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Investigation of Mosquito Survival Associated with *Bacillus thuringensis israelensis* and Aquatic Plant, *Lemna minor*

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Abstract: This research was aimed to study the significance of the mixture from the aquatic plant, duck weed and *Bacillus thuringensis israelensis* as a potent deterrent factor limiting mosquito *Culex pipiens* populations in the field. *C. pipiens* mosquito appeared highly sensitivity for the higher concentrations (ppm) of *Bacillus thuringensis israelensis* (*Bti*); 100% mortality was recorded in the third and fourth instar larvae after treated by 90 and 9 ppm, respectively. The third and fourth instar larvae were appeared high susceptible to the allelochemicals of duckweed, *Lemna minor*. Water half covered by duckweed increase the efficacy of *Bti*. on the third and fourth instar larvae of mosquito.

Key words: *Bacillus thuringensis israelensis*, *Culex pipiens* mosquito, *Lemna minor*, biological control of mosquito

INTRODUCTION

Since the discovery of *Bacillus thuringensis israelensis* in 1976, extensive literature has proved its efficacy to control mosquitoes and black flies, of which many species are known as important vectors of diseases or simply as pests of humans and animals. Since 1978, *Bti* has been used in many countries on all continents and numerous studies have been made on target mosquitoes and black flies. Effect of *Bti* on the survival of immature stages of mosquitoes was studies by many authors[1-6]. *Bti* is known to be highly toxic to mosquitoes. *Bti*. subspecies *thompsoni* exhibit high toxicity to the larvae of the different species of *Culex* and *Aedes*[7].

The presence of floating aquatic plant may act as mechanical barrier to mosquitoes, duck weed and similar floating plants, *Azolla* and *Wolffia*, respectively when they form dense mats on the water surface limit mosquito production[8,9]. Apart from this mechanical effect, aquatic plants may also inhibit the survival of immature stages[10,11]. This research was carried out to study the significance of the mixture from the aquatic plant, duck weed and *Bti* as a potent deterrent factor limiting mosquito *C. pipiens* populations in the field.

MATERIALS AND METHODS

Materials: *Bacillus thuringensis israelensis* formulation based on the crystalliferous spore-forming bacteria was used in the present study against larvae of *Culex pipiens*. All formulations have been kindly supplied by the special program for Research and Training in Tropical Disease, World Health Organization.

The duck weed, *Lemna minor*, was classified by Vivi and Drar as belonging to the family *Lemnaceae*[12]. It has a medium leaf like frond, green beneath and single root.

Mosquito, *Culex pipiens* a laboratory colony was established from the offspring of some females catching from the field Taif area. The rearing was carried according to Elsayed[13]. Larvae were fed on the mixture from yeast, dried milk and powder of bread.

Methods of study

Colonization of the mosquito under field conditions: Inspections of immature stages (larvae and pupae) of *Culex pipiens* in the lakes from different regions in Taif, were practiced during October to November 2003. Weekly samples were taken by one liter glass and the larvae or pupae were counted. The temperature of water lake was recorded during the samples taking.

Effect of *Bti* on the immature stages: In general, 3rd and 4th instar larvae of *C. pipiens* served as test insects, distilled water was used for preparing the bacterial dilution (ppm). Laboratory temperature ranged between 27-30°C[1].

Concentrations of *Bti*.: Eleven concentrations (5, 7, 8, 9, 10, 20, 40, 50, 60, 70, 80 and 90 ppm/100 mL distilled water), were tested against third instar larvae and six concentrations 1, 2, 3, 5, 7 and 9 ppm/100 mL distilled water, for the fourth instar larvae.

Test insects: Larvae of 3rd or 4th instar larvae of mosquito were used in this series of tests. Sixty larvae used for each concentration were divided into six

replicates. Ten larvae from the same age were transferred simultaneously to the treatment bowls. All bowls were provided once by dry mixture of ground bread and dry yeast (2:1) as larval food[14].

Mortality readings: Mortality data were recorded after 24 and 48 h of application by counting both dead and living larvae.

Effect of plant density on survival mosquito: Distilled water was covered by the naturally duck weed or covered with well washed duckweed. Three densities of duck weed well washed or not washed mounted on distilled water (100 mL). In the first density the surface was covered with a continuous mat of duck weed; in the second it was half covered and in the third treatment the surface was slightly covered[15]. Blank one containing only distilled water. Each treatment and control was provided with ten larvae from the same age of fourth instar larvae. Survival data were recorded after 24, 48 and 72 h of application. All density was repeated six times.

Effect of *Bti.* and duck weed plant mixing on the survival of mosquito: Lower concentrations of *Bti.* in 100 mL distilled water covered by half density from duckweed, were tested on the survival of third or fourth instar larvae of mosquito. The concentrations used on the third instar were 5, 10 and 40 ppm but the concentrations used on the fourth instar larvae were 1,3 and 5 ppm[16]. All treatments were repeated six times and the mortality data were recorded after 24, 48 and 72 h of applications.

RESULTS AND DISCUSSION

Colonization of the mosquito under field conditions: Number of larvae and pupae of *Culex pipiens* collected from the different zone in Taif, were decreased gradually during the period of collection from October to November (Table 1). It may be attributable the decreasing in the water temperature. Differentiation in the number of the larvae and pupae in the lakes in the same zone may affect by another environmental factors in the mosquito habitat such as: aquatic plant types, water pH and water salinity (Table 1). pH and salinity water were not seemingly limiting colonization of the mosquito in the sewerage water duck weed free[15]. Oviposition by Culex mosquitoes can be enhanced by alkaline pH[17,18]. *Culex pipiens* tolerate the salinity of the sewerage water[19]. However, in other mosquito species salinity plays a limiting factor in mosquito population[20].

Effect of duck weed on *Culex pipiens* under laboratory conditions: Table 2 indicate that water covered by different densities of *Lemna minor* vegetations. Survival of the third instar larvae as emerged adults related to their initial number, showed that the presence of the duck weed by different densities on water reduced the survival pronouncingly (Table 2). The percentage of adult emergence from the water covered completely or half density by duck weed was 0.0%, but it was 26.6% from the water slightly covered by duck weed. In the present work the effect of duck weed was found firstly to be plant density independent; secondly the duck weed has a chemicals effect on the survival of the immature stages of *Culex pipiens*. Duck weed and similar floating plants especially when they form dense mats on water surface limit mosquito production[9,21]. Duck weed producing the synomones chemicals, which have ability to preventing the oviposition by mosquito[15]. Larvae of *Culex pipiens* could not withstand the presence of the duck weed vegetation's and died[9,11].

Bioassay of *Bti.* on the immature stages of mosquito: The percentage of mortality of the third instar larvae decreased as the concentration (ppm) of *Bti.* was increased. Table 3 indicate that, the effect of *Bti.* on the survival of the third instar larvae of mosquito. It is clear that the highly percentage of mortality (100%) was recorded with 90 ppm, but the concentration of 5 ppm was not caused mortality even after 48 h. Concentrate of *Bti.* (9 ppm) was very effective in survival prevention of the fourth instar larvae (Table 4). But one ppm it have not

Table 1: Number of immature stages of mosquito *C. pipiens* collected weekly from lake water in the field during the period from October-November 2003

	First week		Second week		Third week		Fourth week		First week		Second week		Third week		Fourth week	
Lake water	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.
Galiel	21	6	19	4	17	4	13	3	12	2	8	3	5	1	4	1
Alarag	19	7	16	6	13	5	9	3	8	3	8	2	7	2	5	0
Alrehab	17	6	13	4	11	5	8	4	6	3	7	3	5	2	2	1
Gabra	25	7	22	6	17	5	14	4	8	1	5	0	2	0	0	0
Gabageb	23	6	21	5	16	4	12	2	6	1	2	0	0	0	0	0
Ghader	25	9	22	9	19	7	17	5	14	5	12	3	10	3	7	1

Table 2: Means±SD of effect of aquatic plant *Lemna minor* on the survival % fourth instar larvae of mosquito, *Culex pipiens*

Time (h)	Water covered by a mat of duck weed (%)	Water half-covered by duck weed	Water slightly covered by duck weed
24	0.0	26.7±0.3	46.6±0.4
48	0.0	26.7±0.3	46.6±0.4
72	0.0	0.0±0.0	26.6±0.3

Table 3: Means±SD of effect of *Bti.* on susceptibility of the third instarlarvae of *Culex pipiens*

	Mortality %	
Concentration (ppm)	After 24 h	After 48 h
90	100.0±0.0	100.0±0.0
80	80.0±0.7	80.0±0.7
70	73.3±0.2	73.3±0.2
60	60.0±2.4	73.3±0.2
40	53.3±0.5	66.6±0.5
20	46.7±0.5	60.0±2.4
10	40.0±1.8	46.7±0.5
9	33.3±2.8	40.0±1.8
7	0.0±0.0	26.7±0.3
5	0.0±0.0	0.0±0.0
Control	0.0±0.0	0.0±0.0

Table 4: Means±SD of effect of *Bti.* on susceptibility of the fourth instar larvae of *Culex pipiens*

	Mortality %	
Concentration (ppm)	After 24 h	After 48 h
9	100.0±0.0	100.0±0.0
7	81.7±0.2	81.7±0.2
5	60.0±2.4	60.0±2.4
3	31.7±0.5	31.7±0.5
2	18.3±1.2	18.3±1.2
1	0.0±0.0	0.0±0.0
Control	0.0±0.0	0.0±0.0

toxicity effect on the fourth instar larvae. Generally data in Table 3 and 4 of the fourth instar larvae were appeared that this instar was more sensitivity for *Bti.* than the third instar larvae. This result may be attributed to the fourth instar larvae metamorphosis to the pupae and its become could not be able to resistance the toxicity of *Bti.*

The results of laboratory evaluation revealed 100% mortality of *Aedes aegypti* larvae treated by Bt.H.14[22]. Culinex *Bti.* killed 100% of the larvae after 24 h and was active for 5 days after application[2]. Laboratory tests on *Bti* showed that the aqueous suspension was relatively more effective against *Culex quinquefasciatus* than *Aedes aegypti* and *Anopheles stephensi*[1]. In contrast, in feeding experiments with aquatic bacteria such as *Bt. Medellin*, it was shown that *Culex quinquefasciatus, Aedes aegypti* and *Anopheles albimanus* larvae were able to survive on a diet based on this wild bacterium[23].

B.t. thompsoni exhibit high toxicity towards the larvae of the species of *Culex quinquefasciates, C. tritaeniorhynchus, C. sitiens, A. stephensi* and *Aedes aegypti. Bti* is known to be highly toxic to mosquitoes[7]. The hyper toxic paraent strains of *Bti.* and the sporulation deficient, hypertoxic mutant stains showed almost indential larvicidal activity[24]. Cry 27A protein (*Bt*) showed larvicidal activity highly specific for Anopheles stephensi, but laked the toxicity against *C. pipiens*[3]. Mosquito control with biological insecticide's, such as *Bacillus sp.* Toxins, has been used widely in many countries. Use of these formulations is better and more user-friendly. Some modifications of *Bti.* use against mosquitoes, guided by research, is probably the best of these independent on the pesticides.

Data of effect of the mixture of *Bti.* and duck weed in half density on the survival of the third and fourth instar larvae of mosquito *C. pipiens* Table 5 shows that, the aquatic plant duck weed was increased the efficacy of the lower concentrations of *Bti.*, which have not effect on the survival of mosquito in Table 3 and 4. The 5 and 1 ppm concentrations of *Bti.* with duck weed plant were caused 85 and 86.6% mortality after 72 h in the third and fourth instar larvae, respectively. *Bti.* with methylated soybean oil formulation provided 71% mortality in larvae of *A. quadrimaculatus*[5]. Mixture of *Bti.* with cypermethrin was most efficient to control of *C. quinquefaciatus*[25].

The aim of using the duck weed plant with the *Bti.* in mosquito control, it's reducing the fund of mosquito control by *Bti.* or alternative the *Bti.* by duck weed when *Bti.* formulation is difficult and costly to be used.

Table 5: Means±SD of mortality % of the third and fourth instar larvae of *Culex pipiens* in water half covered by duck weed treated by *Bti*

	Third instar larvae			Fourth instar larvae		
Time (h)	40 ppm	10 ppm	5 ppm	40 ppm	10 ppm	5 ppm
24	88.3±0.20	85.0±0.04	75.0±0.03	90.0±0.0	75.0±0.03	75.0±0.03
48	91.6±0.07	86.6±0.20	75.0±0.03	90.0±0.0	83.3±0.03	75.0±0.03
72	100.0±0.00	93.3±0.07	85.0±0.04	95.0±0.06	90.0±0.00	86.6±0.07

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