Israel Journal of Entomology

Vol. XXIII(1989) pp. 63-68

POTENTIAL OF BACTERIAL CONTROL OF MOSQUITOES IN TURKEY

A. Matur and K. Ceber
Cukurova University, Medical School, Dept. of Medical Biology, Adana, Turkey

ABSTRACT

Several strains of the microbial control *agent Bacillus thuringiensis* (H-14) and *Bacillus sphaericus* were evaluated in the laboratory and field against 4th-instar larvae *of Culexpipiens*. All unformulated compounds tested exhibited activity against *Cx.pipiens* larvae. Fourth instars larvae of *Culexpipiens* exposed for48 hr to t*heBacillus thuringiensis 73-E-10-2* and 73-E-10-16 responded wilhLC5O values of 4.0 and 4.2 viable spores x $1O^4$/ml of unformulated compounds respectively. No activity with the 74-E-37-14 strain *of Bacillus thuringiensis* was detected. The LC50 values for *B. sphaericus* 1593, 2297 and 2362 isolates were obtained to be 1.0, 2.0 and 3.4 viable spores x 10^5 respectively. A correlation between results *from* the laboratory and field trials were observed.

INTRODUCTION

After the discovery *of Bacillus thuringiensis* var. *israelensis* (H-14) or *"Bti"* which was isolated by Goldberg and Margalit (1977) and serologically characterized by deBarjac (1978) several other *Bacillus thuringiensis* (*Bt*) strains were isolated and tried as larviciding agents. Three of those isolates, 73-E-10-2, 73-E-10-16 and 74-E-37-14, were isolated from silkworm litter in Ehime Prefecture, Japan, by Ohba et al. (1979). Although first 2 of these *Bt* isolates were reported to be effective against larvae of several mosquito and blackfly species, the last one was found to have no activity (Finney and Harding, 1982; Lacey and Oldrache, 1983; Padua et al., 1980).

Several strains of another spore-forming bacterium, *Bacillus sphaericus* Neide were isolated which also had larvicidal activity against mosquito species. Apart from its lower activity in comparison with *Bti* against certain species of mosquitoes, *B. sphaericus* persisted longer under natural conditions (Mulligan et al., 1980; Hornby et al., 1981) and might provide the additional benefit of recycling in nature (Hertlein et al., 1979). Certain strains and isolates are known for *B. sphaericus* covering a range of larvicidal potencies (Singer, 1980).

The projcct area, so-called Cukurova or Lower Seyhan Plain (LSP) as shown in Fig. 1, was described earlier (Matur and Ceber, in press). In brief, it is one of the most appropriate places in the world for mosquitoes to breed and survive throughout the year. Furthermore, it could be considered that the LSP is a place where many parasitic diseases originate in Turkey.

The area suffers severely from the mosquitoes as both annoyance and potential carriers of malaria. Although bioassays of bacterial larvicides against mosquitoes have been conducted in almost all related countries, little had been reported on the development of suitable control system in LSP so far (Matur and Ceber a,b, in press; Ramsdale, 1981).

This paper presents the part of our project on the larvicidal efficacy of different formulations and isolates of *Bti, Bt* and *B. sphaericus* against mosquito species.

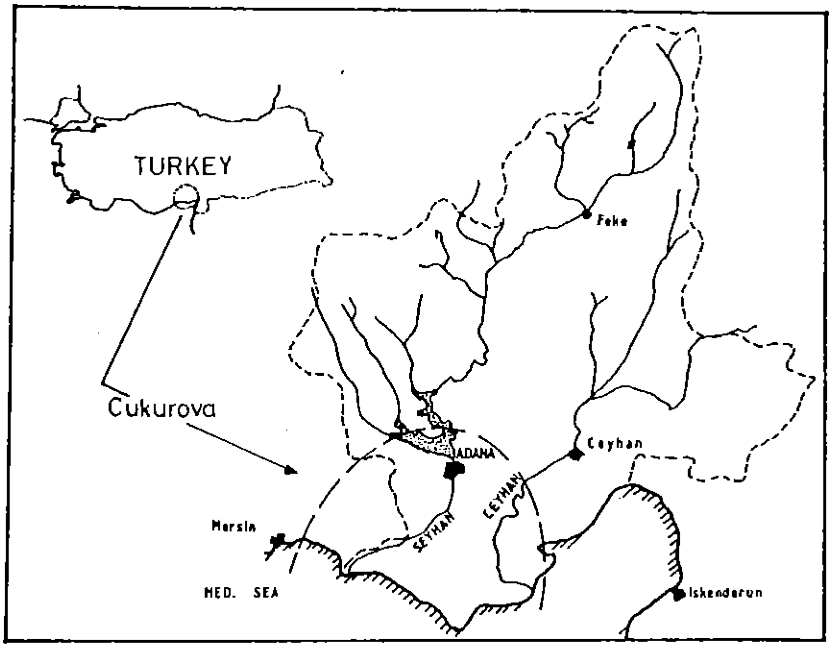

Fig. 1. The location of Cukurova in Turkey.

MATERIALS AND METHODS

The unformulated compounds of *Bacillus thuringiensis* 73-E-10-2, 73-E-10-16 and 74-E-37-14 were provided by Dr. Aizawa, Japan, and the *Bacillus sphaericus* isolates, 1593, 2297 and 2362, and a powder preparation (RB–80) were obtained from Pasteur Institute, Paris. The isolates were provided on agar slants and were then grown in nutrient media, pH 7.4 at 28°C in a shaker (Dedeoglu, Istanbul), harvested after 4 days, counted by serial dilutions, and stored in distilled water at 10°C for 24 hr prior to use in tests. RB–80 reference preparation was prepared according to the directions of Pasteur Institute.

The first part of the work was conducted under insectary conditions (temperature 28 ± 1°C and relative humidity 80 ± 5%). All exposures and controls were conducted with 25 larvae in 150 ml dechlorinated tap water in plastic cups (SSYB Teknik plastik, Istanbul). A dose of 10^4–10^7 spores/ml of one of the isolates introduced into each of three cups, 3 cups were controls and received food only. Each experiment as a whole was replicated 3 or 4 times for each isolate.

Field trials were performed during June and October, 1987, in a swampy ditch in the University Campus about 10 miles away from domestic sites. The ditch was approximately 30 m^2 in size and partly vegetated. Water depth was 10–1000 cm throughout the ditch. Tin pots (dia. 25 cm) open at both ends were used as confined sites. Each pot was plunged into the bottom of water at one end and covered by cheese cloth on top. 50 Fourth instars larvae of *Cx. pipiens* were added to each pot for bioassay, which were prepared in a nearby outdoor station and the water depth in each pot was recorded. Each trial consisted of two replicates at concentrations the same as above. The stock solution of each unformulated compound was prepared in ditch water and the same water was also used for serial dilutions.

Mortality counts were recorded at 48 hr after the addition of each compound. Larvae that had pupated were not included in mortality readings. Date were computer analysed using a maximum likelihood probit analysis programme in BASIC on a Commodore-64 microcomputer. The LC50 and LC90 values were read from log dose/mortality graphs after computer analysis.

RESULTS AND DISCUSSION

Probit analysis of each of the *Bt* and *B. sphaericus* unformulated compounds are graphically depicted in Figs. 2 and 3. The LC50 and LC90 values and their respective 95% confidence intervals are presented in Table 1. The unformulated *Bt* compounds were more effective than *B. sphaericus* compounds. The results from insectarium and field tests showed that the compounds in the field were less effective against *Cx. pipiens* compared to the results from temperature controlled indoor trials, due most probably to the severe fluctuations in temperature during the day in the field. The temperature was about 31–36°C during the day and 25.5–27°C during the night, recorded by a maximum-minimum thermometer.

It can be seen that the two *Bt* strains, 73-E-10-2 and 73-E-10-16 (but not 74-E37-14) were effective over the dose range tested. These two *Bt* strains were the same isolates that Padua et al. (1980) and Finney and Harding (1982) had shown to have toxicity for mosquitoes. Similar activities of these two isolates against fourth instars larvae of *Cx. pipiens* can be seen by reference to LC50 and LC90 values obtained in Table 1. For *Cx. pipiens*, the dose mortality regression lines appeared to have almost the same slope with both compounds tried in laboratory and field. When a comparison is made between LC50 values for *Cx. pipiens* obtained in these experiments and those obtained by Padua et al. (1980), Finney and Harding (1982) and Lacey and Oldrache (1983) who used the same strains, discrepancies arise.

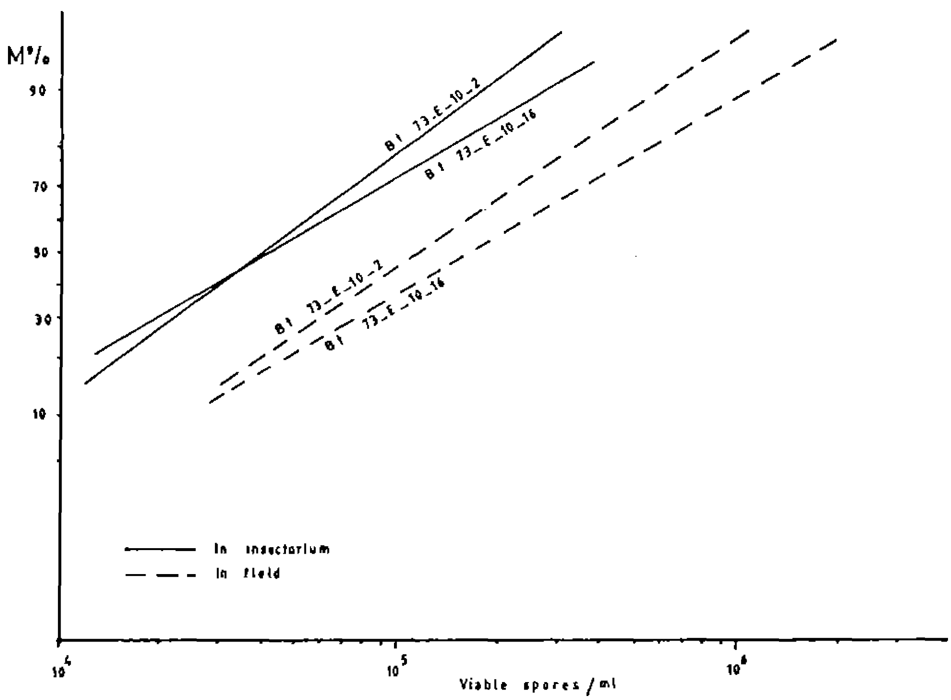

Fig. 2. Dose/mortality response of *Cx. pipiens* to two unformulated *Bt* compounds under insectarium and field conditions.

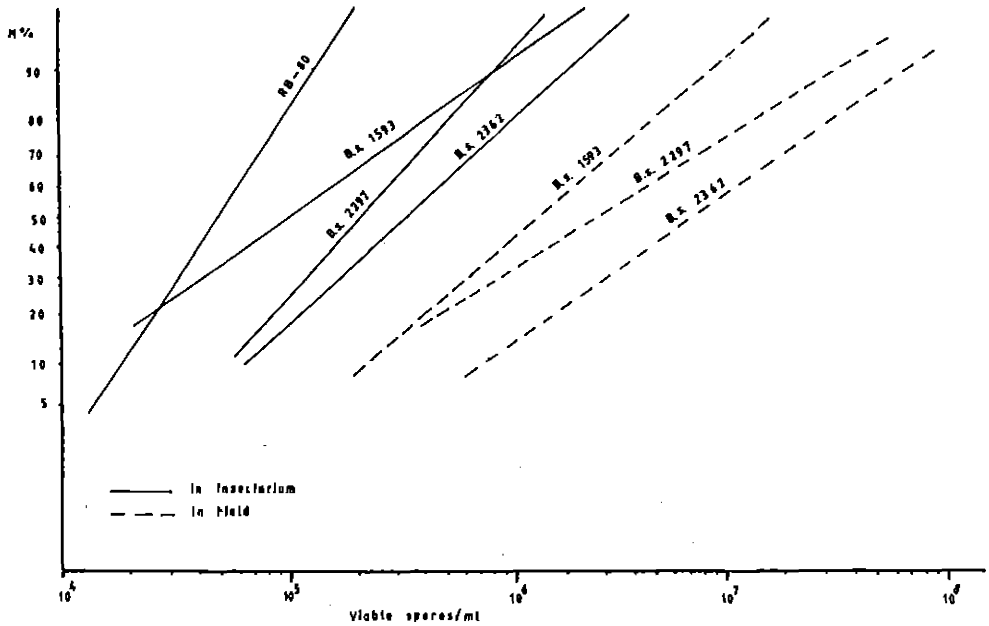

Fig. 3. Dose/mortality response of *Cx. pipiens* to three unformulated *B. sphaericus* compounds together with RB-80 reference preparation under insectarium and field conditions.

The LC50 values for 73-E-10-2 obtained by Padua et al. (1980) and Finney and Harding (1982) were 3.0×10^5 and 1.0×10^5 spores/ml respectively, against *Ae. aegypti*, compared to a value of 4.0×10^4 spores/ml against *Cx. Pipiens*, (Table 1). The LC values, on the other hand, for 73-E-10-16 were 3.40×10^4 for *Cx. quinquefasciatus* and 39.65×10^4 for *Ae. aegypti* (Lacey and Oldrache, 1983) and 1.0×10^5 for *Ae. aegypti* (Finney and Harding, 1982) compared to 4.2×10^4 for *Cx. pipiens* (Table 1). From data upon *Bt* isolates, it can be seen that the 73-E-10-2 isolate shows greater activity against *Cx. pipiens* (Table 1) than against *Ae. aegypti*, about 2.5 (Finney and Harding, 1982) — 7.0 (Padua et al. 1980) times. The 73-E-10-16 isolate also shows greater activity than does against *Ae. aegypti*, about 2.3 (Finney and Harding, 1980) — 10 (Padua et al., 1980; Lacey and Oldrache, 1983) times, and a similar level of activity with *Cx. quinquefasciarus* (Lacey and Oldrache, 1983).

In a study conducted in this laboratory (Matur and Ceber b, in press), the LC50 of IPS-82 standard preparation of *Bti* (Pasteur Institute, Paris) against 4th instar larvae of *Cx. pipiens* was 0.05 ppm. The number of viable spores/mg of IPS-82 was 4.62×10^7. The number of spores/ml of working volume at the LC50 concentration of this preparation was $5.50–5.75 \times 10^3$. Therefore, *Bti* preparation IPS-82 is clearly more active against *Cx. pipiens* than both 73-E-10-2 and 73-E-10-16.

The studies with *B. sphaericus* isolates showed that their activities were lower than activities of *Bt* isolates under both insectarium and field conditions (Table 1). Although the reference preparation of *B. Sphaericus* (RB-80) showed the highest level of activity, it was still lower than the activities of the *Bt* isolates.

The data presented here indicate that, *Bti* has greater potential for the control of culicine mosquitoes in LSP than *Bt* and *B. sphaericus*. However, further field trials are needed to overcome the mosquito problem in LSP.

TABLE I

Susceptibility of fourth instars of *Cx. pipiens* to *Bt* and *B. sphaericus* isolates*

	In insectarium (concentrations and 95% fiducial limits); spores/ml		In field (concentrations and 95% fiducial limits); spores/ml	
	LC50	LC90	LC50	LC90
Bt 73-E-10-2	4×10^4 (2.84–5.45×10^4)	1.8×10^5 (0.33–2.52×10^5)	1.15×10^5 (0.86–2.22×10^5)	5.8×10^5 (3.48–7.12×10^5)
Bt 73-E-10-16	4.2×10^4 (3.15–5.72×10^4)	2.8×10^5 (1.24–3.46×10^5	1.18×10^5 (0.64–2.12×10^5)	1.01×10^6 (0.92–1.86×10^6)
Bs 1593	10^5 (0.94–1.65×10^5)	8.4×10^5 (5.85–11.6×10^5)	1.35×10^6 (0.68–1.54×10^6)	9.4×10^6 (7.36–13.44×10^6)
Bs 2297	2×10^5 (1.31–2.84×10^5	8.2×10^5 (6.24–12.68×10^5)	2.65×10^6 (1.34–3.42×10^6)	3.7×10^7 (1.42–4.64×10^7)
Bs 2362	3.4×10^5 (2.84–4.15×10^5)	1.2×10^6 (0.12–4.42×10^6)	7.1 m 10^6 (5.86–8.96×10^6)	7.8×10^7 (6.26–9.92×10^7)
RB-80	6×10^4 (5.44–7.12×10^4)	1.4×10^5 (0.98–1.84×10^5)	Not tested	Not tested

*The values for 74-E-37-14 were higher than 1.5–$15.O \times 10^7$.

REFERENCES

deBarjac, H. 1978. Une nouvelle variete de *Bacillus thuringiensis* tres toxique pour les moustiques. *B. thuringiensis* var. Israelensis serotype 14. *C. R. Acad. Sci.* (Ser. D) 286:797–800.

Finney, J.R. and Harding, J.B. 1982. The susceptibility of *Simulium verecundum* (Diptera: *Simulidae) to three isolates of Bacillus thuringiensis* serotype 10 (*darmstadiensis*). *Mosq. News* 3:434–435.

Garcia, R. and DesRochers, B. 1980. Preliminary field trials with *Bacillus thuringiensis* var. *israelensis* against *Aedes dorsalis* and *Culex tarsalis* in salt marshes. *Proc. Am. Mosq. Cont. Assoc.*

Goldberg, L.J. and Margalit J. 1977. Bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranotenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens. Mosq. News.* 37:355–358.

Hertlein, B.C., Levy, R. and Miller, T.W., Jr. 1979. Recycling potential and selective retrieval of *Bacillus sphaericus* from soil in a mosquito habitat. *J. Invertebr. Pathol.* 33:217–221.

Hornby, J.A., Hertlein, B.C., Levy, R. and Miller T.W., Jr. 1981. Persistent activity of mosquito larvicidal *Bacillus sphaericus* 1593 in fresh water and sewage. *Wld. Hlth. Org. mimeo. doc. WHO/VBC/81.*830 8 pp.

Lacey, L.A. and Oldrache, S.H. 1983. The effect of temperature larval age, and species of mosquito on the activity of an isolate of *Bacillus thuringiensis* var. *darmstadiensis* toxic for mosquito larvae. *Mosq. News* 43:176–180.

Matur, A. and Ceber, K. a. The utilization of *Bacillus* sp. as larvicidal agent against anopheline and culicine mosquitoes in Turkey. I: Larvicidal activity of *Bacillus thuringiensis* var. *israelensis. J. Trp. Med. Hyg.* (in press).

Matur, A. and Ceber, K. b. The larvicidal activity of *Bacillus thuringiensis* var. *israelensis* (H-14) against *Culex pipiens* in Turkey. *Trop. Biomed.* (in press).

Mulligan, F.S. III, Schaefer, C.H. and Wilder, W.H. 1980. Efficacy and persistence of *Bacillus sphaericus* and *B. thuringiensis* H-14 against mosquitoes under laboratory and field conditions. *J. Econ. Entomol.* 73:684–688.

Ohba, M., Aizawa, K. and Furusawa, T. 1979. Distribution of *Bacillus thuringiensis* serotypes in Ehime Prefecture, Japan. *Appl. Entomol. Zool.* 14:340–345.

Padua, L.E., Ohba, M. and Aizawa, K. 1980. The isolates of *Bacillus thuringiensis* serotype 10 with a highly preferential toxicity to mosquito larvae. *J. Invertebr. Pathol.* 36:180–186.

Undeen, A.H. and Berl, D. 1979. Laboratory studies on the effectiveness of *Bacillus thuringiensis* var. *israelensis* deBarjac against *Simulium damnosum* (Diptera: *Simuliidae*) larvae. *Mosq. News* 39:742–745.

Ramsdale, C.D., Herald, P.R.J. and Davidson, G. 1980. Recent development of insecticide resistance in some Turkish anophelines. *Roy. L. Trop. Med. Hyg.* 83:11–19.