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Larvicidal action of some bacterial formulations against *Culex pipiens* mosquito larvae

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Abstract

Culicine mosquitoes are vectors of filaria larvae that causes elephantiasis. This study comprised the effect of some bacterial formulations of *Bacillus thuringiensis israelensis* (*B.t.i.*) on 2nd, 3rd and 4th larval instars of *Culex pipiens* larvae. One formulation was the International Pasteur Standard of *B.t.i.* (IPS-78) and the other three were commercial formulations (Bactimos, Vectobac and Bactoculicide). The most potent formulation was Bactoculicide of which the LC_{50} values were 0.019-0.107 ppm and LC_{95} values were 0.097-0.175 ppm; followed by IPS-78 which showed LC_{50} values of 0.038-0.077 ppm and LC_{95}s were 0.086-0.159 ppm. Bactimos occupied the third rank of potency followed by Vectobac. The high decrease in potency of the latter both formulations may be due to the long storage period.

Key words: *Culex pipiens*, *Bacillus thuringiensis israelensis*, bacteria, larvicides

Introduction

In recent years, research on the development of eco-friendly and target-specific biocontrol agents has been given much importance. The microbial insecticides are selective in their action on mosquitoes and blackflies. *Bacillus thuringiensis israelensis* (B.t.i.) serovar. H-14, discovered in the 1970s, was found to be effective against larvae of several species of mosquitoes **(de Barjac, 1978)**. The efficacy of *B.t.i.* and its safety to its nontarget organisms have been reviewed extensively **(Das and Dominic Amalraj, 1997)**.

Microbial control of insect vector populations can be highly effective and generally has advantages over chemical control because many are host specific and safe for non-target organisms. Entomopathogenic bacteria used successfully in microbial control programs to decrease mosquito larval populations are *Bacillus thuringiensis* and *Lysinibacillus sphaericus* **(WHO, 1999)**. During the 1990s, a worldwide program for isolation of entomopathogenic *Bacillus* was encouraged by the World Health Organization (WHO). In Brazil, studies which were made in the Southeast region and focused on Simuliidae (black fly) and Culicidae (mosquito) larvae from which 18 strains of *B. thuringiensis* and one of *L. sphaericus* were isolated **(Cavados *et al.*, 2001)**. Among the isolates, at least one that had high toxicity against *Simulium pertinax* and *Aedes aegypti* larvae, was shown to be more effective than the strains used in the commercial production of bio-insecticides **(Cavados *et al.*, 2005)**. As part of this research program, the histopathological effects of these toxins in the posterior midgut cells of *S. pertinax* larvae provided additional knowledge on the mechanisms of δ-endotoxins in dipteran larvae **(Cavados *et al.*, 2004)**.

The larvicidal activity of *B. thuringiensis* is due to the toxins found in the parasporal inclusions that are produced at the time of sporulation. Collectively referred to as δ-endotoxins that comprise a diverse group of proteinaceous toxins that have highly specific activity against larvae, especially those of the Lepidoptera, Diptera and Coleoptera **(Feitelson *et al.*, 1992)**. The toxins with high larval activity for Diptera (Cry4A, Cry4B, Cry11A and Cyt1A) are found in *Bti* and in some other serovarieties that produce the same or similar toxins **(Lacey *et al.*, 1982)**. Invertebrates provide a rich habitat for micro-organisms. In this study, special attention is paid for testing the larvicidal effect of bacterial bioinsecticide formulations from *Bacillus thuringiensis israelensis* against *Culex pipiens* larvae.

Materials and methods

1. Rearing and maintenance of *Culex pipiens* culture

Mosquito larvae were purchased from Research Institute of Medical Entomology, Dokki, Giza, Egypt. The culture was reared under laboratory conditions of about 30°C and photoperiod ranged from 12-15 h. Crowding of larvae was avoided in the plastic rearing pans, which were half-filled with dechlorinated water. The larvae in each pan were provided daily with 0.01-0.02 g/pan of dry yeast pellets. The culture was continuously cleaned and water was regularly replaced by fresh supply. The pupae were collected and placed in plastic cups half-filled with dechlorinated water kept in a cage of 20 × 25 × 25 cm covered with wire mesh. A piece of cotton saturated with 10% sugar solution was suspended from the roof of the cage for feeding adult males. The cage was provided with a partially feathers-removed pigeon as a source of blood meals for females. The floating oviposited egg rafts were

collected and placed in water pans to begin a new life cycle.

2. Bacterial formulations of *B. thuringiensis israelensis*

a) International Pasteur Standard (IPS-78), is an international standard powder with 1000 International Toxic Units (ITU/mg), against *Aedes aegypti*, produced by Pasteur Institute, Paris, France.

b) Bactoculicide, is a lyofilizat, 266/1, containing 30×10^{10} spores/mg and 1800 ITU/mg (Allunion Institute of Agricultural Microbiology, Leningrad, USSR, 1979).

c) Bactimos, is a commercial wettable powder with 6000 ITU/mg produced by Biochem. Products, Avenue Louise 479, Bte 53, Bruxelles, Belgium.

d) Vectobac, is a commercial formulation powder produced by Abbot Laboratories, North Chicago, Illinois, U.S.A.

3. Bioassay protocol

The bioassay was carried out according to the protocol procedures for *B.t.i.* proposed by **WHO (1981)**. The 2nd, 3rd and 4th instar larvae of *Culex pipiens* were used in the bioassay after starvation for 12 h to ensure ingestion of bacteria. Two hundred mg of dry bacterial formulation were homogenized with 5 ml dechlorinated water in a 10 ml sterile test tube with an electric homogenizer untill breakdown of all clumps of the product. This was checked by examining a droplet of the suspension under the phase contrast microscope. The homogenized suspension to be bioassayed was prepared in serial dilutions. According to the orientation tests, only dilutions which gave mortalities distributed around 50% were used. For each dilution, 150 ml were put into a plastic cup and 25 larvae of the tested instar were transferred into it. The experiment was kept at controlled temperature of 30 ± 2°C. Four replicates of cups (100 larvae) were used per each concentration and a similar number was used in same volume of dechlorinated water as a control for each bioassay. The dead larvae were counted and the mortality percentage was calculated after 24 and 48 h. If the mortality percentage of control exceeded 10%, the bioassay was discarded. Every bioassay was repeated three times and the averages of mortality percentages, LC_{50} and LC_{95} were calculated.

4. Statistical analysis

The data were statistically analyzed with probit analysis test. The used program is SPSS 15.0 for Windows Evaluation Version Release, Copyright © SPSS Inc., 1989-2006.

Results

1. Bioassay of IPS-78

After 24 h exposure to IPS-78, the LC_{50} values were 0.046, 0.072 and 0.077 ppm and after 48 h, were 0.038, 0.055 and 0.059 ppm for 2nd, 3rd and 4th instar larvae, respectively. After 24 and 48 h, LC_{50} ratios were 1 : 1.57 : 1.67 and 1 : 1.45 : 1.55 for the three larval instars, respectively. This indicated that the tolerance of larvae towards the pathogen increases with the increase of larval age. The LC_{95} values were 0.116, 0.151 and 0.159 ppm after 24 h and 0.086, 0.127 and 0.133 ppm after 48 h for 2nd, 3rd and 4th instar larvae, respectively. The LC_{95}s are higher than LC_{50}s with 2.52, 2.09 and 2.07 folds after 24 hours and 2.26, 2.31 and 2.25 folds after 48 h for 2nd, 3rd and 4th instar larvae, respectively. This meant that reaching 100% mortality of larvae required about 2-3 folds of the LC_{50} values (Table 1). LC_{50}s and LC_{95}s after 24 h are higher than those after 48 h and this indicates that the prolonged exposure time needed for the bacterial pathogen to increase its larvicidal action against the three larval instars.

Table 1. Susceptibility of *Culex pipiens* larvae to the standard formulation of *B.t.i.* (IPS-78) after 24 and 48 hours.

Concentration (ppm)	Percentages of mortality					
	Second larval instar		Third larval instar		Fourth larval isntar	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Control	1	4	2	5	0	2
0.03	29.00	38.66	-	-	-	-
0.04	42.66	57.00	18.33	30.33	13.66	21.00
0.06	74.33	82.00	37.33	64.00	43.33	49.00
0.08	81.66	92.33	56.00	78.33	59.66	66.33
0.10	88.00	100.00	82.00	89.66	75.66	78.33
0.14	100.00	-	91.66	97.66	81.00	97.66
0.16	-	-	99.33	100.00	96.33	100.00
0.20	-	-	100.00	-	100.00	-
LC_{50}	0.046	0.038	0.072	0.055	0.077	0.059
LC_{95}	0.116	0.086	0.151	0.127	0.159	0.133
Increase	2.52	2.26	2.09	2.31	2.07	2.25

2. Bioassay of Bactoculicide

After 24 h, LC_{50}s of Bactoculicide were 0.054, 0.088 and 0.107 ppm and after 48 h, were 0.019, 0.036 and 0.079 ppm for 2nd, 3rd and 4th instar larvae, respectively. After 24 and 48 h, LC_{50} ratios were 1 : 1.63 : 2.72 and 1 : 1.89 : 4.16 for the three larval instars, respectively. This indicated that the tolerance of larvae towards the pathogen increased as the treated larvae grew older. The LC_{95} values were 0.128, 0.136 and 0.175 ppm after 24 h and 0.097, 0.113 and 0.166 ppm after 48 h for 2nd, 3rd and 4th instar larvae, respectively. The recorded LC_{95}s are higher than LC_{50}s by 2.37, 1.55 and 1.19 folds after 24 hours and 5.11, 3.14 and 2.10 folds after 48 h for 2nd, 3rd and 4th instar larvae, respectively. According to the obtained results, mortality of all the treated larvae required about 2-5 folds of the LC_{50} values (Table 2). LC_{50}s and LC_{95}s after 24 h are higher than those after 48 h. Thus, indicating that the effectiveness of the pathogen increases against the three larval instars as the time after treatment was prolonged.

Table 2. Susceptibility of *Culex pipiens* larvae to the commercial formulation of *B.t.i.* (Bactoculicide) after 24 and 48 hours.

Dose (ppm)	Percentages of mortality					
	Second larval instar		Third larval instar		Fourth larval isntar	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Control	0	1	1	3	0	2
0.01	23.33	29.66	12.66	25.33	-	-
0.02	41.33	52.00	17.66	33.00	-	-
0.04	48.00	65.33	31.00	54.66	11.66	32.00
0.08	57.33	84.33	42.33	69.33	34.99	51.33
0.10	78.66	97.00	64.33	86.00	45.33	66.33
0.12	92.33	100.00	90.66	100.00	62.66	71.66
0.14	100.00	-	100.00	-	70.66	89.00
0.16	-	-	-	-	93.33	100.00
0.18	-	-	-	-	100.00	-
LC_{50}	0.054	0.019	0.088	0.036	0.107	0.079
LC_{95}	0.128	0.097	0.136	0.113	0.175	0.166
Increase	2.370	5.105	1.545	3.139	1.190	2.101

3. Bioassay of Bactimos

As shown in table (3), 24 h after treatment of mosquito larvae with Bactimos, the LC_{50}s of were 0.090, 0.115 and 0.163 ppm and after 48 h, were 0.047, 0.077 and 0.119 ppm for 2nd, 3rd and 4th instar larvae, respectively. The LC_{50} ratios were 1 : 1.28 : 1.81 after 24 hours and 1 : 1.64 : 2.53 after 48 hours for the three larval instars. The data revealed the increase in tolerance of larvae towards the pathogen with the increase of larval age. The LC_{95} values were 0.199, 0.239 and 0.351 ppm after 24 h and 0.152, 0.235 and 0.347 ppm after 48 h for the three larval instars, respectively. The LC_{95}s proved 2.21, 2.08 and 2.15 folds higher than the LC_{50}s after 24 hours and 3.23, 3.05 and 2.92 folds after 48 h for the three larval instars, respectively. This meant that reaching mortality of all treated larvae required about 1-5 folds of the LC_{50} values (Table 3). LC_{50}s and LC_{95}s after 24 h are higher than those after 48 h and indicating that the prolonged exposure period to the bacterial pathogen increased its larvicidal effect against the three larval instars.

4. Bioassay of Vectobac

After 24 h of mosquito larval treatment by Vectobc, the LC_{50}s were 2.361, 14.830 and 19.931 ppm while after 48 h, those were 0.638, 8.119 and 13.810 ppm for 2nd, 3rd and 4th instar larvae, respectively. The LC_{50} ratios were 1 : 6.28 : 8.44 after 24 hours and 1 : 12.73 : 21.65 after 48 hours for the three larval instars, respectively. Data confirmed higher tolerance of older larvae towards the pathogen than younger ones. The LC_{95} values were 12.72, 30.027 and 33.962 ppm after 24 h and 5.073, 12.881 and 31.684 ppm after 48 h for 2nd, 3rd and 4th instar larvae, respectively. The LC_{95}s are higher than LC_{50}s by 5.39, 2.03 and 1.70 folds after 24 hours and by 7.95, 1.59 and 2.29 folds after 48 h for 2nd, 3rd and 4th instar larvae, respectively. Data revealed that reaching 100% mortality required about 1.5-8 folds of the LC_{50} values (Table 4).

Table 3. Susceptibility of *Culex pipiens* larvae to the commercial formulation of *B.t.i.* (Bactimos) *after* 24 and 48 hours.

Dose (ppm)	Percentages of mortality					
	Second larval instar		Third larval instar		Fourth larval isntar	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Control	2	4	4	7	0	3
0.02	12.66	23.33	-	-	-	-
0.04	27.33	48.33	24.33	32.33	-	-
0.08	45.00	60.00	39.00	55.00	18.00	34.00
0.12	68.33	87.66	56.66	71.00	29.33	51.66
0.16	87.66	100.00	63.00	68.00	44.00	58.00
0.18	100.00	-	79.00	91.66	68.00	74.33
0.24	-	-	95.33	100.00	71.66	83.00
0.30	-	-	100.00	-	83.00	93.00
0.36	-	-	-	-	97.66	100.00
0.40	-	-	-	-	100.00	-
LC_{50}	0.090	0.047	0.115	0.077	0.163	0.119
LC_{95}	0.199	0.152	0.239	0.235	0.351	0.347
Increase	2.211	3.234	2.078	3.052	2.153	2.916

Table 4. Susceptibility of *Culex pipiens* larvae to the commercial formulation of *B.t.i.* (Vectobac) after 24 and 48 hours.

Dose (ppm)	Percentages of mortality					
	Second larval instar		Third larval instar		Fourth larval isntar	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Control	1	2	0	2	0	1
0.50	29.00	35.33	-	-	-	-
1.00	41.33	47.00	-	-	-	-
3.00	55.00	63.00	-	-	-	-
5.00	60.66	68.33	16.66	28.00	-	-
7.00	88.66	94.00	33.66	39.33	11.66	23.33
10.00	91.33	100.00	39.00	47.66	37.33	40.33
15.00	100.00	-	52.33	65.00	43.00	54.66
20.00	-	-	61.00	73.00	52.00	65.33
25.00	-	-	83.33	89.33	76.66	82.00
30.00	-	-	95.33	100.00	91.33	94.00
35.00	-	-	100.00	-	98.66	100.00
40.00	-	-	-	-	100.00	-
LC_{50}	2.361	0.638	14.830	8.119	19.931	13.810
LC_{95}	12.72	5.073	30.027	12.881	33.962	31.684
Increase	5.388	7.951	2.025	1.587	1.704	2.294

Discussion

The results showed the susceptibility of 2nd, 3rd and 4th larval instars of *Culex pipiens* to all bacterial formulations of *Bacillus thuringiensis israelensis*. The most potent one was the *B. t. i.* commercial formulation Bactoculicide of which the LC_{50} values ranged from 0.019 to 0.107 ppm and LC_{95} values from 0.097 to 0.175 ppm. In general, these results agreed with those of **Rettich (1987)** who recorded that the LC_{90} values of Bactoculicide and Moskitur (Czechoslovakian formulation of *B.t.i.*) against *Culex* were 0.11-0.41 and 0.14-0.31 mg/L, respectively. He recorded higher values on *Anopheles messeae* being 1.6 and 6.4 mg/L, respectively.

The second rank of potency was for the standard bacterial formulation of *B.t.i.* (IPS-78). LC_{50} values ranged from 0.038 to 0.077 ppm and LC_{95} from 0.086 to 0.159 ppm. These results agreed with those of **Wraight *et al.* (1981)** who reported a decline in the bacterial efficacy of IPS-78 (1000 ITU *Aedes aegypti*/mg) against *Aedes stimulans*, with increasing the age of larvae. Their results showed also higher toxicity of IPS-78 on *Aedes stimulans*, the LC_{50} values were 58 (46-73), 83 (69-100), and 119 (91-155) ppb for 2nd, 3rd and 4th instar larvae, respectively; and the LC_{90} values were 94, 125, and 238 ppb. In this concern, the present results agreed with those of **Lacey and Singer (1982)** who bioassayed (IPS-78) against *Aedes aegypti* and the LC_{50} value was

0.028 μ g/ml for the 4th larval instar. While, in contrast to the present results, **Misch *et al.* (1992)** found that the LC_{50}s of the *B. t. i.* formulation IPS-82 against the early 4th instar larvae of *Aedes aegypti* were 11 and 9 μ g/liter after 24 and 48 h, respectively, being much higher than values recorded in the present study.

The third potent formulation was Bactimos that resulted LC_{50}s from 0.047 to 0.163 ppm and LC_{95}s from 0.152 to 0.351 ppm. On the same *B. t. i.* formulation Bactimos, **Majori and Ali (1984)** recorded that the LC_{50}s were 0.024 (0.016-0.036) ppm and the LC_{90}s were 0.088 (0.018-0.098) ppm against the late 3rd instar larvae of *Culex pipiens* in laboratory. **Holck and Meek (1987)** reported that LC_{50} of Bactimos after 72h was 0.037 ppm against *Culex salinarius*. The lowest recorded potent formulation was Vectobac of which LC50 values ranged between 0.638 and 19.931 ppm and LC_{95} values ranged between 5.073 and 33.962 ppm. This weakness in efficiency may be attributed to the long storage period of the product. The present study differ greatly from those of **Majori and Ali (1984)** who recorded that the LC_{50} values of Vectobac were 0.061 (0.051-0.073) ppm and LC_{90} values were 0.179 (0.0127-0.0251) ppm for *Culex pipiens* in laboratory. The laboratory tests of **Dominic *et al.* (2000)** on larvicidal activity of Vectobac against *Culex quinquefasciatus* showed that the LC_{50} was 0.046.

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الملخص العربى

التأثير الإبادى لبعض المستحضرات البكتيرية على يرقات البعوضة كيوليكس بيبينز

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البعوض واحد من أخطر الحشرات ناقلات الأمراض للإنسان ومن أهم مسببات الأمراض التى تنقلها هى يرقات الفيلاريا المسببة لداء الفيل والتى تنقلها بعوضة الكيوليكس بيبينز. تعتبر البكتريا الممرضة للبعوض من أهم طرائق المكافحة الميكروبية لتخصصيتها العالية وتأثيرها الآمن على البيئة. اشتملت الدراسة على تجربة التأثير الإبادى لأربع مستحضرات بكتيرية من نوعية باسيللس ثورينجينسيس إسرائيلينسيز، أحدها قياسى وهو (IPS-78) والثلاث الأخريات تجارية وهى (Bactoculicide) و(Bactimos) و (Vectobac) على الأعمار الثانى والثالث والرابع من يرقات بعوضة الكيوليكس بيبينز. أسفرت النتائج عن أن المستحضر التجارى (Bactoculicide) كان له التأثير الأقوى حيث تراوح التركيز القاتل لـ 50٪ من اليرقات المعاملة (LC_{50}) من 0.019 إلى 0.147 جزء من المليون بينما تراوح التركيز القاتل لـ 95٪ (LC_{95}) من 0.097 إلى 0.105 جزء من المليون. وجاء المستحضر البكتيرى القياسى (IPS-78) فى الترتيب الثانى حيث تباينت التركيزات القاتلة لـ 50٪ من 0.038 إلى 0.077 جزء فى المليون والقاتلة لـ 95٪ من 0.086 إلى 0.159 جزء من المليون. على العكس من ذلك كانت هذه التركيزات أعلى بكثير فى حالة المستحضرين التجاريين (Bactimos) يليه (Vectobac) وربما يعزى ذلك لفترة التخزين الطويلة لهذه المستحضرات البكتيرية.