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THE TOXIC EFFECT OF *BACILLUS THURINGIENSIS* ON PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* AS BIOLOGICAL CONTROL AGENT AND ITS SIDE EFFECT ON APHID LION, *CHRYSOPERLA CARNEA*

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ABSTRACT: *Trials were conducted to evaluate the effect of Bacillus thuringiensis isolate against both pink bollworm Pectinophora gossypiella and Chrysoperla carnea, it had been found that the total P. gossypiella larval mortality was recorded as 35 and 74% at 1.25 and 10 CFU/ml of B. thuringiensis isolate , respectively. At any of the tested concentrations, the highest percentage of mortality occurred within the first three days following the bacterial application, then the larval mortality started to decrease. Whereas, the total larval death recorded 5% in control trials. In case of ant lion C. carnea, trials revealed that both indirect and direct effects of Bt isolate did not statistically differ from that recorded in case of control trials i.e., there were no significant difference in larval mortality. The extending pathogenic effect of the tested bacterial isolate showed that the larval duration gradually increased with increasing isolate concentrations in indirect method. On contrast in direct method isolate caused non- significant change in the larval period at any of the tested concentrations. In case of indirect effect the larval period duration gradually increased with increasing isolate concentration from 1.98 days at 1.25 CFU/ml. to 21.5 days at 10 CFU/ml. compared to 19.5 days for the control. On the other hand, no change occurred in the larval duration at any of the tested Bt concentrations.*

Key words: *Aphid lion, biological control, Bacillus thuringiensis, Chrysoperla carnea, Pectinophora gossypiella, Pink bollworm.*

INTRODUCTION

Natural enemies (predators and parasites) play a very important part in controlling pest populations. Chrysoperla carnea (Stephens, 1836) which known as the common aphid lion or green lacewing, is an insect predator in the family Chrysopidae. The adults feed on nectar, pollen and aphid honeydew and are not predatory , but the larvae are active predators and feed on aphids and other small insects. It has been used in the biological control of insect pests on crops.

Pink boll worm (PBW) larvae , Pectinophora gossypiella, burrow into cotton bolls to feed on the cotton seeds. In the process they destroy the cotton lint. This feeding damage allows other insects and fungi to enter the boll and cause additional damage.

For a long time, pesticide application was the effective control method of this pest. Many problems have been encountered as a result of the extensive use of synthetic pesticides. Increasing problems concerning the application of such pesticides include pest resistance, residue contamination of human foods, mammalian toxicity and pollution of environment.

So, many workers used the microbial control agents against this pest

Bacteria infect insects through the mouth and digestive tract, and less commonly through the eggs, integument, and trachea. They may also enter an insect by means of parasitoids and predators.

Bacterial infections in insects can be broadly classified as bacteremia, septicemia, and toxemia. Bacteremia

occurs when the bacteria multiply in the insect's hemolymph without the production of toxins. This situation occurs in the case of bacterial symbionts and rarely occurs with bacterial pathogens (Durasula et al., 1997). Septicemia (blood poising) occurs most frequently with pathogenic bacteria, which invade the hemocoel, multiply, produce toxins, and kill the insect (Wang et al., 1993). Toxemia occurs when the bacteria are confined to the gut lumen and produce toxins (Garczynski et al., 1991).The spore forming bacilli have received the most attention as biological control agents. Many of them produce proteinaceous insect selective protoxins during sporulation. One member, (Bt), has been used as a microbial pesticide against several insect pests, particularly lepidopterans. Bacillus thuringiensis is a gram-positive spore forming bacterium that produces a parasporal crystal protein inclusion during its sporulation. Bacillus thuringiensis has become the leading biopesticide since the beginning of the 1960s. The toxicology of Bt is complex and its potency against particular insects varies with the strain of Bt used.

MATERIALS AND METHODS

1- Soil Samples:

Soil samples were collected randomly from different fields in El-Bahariya Oases, Surface materials of the soil was removed; and with a sterile spatula, about 100 gram sample of soil was taken from at least 5 cm in depth. The soil samples were preserved in sterile plastic bags and stored for 2 - 12 months at 4°C until analyzed. The collection sites had no history of treatment with *Bt* .

2- *Bt* Isolation Technique:

Based on the acetate selective method described by (Smith *et al.*, 1991) soil samples (0.5 g) were added, each to 10 ml of LB broth buffered medium with 0.25M sodium acetate buffer at pH 6.8 in a sterile conical flask under aseptic conditions in a laminar flow workstation. The flask was incubated in a controlled environment incubator shaker, Edmund Bühler (TH25) operated at 300 rpm and 30°C for four hours. In this method, germination of *Bt* spores was selectively inhibited by sodium acetate buffer (0.25M), while most of the undesired spore-formers germinated. Then suspensions were allowed to stand for 10 minutes; the upper layer of suspended samples were transferred to a sterile test tube with screw cap followed by heat treatment at 80°C for three minutes in a water bath. Heat treatment was made to eliminate all vegetative cells and non-sporulated soil microorganisms present in the samples. The samples were left to cool at room temperature before inoculating 1 ml of the supernatant using sterile pipettes onto agar plates and distributed over agar surface homogeneously. The plates were incubated overnight at 30°C; then random colonies of *Bt* from agar plates were transferred onto T3 - plates using sterile loop. Transferred colonies were left for 2-3 days at least to allow complete sporulation and crystal formation characteristic for *Bt* isolate. Careful aseptic techniques were done for investigating the germinated colonies using a laminar flow workstation. Examination of germinated colonies was done using stained smears method. The germinated colonies were fixed to clean slides and stained according to (Smirnoff ,1962) stain method, then examined microscopically as follows:

1- Smears were fixed by air-drying followed by flame drying.
2- The slide was stained by admidoschwartz stain for 70 seconds, washed carefully with tap water and then stained by Ziehl's carbol fuchsin stain for 20 seconds.
3- The slide was then washed carefully with tap water, left to dry and then examined under light microscope. The presence of the crystal with its blue staining and spores with purple color, confirms that the isolate is *Bt*
4- *Bt* isolate were purified by a dot-transfer from positive colonies to fresh nutrient agar slants, then incubated for 2 days at

30°C and kept in the refrigerator until used.

5- Stained smears of *Bt* isolate were photographed.

2.1- Culturing of *B. t.* Isolate:

For culturing the obtained isolates, the method of Shake Flask Fermentation described by (Morris *et al.* 1996) was adopted as follows:

1- A single colony of bacterial sample was suspended in sterile water and incubated onto agar media plates overnight at 30 °C.

2- A single colony was transferred, using a sterile loop, from agar media to 250 ml conical flask containing 50 ml tryptose phosphate medium (dissolve 29.5 g tryptose phosphate in 1000 ml distilled water to be sterilized using (JPS Electa Co.) autoclave for 15 minutes at 1.5 pounds pressures, and 121 °C) for the first passage broth and incubated in controlled environment incubator shaker, operated at 340 rpm and 30 °C for 16-24 hours.

3- One ml of the first passage was inoculated to another 250 ml conical flask containing 50 ml of fresh tryptose phosphate broth medium (second passage broth) and incubated in controlled environment incubator shaker, operated at 340 rpm and 30°C for 16-24 hours.

4- Two ml of the second passage broth were inoculated to 100 ml of sterile fermentation broth media (M2 media). To prepare 1 liter from this media, Proflo 10g, peptone 2g, dextrose 15g, yeast extract 2g, $MgSO_4$ 7 H_2O 0.3 g, $FeSO_4$ 7 H_2O 0.02g, $ZnSO_4$ 7 H_2O 0.02 g, $CaCO_3$ 1 g, NaCl 5 g, and 1 ml Tween 60 were dissolved in 1 liter distilled water in 2L flask and pH adjusted to 7.0 then sterilized using an autoclave for 15 minutes at 1.5 pounds pressures, and 121°C. The sterile medium was divided under aseptic conditions into 2 L flasks, each one containing 100 ml of the previous sterile medium.

5- Flasks were incubated for 48 - 72 hours in a controlled environment incubator shaker, operated at 340 rpm and 30°C, depending on the time necessary to complete lyses.

6- The bacterial culture broth was harvested using cooling centrifuge at 5000 rpm for 40-50 minutes and the supernatant broth was collected and measured at the end of centrifugation. The resulting pellet was weighed.

Small quantities of *Bt* can easily be recovered by the lactose-acetone co-precipitation procedure of (Dulmage *et al.*, 1970) as follows:

1- The resulting pellet at the end of centrifugation was collected and washed twice by sterile distilled water and suspended in 5 % lactose solution (the total amount of lactose is 10 % of the total amount of fermentation broth).

2- The suspension was stirred using a magnetic stirrer for 30 minutes at room temperature and slowly; 4 times the volume of acetone was added and stirring for another 30 minutes.

3- The lactose-acetone precipitate stands undistributed for another 30 minutes followed by filtration using a Buckner funnel and # 1 Whatman filter paper. This is hooked up to a vacuum.

4- The *Bt* on the filter paper was washed twice with small amounts of acetone and suction continued for about 10 minutes.

5- *Bt* precipitate was scraped off and left to dry enough overnight then grinding in a mortar and the resulting powder was weighed and stored in a vial to be kept in the refrigerator.

In this concern, the most common method of maintaining *Bt* culture on nutrient agar slants was used. The slants were subcultured at approximately 6 months intervals when stored at 4°C in the refrigerator.

2.2- Enumeration of Bacteria:

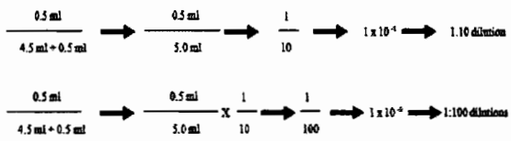
The method described by (Dulmage 1971) was used to determine the number of bacteria that are present in the isolates. Serial dilutions of a solution containing an unknown number of bacteria were made. The diluted bacteria were plated on media that supported the growth of the micro-organisms. Moreover,

determine the total number of bacteria in the original solution was determined by counting the number of colony forming units (CFU's) and comparing them to the dilution factor. Each colony forming unit represents a bacterium that was present in the diluted sample. The numbers of colony forming units are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per ml that were present in the original solution.

2.2.1-Serial dilutions:

Ten small, sterile test tubes were labeled from 1 to 10 and then 4.5ml deionized, distilled water was added, to each test tube. Then 0.5ml of the original solution was pipitted into test tube (1). This bacterial suspension was mixed thoroughly before proceeding to the next step. By a clean pipette 0.5 ml of the diluted bacterial suspension from the first test tube was withdrawn and pipetted in the second test tube. The same process was preceded in such manner that serial dilutions from the original bacterial suspension were obtained.

Tube 1 contains 4.5 ml of water; in addition to 0.5 ml of the undiluted bacterial suspension to yield a total volume of 5.0 ml.



2.2.2- Plating the serially diluted cells:

Ten plates were obtained; each plate should be labeled with initial concentration and the dilution factor. The plates had the following dilutions:

1×10^{-1}, 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, 1×10^{-8}, 1×10^{-9} and 1×10^{-10}. Then, 0.5 ml of the diluted suspension from the appropriately diluted test tube was pipetted onto the surface of the plate.

A sterilized loop was used to spread the bacterial suspension evenly over the entire surface of the plate. This process was continued with the remainder of the bacterial dilutions (one dilution for one plate). The numbers of colony forming units (CFU) for each dilution was counted and the number of bacteria in the original suspension was calculated.

For each dilution, the number of colony forming units (CFU) on the plates was counted. Typically, numbers between 30 and 800 are considered to be in the range of statistically accurate data. To calculate the number of bacteria per ml of diluted sample, the following equation was used:

$$\frac{\text{Number of CFU}}{\text{Volume plated (ml) x total dilution used}} \longrightarrow \frac{\text{Number of CFU}}{\text{ml}}$$

3- Origin and maintenance of insect culture:

The mass rearing of the PBW larvae occurred on the kidney bean diet that previously described by (Abdel-Hafez *et al.* 1982). Such artificial diet consists of 215 g dried kidney beans boiled in water, 32 g dried active yeast, 3 g ascorbic acid, 1.5 g methyl-p-hydroxy benzoate, 1.5 g ascorbic acid and 12 g. agar, to which 150 ml. water was added.

The kidney bean diet was placed in glass tubes (2 x 7.5 cm.) at rate of 4 g diet/ tube then about 7 neonate larvae were placed into each tube using fine hair brush and capped by cotton wool. All tubes were kept at 27±1°C and 80±5 % R.H. As the larvae completed their development (about 14 days) the full-grown larvae found their way to the cotton wool to pupate. The newly emerged adult moths were sexed and kept in a glass chimney. To avoid stress effects of crowding, the male and female moths were distributed at the rate of 10-males: 15-females/ chimney.

The upper and lower surface of each chimney was covered with muslin secured by rubber bands. Moths were fed on sucrose solution by providing each

cage with soaked cotton wool. The moths normally deposit eggs mostly on the upper muslin cover and lightly on the lower surface. The eggs were incubated in clean glass jars and placed in an incubator at the same conditions suitable for rearing the larvae. The newly hatched larvae were transferred to the kidney bean diet as described above.

The predator, *Chrysoperla carnea* was initially collected from the cotton field and reared on Angoumois grain moth, *Sitotroga cerealella* at the same mentioned laboratory conditions. The adults of *C. carnea* were sexed and 10 pairs of adults were placed in plastic boxes (22x13x10 cm) covered with black muslin for deposited eggs and changed daily. Drops of Semi artificial diet solution consists of 2g yeast extract, 1g fructose and 1cm distilled water were provided on tape stacked on the muslin cover. The deposited eggs were collected daily and kept in glass jars until hatching. The hatched larvae were reared on *Sitotroga cerealella* eggs (Karim *et al.* 2009).

The original colony of the predator and its prey were supplied from the Plant Protection Research Institute, Agriculture Research Centre. Mass rearing was carried out in the laboratory of the Economic Entomology Unit, Plant Protection Department, Desert Research Center.

4-Toxicity Test:

4.1- Method of application on pink bollworm larvae:

Equal weights of artificial media mixed with several concentrations of the bacterial isolate (1.25, 2.5, 5.0, 10.0 CFU/ml) and provide as food to starved newly hatched larvae of PBW. Food in water only was offered as a control, For each concentration,10 replicates of 10 larvae each were tested. Numbers of alive and dead larvae were recorded daily till pupation.

4.2- Method of application on aphid lion larvae:

4.2.1- Direct effect:

The residual film technique was used where 3 ml of the desired concentrations were evenly spread on a Petri dish surface (9 cm in diameter). The solvent allowed being evaporated leaving a film of several concentration of bacterial isolate (1.25, 2.5, 5, 10 CFU/ml), the newly hatched larvae of the aphid lion were exposed to the thin film for 24 hour and offer them the appropriate numbers of PBW larvae as food. Each concentration was replicated 6 times, where sex Petri dishes for each containing three larvae were prepared. The control dishes were treated with water.

4.2.2- Indirect effect:

The newly hatched larvae of aphid lion, were fed on larvae of pink bollworm previously treated with sublethal concentrations of bacterial isolate, PBW larvae were sprayed with different concentrations of bacterial isolate (1.25, 2.5, 5, 10 CFU/ml) then immediately placed with newly hatched larvae of aphid lion. The control specimens were treated with water. Daily inspections were conducted.

5- Statistical Analysis and Assessment of Results:

- Duration of larval stage was assessed for survived larvae.
- Data obtained in different tests were subjected to statistical analysis to evaluate the relative efficiency of the isolates. Mortalities were corrected for the natural mortality according to (Abbot's formula 1925).

The corrected mortality % = (Observed %-Control %) x 100 / (100 -Control %)

- Concentration / mortality regression lines were drawn on probit logarithmic graph according to the method developed by (Finney 1971) .

The LC_{50} and LC_{90} values were calculated according to probane program.

- Standard deviation was calculated according to (Bliss and Stevens 1937) as follow:

$$\text{Standard deviation (S)} = S = \sqrt{\frac{\Sigma(X-M)^2}{n-1}}$$

Where, Σ= Sum of M = Mean of all treatments Variance = S2
X= Individual treatment N = Sample size (Number of treatments)

RESULTS AND DISCUSSION

In the present study, the occurrence and distribution of *Bt* in certain Egyptian soils have been investigated. Soil samples collected from El-Bahariya Oases; have revealed 9 *Bt* isolates of which only one isolate are found effective against the pink boll worm. It should be mentioned that the soil of El- Bahariya Oasis has no back history of applying chemicals for soil fertilization or for insect control (Personal communication with local authorities). In this concern, (De Lucca *et al.* 1981) carried out studies on the existence and distribution of *b. t.* in the soils of United States of America and reported that 0.5% of collected samples are identified as *B. t.* justifying that this organism can exist in the soil as a natural habitat. (Morris *et al.* 1998) isolated many strains of *B. thuringiensis* from the soils of Manitoba-Canada; and 33% of the soil samples are positive for *Bt* (Kim, 2000) reported that 10% of the soil samples collected in Korea is positive for *Bt* Moreover, (De Lucca *et al.* 1981) reported that the distribution of *Bt* is not affected by soil pH.

Moreover,Morris *et al.* 1998, reported that *Bt* is found most frequently in organic-rich soil samples from six different types of Canadian soil. Concerning the existence of non-toxic isolates from the soil samples, (Roh *et al.*, 1995) reported isolates from Korea, which were non-toxic against 10 species of orders Lepidoptera, Diptera and Coleoptera. Also, (Park *et al.* 1998) isolated non-toxic *Bt* strains against 19 insect species belonging to 2 orders, Lepidoptera (e.g. *Bombyx mori* larvae) and Diptera (e.g. *Culex pipiens pallens* larvae) from Korean soil samples.

From the aforementioned discussion, it is appear that *Bt* exists and is distributed in different types of soils in Egypt. Its toxicity, however, may depend on several factors including the type of soil from which it is isolated and its chemical and physical properties, *Bt* strain, endotoxin formation, condition of application, tested insect and may be any other factor.

1- Identification of *Bt* Isolate:

Based on the acetate selective method described by (Smith *et al.*, 1991) positive isolates with *Bt* were identified. The germinated colonies obtained from the soil samples were examined microscopically after staining according to (Smirnoff 1962). The crystals obtained from the positive isolates were characterized by their bipyramidal shape (Figure 1). After 48 hour *Bt* cultures grown in nutrient agar medium, the parasporal inclusion bodies (crystals) appeared blue in color; whereas the spores were oval and purple in color. By using the transmission electron microscope showed the release of spores and bipyramidal crystals from the sporangium of 24 hours cultures.

2- Enumeration of Bacteria:

After identification of *Bt* isolate, the average number of bacteria per ml of water was determined. The results showed that the average number of bacterial isolate was (6.70 x10^6 bacteria /ml); the colony forming units per ml (CFU) which measure the viable bacterial number.

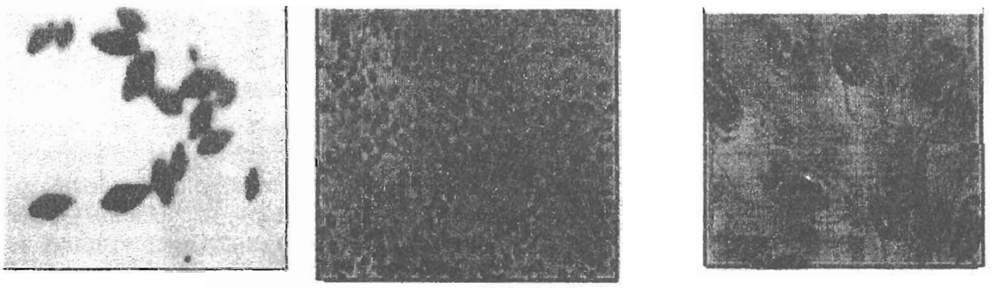
2- Toxic effect of *Bt* isolate:

2.1- on pink bollworm larvae:

Feeding of newly hatched larvae of PBW to *Bt* isolate (Table,1) revealed adverse effects on the total percentage of larval mortality, which was concentration dependent. Whereas the total larval death recorded 5% in control trials, treatment revealed 35 and 74% of larval death at 1.25 and 10 CFU/ml, respectively. At any

of the tested concentrations, the highest percentage of mortality occurred within the first three days following application, then the larval mortality started to decrease. As, for example, at 5 CFU/ml., the larval mortality started by 11% after the first day of treatment then recorded 28,8, 3, and 3 % after the second, third, fourth, and fifth days, respectively.

The standard bioassay procedures were followed according to Dulmage (1973). All bioassays were carried out using newly hatched larvae of PBW. The LC_{50} value of the tested potent isolate were computed from the data obtained on the percentage of larval mortality at each of the tested concentration through probit analyses within 95% confidence limits (Figure 2). The data illustrated indicated that the LC_{50} value of the tested potent isolate was 3.77 CFU/ml; the confidence limits at (95%) were 2.93- 4.91CFU/ml. In addition, LC_{90} value also determined and it obtained 44.23 CFU/ml. and its confidence limits at (95%) were 23.18-149.70 CFU/ml.



Bipyramidal crystals | Staind smear of *B.t.* (100X) | Transmission electron micrograph of *B.t.* grown in nutrient broth medium at 28°C (500

Fig. (1): Transmission electron microscope showed spores and bipyramidal crystals from the sporangium of 24 hours cultures.

Table (1): Mortality percentages of newly hatched larvae of *Pectinophora gossypiella* after different days of *Bt* application at four concentrations

Concentration CFU / ml	Mortality % after indicated days												Total Mor. %	
	1	2	3	4	5	6	7	8	9	10	11	12	obs.	corr.
0	0	0	0	1	0	1	2	0	0	1	0	0	5	0
1.25	6	14	5	2	0	1	0	3	0	4	0	0	35	31.57
2.5	8	19	7	0	0	1	2	0	1	0	3	0	41	37.89
5.0	11	28	8	3	3	0	0	1	0	0	1	2	55	52.63
10.0	12	39	9	4	0	3	0	0	1	5	1	0	74	52.63

Each concentration was replicated ten times with 10 larvae per replicate

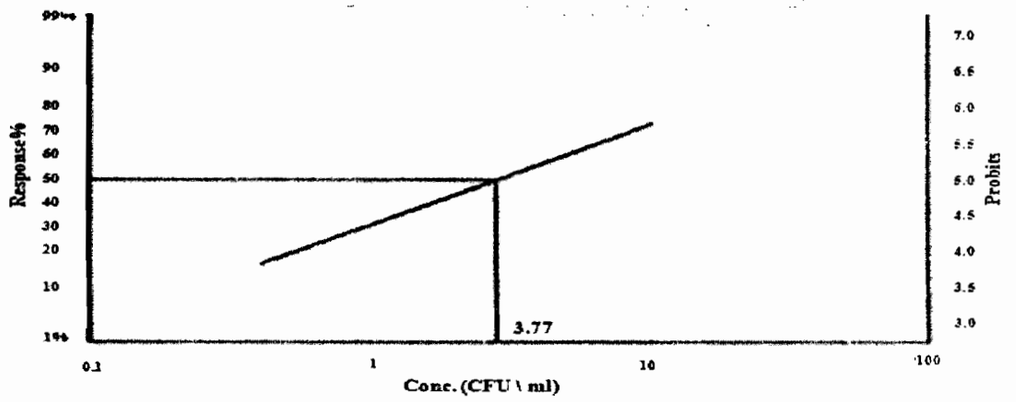


Fig. (2): Concentration / mortality regression lines for newly hatched larvae of Pink bollworm treated with *B.t.* isolate.

According to the recorded data all applied concentrations of *Bt* isolate reduced the larval population of pink boll worm. Larval mortality, according to (Yoshinori and Kaya, 1993), is probably due to either the septicemia in which the bacterial spores invade the hemocoel, multiply, produce toxin and subsequent kill the insect; or due to the toxemia in which the bacteria produce toxin and confined to the gut lumen. Abdel-Aziz (2000) attributed the larval mortality to such septicemia case. Mortality in infected larvae may also be due to the deficiency in the excretory system due to Malpighian tubules infection (Lotfy, 1988). These factors individually or together may explain larval mortality.

The obtained results are in harmony with Abou-Bakr (1997) who tested *Bt* on *Spodoptera littoralis*. Desuky (1998) found that when the 2nd larval instar of the cotton leafworm was fed on both clover and cotton leaves, accumulative mortality percents increased by time elapsed after spraying by Delfin till 24 hrs, then decreased, whereas in case of the 4th larval instar the accumulative mortality percents decreased. El-Sayed *et al.*, (1999) found that the tolerance of *Autographa gamma* to the pathogen slightly or highly increased as the larvae developed from the 2nd to the 3rd or the 4th instars, respectively.

The high percentage of larval mortality, three days post treatment, revealed higher susceptibility level of early larval instars of *Pectinophora gossypiella* to different concentrations of *Bt* isolate. The higher susceptibility of young larval instars may be either due to the binding of the bacterial endotoxin to the brush border membrane of the midgut epithelium (Van Rie *et al.*, 1990) or due to certain physiological differences between the early and late instars, where in late instars certain enzymes are secreted due to which tolerance to the bacterial infection may be developed (Goldberg *et al.*, 1974).

2.2- on Aphid lion larvae:

Both indirect and direct effects of Bt isolate on the aphid lion larvae did not statistically differ from that recorded in case of control trials. There was no significant difference in larval mortality.

The extending pathogenic effect of the tested bacterial isolate showed that the larval duration gradually increased with increasing isolate concentration in indirect method. On contrast in direct method isolate caused non- significant

change in the larval period at any of the tested concentrations. (Table, 2).

As shown in Table (2) in case of indirect effect the larval period duration gradually increased with increasing isolate concentration from 1.98 days at 1.25 CFU/ml. to 21.5 days at 10 CFU/ml. compared to 19.5 days for the control. On the other hand, no change occurred in the larval duration at any of the tested *Bt* concentrations (Figure 3).

Table (2): Larval duration in days of *Chrysoperla carnea* after application of Bt isolate at 25 ± 2 °C and 60 ± 5 % RH

Concentration CFU / ml	Average larval period	
	Indirect method	Direct method
0	19.5 ± 0.5	
1.25	19.98 ± 0.9	19.6 ± 0.19
2.5	20.6 ± 0.3	19.4 ± 0.28
5.0	20.96 ± 0.3	19.32 ± 0.12
10.0	21.5 ± 0.2	19.5 ± 0.5

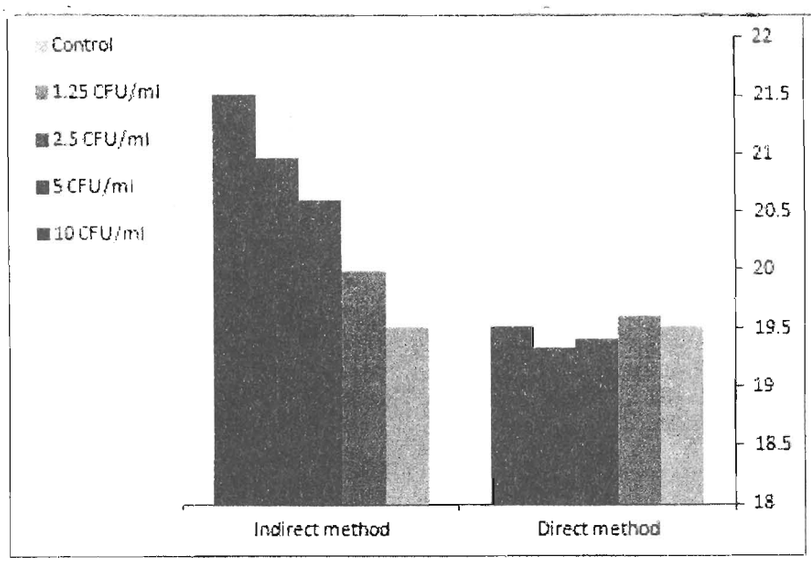


Fig. (3): Effect of *B.t.* isolate on larval duration of *Chrysopela carnea*.

In this concern, Jörg *et al.*, 2004, found that the larvae of the green lacewing predator *Chrysoperla carnea* were negatively affected when preying on lepidopteran larvae and reported that it had been fed with transgenic maize expressing the cry1 Ab gene from *Bacillus thuringiensis*. Angelika *et al.*, 2003 , reported that *Chrysoperla carnea* was affected by Dipel as the developmental time required by larvae which were fed on *Bt* sprayed plants was prolonged when compared to larvae on untreated plants. Negative effects on *C. carnea* larvae were also shown through prey-mediated exposure to Dipel. A significant increase in mortality, a prolonged developmental time and a slight decrease in weight was observed for *C. carnea* fed with *Bt* contaminated' *Spodoptera littoralis* larvae. Furthermore, Anna *et. al* 2003 found that *Spodoptera littoralis* larvae were significantly affected by Dipel as the developmental time required by larvae which were fed on *Bt* sprayed plants , where it was prolonged when compared to larvae on untreated plants. Negative effects on *C. carnea* larvae. Similar opinion is stated by Ana Rodrigo-Simón *et al.*, 2006, who studied the effect of Cry proteins of *Bacillus thuringiensis* on the green lacewing (*Chrysoperla carnea*) and concluded that in vivo and in vitro that the lacewing larval midgut lacks specific receptors for Cry1Ab or Cry1Ac. These results agree with those obtained in bioassays, and it could be concluded that the Cry toxins tested, even at concentrations higher than those expected in real-life situations, do not have a detrimental effect on the green lacewing when they are ingested either directly or through the prey. According to Hilbeck *et Al.*, 1998, the development time of chrysopid larvae was prolonged when *B. thuringiensis* fed *Ostrinia nubilalis* which was given to the predators but not for *B. thuringiensis*-fed *S. littoralis*. Although some unnoticed adverse effects in *S. littoralis* may have occurred because of the *B. thuringiensis*. The prolonged development time of chrysopid larvae raised on *B. thuringiensis*-fed *Ostrinia nubilalis* was probably because of a combined effect of *B. thuringiensis* exposure and nutritional deficiency caused by sick prey. The obtained results suggest that the reduced fitness of chrysopid larvae was associated with *B. thuringiensis*.

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التأثير المميت لسلالة من بكتريا باثليس ثيرونجينسيس كمادة مكافحة بيولوجية على دودة اللوز القرنفلية وتأثيرها الجانبى علي مفترس اسد المن

ايمان ابراهيم امام

وحدة الحشرات الاقتصادية – قسم وقاية النبات – مركز بحوث الصحراء

الملخص العربي

أجريت هذه الدراسة بغرض تقييم فعالية تأثير سلالة من البكتيريا الممرضة للحشرات *باثليس ثيرونجينسيس* والتي تم عزلها من بعض العينات المأخوذة عشوائيا من تربة بعض الحقول في الواحات البحرية، و اظهرت النتائج أن التركيز نصف المميت (LC_{50}) ليرقات دودة اللوز القرنفلية الحديثة الفقس هى ٣,٧٧ CFU/ml ، كما اثبتت التجارب ان هذه العزلة من البكتريا لها تأثير على يرقات دودة اللوز القرنفلية حديثة الفقس وسببت نسب موت تراوحت بين ٣٥ – ٧٤ ٪ عند استخدام تركيزات من ١,٢٥ – ١٠ CFU/ml ، وقد وجد ان اعلى نسبة موت لليرقات بالتركيزات سالفة الذكر كانت خلال الثلاث ايام الاولى من بداية المعاملة، ثم تبدأ نسبة الموت تقل تدريجيا، في حين كانت نسبة الموت فى اليرقات الغير معاملة (كونترول) ٥ ٪. وعلى الجانب الاخر عند معاملة يرقات حديثة الفقس من المفترس اسد المن بالجرعات المختبرة من العزلة البكتيرية بالطريقة المباشرة او الغير مباشره وجد انه لا توجد فروق معنويه في نسب الموت للحشرة المختبرة عن الافراد المقارنة. اما في حالة التأثير الغير مباشر للعزلة البكتيرية علي يرقات حديثة الفقس من المفترس أسد المن فقد طالت فترة الطور اليرقي عن الافراد المقارنة بينما لم تحدث تأثيرات واضحة في حالة التأثير المباشر.