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EFFECTIVENESS OF BACILLUS THURINGIENSIS SEROTYPE H-14 ON CERTAIN EGYPTIAN MOSQUITO SPECIES IN SMALL DITCHES

By

A.I. MERDAN, NAHED M. HILMY and A.A. IBRAHIM

Research and Training Centre on Vectors of Diseases, Ain Shams University, Cairo, and Department of Entomology, Faculty of Sciences, Benha, Egypt.

ABSTRACT

The commercial formulation TEKNAR based on *Bacillus thuringiensis* serotype H-14 in the form of a liqued concentrate was applied against larval populations in two road-side ditches near the Faculty of Science in Benha. Three experimental treatments were carried out at a dose of 2 mg/L. Eight days following each application, treated areas had returned to pre-treatment levels of larvae, but with differences in age distributions. Laboratory bioassays were conducted to study the factors which can influence the efficiency of this larvicide under natural conditions.

INTRODUCTION

The increasing interest of the mosquito biological larvicides *Bacillus thuringiensis* Sertype H-14 led to intensify the research activity to gain more knowledge on the applicability of its produced formulation. Field evaluation of the efficacy of such formulations is one of the most important aspects in this activity. Most of the field evaluation trials were oriented to-

wards relatively large breeding places in rural areas.

The present investigation aimed at evaluating the efficiency of one of the commercial formulations on the larval population in road-side ditches located in an urban area where no larval control measures were carried out.

MATERIAL AND METHODS

1.1 *The Test Sites : B.t.* H-14 was applied to two small ditches formed by water escaped from two underground drinking water pipes, which were about 200 meters apart. Both were roadside ditches in residential area near the faculty of science, Benha University, Benha. An adult population of mosquitoes provided large numbers of continuously hatching egg rafts throughout the test.

Site (A) was, 1 m long, 0,75 m deep at midwidth, contained an estimated 0,45 m^3 of water, the site borders were surrounded by vegetation and completely exposed to direct sunlight. The pH value was about 6.5 and water temperature varied between 25 to 30° during the test periods. Site (B) was 2 m long, 1 m wide, averaged about 0.25 m deep, and contained an estimated 0.5 m^3 of water, the site was protected from direct sunlight most of the time, the site borders were surrounded by vegetations and the water surface always contained algae, the pH value was about 7 and the water temperature varied between 24 to 29° during the test period. A third similar ditch was used as check, this ditch 6 m long, 80 cm wide and 10 cm deep. dense vegetation was present on the site borders.

1.2 The Tested Mosquito Species : Culex pipiens, Culceita longairiolata, Culex antennatus and *Aedes detritus* larvae were encountered in the selected ditches with densities of 50.4%, 41,8%, 6,4% and 1,5% respectively.

1.3 *The Bacterial Formulation :* A liquid concentrate commercial formulation produced by Sanduz Co. under the name of TEKNAR, based on *B. thuringiensis* H-14 (1500 ITU/mg *Aedes aegypti)* was used.

1.4 *Application :* A Hudson x-pert sprayer was used in spraying the larvicidal material on the tested ditches. A stock suspension was prepared and dilutions were used in spraying and the breeding water volumes to reach 2 mg/L. Larval density of each species was estimated in each site in terms of larvae/dip (10 dips) before and 24 hours after application, then continued daily till the end of the experiment. The period from the application to the detection of 1st instar larvae is considered as the period during which the larvicidal action can persist under natural (field) conditions.

1.5 *Laboratory Bioassays :* Water samples of the sprayed ditches were collected daily and bioassayed in the laboratory. Three replicates were made. Often 3rd instar larvae were tested in each replicate with a similar number of unsprayed water sampled from the check ditch and used as control. Mortality readings were carried out after 24 hrs of larval exposure. Samples of water and larvae were collected prior to application of the *B.t.* H-14 material. The effect of ditch water on larvae was mentioned with non-treated ditch water against laboratory-reared 2nd instar larvae. Interference by ditch water upon the effectiveness of *B.t.* H-14 was monitored by addition of a known dose to non-treated ditch water and assayed against 2nd instar larvae. Treated field water was tested without dilution.

RESULTS AND DISCUSSION

Most larval instars (85.7 to 100%) were killed the 1st day post treatment, very few number survived in some cases until the 2nd day post-treatment, but in all cases not even one larva was found alive 48 hours post-treatment. These results are relatively in agreement with what have been found by many authors used the same formulation (TEKNAR), Ramoska et al. (1982), Foo and Yab (1983).

All mosquito species treated during these experiments ; *Culex pipiens, Culesita longairiolata, Culex antennatus* and *Aedes detritus* were susceptible to the formulation.

Very fast reinfection of the treated ditches was observed, 1st instar larvae began to appear 2 or 3 days post treatment and developed naturally into 4th instar larvae and pupae. This quick reinfestation of the treated areas by larvae were found by Elddridge and Callicrate (1982), McLaughlin and Fukuda (1982) and many others.

The appearance of 4th instar larvae of *Culex specics* began the 8th day post-treatments, so that the application of *B.t.* H-14 must be the 9th or 10th day post-treatments to ensure that no larvae will pupate and emerged as adults, these results are in agreement with the results of Eldridge and Callicrate (1982), Rampal et al, (1983) which stated that application of *B.t.* H-14 must be repeated weekly. McLaughlin and Fukuda (1982), Ree et al. (1983) stated that, the oviposition rate, rate of larval development and other populations factors and habitat conditions would influence the timing and frequency of B.t. H-14 application required to prevent emergence of a significant adult population in many specific situation. This may be the reasons why *Culisita Longairiolata* larvae were not found in the 3rd, 4th or pupal stages since the 1st application. Results of the present work indicate that the number of 4th instar larvae and pupae shows the excellent control attained with *B.t.* H-14 large numbers of 4th instar larvae and pupae would normally result from such a population, as is borne out by the data of the untreated ditch (Table C) the treated ditches had no more than 0.1 pupae/dip from *Culex species* and no pupae from *Culesita lorgairiolata* whereas the untreated ditch had an average of 0.75 pupae/dip from *Culex species* and 0.73 from *Culesita longairiolata*. Therefore, the tests, showed that B.t. H-14 has the potential to drastically reduced emerging adult populations even with the presence of extremely large adult oviposition pressure.

Laboratory assay data, summarized in Table (D) indicate the following :

I. Well water has no killing effect on larvae, whereas 2 larvae out of 90 (6.66%) were killed by field water.

II. A known dose (1 mg/L) in pretreatment ditch water

Table (A) effectiveness of Bt H-14 under natural conditions site A

Days after treatment	Average Number of Immature Stages/dip Culex Species							Culesita Species						
	1st	2nd	3rd	4th	pupal	total 4th instar+pupa	% red.	1st	2nd	3rd	4th	pupal	total 4th+Pupae	% red.
pretreatment	8.7	6.4	10.5	6.2	0.9	7.1		2.4	4.2	2.4	2.6	3.2	5.9	
1Day posttreat	0	0	0	0.3	0	0.3	95.8	0	0	0	0.1	0	0.1	98.3
2Day "	0	0		0.2	0	0.2		0	0	0	0	0	0	100
3Day "	9.5	0	0	0	0		100							
4Day "	3.4	0	0	0	0									
5Day "	1.9	1.5	0	0	0									
6Day "	1.4	0.8	0.1	0	0									
7Day "	*	*												
8Day "	1.9	4.9	0.9	0.3	0	0.3								
9Day "	4.3	5.4	3.6	0.4	0	0.4								
10Day "	7.6	4.3	4.9	0.5	0.1	0.6								
2nd application														
1Day posttreat	1.0	0	0	0	0	0	100							
2Day "	1.4													
3Day "	1.2													
4Day "	*	*												
5Day "	2	1.3	1.2											
6Day "	18.2	3	2											
7Day "	9	1.7	0.8	0.1	0	0.1								
8Day "	12.6	3.1	2.7	0.3	0.1	0.4								
3rd application														
1Day posttreat	0	0	0	0	0	0	100							
2Day "	0.3	0	0	0	0	0								
3Day "	0	0	0	0	0	0								
4Day "	2.1	0	0	0	0	0								

* * No Samples were taken during this day.

Table (B) effectiveness of Bt.H-14 under natural conditions site B

Days after treatment	Average Number of Immature Stages/Dip													
	Culex Species							Culesita longairiolate						
	1st	2nd	3rd	4th	pupa	total no. 4th 1. and pupa	% red.	1st	2nd	3rd	4th	pupa	total 4th + pupal	% red.
pretreatment	11.5	9.7	3.3	0.9	1.2	2.1		5.5	7.1	6.3	0.8	3.1	3.9	
1Day posttreat.	0	0	0	0	0	0	100	0	0	0	0	0	0	100
2Day "	0	0	0	0	0	0								
3Day "	0.3	0	0	0		0								
4Day "	8.3	1.6		0	0	0		12.3						
5Day "	11.2	4.5	1.7	0	0	0		7.2	0.7					
6Day "	9	7	0.1	0	0	0		12	1.3					
7Day "	*	*												
8Day "	8.8	1.8	1.8	0.2	0	0.2		3.6	0.8					
9Day "	3.2	4.4	1.3	0.8	0	0.8		3.5	1.6					
10 Day "	6.9	4.2	2.4	0.4	0.2	0.6		16.5	4.3					
2nd treatment														
1Day posttreat.	0	0	0	0	0	0	100							
2Day "	17.2	0	0	0	0	0		4						
3Day "	12	0.9	0	0	0	0		1						
4Day "	3.9	1.1	0	0	0	0								
5Day "	*	*												
6Day "	8.7	3.2	3.9	0	0	0		2	1					
7Day "	6.7	5.4	5.2	0	0	0		7.2	3.1					
8Day "	22	4.7	2.1	0.1	0	0.1		2.1	4					
9Day "	13.7	12.3	9.6	0.6	0.1	0.7		0	0					
3rd treatment														
1Day posttreat.	0	0	0	0.1	0	0.1	85.7							
2Day "	0	0	0	0	0	0	100							
3Day "	0	0	0	0	0	0								
4Day "	6.9	0	0	0	0	0								
5Day "	4.3	0.2	0	0	0	0								
6Day "	16	1.3	0.9	0	0	0								
7Day "	13.9	4.3	3.2	0	0	0								
8Day "	28	7.1	2.7	0.2	0	0.2								
9Day "	21	9.6	3.4	0.9	0	0.9								

** No Samples were taken during this day

killed fewer larvae than it did in well water. This reduced effect may be due to the following : (a) A direct action upon the toxic crystals (McLaughin and Fukuda 1982). (b) Reduction of larval/intake of *B.t.* H-14 due to : (1) Crystals were removed from the feeding zone and (2) Competition for feeding i.e. selection of organic food and decreased intake of crystals (McLaughin and Fukuda 1982, Hougard et al., 1983).

III. Results of the laboratory assays of field water treated with 2 mg/litre at 1, 2 and 3 days posttreatment (Table E) declared that *Bt.* H-14 was effective only for 24 hours, very few mortality was detected 48 hours posttreatment, and no mortality was observed 3 days posttreatments. This low residual effect under different field conditions has been found by many authors (chaefer, 1979; De Barjac et al., 1980; Mulligan et al.,

Table (C) No. of immature stages/dip in the check site (c)

day during experiment	Culex Species			Culesite longairiolar		
	4 th stage	pupae	total	4 th stage	pupae	total
25-4-85	1.5	0.5	2.0	1.4	2.0	3.4
26	0.9	0.2	1.1	2.0	2.2	4.2
27	1.1	0.4	1.5	1.5	2.1	3.6
28	0.7	1.4	2.1	1.7	1.2	2.9
29	0.6	0.2	0.8	1.1	3.0	4.1
30	1.3	0.2	1.5	0.7	2.0	2.7
1-5-85	0.8	0	0.8	2.2	0.9	3.1
2	0.6	0	0.6	1.9	0.3	2.2
3 • •						
4	1.4	0.3	0.7	1.2	0.2	1.4
5	1.8	0.5	2.3	0.7	0.4	1.1
6	0.4	0	0.4	1.4	0.6	2.0
7	0.7	0	0.7	1	1.1	2.1
8	1.0	3	2.3	0.9	2.3	3.2
9	0.6	0.8	1.4	0.6	0.5	1.1
10	1.0	0	1.0	0.5	0.3	0.8
11 • •						
12	2.4	0.7	3.1	1	0.7	1.7
13	0.3	2.1	2.4	2.1	0.9	3.0
14	0.7	0.9	1.6	1.0	1.1	2.1
15		1.3	1.3	0.7	0.2	0.9
16	0.3	0	0.3	0.1	0.1	1.1
17	0.2	0.5	0.7	0.2	0.4	0.6
18	0	0	0	0	0.3	0.3
19	0.1	0	0.1	0.1	0.5	0.6
20	0	0.3	0.3	0	0.2	0.2
21	0.1	0	0.1	0	0	0
22	1.0	0.1	1.1	1	0	1

Table (B) : Percentage mortality of *C. pipiers* exposed to *B.t.*-H-14 (Teknar) in water from ditches in laboratory assay at 24 hours post treatment*.

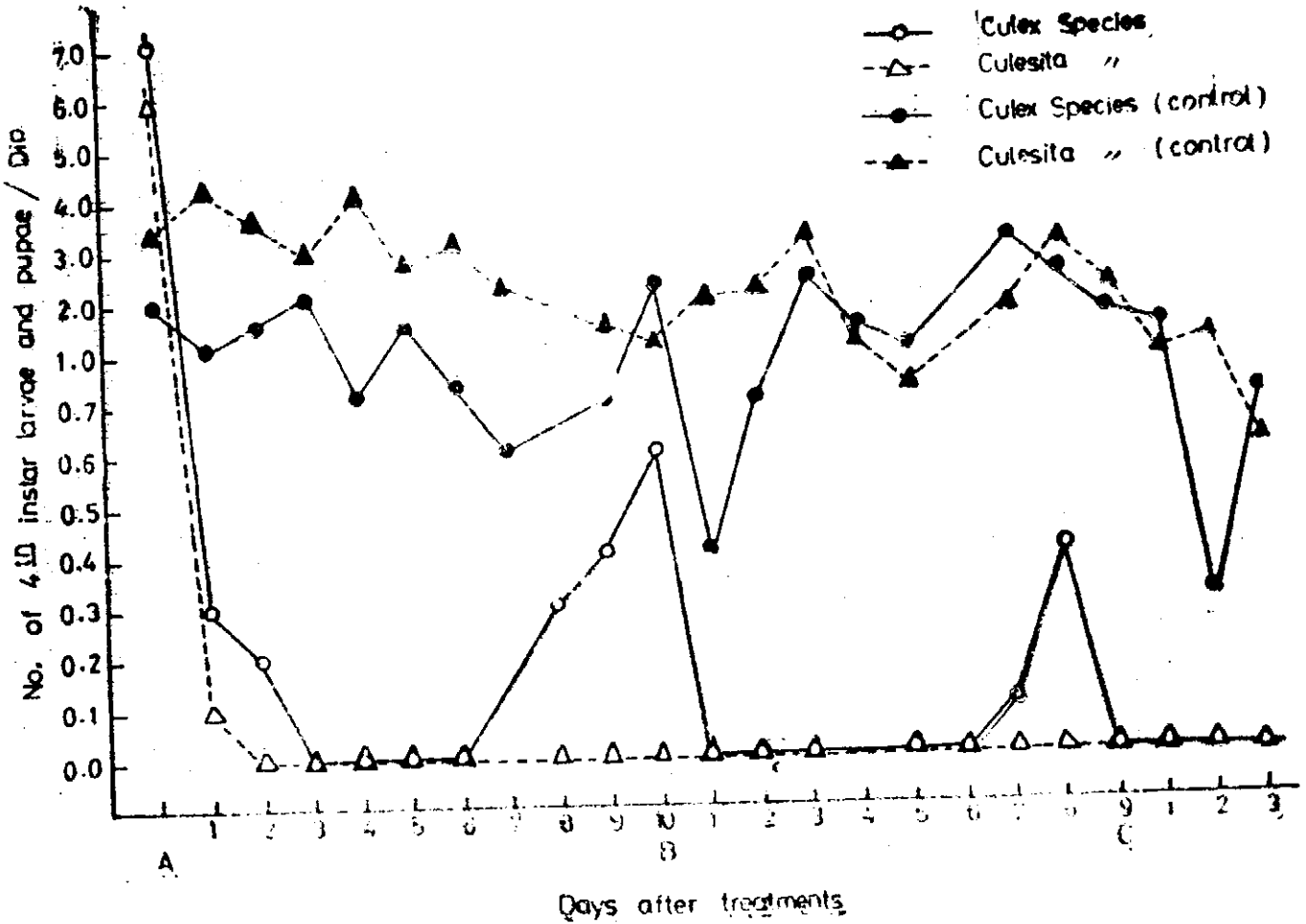
treatment number	untreated		well-water + 1 mg/L *B.t.* H-14	pretreatment field water+ 1 mg/L B.t. H-14		zerotim post-treatment field water + 2 mg/L of B.t. H-14	
	well water	field water		site (A)	site (B)	site (A)	site (B)
1	0	6.66	100	56.66	46.66	83 33	96 66
2	0	—	100	53.33	63.33	100	100
3	0	—	100	73.33	53.33	96.66	100

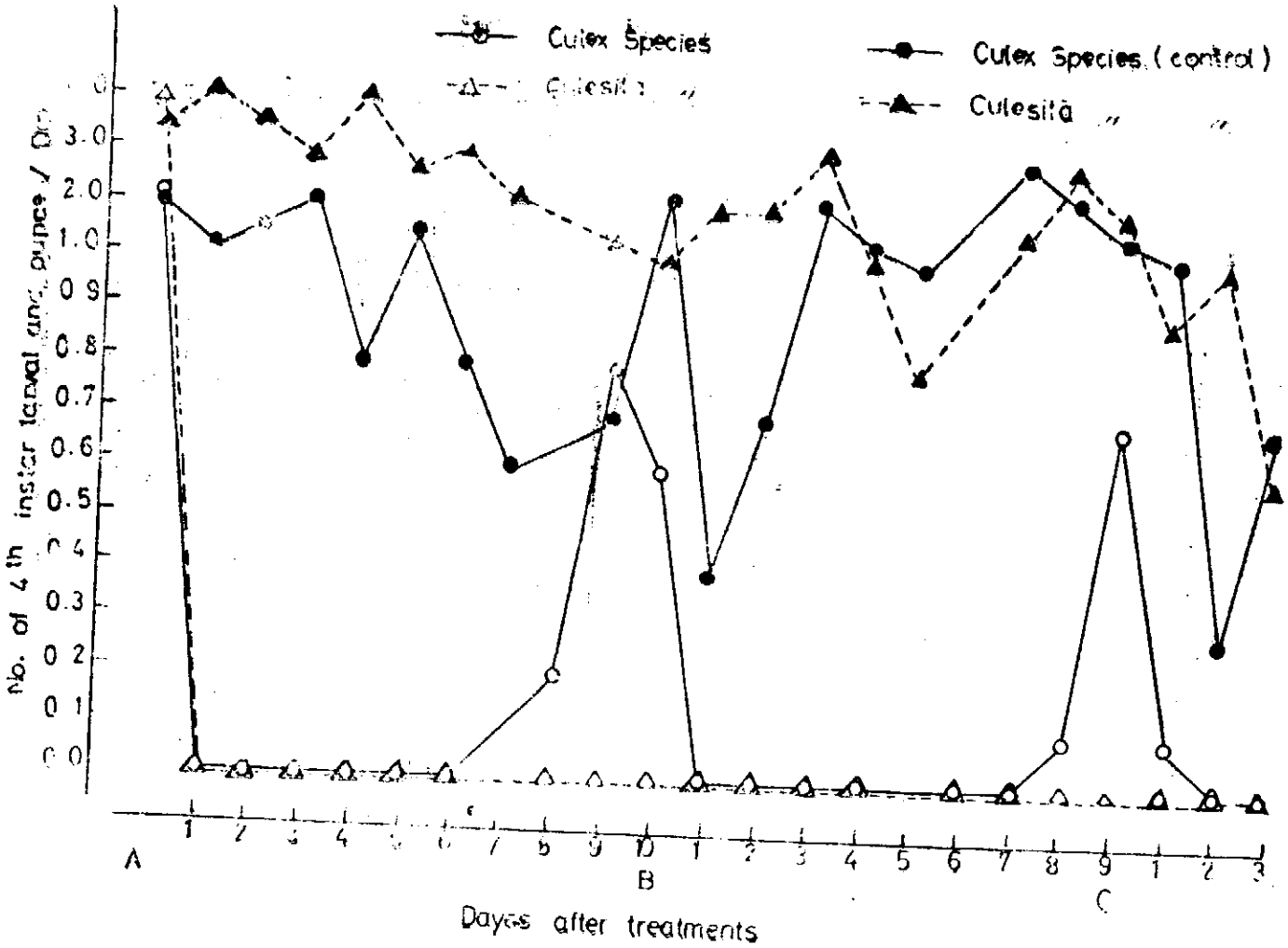
10 2nd instar, laboratory reared larvae were used — for each replication.

Table (E) : Persistance of *B.t.* H-14 under field conditions.

Treatment number	Time after treatment	% Mortality site (A)	% Mortality site (B)
1	Zero time	83.33	96.66
	24 hours	16.66	26.66
	48 hours	0	3.33
	72 hours	0	0
2	Zero time	100	100
	24 hours	10	16.66
	48 hours	0	0
	72 hours	0	0
3	Zero time	98.1	100
	24 hours	16.66	20
	48 hours	0	0
	72 hours	0	3.33

1980; De Maio et al., 1981; Mclaughlin and Fukuda, 1982; and Rampal et al., 1983). Free chlorine content, sunlight, suspended matter, settlement of active ingredient and improper formulation are increminated factors responsible for this low persistance of *Bt.* H-14 under natural conditions in addition to the fact that this bacterium does not recycle in nature, as many other similar spore forming bacteria (De Barjac et al., 1980 and Ignoffo et al., 1981).


Culex Species
Culesita "
Culex Species (control)
Culesita " (control)
No. of 4th instar larvae and pupae / Dip
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A
B
C
Days after treatments


Culex Species
Culex Species (control)
Culesita
No. of 4th instar larval and pupae / Dip
3.0
2.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A
B
C
Dayes after treatments

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