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Effect of *Bacillus thuringiensis* on the biological aspects of the great waxworm *Galleria mellonella*

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Abstract--The research was conducted to study the effect of *Bacillus thuringiensis* on *Galleria mellonella*(L.) at a concentration of 3 and 5%. The results of the study showed that 5% concentration gave the highest rates of killing for the three phases (first, third, and fifth) 88.88, 88.88, and 77.77%, respectively, compared with the control treatment that did not record any killing. The treatment also prolonged the larval life of the same concentration and for the stages mentioned above, where it recorded 24, 17 and 14 days, respectively, compared with the control treatment, which recorded 20, 15 and 11 days, respectively. The results also showed an effect on the percentage of adult emergence, where the concentration was recorded 5% for the phases (first, third, and five) 11, 11, 22 % respectively, compared to the percentage of emergence in the comparison treatment, which was recorded as 100%.The results also showed the presence of deformation in adults with a concentration of 5%, of which the wings are short and wrinkled and their appearance is distorted, as the percentage of deformation for the same stages mentioned above was 11, 11, 0 %, compared with the control treatment that did not record any deformations. As for the concentration of 3%, it gave rates of killing less than the concentration of 5%. The rates of reduction in the first, third, and fifth phases were 88.88, 66.66 and 66.66%, respectively, compared with the control treatment, which recorded a percentage of 0.00%.The treatment also prolonged the duration of the larval life of the same concentration and for the stages mentioned above, which recorded 28, 19 and 15 days, compared with the control treatment, which recorded 20, 15 and 11 days, respectively. The results also showed an effect on the percentage of adult emergence, where the concentration recorded 3% For the phases (first, third and

fifth)11, 33, 33. % respectively, compared with the percentage of emergence in the comparison treatment which was recorded at 100 %. The results also showed the presence of deformation in the adults emerging from the stages treated with a concentration of 3%, including short wings, where the percentage of deformation for the same stages mentioned above was 0.00, 22, 22%, compared with the control treatment that did not record any abnormalities.

Keywords---galleria mellonella, bacillus thuringiensis, honey bee.

Introduction

Honey bee keepers suffer from the infestation of bees inside and outside the hives by many insect and non-insect pests, and the major waxworm *Galleria mellonella* (L.) is a permanent and important economic pest that attacks wax tablets and pollen inside the honeybee hives and in the store (Warhurst and Goebel, 1995). In the United States of America, beekeepers lost 3,300 beehives during 1958 as a result of not using chemical pesticides to control the insect, and this pest caused damage to 13,500 unused tires and caused great damage to weak hives and stored tires, as losses reached approximately 31,000\$ annually in the state of Louisiana (Oertel, 1969). The losses as a result of infestation by this insect amounted to more than 8,000,000 dollars in 1984 (Szabo and Heikel, 1987). As a result of infection with this pest, 1976 beehives were destroyed, with a loss estimated at approximately 4,000,000 dollars in the United States of America during 1974 (Philip, 1982).

The greatest damage caused by this pest is to attack stored tires and stored wax blocks and burrow into the wood from which the cells and tires are made (Warhurst and Goebel, 1995). Many researchers touched on the use of chemical pesticides to combat this insect, as Bailey et al. (1981) and Goodman et al. (1990) found that the use of chemicals such as Paradichlorobenzene, Phosphine, Calcium cyanide or Methyl bromide by fumigating wax tires led to the elimination of the major wax worm and the rest of the insects. others during the storage process. Jay and others (1972) also pointed out the use of carbon dioxide (CO₂) in combating all phases of resistance to other chemicals, in addition to the fact that this gas is safer than other chemicals on honeybees in terms of residues.

In view of the recorded negatives of chemical pesticides and their toxic effects on honey bees, which requires finding a suitable alternative to combat these pests, or at least supporting chemical control methods by other means that lead to rationalization of the consumption of chemical pesticides and reduce their negative effects. Therefore, bacterial pesticides have been widely used in controlling harmful insect pests, including bacteria belonging to the genus *Bacillus*, one of the genus *Bacillaceae*, which is the most important genus in terms of pathogenicity of insects (Lacey, 1997). There are 185 isolates related to it known in the world, of which 61 strains were isolated from hybrid beehives and 21 strains were isolated from wild beehives (Gilliam, 1985). As for the bacteria *Bacillus thuringiensis* that belongs to this genus, it is used specifically in programs to control insect pests that belong to the order Lepidoptera and some

insects belonging to the orders Coleoptera and Diptera. to ranks above (DeBarjac and Frachon, 1990) In the absence of a detailed study on the use of *Bacillus thuringiensis* (Berliner) in controlling the Great Waxworm and its overlap with other control methods in integrated control programs, our study aimed to estimate the effect of the commercial preparation of *Bacillus thuringiensis* on the roles of *Galleria mellonella*.

Materials and working methods

Insect breeding

Wax tires severely infested with the major waxworm were obtained from different apiaries in Baghdad , and the larvae were collected by forceps and placed in breeding bottles, which are 1 kg glass bottles covered with tulle cloth and containing dark wax inside, which were sterilized by cooling after placing them in the freezer for a period of (3) Days at a temperature of (-7 C °) for the purpose of feeding the larvae (6), and they were monitored until it was not possible and the adults came out, then pieces of cotton saturated with 10% sugar solution were placed over the roof of the glass bottles to feed the adults and motivate them to lay eggs, and for the purpose of preparing sufficient numbers of For each larval stage, sufficient numbers of eggs were isolated to obtain the first, third and fifth larval stages for the purpose of conducting subsequent experiments on them(Cantwell and smith , 1970).

Effect of *Bacillus thuringiensis* on the larval stages of *Galleria mellonella* Prepare and isolate larvae

Isolation of newly laid eggs by adults in the farm and placing them in small, sterile 14 cm high glass containers equipped with dark wax, for the purpose of obtaining one-year-old larvae, where 1-day-old larvae (first instar), 8-day-old larvae (third instar) and larvae were isolated. At the age of 15 days (the fifth stage) to carry out transactions on it.

Treatment of first instar larvae

A group of 1-day old larvae were taken and distributed with 3 larvae for each replicate in glass containers, 14 cm high and 8 cm in diameter, containing dark wax inside for feeding purpose. Vertical in the direction of the cans, and the replicates represented by glass cans were distributed with 3 replicates for each treatment that includes *Bacillus thuringiensis* concentrations (2, 5%) and the comparison treatment.

Treatment of third instar larvae

A group of 8-day-old larvae were taken and distributed at the rate of 3 larvae for each replicate, and the same previous steps were performed in the treatment of the first instar.

Treatment of fifth instar larvae

A group of 15-day-old larvae were taken and distributed at the rate of 3 larvae for each replicate, and the same previous steps were performed in the treatment of the first instar.

statistical analysis

The results were analyzed according to a completely randomized factorial (C.R.D) design and using Duncan's multiple range test.(Masonet al.,2003).

Results and discussion

Effect of *Bacillus thuringiensis* on first instar larvae of *Galleria mellonella*

The results showed in Table (1) that the treatment of the first-stage larvae of the moth in *Bacillus thuringiensis* had an effect on the life of the insect, as the percentage of killing in the larvae of concentrations (3, 5%) was 88.88 and 88.88 %, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 0%. The results also showed the effect of bacteria on the length of the larval stage, as it gave the concentrations (3, 5%) 28 and 24 days, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 20 days. As for the percentage of failure, the same concentrations were recorded at 11 and 11 % respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 100%.

The percentage of emergence from larval pupae treated with concentrations (3, 5%) was 11 and 11%, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 100%. The treatment of larvae caused deformations of emerging adults, including short wings, where the percentage of deformation of adults for concentrations (3, 5%) 0 and 11%. The results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 0%.

Through the course of the experiment in bacterial treatments, it was observed that the larvae of the first stage are small so that they can dig in the separating walls between the hexagonal eyes from the top down to the bottom, especially at the top of the wax frame or the areas where the base wax contacts the wood of the frame to feed on the untreated wax, which makes it move away. About the effect of bacteria at first, and then affected by digging the walls separating the hexagonal eyes and taking the necessary doses of bacterial spores when fed on treated wax,

and this explains the survival of some first-stage larvae for a longer period than other treated larvae.

Ali and Watson (1982) showed that the treatment of the larvae of the tobacco shoot worm *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) with the commercial preparation Dipel containing *Bacillus thuringiensis* led to the prolongation of the larval role and the role of the pupa, and that the rates of killing increased with the increase in the lethal dose taken by the larvae. from bacteria, as well as an increase in the duration of exposure to them.

ElcinYm (1995) proved the effectiveness of B.t. bacteria on the first instar larvae, which gave a 98% kill rate. It was reported by Skovmand and Sanog (1999) that the bacterial isolates isolated from Burkitavaso city, compared to the international standard isolate B.t., gave a killing rate of 95% in the control of mosquitoes. *Culex quinquefasciatus* Say and a killing rate of 60-97 % on *Anopheles gambiae* mosquito larvae. These results were proven by researchers Poopathi and Abidha (2010), where they showed a killing rate of 96% of mosquito larvae, and the results of Lee et al. (2003) confirmed that B.t. bacteria give a killing rate ranging from 85 - 100% against *Culex pipiens* larvae.

Table No. (1): The effect of treating the first-stage larvae of the larger waxworm with *Bacillus thuringiensis* on the insect's life

Transaction	Concentrations	the kill %	Larval longevity (day)	Unable %	emergence %	distortions %
Control	Zero	0.00 c	20 c	100 a	100 a	0 b
Bacillus thuringiensis	3	88.88a	28 a	11 b	11 b	0 b
	5	88.88a	24 b	11 b	11 b	11 a

a, b, c...: the lowercase letters indicate the presence of significant differences between the concentrations and the comparison treatment

Effect of *Bacillus thuringiensis* on third instar larvae

The results showed in Table (2) that treatment of third instar larvae with *Bacillus thuringiensis* had an effect on the life of the insect, as the percentage of killing in larvae of concentrations (3, 5%) was 66.66 and 88.88 %, respectively, and the results of the statistical analysis confirmed the existence of significant differences between concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 0%. The results also showed the effect of bacteria in prolonging the duration of the larval stage, as it gave concentrations (3, 5%) 19 and 17 days, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 15 days. As for the percentage of failure, the same concentrations were recorded 33and 11%, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 100%.The percentage of emergence from larval pupae treated with concentrations (3, 5%)

was 33 and 11%, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 100%. The treatment of the larvae caused deformations of the emerging adults, including shortening of the wings, wrinkling and their appearance in a distorted way, as the percentage of deformation of adults of concentrations (3, 5%) was 22 and 11%. The results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 0%. Lima et al. (2005) confirmed that the killing rate within 24 and 48 hours was 95% for *Cx. quinquefasciatus*, and also (Sharma et al., 2003) a 100% kill rate when using B.t. On larvae of *Culex quinquefasciatus* mosquitoes, *Aedes aegypti* in vitro.

Table No. (2): The effect of treating the third instar larvae of the Great Waxworm with *Bacillus thuringiensis* on the insect's life

Transaction	Concentrations	% the kill	Larval longevity (day)	Unable %	emergence %	distortions %
Control	Zero	0 c	15 c	100 a	100 a	0 c
Bacillus thuringiensis	3	66.66b	19 a	33 b	33b	22 a
	5	88.88a	17 b	11 c	11 c	11 b

a, b, c...: the lowercase letters indicate the presence of significant differences between the concentrations and the comparison treatment

Effect of *Bacillus thuringiensis* on fifth instar larvae

The results showed in Table (3) that treatment of the fifth instar larvae with *Bacillus thuringiensis* had an effect on the life of the insect, as the percentage of killing in larvae of concentrations (3, 5%) was 66.66 and 77.77%, respectively, and the results of the statistical analysis confirmed the existence of significant differences between concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 0%. The results also showed the effect of bacteria in prolonging the duration of the larval stage, as it gave concentrations (3, 5%) 15 and 14 days, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 11 days. As for the percentage of failure, the same concentrations were recorded 33 and 22%, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 100%. The percentage of emergence from larval pupae treated with concentrations (3, 5%) was 33 and 22%, respectively, and the results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 100%. The treatment of the larvae caused deformations of the emerging adults, including shortening of the wings, wrinkling and their appearance in a distorted way, as the percentage of

deformation of adults for concentrations (3, 5%) was 22 and 0%. The results of the statistical analysis confirmed the existence of significant differences between the concentrations, and a significant difference was found between the concentrations and the comparison treatment, which recorded 0%.

The length of time to achieve killing in the treatment of local bacteria and the commercial preparation may be attributed to the need to provide sufficient time for the bacteria to reach the digestive system of the larvae and to have a toxic effect, in addition to the fact that the infection of the larvae with bacteria leads to a lack of nutrition and a delay in growth, as the growth of the larvae took about 22-25 days, While the period of the fifth larval stage of the Great Waxworm takes 4.8 days at a temperature of 30 ± 1 Celsius and a relative humidity of 70-75% (Al-Yasiri 1977). Also, Snch et al. (1981) reported when controlling the cotton leafworm *Spodoptera littoralis* (Boisduval) using *Bacillus thuringiensis* that any larva that survives feeding on the bacteria delays its growth and reduces its appetite. Govindarajan et al. (1979) showed that mixing *Bacillus thuringiensis* with the chemical pesticide Nocose (DDVP) led to a higher toxicity of bacteria when *Spodoptera littoralis* (Boisduval) larvae were treated with the mixture compared to using the bacteria alone.

Table No. (3): The effect of treating the fifth instar larvae of the major waxworm with *Bacillus thuringiensis* on the insect's life

transaction	Concentrations	the kill %	Larval longevity (day)	Unable %	emergence %	distortions %
Control	Zero	0 c	11 c	100 a	100 a	0 b
Bacillus thuringiensis	3	66.66b	15 a	33 b	33b	22 a
	5	77.77a	14 b	22 c	22c	0 b

a, b, c...: the lowercase letters indicate the presence of significant differences between the concentrations and the comparison treatment

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