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FIELD EVALUATION OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* PRODUCED ON MEDIUM MADE FROM NIGERIAN AGRICULTURAL PRODUCTS

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ABSTRACT

Locally produced primary powder *of Bacillus thuringiensis* var. *israelensis* (de Barjac) was applied , to the natural breeding sites of mosquitoes in the fields and in the domestic clay pots used for water storage in homes in rural areas of Nsukka, Nigeria. The powder was applied at doses of a laboratory determined $LC_{100,}$ 2 x LC_{100} and 4 x LC_{100}. The predominant larvae in the fields were *Anopheles* spp. which transmit malarial parasites and *Culex* spp., common vectors of filarial worms. The predominant larvae in the pots were *Aedes* spp., potential vectors of yellow fever, dengue and mosquito-bome encephalitic viruses. Anopheles larvae were controlled with 4 x LC_{100} (5 ppm) while *Culex* larvae were controlled with 2 x LC_{100} (2.5 ppm). *Aedes and* other mosquito spp. larvae were controlled with 2 x LC_{100} (2-2,5 ppm).

INTRODUCTION

In recent years efforts have been directed to the use of biological agents for the control of mosquitoes and other insect pests and vectors of diseases. A very potent bacterial control agent of mosquito larvae, *Bacillus thuringiensis* var. *israelensis* (de Barjac, 1978) was isolated by Goldberg and Margalit (1977). In the intervening years, the high larvicidal activity of *B. thuringiensis* var. *israelensis (B.t.i.)* has been demonstrated in many countries in the laboratory and field by various workers (Goldberg and Margalit, 1977; de Barjac and Coz, 1979; Garcia and DesRoches, 1979; Mulligan et al., 1980; Davidson et al., 1981; De Maio et al., 1981; Majori and Ali, 1984).

The use of high doses of *B.t.i.* for vector control has shown no adverse effects on most nontarget aquatic organisms (Ali, 1981). Consequently *B.t.i.* has been produced on large scale and used extensively for vector control in many developed countries. Production and application of *B.t.i.* are however lagging behind in most Third World countries, especially in Africa, even though mosquitoes pose greater health problems in the latter countries. Laboratory scale production of *B.t.i.* has been reported in Ghana and Nigeria using blood-based medium as well as agricultural products (Anonymous, 1985; Obeta and Okafor, 1984), but reported field trials are scanty in both countries.

This paper reports small scale field evaluation of locally produced B.t.i primary powder in natural breeding sites of mosquitoes.

MATERIALS AND METHODS

Bacillus thuringiensis var. *israelensis* (de Barjac, 1978) was obtained from the laboratory of H. de Barjac of Institut Pasteur, Paris. The bacterium was grown on a medium prepared from ground seeds of bambara beans *(Voandzeia subterranea* Thours) and dried cow blood as described by Obeta and Okafor (1984). The primary powder was prepared from the broth cultures by the acetone-lactose coprecipitation method of Dulmage et al. (1970). The powder was tested against mosquito larvae in selected natural breeding sites in Nsukka, Southern Nigeria.

Selection of test site

Blocked drainage systems, quarry site ditches, flooded areas, a roadside ditch and a reservoir containing approximately 1240–6930 litres of water and an average number of 16–25 mosquito larvae per dip of 400 ml enamel dipper were selected as major test and control sites. The selection of the sites and the determination of the average number of mosquito larvae in each site were carried out as described by Obeta (1986).

Domestic clay pots used for water storage in homes in rural areas were also selected as minor test and control sites. The pots were divided into six groups; each group consisted of five pots and was designated as one test or control site. The volume of water in each pot was made up to 70 litres with tap water two weeks prior to treatment. The average number of larvae ranged from 11–13 per dip of 400 ml enamel dipper. The number was determined by making two dips per pot; the larvae collected were separated into *Aedes* spp. and other mosquito spp. They were counted immediately after taking the second dip. The larvae were then returned to the pot.

The predominant larvae in the major test and control field sites were *Anopheles* spp., followed by *Culex* spp. *Culex* larvae were predominant in few relatively polluted sites. Most sites were exposed to the direct sunlight and contained moderately clear water at the top with vegetation and mud at the bottom. *Aedes* larvae were predominant in the clay pots.

The larvae were not all identified to the species level but most of the *Anopheles* were *A. gambiae* Giles, potential vectors of malarial parasites. The majority of *Culex* larvae were *C. quinquefasciatus* Say, potential vectors of filarial worms and mosquito-borne encephalitis. The larvae in the pots were mainly *Ae. aegypti* (L.), the potential vectors of yellow fever, dengue and mosquito-borne encephalitic viruses.

Laboratory bioassay and determination of field test doses

Third instar larvae of *Anopheles*, *Culex* and *Aedes* spp., collected from breeding sites, were assayed against *B.t.i.* powder; other mosquito larval types were not assayed because they were very few. One per cent bacterial suspension was prepared in distilled water and serially diluted with clear water collected from the test sites. Twenty larvae were added to each 250 ml white plastic cup containing 150 ml dilution of bacterial suspension. Three cups were used per serial dilution and the control was made up of three cups per assay each containing 20 larvae in 150 ml clear water from test site. The assay cups were incubated at room temperature (*ca.* 28°C) for 24 h. The assay results were used to calculate the LC_{100} of each larval type.

Field test doses were calculated for the most tolerant larval group by the following formula of Singer and Ramoska (Protocol–Stage III, field testing with *Bacillus sphaericus* powder, unpublished):

$$\frac{\text{Wt (g) bacterial powder}}{\text{Vol. of water in lab. assay}} = \frac{\text{x (g) bacterial powder in field plot}}{\text{vol. of water in field plot}}$$

where x = field test dosage.

Application of *B.t.i.* powder to the test sites

Powder of *B.t.i.* equivalent to *ca.* 1, 2 and 4 times the laboratory LC_{100} were applied to test sites. Three sites were treated at each dosage while one was left untreated (control). In clay pots only two replications were made because of the difficulty in obtaining permission from the owners of the pots. The powder of *B.t.i.* was prepared for application by using the principle described by Goldberg (1979). Four millilitres of dewaxed corn oil (Archer Daniels Midland Company, Illinois) were put into solution with 30 ml dioxane (Riedel-DeHaen AG Seelze, Hanover) in 100–250 ml flask with stopper. The bacterial powder (*ca.* 11.76% w/v) was added to the oil/dioxane solution to form a

colloidal suspension. The powder readily suspended in solution with little agitation by shaking or stirring. Four per cent (v/v) Tween 20 (Fluka AG Chemische Fabrik, Switzerland) was added to the suspension and stirred for 1½ min. The mixture was quickly poured into 1,000 ml distilled water in 2–litre beaker and stirred. The resulting buoyant colloidal suspension was carried in 2–litre flasks to the test sites where further dilution of up to 1–2 g/litre was made in non-chlorinated tap water. The suspension was poured into a 20–litre knapsack sprayer (Horst, Holland) and sprayed evenly on the surface of water. Suspension of *B.t.i.* was sprayed on the surface of water in the pots with 0.6 litre continuous Flyol sprayer (Metropolitan Chemicals, London).

Fifty third instar larvae (25 *Anopheles* and 25 *Culex* spp.) collected from the test sites were suspended in floating plastic cages (8 cm high and 13 cm diameter) with two 3.5 × 11.5 cm windows screened with galvernized wire gauze (30 mesh). The cages allowed free movement of water over the larvae but prevented their escape. Two cages were placed at the centre of each test and control site before treatment except in the clay pots.

Pre-treatment counts were made prior to treatment while post-treatment counts were made 24 h after treatment as previously described (Obeta, 1986). Surviving larvae in the cages were also counted after 24 h and the average mortality was obtained after correction for the control mortality with Abbott's formula (1925). The percentage reduction in larval population in test sites after treatment was calculated by the following formula of Mulla et al. (1971):

$$\% \text{ Reduction} = \left(\frac{C_1}{T_1} \times \frac{T_2}{C_2}\right) 100$$

where C_1 = average number of larvae in the control prior to treatment; C_2 = average number of larvae in the control after treatment; T_1 = average number of larvae in treated sites prior to treatment; T_2 = average number of larvae in treated sites after treatment.

Location of test sites, volumes of water contained, amount of *B.t.i.* powder applied, date of treatment, pH and temperature of water during treatment are shown in Table 1.

RESULTS AND DISCUSSION

The average laboratory LC_{100} for *Aedes* and *Culex* larvae was 1.02 µg/ml while that for *Anopheles* larvae was 1.24 µg/ml. Field test doses were determined from the LC_{100} of *Anopheles* larvae which were the most tolerant larvae. Test doses for the clay pots were calculated from the LC_{100} of *Aedes* larvae because they were predominant. Table 2 illustrates the mean number of surviving larvae observed in the post-treatment counts and the calculated % reduction in larval population after treatments. Treatment with LC_{100} dose in test sites 2, 3 and 4 reduced the total population of all larval types by 72–80%. *Anopheles*, *Culex* and other mosquito spp. were reduced by 47–72%, 82–88% and 72–80% respectively. Application of $2 \times LC_{100}$ doses to test sites 6, 7 and 8 produced a total larval reduction of 87–91%. Reduction in larval types were *Anopheles* spp. 82–88%, *Culex* spp. 92–99% and other mosquito spp. 87–100%. Treatment with $4 \times LC_{100}$ dose in test sites 10, 11 and 12 produced a reduction in the population of all larval types by 97–99%. *Anopheles* and *Culex* larvae were reduced by 96–98% and 99–100%, respectively, while larvae of other mosquito spp. were completely destroyed (100% reduction). The average mortality in the caged larvae in the test sites at doses of LC_{100}, $2 \times LC_{100}$ and $4 \times LC_{100}$ for *Anopheles* were 67, 81 and 97% respectively while for *Culex* it was 81% at LC_{100}, 96% at $2 \times LC_{100}$ and 100% at $4 \times LC_{100}$ doses.

Table 3 shows the mean number of the surviving larvae observed in domestic clay pots during the post-treatment counts and the calculated % reduction in larval population after treatments. A dose of LC_{100} applied to groups 2 and 3 pots reduced the population of all the larvae by 78–80%. *Aedes* spp. were reduced by 77–81% while other mosquito spp. were reduced by 70–83%. Treatment

TABLE 1
Location of test sites, amount of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) applie, date of treatment, water temperature, and pH during treatment

Test site	Location	Approximate volume of water (litres)	Amount of *B.t.i.* powder applied (g)	Date of 1985	Water temperature (°C)	pH of water
1	Water reservoir — abandoned concrete water tank at State Water Board construction site *ca.* 3.4 km from Urban Girls Secondary School (UGSS), Nsukka	4012	Control	–	29.6	6.98
2	Blocked drainage system adjacent, UGSS	1313	1.63 (LC_{100})	Aug. 29	27.0	7.31
3	Blocked drainage system opposite UGSS	1240	1.54 (LC_{100})	Aug. 29	27.1	7.26
4	Blocked drainage system along Umuakashi Road *ca.* 1.8 km from UGSS	1405	1.74 (LC_{100})	Aug. 29	27.6	7.40
5	Flooded area in a building site opposite abandoned Tectonic camp. *ca.* 2 km from UGSS	4981	Control	–	27.9	7.11
6	Quarry site ditch near Ikenga Hotel *ca.* 2.4 km from UGSS	3872	9.60 ($2 \times LC_{100}$)	Sept. 3	28.4	6.92
7	Quarry site ditch *ca.* 2 m from TS 6	4035	10.01 ($2 \times LC_{100}$)	Sept. 3	28.1	7.01
8	Quarry site ditch opposite Teacher Training College (TTC) Nsukka	6316	15.66 ($2 \times LC_{100}$)	Sept. 3	27.8	6.85
9	Quarry site ditch *ca.* 4 m from TS 8 (TTC, Nsukka)	3952	Control	–	28.4	7.01
10	Roadside ditch *ca.*20 m from abandoned Tectonic camp and *ca.* 1 km from UGSS	4312	21.39 ($4 \times LC_{100}$)	Sept. 25	29.0	7.03
11	Flooded area in the abandoned army barracks *ca.* 1.1 km from UGSS	4801	23.81 ($4 \times LC_{100}$)	Sept. 25	27.8	6.90
12	Quarry site ditch along Ibagwa-Enugu-Ezike Road *ca.* 13 km from UGSS	6930	34.37 ($4 \times LC_{100}$)	Sept. 25	28.2	7.14
	Domestic clay pots were at Ihuama, Obukpa *ca.* 12 km from UGSS and at Umundu *ca.* 25 km from UGSS	70 per pot	$LC_{100} = 0.0714$ $LC_{200} = 0.143$	Oct. 13 and 15	26.8 27.4	6.94 7.02

TABLE 2
Mean number of surviving mosquito larvae and percentage reduction after treatment with LC_{100}

	LC_{100} test site			
Mosquito types	1 (control)	2	3	4
Anopheles	20.17	1.92[a]	1.58[a]	1.25[a]
	(0)	(72)	(47)	(65)
Culex	5.00	3.25[a]	3.67[a]	4.00[a]
	(0)	(82)	(85)	(88)
Other spp.	1.58	0.17[a]	0.08[a]	0.25[a]
	(0)	(80)	(72)	(79)
Total	26.75	5.34[a]	5.33[a]	5.50[a]
	(0)	(80)	(72)	(79)
	$2 \times LC_{100}$ test site			
Mosquito types	5 (control)	6	7	8
Anopheles	17.08	2.17[a]	3.08[a]	1.58[a]
	(0)	(88)	(82)	(87)
Culex	5.83	0.25[a]	0.17[a]	0.08[a]
	(0)	(92)	(98)	(99)
Other spp.	0.92	0.17[a]	0.17[a]	0.09[a]
	(0)	(88)	(87)	(100)
Total	23.83	2.59[a]	3.42[a]	1.75[a]
	(0)	(89)	(87)	(91)
	$4 \times LC_{100}$ test site			
Mosquito types	9 (control)	10	11	12
Anopheles	12.67	0.17[a]	0.25[a]	0.58[a]
	(0)	(98)	(97)	(96)
Culex	5.75	0.08[a]	0.00[a]	0.00[a]
	(0)	(99)	(100)	(100)
Other spp.	0.58	0.00[a]	0.00[a]	0.00[a]
	(0)	(100)	(100)	(100)
Total	19.00	0.25[a]	0.25[a]	0.58[a]
	(0)	(99)	(99)	(97)

Note: Means in a row under each treatment and control followed by the same letter are not significantly different at the 5 and 1% levels from each other when subjected to analysis of variance and Duncan's multiple range test. Numbers in parentheses indicate mean percentage larval reduction after treatment.

TABLE 3
Mean number of surviving mosquito larvae and percentage reduction after treatment with LC_{100} and $2 \times LC_{100}$ of *Bacillus thuringiensis* var. *israelensis* powder in domestic clay pots

	LC_{100} test site (Group 1–3 pots)		
Mosquito types	1 (control)	2	3
Aedes spp.	11.50	2.10[a]	2.70[a]
	(0)	(81)	(77)
Other spp.	3.10	0.40[a]	0.30[a]
	(0)	(70)	(83)
Total	14.60	2.50[a]	3.00[a]
	(0)	(80)	(78)
	$2 \times LC_{100}$ test site (Group 4–6 pots)		
Mosquito types	4 (control)	5	6
Aedes spp.	10.20	0.40[a]	0.10[a]
	(0)	(96)	(99)
Other spp.	2.70	0.20[a]	0.20[a]
	(0)	(92)	(91)
Total	12.90	0.60[a]	0.30[a]
	(0)	(95)	(98)

Note: Means in a row under each treatment and control followed by the same letter are not significantly different at the 5 and 1% levels from each other when subjected to analysis of variance and Duncan's multiple range test. Numbers in parentheses indicate mean percentage larval reduction after treatment.

with $2 \times LC_{100}$ in groups 5 and 6 pots results in the reduction of all the larval types by 95–98%. *Aedes* larvae were reduced 95–98% while larvae of other mosquito spp. were reduced by 91–92%.

A total reduction of 82–88% and 97–99% were observed in larval population of mainly *Anopheles* and *Culex* spp. in natural breeding sites containing vegetation, mud and debris after treatment with $2 \times LC_{100}$ (2.5 ppm) and $4 \times LC_{100}$ (5 ppm) of the locally produced *B.t.i.* powder. These figures compare favourably well with 95–100% obtained by Davidson et al. (1981) in a larval population of similar mosquito types in artificially constructed ponds with $2 \times LC_{100}$ and $4 \times LC_{100}$ of commerically produced *B.t.i.* powder. Using Roger Bellon WDP preparation of *B.t.i.* in a roadside ditch and laterite ponds located in Kaduna, Northern Nigeria, Prasertphon and Knudsen had earlier (1980) reported 65–100% reduction in larval population of *Anopheles* and *Culex* spp. at a concentration of 10 mg/l or 10 ppm (unpublished report to WHO). The reduction in larval population also compares well with 82–100% obtained for *Anopheles* and *Culex* in this work with $2 \times LC_{100}$ and $4 \times LC_{100}$ which are approximately 2.5–5 ppm.

A larval population of mainly *Aedes* spp. was reduced in the clay pots by 77–81% and 95–98% with LC_{100} (1 ppm) and $2 \times LC_{100}$ (2 ppm) of the same *B.t.i.* powder. These figures are also fairly close to the work of De Maio et al. (1981) who reported 85–100% reduction in a population of *Ae.*

triseriatus Say in tree holes (oak and beeches) and tyres after application of 1 mg/l or 1 ppm of *B.t.i.* (Abbott powder ABG 6108).

A total reduction in the larval population of *Anopheles* spp., *Culex* spp. and *Aedes* spp. in their natural breeding sites by 96–98%, 99–100% and 95–98% respectively, after treatment with locally produced *B.t.i.* powder, is quite encouraging in a region where the diseases transmitted by these three important mosquito species constitute serious health hazard resulting in economic losses in terms of morbidity, mortality and man power.

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