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Biological control of the larval mosquito *Culex pipiens* Say using bio-pesticide *Bacillus thuringiensis israelensis* and growth regulator Dimilin

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ABSTRACT: This study was conducted at the Research Laboratory / Animal House Animal / College of Science / Mustansirhiya University to evaluate the efficiency of the growth regulator Dimilin chitin synthesis inhibitor by three different concentrations of 0.5, 1.0 and 1.5 ppm and efficiency of bacteria *Bacillus thuringiensis israelensis* concentrations 6.25×10^9, 6.25×10^{10} and 6.25×10^{11} spores / ml at different instars larval mosquito *Culex pipiens*. Highest mortality was 100% of the growth regulator at 1.5 ppm for a period of 48 hours and lowest mortality was 26.67 % for the 12-hours period of concentration of 0.5 ppm for the first instar larval, respectively. The highest mortality was 86.67 % of the growth regulator at 1.5 ppm within 48 hours and lowest mortality was 20 % during the 12-hours concentration of 0.5 ppm for the second instar larval, respectively. The results showed that the highest mortality was 73.33 % during the 48-hours concentration of 1.5 ppm and lowest mortality was 33.33 % of the concentration of 1.0 ppm during the 12 hours of third instar larval respectively. The highest mortality of the fourth instar larval reached 80% within 48 hours and lowest mortality was 26.67 % of the two concentrations 0.5 and 1.0 ppm for growth regulator.

The results showed that the highest mortality of bio-pesticide Bti was100% of the first instar larval at 6.26×10^{11} spores / ml within 24 to 48 hours and lowest mortality was 40% of the concentration of 6.26×10^9 spore / ml within 12 hours. The highest mortality of second instar larval was 100% for two concentrations 6.25×10^{10} and 6.25×10^{11} spores / ml within 24 and 48 hours, respectively, and lowest mortality was 53.33% of the concentration of 6.25×10^9 spore / ml within 12 hours and the highest mortality of the third and fourth instar larval 100%, respectively, during the 48-hours concentration of 6.25 × 1011 spores / ml, and lower ratios for killing concentration 6.25 × 109 spore / ml was 40% and 33. 33% for the two ages, respectively, within 12 hours.

KEYWORDS: Biological control, larval mosquito Culex pipiens Say, bio-pesticide Bacillus thuringiensis israelensis, growth regulator Dimilin.

INTRODUCTION

The mosquitoes of the enemies of man as the father of his transmission serious diseases such as malaria and Filarial, yellow fever and other and led to death. In 2008, the number of people diagnosed with malaria in the world's 243 million people, almost 863,000 people has died (Howard et al; 2010).

Mosquitoes spread mainly in Iraq from the far north and south, even as there are in Iraq 16 species of Anopheles. Some of *Anopheles spp* which have not previously registered in Iraq and 18 species of Culex belongs to the family of Culicidae, one of the most important families in the rank of bilateral wings Diptera. The family includes under Culicinae belongs to this family of many races and is Aedes, Anopheles, Culex As for the genus Culex fitted with many types of the most important of Culex pipiens and called this kind *Cx. Complex* because it has three subspecies are:

Cx.pipiens pipiens, Cx.pipiens molestus, Cx pipiens quinquefasciatus, spreading mosquitoes *Cx.pipiens quinquefascitus* in Iraq in abundance in the central and southern regions, either *Cx.pipiens molestus* Venture in northern Iraq, particularly in the area of Mosul, *Cx.pipiens pipiens* it spreads different areas of Iraq, including Baghdad, especially in homes and breed in

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different environments, such as waste water and sewer openings and small ponds and pots and cans and abandoned pools of rain water and tunnels and wells (Abu Alhab.1979).

Have used many chemical pesticides to combat mosquitoes and various insects and harmful aquatic including mosquitoes despite chemical pesticides have the great successes in eliminating the causes of insect-borne diseases to humans , animals and plants and kill other types of beneficial insects (Al-Adel.1979) , but the control these diseases has become very difficult due to increased insect resistance to pesticides in spite of its significant contribution to the control of the disease , they have the ability to water pollution , soil and food their presence to change the attributes of quality to the atmosphere of the Biosphere (Rajkumar and Jebanesan, 2005).

Can get rid of the larvae and adult mosquitoes also through prevention and control , prevention is meant to reduce mosquito breeding by removing the drinking water for mosquito breeding and filling ponds and poured the remaining water in pots and drums and not allowed to assemble and disposal and cleaning of beaches , sewage and streams and the edges of the channels , while the adult can be taken away and to prevent its arrival to humans and animals , using metal grille with small openings for windows and doors and exposing rooms to the light and prevent moisture and fogging squares and large halls (Abu Alheb.1979) .

The failure to find new groups of pesticides has led many scientists to the discovery of new studies to look for ways to the vitality and economic alternative (Shalaan et al; 2006).

THE AIM OF THE STUDY

The present study aims to use unconventional means to combat the mosquito larvae of *Culex pipiens* by examining :

1- Effect of growth regulator Dimilin on the larval instars of mosquito *Culex pipiens* .
2- Effect of growth regulator Dimilin on larval different ages and compared with bio-pesticide *Bacillus thuringiensis israelensis*.
3- Study the impact of bio-pesticide as means anti-chemical alternative for reconstruction of different larval mosquito Culex pipiens using bio-pesticide *Bacillus thuringiensis israelensis*

MATERIALS AND METHODS

Collecting and breeding mosquito *Culex pipiens* Say:

Collected larvae of the mosquito Culex pipiens Say from Center for Disease transitional / Ministry of Health / Andalus Square / Baghdad- Iraq in the month of April 2013. Has larval rearing in laboratory/ House Animal / Department of Life Sciences / College of Science / Mustansirhiya University where developed larvae in the basins of the plastic 500 mL container over water tap 150 ml exposed to the sun up to 72 hours to get rid of the chlorine in the water normal (Hill and John 2007).

These larvae feed and fishmeal Fish consisting of (protein 35%, fat 2.5%, fiber 2.5%, 0.9-2% calcium, phosphate 0.8 -1.8%) with water basin notice Substituted from 4-3 days in order to avoid rot water and after turning the larvae to pupae and placed in wooden cages cubic shape, especially for the breeding of insects., and adult placed in the pot 500 mL of 150 ml tap water for up to 72 hours, and put pots inside cages breeding wooden dimensions (30 × 30 × 30 cm) closed Clamp metal from four sides and is covered with a cloth cylinder from one side, and the base of the timber and put cages in incubator at temperature 27 ± 2 C˚, and Lighting 12 hours a day, after the emergence of adult fed males using cotton swabs saturated with a solution of 10% concentration of sugar and placed in a glass dish inside the cage,

The females were fed using a dove (Pigeon) feather plucking them from the chest area and put the dove above the cage after tying their wings, and legs all night (Overnight) so that the female can get blood meal easily (Sivagnanme and Kalyanasundaram, 2004).

After 2-3 days collected Boats eggs (Egg rafts) set by the female feeders on blood transferred boats by a soft brush to basins clean glass with water forsaken for 72 hours, taking into account not move the boat to avoid the disintegration and break it, reared colony for three generations before initiate a treatments for the purpose of getting rid of the remnants of chemical and biological pesticides used to control them (al-Bandar 2013).

Preparation peptone water:

Peptone water attended peptone water of 15 grams of powder peptone to 1 liter of distilled water after sterilization by autoclave for 15 minutes under the temperature of 121 ° C and pressure of 1 atmosphere pH has 7.2 consisting of 10.0 g of peptone and 5.0 g sodium chloride.

Prepare nutritious media Nutrient agar:

Attended media appropriate and nutritious is Nutrient Agar by the manufacturer's recommendations (England, Basingstoke, Hampshire) using 28 g of the media in one liter of distilled water and then sterility device Autoclave at 121 ° C for 15 minutes, and pressure 1 atmosphere, and poured in Petri dishes, Bti planted isolation by way of strip by sterile Loop, and incubated at 37 ± 2C° for a period of 24 hours for bacterial growth was observed in all pure isolates.

Development and activation of the bacterium *Bacillus thuringiensis israelensis:*

The process of activating the bacteria by adding 1 g of powdered bacteria to 9 ml of peptone water, and planting nutrient agar dishes, sterile Petri to feed the bacteria and then planted in a manner of striking by Loop sterile, and put the sterilizer dishes in an incubator at a temperature of 37 $\pm$ 2 C° for 24 hours, observed the growth of bacteria and harvested by Loop sterile L-shaped to prepare different concentrations for the treatment of mosquito larvae of *Culex pipiens*, where isolation photographed using a compound microscope and the use of a digital camera.

Prepare concentrations and calculate the number of spores of the bacteria Bti:

Added 5 ml of distilled and sterilized water to the bacterial prepared on the media nutritious Nutrient agar in dishes with the addition of 0.2 ml of Tween-20 concentration of 0.20%, and harvested spores by the glass rod-shaped L, and filtered the contents by the suppression of a glass contains a piece of sterilized gauze with the addition of 5 ml of distilled and sterilized water to ensure filtering of all bacteria spores. And collect the filtrate in a glass beaker containing 10 ml of distilled water and suspended bacterial spores, is a suspended Stock suspension.

To calculate the number of spores bacterial been using a slide count Haemocytometer own to count the white blood cells where Added 1 ml of the stock solution to 99 ml of sterile distilled water. Then put 0.1 ml of it on a slide count after placing cover slide was counting the number of spores, according to the following equation (Aube and Gagnon, 1969).

Number of spores (spore / ml) = rate of spores × dilution factor × 25 x 10^4

Study the effect of the concentrations of spores' bacteria Bti in the different instars of *Culex pipiens* larvae:

To study the effect of three concentrations of bacteria Bti on first, second, third, fourth stage larvae and concentrations were(6.25×10^9, 6.25×101^{10}, $.256 \times 10^{11}$ spore / ml) where took 5 larvae of every stage and put in a pot winy 250 mL put 150 ml tap water up to for 72 hours, add 3 ml of each concentration of suspended bacterial Bti ml, with the addition of 1.5 g of fish diet for the purpose of feeding larvae repeated the experiment with three replicates per concentration.

Covered all the treated pots with pieces of cloth tulle linked bond rubber, and took reading after 12, 24 and 48 hours, to determine the percentage of mortality for each stage of larvae and Note distortions occurring in the larvae and the proportion of the emergence of the adult (Ishii and Ohba 1993).

The control was placed 5 larvae for each stage in the basin winy 250 mL, put 150 ml tap water up to 72 hours with the addition of an aqueous solution with Tween-20 concentration of 0.02% and with three replicates for each instars larval and each concentration.

Study the effect of growth regulator Dimilin in mosquito larvae *Culex pipiens*:

Obtained growth regulator Dimilin from the College of Agriculture - University of Baghdad to control mosquito larvae *Culex pipiens* after transferred 5 larvae for each stage and placed in a pot of wine 250 mL, put 150 ml of water, the usual exhibition of the sun and prepared already, and added different concentrations of this growth regulator which were 0.5, 1.0 and 1.5 ppm for all larval instars, With the addition of 1.5 g of diet fish in order to feed the larvae and repeated the experiment three times for each concentration and each instar larval, either the control treatment and placed 5 larvae of every instar in the pot of 250 ml, put 150 ml of water normal sun-exposed in advance with the addition of aqueous solution with Tween-20 concentration of 0.02%, and repeated treatment to control three times for each instars larval and each concentration and covered all the treatments and control cover cloth tulle and tied in the bond of rubber were followed larvae were recorded after 12, 24 and 48hours, to determine the percentage of mortality for each larvae instars, Note distortions occurring in the larvae and The percentage of adult emergence.

RESULTS AND DISCUSSION

1 - The effect of different concentrations of growth regulator Dimilin in first larvae instar of the *Culex pipiens*:

Showed results in Table (1) the effects of the concentrations of growth regulator Dimilin 0.5, 1.0 and 1.5 ppm and the time in the mortality of first instar larvae *Cx. pipiens* and notes that the highest mortality was100% for concentration1.5 ppm after 48 hours and lowest mortality for the same concentration reached 73.33% ppm after 12 hours, The highest mortality was 73.33% for the concentration0.5 ppm after 48 hours of treatment and lowest mortality was 26.67% after12 hours while the highest mortality was 86.67% at concentration 1.0 ppm either lowest mortality was 53.33% for the same concentration during the 12 hours of treatment.

The table shows the sensitivity of first instar was increasing according to increasing the concentration of Dimilin and increasing the period of treatment, as is evident in the picture (1) the highest mortality Compatible to the highest concentration 1.5 ppm and for long a period 48 hours and lowest mortality harmonious with lowest concentration 0.5 ppm and with less period 12 hours and Affect growth regulator on the occurrence of abnormalities in the larvae deformation of the wall of the body and chitin layers as set out in the picture (1). Statistical analysis showed significant differences between the concentrations and time of the mortality.

The results come consistent with (Hadley et al 1987) when exposed to the immature stages to growth regulator Dimilin get distortions of the larvae's body, which depends on the concentrations as the first and second larval instars more sensitive than third and fourth larval instars. And agree with (Najwa et al 2004) that the mortality for mosquito larvae *Cx.pipiens* commensurate with the concentration and reached mortality to 90% at concentration of 40 ppm after 3 days of treatment.

Table1: Effect of times and different concentrations of growth regulator Dimilin in mortality to the first instar larvae of Culex pipiens.

LSD	The periods / Hours			Concentrations Ppm
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 23.07	6.67 ± 73.33	6.67 ± 53.33	6.67 ± 26.67	0.5
* 23.07	6.67 ± 86.67	6.67 ± 73.33	6.67 ± 53.33	1.0
* 18.83	0.0 ± 100.00	6.67 ± 86.67	6.67 ± 73.33	1.5
---	* 18.83	* 23.07	* 23.07	LSD
(P<0.05)				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

2 - Effect of time and different concentrations of growth regulator Dimilin in second instar of the *Culex pipiens*:

Results in table (2) Showed the highest mortality at concentration of 0.5 ppm was 73.33% within 48 hours and less mortality was 20% within 12 hours, and the highest mortality at concentration 1.5 ppm was 86.67% within 48 hours and less mortality reached 60% within12 hours after treatment, Statistical analysis explained significant differences between the various above concentrations and the times, either at 1.0 ppm concentration reached highest mortality 60% and less mortality reached 46.67%, The statistical analysis shows no significant differences for different times. We note little resistance to the second instar larvae meaning it is less sensitive to the first instar larvae to growth regulator Dimilin notes have not been achieved mortality of 100%.

These vary results to (Mohsen et al 1989), where the sensitivity of larvae increases with exposure to growth regulator as well as the highest mortality larvae *Cx.pipiens* was 96% and 100% at concentrations of 1.3 and 3.23 ppm for growth regulator Dimilin after 3 days of exposure respectively.

These findings are consistent with Mulla (1995) in the same effect on *Cx. quinquefasciatus* where the mortality was 74.4% at the concentrations of 0.1 ppm, which causes a delay in the development of the immature stages.

Table 2: Effect of times and different concentrations of the growth regulator Dimilin in the mortality of second instar larvae of Culex pipiens.

LSD	The periods / Hours			Concentrations ppm
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 18.63	6.67 ± 73.33	6.67 ± 53.33	0.00 ± 20.00	0.5
29.78	11.5 ± 60.00	6.67 ± 46.67	6.67 ± 46.67	1.0
* 18.63	6.67 ± 86.67	6.67 ± 66.67	0.00 ± 60.00	1.5
---	* 29.78	* 23.07	* 13.31	LSD
(P<0.05)				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

3: the effect of time and the different concentrations of growth regulator Dimilin in the mortality to the third instar larvae of the *Culex pipiens*:

The table (3) show the results to the highest mortality of third instar larval when the concentration 0.5 ppm the mortality was 53.33% of the time 48 hours and less mortality was 40% of the time 12 hours and shows the statistical analysis, no significant differences for different times, The highest mortality was 73.33% at the concentration of 1.5 ppm for the time of 48 hours and less mortality was 46.67% for the 12 hours' time, and the highest mortality was 66.67% and the lowest mortality was 33.33% for the 12 hours' time when the concentration 1.0 ppm shows the statistical analysis to the presence of significant differences of concentrations 1.5 and 1.0 ppm and of different times.

These results are consistent with what it says (Duel et al 1978) that the compounds of growth regulators Not occur a quick death, but urges the inhibition of the synthesis of chitin and separated from the Epidermal through a process of molting.

These results differ to the findings by (Najwa et al 2004) show that growth regulator had a fatal effect against larvae *Cx.pipiens*. Had attributed the difference in results to the time difference and different environmental conditions and method of making treatments.

Table 3: Effect of times and different concentrations of growth regulator Dimilin in the mortality of the third instar larvae of the Culex pipiens.

LSD	The periods / Hours			Concentrations ppm
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
18.63	6.67 ± 53.33	6.67 ± 53.33	0.00 ± 40.00	0.5
* 23.07	6.67 ± 66.67	6.67 ± 46.67	6.67 ± 33.33	1.0
* 23.07	6.67 ± 73.33	6.67 ± 66.67	6.67 ± 46.67	1.5
---	* 23.07	* 23.07	* 18.83	LSD
(P<0.05)				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

4 - Effect of times and different concentrations of growth regulator Dimilin in mortality of fourth instar larvae of *Culex pipiens*:

Shown in Table (4) that the highest mortality was 80% at the concentration of 1.5 ppm in 48 hours and less mortality was 53.33% for the 12-hour time and the statistical analysis, there were no significant differences to concentrations and times, either higher mortality to the concentrations 1.0 and 0.5 ppm were 66.67 and 53.33%, respectively, within 48 hours, and lowest mortality was equal to the above concentrations was 26.67% during 12 hours, and the statistical analysis shows that there was significant differences for two concentrations and different times. Note for abnormalities superficial and obvious change such as in the trunk of the body of treatment adult emerging and deformation of the head and be thinner than the adult of non-treatment for third and fourth instars larval, as shown in the picture (5) (B, C, E). These results conform to the findings by(Prakash 1993) proved that where the growth regulator toxic effect used to control mosquito larvae *Cx.pipiens*.

(Mulla 1995) found that the compounds of growth regulators urging quick death, and when the larvae treatment with a growth regulator will lead to kill larvae during the moult and this note the absence of pupae Incidence distortions to both the larvae and pupae and adult, as shown in the picture (1), And thus will fail larvae and pupae in the removal of the old cuticle, and He explained the mortality of *Cx.quinquefacium* larvae reached 74.4% at a concentration of 0.1 ppm for growth regulator Dimilin.

This may be attributed the difference in the results of this study to the method of testing and the difference in the type of mosquitoes as well as the difference in the factors of environmental conditions.

Table 4: Effect of times and different concentrations of growth regulator Dimilin in mortality of the fourth instar larvae of Culex pipiens.

LSD	The periods / Hours			Concentrations ppm
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 23.07	6.67 ± 53.33	6.67 ± 46.67	6.67 ± 26.67	0.5
* 29.78	6.67 ± 66.67	11.5 ± 40.00	6.67 ± 26.67	1.0
35.24	11.5 ± 80.00	11.5 ± 60.00	6.67 ± 53.33	1.5
---	* 29.78	* 35.24	* 23.07	LSD
(P<0.05)				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

(B)

(A)

(D)

(C)

Image (1) (A): describes the larva is a natural not treatment with growth regulator Dimilin
B): larva growth regulator treatment note biodegradable layer cuticle
C): Image shows the decomposition of the larva's body is fully
D): A photo showing the deformation of the head of the larva treatment
Power magnification (40X)

5- The effect of times and different concentrations of the bio-pesticide *Bacillus thuringiensis israelensis* (Bti) in the mortality of the first instar larvae of *Culex pipiens*:

The results showed in Table (5) to the highest mortality reached 100% at the concentrations of 6.25×10^{11} spores / ml of the time 48 hours, and less mortality of the same concentration was 73.33% for the 12 hour time, and the highest mortality of two concentrations 6.25×10^{9} and 6.25×10^{10} spores / ml was 86.67% for the 48hour time, Less mortality reached 40% at a concentration of 6.25×10^{9} spore / ml, and less mortality was 53.33% at the concentration 6.25×10^{10} spores / ml for 12hour time, and statistical analysis shows significant differences between the different treatments and different times.

These effects as a result of the deadly toxic effects caused by bacteria Bti and agree this result to (Ibrahim 1990) larvae mortality of *Culex pipiens* reached to100% within 3 days when the bacteria Bti concentration 1.6×10^{3} spore / ml.

As well as agree on what it says (Shoukry 1986) to the deadly toxin produced by the bacteria Bti treatment where larvae affect the physiological processes within the gastrointestinal tract, leading to kill the larvae.

Table 5: Effect of times and different concentrations of the bio pesticide Bacillus thuringiensis israelensis (Bti) in the mortality of first instar larvae of Culex pipiens.

LSD	The period / Hours			Concentration Spore/ml
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 29.78	6.67 ± 86.67	6.67 ± 73.33	11.5 ± 40.00	6.25×10^{9}
* 23.07	6.67 ± 86.67	6.67 ± 73.33	6.67 ± 53.33	6.25×10^{10}
* 13.31	0.0 ± 100.0	0.0 ± 100.00	6.67 ± 73.33	6.25×10^{11}
---	* 18.83	* 18.83	* 29.78	LSD
.(P<0.05) *				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

6- The effect of times and different concentrations of the bio-pesticide *Bacillus thuringiensis israelensis* (Bti) in the mortality of the second instar larvae of *Culex pipiens*:

Indicates Table (6) to the lower resistance and high mortality to second instar larval, the mortality was100% for two concentrations 6.25×10^{10} and 6.25×10^{11} spore / ml within 24 hours and less mortality was53.33% at the concentrations of 6.25×10^{9} spore / ml during the 12hour and 93.33% at concentration 6.25×10^{11} spore / ml during the same period.

The statistical analysis indicates significant differences for two concentrations 6.25×10^{9} and 6.25×10^{10} spore / ml and the no significant differences for the concentration of 6.25×10^{11} spore / ml .

The results were consistent with the results of (Lee et al 2003) that the bacteria Bti cause mortality ranging from 85-100% against larvae of *Culex pipiens*, and the results had proven the researchers (Poopathi and Abidha 2010) which showed mortality was 96% of the *Culex pipiens* larvae.

Table 6: Effect of times and different concentrations of the bio pesticide Bacillus thuringiensis israelensis (Bti) in the mortality of second instar larvae of Culex pipiens.

LSD	The period/ Hours			Concentration Spore /ml
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 18.83	0.0 ± 100.0	6.67 ± 93.33	6.67 ± 53.33	6.25×10^9
* 23.07	0.0 ± 100.0	0.0 ± 100.0	11.5 ± 60.00	6.25×10^{10}
13.31	0.0 ± 100.0	0.0 ± 100.0	6.67 ± 93.33	6.25×10^{11}
---	* 10.00	* 13.31	* 29.78	LSD
P<0.05				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

7- The effect of times and different concentrations of the bio-pesticide *Bacillus thuringiensis israelensis* (Bti) in the mortality of the third instar larvae of *Culex pipiens*:

Table 7 indicates significant differences for the concentrations and different periods. The highest mortality reached 100% at the low concentration of the bio pesticide 6.25×10^9 spore / ml for a period of 48 hours exposure, as well as for two concentrations 6.25×10^{10} and 6.25×10^{11} spores / ml for the same period of exposure.

Less mortality was 40% for the concentration of 6.25×10^9 spore / ml within 12 hours, and the less mortality for two concentrations 6.25×10^{10} and 6.25×10^{11} spores / ml were 53.33 and 80% within 12 hours., And equal mortality 73.33% for two concentrations 6.25×10^9 and 6.25×10^{10} spores / ml for 24 hours' time.

We notice the results of the effect for the Bti bacteria in third instar larvae these results were compatible with the (Jamil 2007) found the mortality was within the range 91-100% using different concentrations of local isolates of Iraq against the third instar larvae of the mosquito *Cx pipiens*.

Table 7: Effect of times and different concentrations of the bio pesticide Bacillus thuringiensis israelensis (Bti) in the mortality of third instar larvae of Culex pipiens.

LSD	The period / Hours			Concentration Spore / ml
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 13.31	0.0 ± 100.0	6.67 ± 73.33	0.00 ± 40.00	6.25×10^9
* 18.83	0.0 ± 100.0	6.67 ± 73.33	6.67 ± 53.33	6.25×10^{10}
* 13.31	0.0 ± 100.0	6.67 ± 93.33	0.00 ± 80.00	6.25×10^{11}
---	* 10.00	* 23.07	* 13.31	LSD
P<0.05				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*

8- The effect of times and different concentrations of the bio-pesticide *Bacillus thuringiensis israelensis* (Bti) in the mortality of the fourth instar larvae of *Culex pipiens*:

The results shown in the table (8) for the fourth instar larval that the highest mortality was 100% of the two concentrations 6.25×10^9 and 6.25×10^{11} spore / ml for 48 hours' time. The lowest mortality was 33.33 and 73.33% of the

concentrations 6.25×10^9 and 6.25×10^{11} spore /ml respectively, within 12 hours. We note at the time 24 hours had equal mortality for two concentrations 6.25×10^{10} and 6.25×10^{11} spore / ml while the highest mortality was 93.33% of the concentration of 6.25×10^{10} spore / ml and 48-hour time.

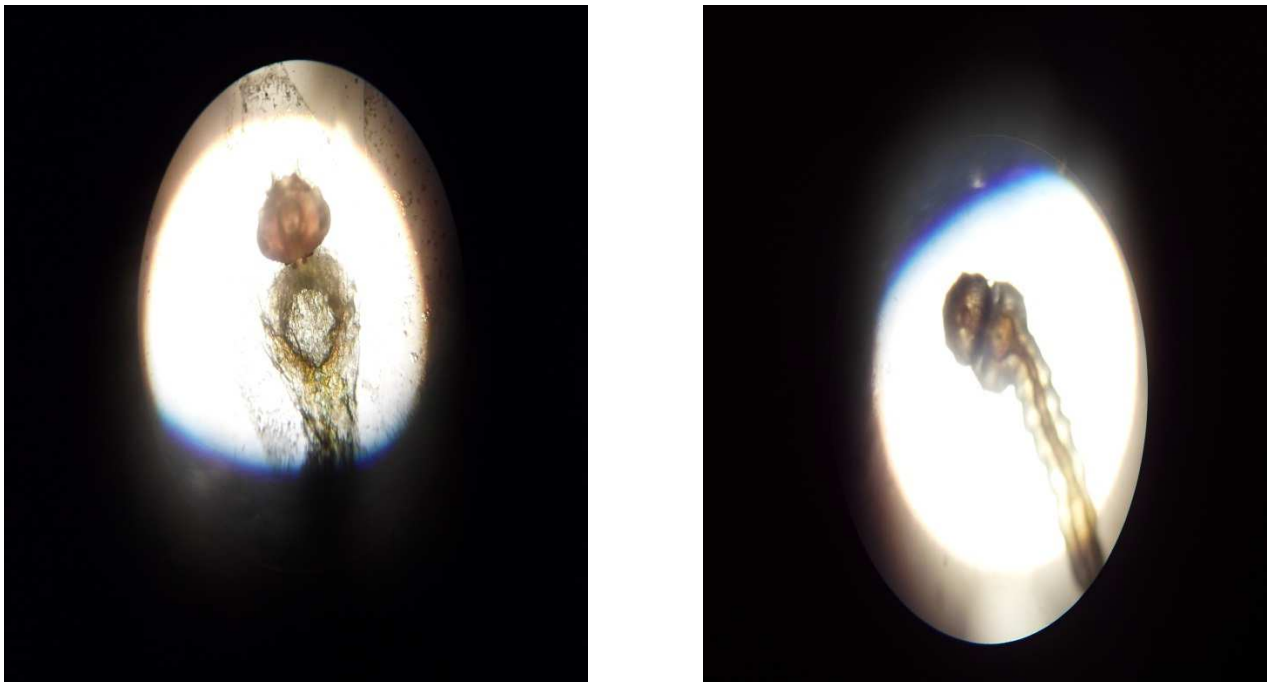
The larvae treated with bacteria Bti leads to a deformation in the body and deformation of the gastrointestinal tract as shown in the picture (2), The larvae remaining and converts it to the pupae they die when developed to adults as a result of the deformation, which happens to body of the adults, and this result was conformity with (Zayed and Bream 2004), where they noticed the effect of bacteria Bti on the pupae at the emergence of adults where noted deformities size for adults where the size of the adult abnormal, leading to her death when emerge directly.

The results identical with each of (Hafez 2000) and (Lima et al 2005) where the mortality was 95% of the mosquitoes *Cx.quinquefasciatus* during periods 24 and 48 hours when exposed to bacteria Bti during the fourth instar larvae.

Table 8: Effect of times and different concentrations of the bio pesticide Bacillus thuringiensis israelensis (Bti) in the mortality of fourth instar larvae of Culex pipiens.

LSD	The period / Hours			Concentration Spore / ml
	48	24	12	
	Mortality %			
0.00	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0
* 18.83	0.0 ± 100.0	6.67 ± 66.67	6.67 ± 33.33	6.25×10^9
* 18.83	6.67 ± 93.33	0.0 ± 80.00	6.67 ± 66.67	6.25×10^{10}
* 13.31	0.0 ± 100.0	0.0 ± 80.0	6.67 ± 73.33	6.25×10^{11}
---	* 13.31	* 13.31	* 23.07	LSD
P<0.05				

** Indicate the presence of significant differences between the different treatments at the level of the probability (P <0.05), according to test less significant difference (LSD)*



(B) (A)

Image (2): (A): larva does not treatment of bio-pesticide Bti (Control).
(B): larva treatment of bio-pesticide Bti note decomposition guts.
The power of magnification (40 x)

CONCLUSIONS

1. The study showed the effect of growth regulator Dimilin on the mortality of *Culex pipiens* larvae, especially for the high concentration of 1.5 ppm and the mortality was100%.
2. Effect of growth regulator on the composition chitin layers where Dimilin chitin synthesis inhibitor treatment is during larval development, leading to deformation of the larvae of different instars larval and thus deformation for pupae resulting from treated larvae and the adult emerging from pupae.
3. The results show that all concentrations of biological pesticide *Bacillus thuringiensis israelensis* used in the study are more effective than the effect of growth regulator Dimilin.

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