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RESEARCH ARTICLE

The Effect of *Bacillus thuringiensis israelensis* (Bti) as a Microbial Control Agent against *Musca domestica* in Makkah Region

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Abstract

***Bacillus thuringiensis* (*Bt*) is a gram-positive spore forming bacteria. It forms a spore in an adverse condition i.e. when nutrients are limited. *Bt* produces protein crystals in the cytoplasm of the mother cell during sporulation. The protein crystals are insoluble protoxins, when synthesized the ä- endotoxins consist of two multigenic families, Cry and Cyt. Cry proteins are toxic to different insect orders. They are toxic to Lepidoptera, Coleoptera, Hymenoptera, Diptera and also to nematodes.The use of manufactured high toxic pesticides with their harm environmental effect led to search for non-traditional means of control. *Musca Domestica* plays an important role in the transmission of many pathogens such as cholera, typhoid, trachoma, diarrhea, tuberculosis, salmonella and intestinal sedimentation.The results recorded illustrated the efficiency of Bti bacteria at concentrations (0.5, 1.0, 1.5 and 20%) against the second instar larvae of the domestic fly *M. domestica*. The results of this study show that the different concentrations of Bti Clearly indicate the percentage of larvae where they increase directly and gradually by increasing the concentration (16, 52 and 80%) of the concentrations used respectively. The results recorded potential effects of the laboratory biocide on the second larval life span of *Mucosa domestica*. The life span of the mature phase, the number of eggs per female (fertility) and the percentage of hatching, and the number of mutilated individuals were recorded. The results shown that the bacterial pesticide causes a significant decrease in larval life span compared with the control group (10.36±0.027) since the minimum life span is 2.45±0.251 days at concentration of 0.5%, maximum life span is (8.68± 0.158) Days at concentration of 20%.**

Keywords: *Bacillus thuringiensis israelensis,* Microbiol control agent, Makkah.

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INTRODUCTION

Musca domestica plays an important role in the transmission of many pathogens such as cholera, typhoid, trachoma, diarrhea, tuberculosis, salmonella and intestinal sedimentation [1]. The Muscidae family is one of the most numerous and widespread in the world, where they multiply around houses in decomposing food and garbage collection sites, in poor communities and cities. There are experiments aimed at reducing the spread and reproduction of this serious insect. The use of chemical pesticides has been widespread in many countries of the world[2]. The results were not satisfactory. It was noted that the control programs adopted only on chemical pesticides led to natural imbalance and the appearance of immunity in many of the target pests [3-5], as well as pollution of the entire ecosystem, which led the world to limit the use of chemical pesticides in the control of insects in general and Musca Domestica in particular and the search for new high efficiency means to be introduced in the control system without disturbing the natural balance or pollution of the environment [6]. The most important of these was the use of highly specialized bactericides in the eradication of medical pests [7]. The most common insecticide of pests species classified as safe bacterial pesticides and used as substitutes for chemical pesticides is (*Bacillus thruingiensis*), whose toxicity is attributed to the formation of protein originating crystals in the body of the insect from an ineffective primary poison which is due to the high alkalinity of the insects intestine. Sensitivity to these crystals [8,9], parts of these proteins have receptive sites on the cellular walls of the middle intestine in the pests [10-13]. These proteins act as cell keys to alter many of their properties, most notably the permeability of cellular membranes and cell loss to normal formation, their association with adjacent cells, and sometimes the cytoplasm to enter the intestinal cavity [14]. The current study aims to find a suitable biological control method to eliminate *Musca Domestica* that transmit diseases and to be highly efficient to be included in control systems without disturbing the natural balance or pollution of the environment and to avoid the dangers of chemical pesticides through studying the sensitivity of the second larval life of the *Musca domestica* using various concentrations of *Bacillus Thuringiensis* (Bti) as well as evaluating the effect of these concentrations on some biological aspects.

MATERIALS AND METHODS

Rearing of *Musca domestica*

The mature stages of the *Musca domestica* were collected from their respective breeding sites from different areas of Jeddah governorate using the Japanese network. They were arranged for (15 generations) to avoid any residual effect of using any pesticides in the environment to obtain a laboratory strain of the relative temperature and humidity 27 ± 2°C and 60 ± 5% RH.

Mature Phase Breeding

The mature phase is raised in cubic cages of dimensions (35, 35 and 35 cm) made of a wooden frame covered with iron mesh, the base is consisted of a movable part for easy cleaning and the front part is equipped with a wooden façade with a muslin cover to handle the insects inside the cage and provide food Mature insects were supplied with a submerged cotton in a solution of milk and sucrose (2:10), placed in a petri dish for feeding and laying eggs.

Non-Mature Phase Breeding

Development of the egg

The mature stages lay eggs on the submerged cotton in a solution of milk and sucrose, eggs placement was daily examined, and egg blocks (150-200 eggs) were isolated and placed daily on larval culture in preparation for hatching.

Larval Phase

Egg blocks were transferred to the larval culture environment according to [15], which consists of wheat bran + yeast + milk powder + water with the following proportions (20: 1: 2:20) respectively. All glass utensils and food media used in the study were sterilized using a sterilizing device (2)

Pupa Phase

The dry upper layer was removed from the larval food environment since the larvae were placed in an aluminum dish and the air stream was transferred to separate the pupae from the nutritional environment and then transferred the pupae to the mature pupae cages.

Bacterial Pathogen Source

Bacillus thuringiensis israelensis was

selected for this wide-scale study as a biological control agent for the biliary dendritic system obtained from Valeant Biosciences, USA.

Sensitivity of *Musca domestica* to Bacterial Pathogen

The second-instar larvae of the *Musca domestica* was collected in Petri dishes (diameter 12cm) and starved for 24 hours.

Preparation of Bacterial Suspension

Four concentrations of bacterial suspension (0.5,1.0,1.5,2.0%) were used and prepared by shaking of the bacterial powder in distilled water.

Preparation and Processing of Larvae

20gm of the larvae was prepared and mixed with pre-processed bactericidal doses and left for 24 hr incubated in an incubator at 27°C and then placed 20 larvae (repeated) for each concentration and left for 24 hr of feeding. The remaining food, dead larvae and residues were isolated, the larvae were then placed on a natural food environment under the previously established laboratory conditions. Four replicates were made for each concentration and all larvae were left to complete their life cycle after calculating the number of dead larvae[16].

Comparison Experiment

All previous steps were followed in the comparison experiment except that the larvae were fed to a non-bacterial processed environment.

Death Ratio

The final death ratios were recorded for both treated and non-treated experiments (Comparison) after 24 hours of treatment[17].

Biological Studies

Biological Considerations

To determine the effect of bacterial disease on some biological considerations of *Musca domestica*, 4 concentrations (0.5,1.0, 1.5,2.0%) were processed and placed on the larval environment such as the previous experiment, 20 larvae were treated from each concentration and repeated 5 times. In the comparison experiment without the bacterial pesticide, the larvae were left to feed for 24 hr on the food environment. The daily notes are recorded until the complete insects are emerging from the pupae stage and the number of (larvae, pupae, mature phase) is recorded for each concentration as well as the comparison experiment, all experiments are put under the previous constant laboratory conditions[18].

Non-Mature Phase Studies

The period of larval life, pupation, the age of pupae, the weight of the pupae and the number of mutilated pupae were calculated.

Mature Phase Studies

The mature phase out rate was calculated, the life span of the mature phase, the fertility of both female and male (the number of eggs laid by the female, the rate of hatching) and the existing mutilated. The life span and fertility were determined by placing one male and one female together in a glass tube supported by cotton piece immersed in mature phase food. A small piece of dissection paper is placed as a place to lay the eggs and the experiment is repeated 5 times for each concentration. The daily procedure is used to calculate the number of dead flies, calculate the life span of the mature phase and the number of eggs to determine the fertility. Three masses of eggs were collected not less than one for 100 eggs and left until hatching under the above laboratory conditions and the percentage of hatching is calculated[19].

RESULTS

Susceptibility of 2nd Instar Larvae of *M. domestica* to Different Concentration of *Bacillus thrugingiensis* Isrelensis (Bti) After 24 hr. of Treatment

The results recorded in Table (1), illustrated in Figure (1), show the efficiency of Bti bacteria at concentrations (0.5, 1.0, 1.5 and 20%) against the second instar larvae of the domestic fly *M. domestica*. The results of this study show that the different concentrations of Bti Clearly indicate the percentage of larvae where they increase directly and gradually by increasing the concentration (16, 52 and 80%) of the concentrations used respectively.

Impact of The Tested bio Insecticide (B.ti.) at Different concentration on some Biological Attributes of The 2nd Larval *Mucosa domestica* Life Span

The results recorded in Tables (2, 3) and illustrated in Figures (2,3,4,5,6,7,8,9,10,11) show the potential effects of the laboratory biocide on

Table 1. Susceptibility of 2nd instar larvae of *M. domestica* vicna to different concentration of *Bacillus thruingiensis israelensis* after 48 hrs from treatment

Conc.%	Observed % of Mortality	Expected % of Mortality
Control	-	-
0.5	16	10.745
1.0	26	36.525
1.5	52	57.125
2.0	80	70.934

Fifty larvae were used for each conc.
P- Value = 0.001
Slop of the regression line = 2.97%
LC_{30}= 0.87 LC_{50}= 1.305

the second larval life span of *Mucosa domestica*. The life span of the mature phase, the number of eggs per female (fertility) and the percentage of hatching, and the number of mutilated individuals were recorded

Total larval life span

The results shown in Table (2) illustrated in Figure (2) show that the bacterial pesticide causes a significant decrease in larval life span compared with the control group (10.36±0.027) since the minimum life span is 2.45±0.251 days at concentration of 0.5%, maximum life span is (8.68± 0.158) Days at concentration of 20%.

Percentage of Pupation

The percentages of the pupation were significantly affected by all concentrations applied as shown in Table (2) and illustrated in Figure (3). Our results clearly show the inversely proportional relationship between different concentrations and percentages of pupation since were (78.57,51.43, 27.14) at concentrations of (0.5, 1.0, 1.5) respectively when compared (100%).

Weight of Pupae

All pupae weights have reduced at all the concentrations used in the experiments as recorded in Table 2 and illustrated in Figure (4). There was a significant reduction in all larvae of the second life span, for example, at (0.5,1.0,2.0%) pupae recorded the following mean weights: (54.7±12.20mg), (55.1± 12.20), (66.4±10.01) and (70.2± 12.80) respectively when compared to the trial experiment, since recorded (73.5±8.83mg).

Table 2. Latent iffect of different constrations of *Bacillus thuringiensis israelnsis* on some biological aspects of immature stage

Con. (%)	Larval duration (days) Means± S.E	+ or – (%)	Pupation (%)	+ or- (%)	Pupal weight Means± S.E	+ or-(%)	Pupal malformation (%)	Pupal Duration (days) Mean±S.E	+or-(%)
Control	10.36±0.127	-	100	-	73.5±8.85	-	0	3.04±0.115	-
0.5	2.45±0.363**	20.17	78.57	-21.43	70.2±12.80	-1.21	5.46	5.12±0.119	0.81
1	4.92±0.363**	44.02	51.43	-48.57	99.4±10.01*	-2.60	16.67	4.19±0.337	23.72
1.5	8.58±0.666*	79.34	47.14	-52.86	55.1±12.20**	-6.73	21.21	5.68±0.861*	73.40
2	8.68±0.158**	80.31	27.14	-72.86	54.7±12.20*	-6.87	21.15	3.67±0.836	40.12

Data Expressed as Mean ± Standard Error (S.E)
* significant (P< 0.05)
** Highly Significant (P< 0.01)

$$+ \text{or} - (\%) = \frac{\text{Treated} - \text{control}}{\text{control}} \times 100 = \text{percentage og increase or decrease as compared to the control}$$

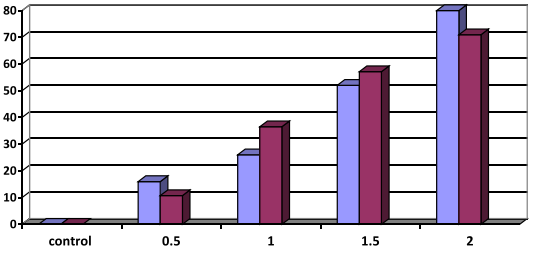
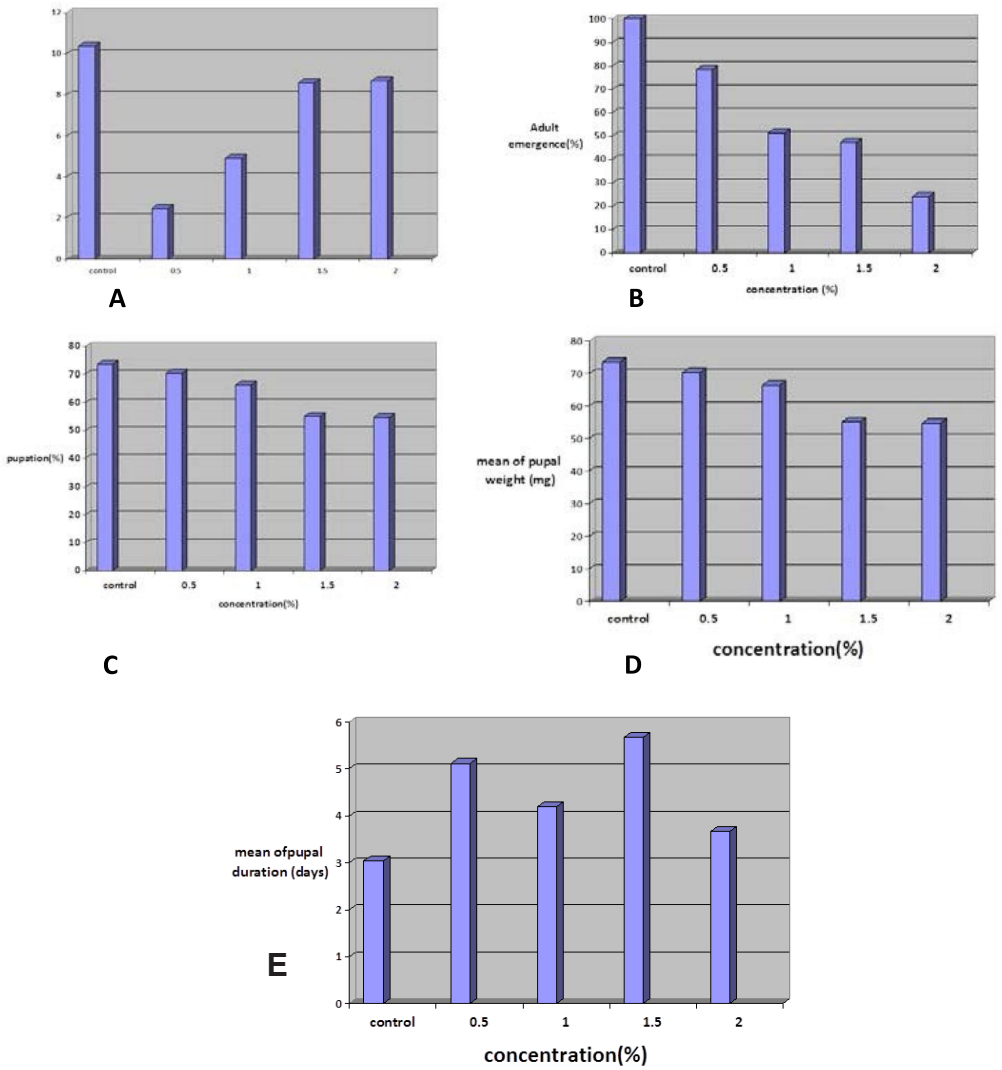


Fig. 1. Susceptibility of 2nd Larval Instar of *M. domestica* Vicina to Different Concentration of *Bacillus thruingiensis isralensis* After 48hrs of Treatment.



A: Fig. 2. Effect of Different Concentrations of *Bacillus thruingiensis israelensis* on Larval Life Span (Days) of *M. Domestica* Treated as 2nd Larval Instar.

B: Fig. 3. Effect of Different Concentrations of *Bacillus thrringiensis israelensis* on the Percentage of Pupation of *M. domestica* Treated as 2nd Larval Instar.

C: Fig. 4. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on the Percentage of Pupation of *M. domestica* Treated as 2nd Larval Instar.

D: Fig. 5. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on Mean of Pupation Weight (mg) of *M. domestica* Treated as 2nd Larval Instar.

E: Fig. 6. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on Pupation of *M. domestica* Treated as 2nd Larval Instar.

Life span of Pupae

The results recorded in Table (2) illustrated in Figure (5) show that the life span of the pupa phase was affected by the increase when feeding the larvae on a bacterial treatment environment at different concentrations, ranging from (3.67 ± 0.8.36) and (5.12 ±0.119) days compared to control group as it was (3.04±0.115).

Mature phase exit rate and sexual ratios

The results recorded in Table (3), illustrated in Figure (7), indicate that the mature phase-out rates were affected by a non-significant effect, since there was a decrease with increased concentrations, for example, (94.55, 88.89, 84.85, 84.21) at concentrations (0.5, 1.0, 1.5 2.0) Respectively when compared with (100%) in the control experiment. In the calculation of sexual ratios, the trend of the sex ratio was observed in terms of female production when the larvae were treated with concentrations (1.0 and 2.0%) The affected mature insects were observed slightly at (1.5%), Figure (10).

The results recorded in Table 3 illustrated in Figure (9)showed a significant reduction in the mature life span of the males and females. The males produced from bacterial larvae (Bti) showed reduction in life span compared to the control group, for example (7.6±1.3, 7.6±0.68, 8.4±0.40, 7.4±1.21) days at treatment with concentrations (0.5,1.0,1.5) respectively, compared to control group (9.6 ±0.51) days, females recorded (8.8 ±0.49,9.0±1.18, 8.8±1.02) days for the same concentrations used respectively compared with the control group that was (11.2 ± 0.37) days.

Fertility number of eggs laid by females

As recorded in Table 3, illustrated in Figure (8) it is shown that the number of eggs laid by female larvae fed to a (Bti) treated environment has decreased below the normal non-treated rates. The coefficients were significantly lower than the non-treated normal rates and the (P <0.01). This effect is increased by increasing the concentration of the bacterial pesticide in each treatment that the normal female laid (2142.00 ± 159.80 eggs / female). Treatment with a 2.0% concentration resulted in the lowest decrease in the number of eggs (964.50 ± 111.06 eggs / female) in the control group, while the treatment with (0.5%) concentration led to obtain a decrease of (1552.50 ± 157.80 eggs / female).

Table 3. Latent effect of different constrations of *Bacillus thuringiensis israelnsis* on some biological aspects of adult stage of *M. domestica* vicina treaded as 2nd larval instrar

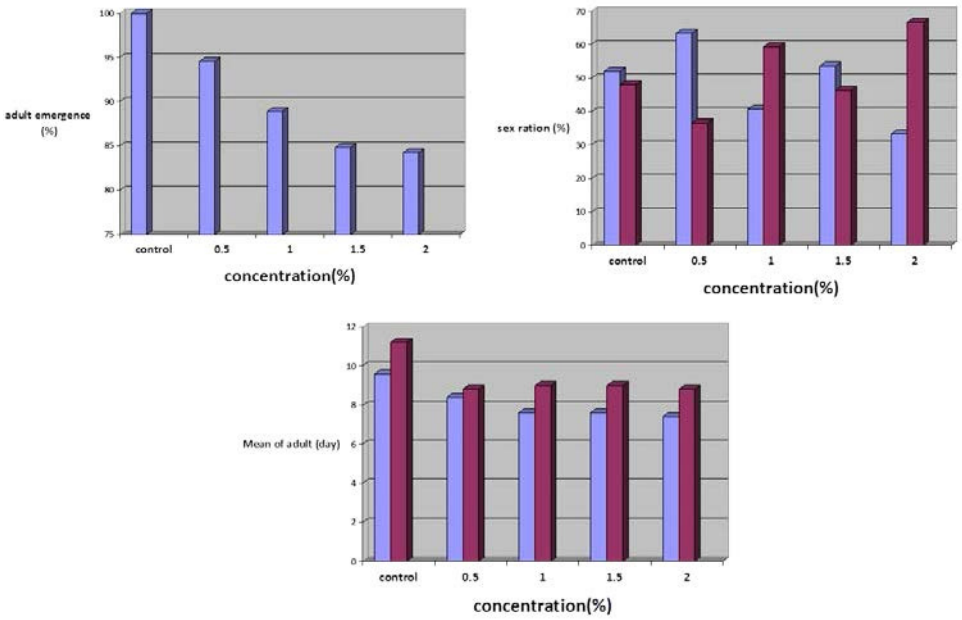
Con.(%)	Adult emergence (%)	+ or- (%)	Sex ration (%)		Adult longevity (days) Means ± SE			Fecundity (egg/female) mean ±SE	+ or – (%)	Fertility (%)	+ or – (%)	Adult malformation (%)	+ or (%)
			Male	Female	Male	+ or – (%)	Female	+ or – (%)					
Control	100	-0	52	48	9.6±0.51	-	11.2±0.37	-	2142±159.80	-	100	-	4	-
0.5	94.55	-5.46	63.46	36.54	8.4±0.40	-12.50	8.8±0.49	-21.43	1552.50±157.80**	-27.50	100	0	17.31	332.75
1	88.89	-11.11	40.63	59.38	7.6±0.68**	-20.83	9.0±1.18**	-19.64	1339.80±40.03**	-37.45	97.12	-2.88	21.88	447.00
1.5	84.85	-15.15	53.57	46.43	7.6±1.30**	-20.83	9.0±0.55**	-19.64	1289.75±40.03	-39.79	74.49	-25.51	28.57	614.25
2	84.21	-15.79	33.33	66.67	7.4±1.21**	-22.92	8.8±1.02	-21.43	964.50±111.06	-54.97	51.70	-48.30	37.50	837.50

Data Expressed as Mean ± Standard Error (S.E)
* significant (P< 0.05)
** Highly Significant (P< 0.01)

$$+ \text{ or } - (\%) = \frac{\text{Treated} - \text{control}}{\text{Control}} \times 100 = \text{percentage og increase or decrease as compared to the control}$$

Percentage of Eggs Hatching

We take into consideration that larval treatment at different concentrations on the percentage of eggs hatching (Table 3) (Figure 11) from the *Musca domestica* ranged from (51.70%) when processed with the concentration of (2.0% to 97.12%) and at the 1.0% concentration. The egg hatching rate is 0.5% relative to the control group (100%).



A: Fig. 7. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on The Percentage of Mature Emergence *M. domestica* Treated as 2nd Larval Instar.

B: Fig. 8. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on Sex Ration of *M. domestica* Treated as 2nd Larval Instar.

Fig. 9. Effect of Different Concentrations of *Bacillus thuringiensis Israelensis* on Mature Life Span *M. domestica* Treated as 2nd Larval Instar.

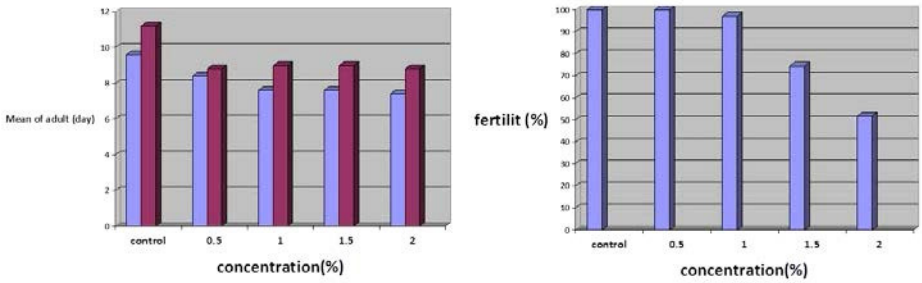


Fig. 10. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on Eggs Female (days) *M. domestica* treated as 2nd larval Instar.

Fig. 11. Effect of Different Concentrations of *Bacillus thuringiensis israelensis* on Eggs Female (days) *M. domestica* treated as 2nd larval Instar.

DISCUSSION

The use of manufactured high toxic pesticides with their harm environmental effect led to search for non-traditional means of control. The purpose of current thesis is to study the possibility of applying one of these methods, such as the extraction of substances and compounds that have the characteristic of insect toxicity such as pesticides to safe fight a dangerous insect to the environment and adversely affect the environment and Human health, *Musca domestica* Vicina, The bacterial pesticide *Bacillus thuriniensis* israelensis (Bti) was used as an alternative to the use of conventional chemical pesticides. The hemi lethal LC50 dose of the bacterial pesticide and the LC30 effect of the same pesticide were determined. Some biological considerations as the physiological and biochemical effects of Musca domestica Studies by [20], which supports the development of bacterial pesticides, continued with the study and production of new compounds such as Bacthuricin F103. They continued their research to develop bacteria-derived antibodies using protein, lipid chromatography to purify bacterial compounds and obtain highly efficient pesticides when penetrating the

Cellular wall in the middle intestine of insects, [21]protected the corn crop from the *Dibrotica virgiefra* virgifer worm by producing strains of a genetically engineered plant and supplying it with Cry3Bb derived from Bt. This protein affected the insect's life when fed in the **Bt** treated plant where The insect lost its ability to continue its life cycle by increasing the mortality rate in the larvae fed on Bt plant and this contributed to the reduction of fertility by inhibiting bacteria for eggs. [22] separated three new proteins with a clear toxicity on the *A. egypti* dendritic vector. These proteins are CrylA (B) CryIB and the possibility of using this microbial agent in the control of medical dipteral insects.

Bacillus bacteria produce different types of toxins during their life cycle, including external toxins(Alpha, Beta , Gamma) and one type of internal toxin known as the internal poison (Sigma). The ability of these toxins to cause the death of the insect larvae of the dipteral and scalp insects [23] Many scientists have been interested in studying the toxicity, biochemical, structure, and pathological and histological influence of this toxic protein molecule on insect larvae, especially dipteral. [24] studied the environmental activity of a number of **B.T** bacterial isolates against some *Aedes aegypti Drosphila melangaster* and *Musca domestica* larvae and demonstrated that all isolates had a named activity against the tested larvae and agreed with our results that **B.T** as a bio component against *Musca domestica* larvae.

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