On the other hand, the LC_{50} of methomyl for 1st instar larvae was 3394 ppm. The slope for 1st instars larvae of *S. cretica* when treated by methomyl was 2.0428. Ebieda

et al. (1998) evaluated the toxic effect of three insecticidal groups included six insecticides namely, Methomyl (90 % SP), Carbofuran (10% G), as Carbamate insecticides; Monocrotophos (40 % EC), Chlorpyrifos (48 % EC), Fenitrothion (50% EC), as Organophosphorus insecticides and Hexaflumuron (5 % EC), as Insect Growth Inhibitor (IGI), against *Sesamia cretica* Led., *Chilo agamemnon* Bles. and *Saccharicoccus sacchari* Ckll. as individual or concurrent infestations during the two successive seasons, 95/1996 and 96/1997 in Shandawil and Sohag Governorates. For selecting the effective insecticides against sugarcane insects, suspension (S.P.) and granular formulations (G.) were more effective ones against *S. cretica* and *S. sacchari* than emulsifiable concentrate (E.C.).

Table 2: Toxicity of different concentrations of methomyl against, 1st and 2nd instars larvae of *S. cretica*.

Larvae instars	Concentrate (ppm)	Mortality %		LC_{50} (ppm)	Slope
		Responded	Corrected		
1st	14062.5	92.4	91.56	3394	2.0428
	7031.25	76	73.33		
	3515.6	48	42.22		
	1757.8	34	26.67		
	878.9	30	22.22		
	439.5	12	2.22		
	Control	10			
2nd	14062.5	92	91.67	5481	3.0148
	7031.25	62	60.42		
	3515.6	22	18.75		
	1757.8	14	10.42		
	Control	4			

Results in Table (2) indicated that the toxic effect of methomyl increased by increasing of used concentration, the mortality % were 91.67, 60.42, 18.75 and 10.42 % when the methomyl concentrations were 14062.5, 7031.25, 3515.6 and 1757.8ppm, respectively. Where as the LC_{50} of methomyl against 2nd instar larvae was 5481 ppm. The slope for 2nd instar larvae of *S. cretica* when treated by methomyl was 3.0148**.**

3- Toxicity of *B. thuringiensis* and methomyl on some biological aspects of *S. cretica*

This part of study aims to investigate the latent effect of LC_{50} of tested compounds on certain biological aspects; larval duration, pupation, larval mortality, pupal weight, pupal duration, pupal mortality and emergence percentage of the *S. cretica*. Such investigations may throw a light to complete the picture on the mode of actions of *B. thuringiensis* and methomyl during the 1st generation.

1- On 1st instars larvae

Data in Table (3) showed that, after treatment the 1st instar larvae of *S. cretica* with LC_{50s} of *B. thuringiensis* and methomyl the average larval duration of *S. cretica* were 36.53, 31.42 and 24.69 days for *B.t*, methomyl and untreated, respectively. The pupation percentage was 47.0, 34.0 and 92.0 for *B.t*, methomyl and untreated, respectively. The larval mortality percentage was (53.0, 66.0 and 8.0).

Data in Table (3) indicated that, the pupal weight for treated and untreated *S. cretica* was 0.1887, 0.1626 and 0.184 gm for *B. thuringiensis*, methomyl and untreated, respectively. When it treated as 1st instars larvae, respectively. On the other hand, the pupal duration was 12.14, 12.41 and 10 days for *B. thuringiensis*, methomyl and untreated, respectively. For pupal mortality percentage,

it was 6.67, 0.0 and 0.0 for *B. thuringiensis*, methomyl and untreated, respectively.

Table 3: Effects of *B. thuringiensis* and methomyl on larval and pupal stages of *S. cretica* after treated as 1st instar larvae with LC_{50} concentrations.

Biological aspects	Larval stage		
	B. thuringiensis	methomyl	Control
Larval duration (Days ± S.E.)	36.53 ± 2.28 a	31.41 ± 1.28 b	24.69 ± 0.45 c
Pupation %	47	34	92
Larval mortality %	53	66	8
	Pupal stage		
Pupal weight (gm)	0.1887 ± 0.01 a	0.1626 ± 0.01 b	0.184 ± 0.01 a
Pupal duration (Days ± S.E.)	12.14 ± 0.22 a	12.41 ± 0.24 a	10 ± 0.12 b
Pupal mortality %	6.67	0.0	0.0

*- The values have the same letters horizontally are non-significant different.

2- On 2nd instar larvae

Data in Table (4) showed that, after treatment of 2nd instar larvae of *S. cretica* with LC_{50} concentrations of *B. thuringiensis* and methomyl, the average larval duration of *S. cretica* were 34.8, 33.87 and 20.31 days for *B. thuringiensis*, methomyl and untreated, respectively. The pupation percentage was 18, 28 and 84 for *B. thuringiensis*, methomyl and untreated, respectively. The larval mortality percentage was 82, 72 and 16 for *B. thuringiensis*, methomyl and untreated, respectively. Data in Table (4) indicated that, the pupal weight for (treated & untreated) *S. cretica* were 0.1842, 0.1994 and 0.1601 gm for *B. thuringiensis*, methomyl and untreated, respectively. On the other hand, the pupal duration 10.44, 10.83 and 9.83 days for *B. thuringiensis*, methomyl and untreated, respectively. For pupal mortality percentage, it was 0.0, 14.3 and 0.0 for *B. thuringiensis*, methomyl and untreated, respectively.

El-Halim (1993) evaluated the insecticidal activity and the latent effect of Dipel 2X, a commercial preparation of *B. thuringiensis* subsp. *kurstaki* in the laboratory on *S. littoralis* larvae. Dipel 2X had slight insecticidal activity against the cotton leafworm. The ability of larvae to recover decreased with the increase in concentration and/or feeding time. Both larval and pupal duration markedly prolonged with dose increase, while percentage pupation reduced. Marked latent adverse effects detected on adult emergence, fecundity and egg viability, particularly with doses above 320 IU/ml.

Table 4: Effects of *B. thuringiensis* and methomyl on larval stage of *S. cretica* after treated as 2nd instar larvae with LC_{50} concentrations.

Biological aspects	Larval stage		
	B. thuringiensis	Methomyl	Control
Larval duration (Days ± S.E.)	34.8 ± 3.56 a	33.78 ± 1.62 a	20.31 ± 0.54 b
Pupation %	18	28	84.0
Larval mortality %	82	72	16.0
	Pupal stage		
Pupal weight (gm)	0.1842 ± 0.01 b	0.1994 ± 0.011 a	0.1601 ± 0.01 b
Pupal duration (Days ± S.E.)	10.44 ± 0.4 a	10.83 ± 0.5 a	9.83 ± 0.1 a
Pupal mortality %	0.00	14.3	0.00

The values have the same letters horizontally are non-significant different.

Data in Table (5) and Figs. (1 and 2) showed the total emergence %, sex ratio % and malformed pupa and adult for 1st and 2nd instars larvae were 94, 100, 100 100.0, 85.7and 100.0 for *B.t*, methomyl and untreated, respectively.

Table 5: Effects of *B. thuringiensis* and methomyl on adult stage of *S. cretica* after treated as 1st instar and 2nd instar larvae with LC_{50} concentration

Larvae instars	Insecticide used	Biological aspects				
		Emergence %			Sex ratio %	
		Total emergence	Normal adult	Malformed adult	♂	♀
1st instar	*B.t*	94.00	100.00	0.00	28.75	71.43
	methomyl	100.00	94.12	5.88	29.41	70.59
	control	10.00	100.00	0.00	48.5	51.5
2nd instar	*B.t*	100.00	85.70	0.00	22.22	77.78
	methomyl	85.70	100.00	0.00	50.00	50.00
	control	100.00	100.00	0.00	58.54	41.46

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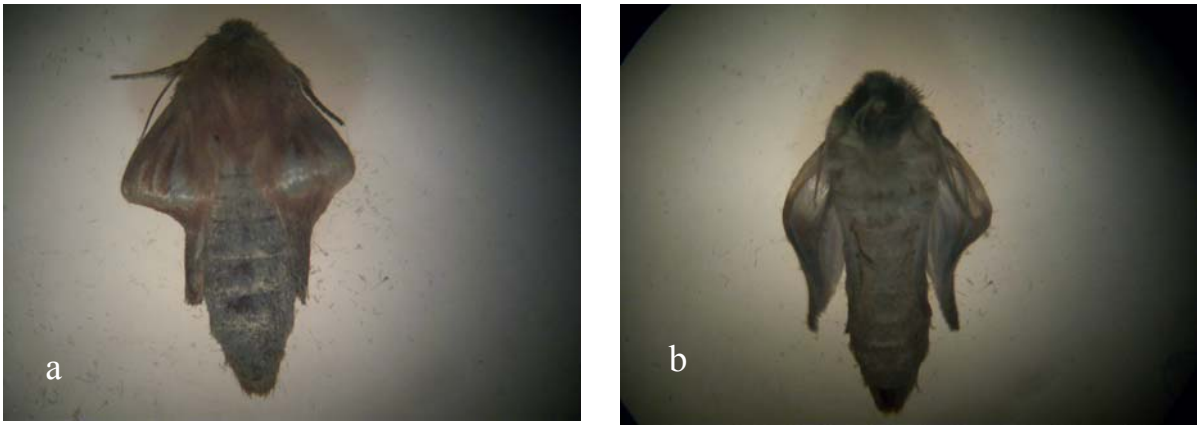

Fig 1: Malformed adult of *S. cretica* following treatment with methomyl as 1st instar

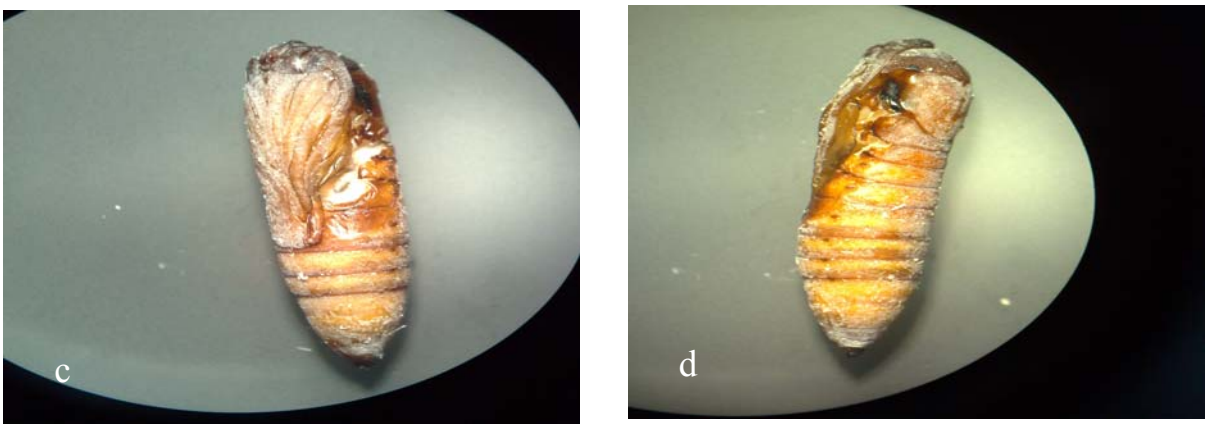

Fig. 2: Malformed pupa of *S. cretica* following treatment with methomyl as 2nd instar.

ARABIC SUMMARY

تقييم التأثيرات السامة لبكتيريا *Bacillus thuringeinsis* الممرضة للحشرات والمبيد الحشري الميثوميل على يرقات حشرة ثاقبة القصب الكبيرة *Sesamia cretica* (Lederer)

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كان الهدف من الدراسة التي أجريت لتقييم فعالية المبيد الحيوي (Dipel 2x® 6.4 % WP) حيث أن المادة الفعالة فيه هي *Bacillus thuringeinsis* مقارنة بفاعلية المبيد الحشري الميثوميل الذي يعرف بالاسم (Lannate 90 % SP) على العمر الأول والثاني لحشرة ثاقبة القصب الكبيرة *Sesamia cretica* (Lederer) وكانت أهم النتائج المتحصل عليها كما يلي:

بعد ٤٨ ساعة من المعاملة بمستحضر الـ*B.t.* كانت قيمة الـLC_{50} ٠.٠٠٥٢٦ و ٠.٧ جم للعمر اليرقى الأول والثاني على الترتيب بينما كانت القيمة بعد ٢٤ ساعة من المعاملة بالميثوميل ٣٣٩٤ ، ٥٤٨١ جزء في المليون على الترتيب للعمر اليرقى الأول والثاني.

تم معاملة اليرقات بقيمة الـLC_{50} بمستحضر الـ *B.t.* لمعرفة التأثير على فترة العمر اليرقى فكان متوسط العمر لليرقات المعاملة ٣٦.٥٣، ٣٣.٧٨ يوم وكانت ٢٤.٦٩ ، ٢٠.٣١ يوم لليرقات غير المعاملة للعمر اليرقى الأول والثاني على الترتيب.

تم معاملة اليرقات بقيمة الـLC_{50} من مبيد الميثوميل لمعرفة التأثير على فترة العمر اليرقى فكان متوسط العمر لليرقات المعاملة ٣١.٤١ ، ٣٣.٨٧ يوم وكانت ٢٤.٦٩، ٢٠.٣١ يوم لليرقات غير المعاملة للعمر اليرقى الأول والثاني على الترتيب.

وكان من النتائج المتحصل عليها أيضا في هذه الدراسة حدوث تشوهات في بعض العذارى وذلك بعد معاملة العمر اليرقى الثاني بالجرعة النصفية من مبيد الميثوميل وكذلك نتجت فراشات مشوهة نتيجة معاملة العمر اليرقى الأول بالجرعة النصفية.