EVALUATION OF *BACILLUS THURINGIENSIS* SEROTYPE H-14 FORMULATIONS AS LARVICIDES FOR *ANOPHELES ARABIENSIS* (SPECIES B OF THE *AN. GAMBIAE* COMPLEX)

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ABSTRACT. Formulations of *Bacillus thuringiensis* serotype H-14 were evaluated in the laboratory for larvicidal potency against the Afro-tropical malaria vector *Anopheles arabiensis*. The 3 formulations tested were: standard IPS-78 water-dispersible powder; an experimental water-dispersible powder (ABG-6108); a water-dispersible concentrate (San 402–1). Mortality was recorded for batches of 25 second instars exposed for 24 hr, giving LC_{50} values of 0.159, 0.211 and 0.163 ppm, and for 48 hr giving LC_{50} values of 0.113, 0.119 and 0.099 ppm, respectively, for the 3 formulations. When compared with the IPS-78 standard wdp the relative activity at LC_{50} level was 0.75 for 24 hr and 0.94 for 48 hr exposure to ABG-6108 wdp; 0.97 for 24 hr and 1.14 for 48 hr exposure to San 402–1 wdc. It is concluded that wdp formulations are less efficacious than emulsion which has prolonged availability for ingestion by surface-feeding anopheline larvae.

Anopheles arabiensis Patton is the most widespread species belonging to the *An. gambiae* complex of sibling species of mosquitoes in the Afro-tropical region, i.e. tropical Africa, southern Arabia, Madagascar, Pemba, Zanzibar and other islands. Throughout this range *An. arabiensis* is an important vector of human malaria and contributes to local transmission of arboviruses and filarial parasites affecting man and domestic animals (White 1974). Thus *An. arabiensis* should be controlled for the protection of people and livestock.

House spraying with residual insecticides has not been sufficiently effective for the reduction of adult anopheline survival and interruption of malaria parasite transmission in situations where *An. arabiensis* and *An. gambiae* are the main vectors, e.g. in the Sudan savanna belt (Molyneaux and Gramiccia 1980). This is because man-biting females of these species may be partially or completely exophilic (i.e. outdoor-resting) and so fail to pick up lethal doses of insecticide from the deposits on sprayed surfaces indoors. Behavioral variability among adult female mosquitoes seems to be correlated with inherent polymorphisms (Coluzzi et al. 1979) endowing *An. gambiae* and especially *An. arabiensis* with adaptability that complicates their control. Therefore we are considering the use of larvicides as well as adulticides and other management strategies for the control of *An. arabiensis* in Sudan where this species is the main vector of malaria. Since resistance to organochlorine and organophosphate insecticides has already developed in *An. arabiensis* of the Gezira Irrigated Area (Haridi and Nugud 1979), alternative larvicidal agents are needed. The biological control agent *Bacillus thuringiensis* serotype H-14 seems to be worth consideration for use against *An. arabiensis* larvae.

The strain of *Bacillus thuringiensis* Berliner isolated by Goldberg and Margalit (1977) was subsequently designated serotype H-14 and named var. *israelensis* (de Barjac 1978a). Laboratory studies showed that *Bacillus thuringiensis* var. *israelensis* (serotype H-14), for which we adopt the abbreviation *Bti*, has larvicidal activity against several genera and species of mosquitoes (Goldberg and Margalit 1977, de Barjac 1978b, de Barjac and Coz 1979, Lacey and Lacey 1981) and

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blackflies (Undeen and Nagel 1978), as well as Lepidoptera larvae (Ignoffo et al. 1981). This serotype H-14 differs from other *B. thuringiensis* strains which have been employed for controlling caterpillars in its high toxicity to mosquito and blackfly larvae (de Barjac 1978b, Ignoffo et al. 1981). During sporulation each serotype of *B. thuringiensis* produces parasporal crystals of a particular protoxin (Tyrell et al. 1979), usually known as the δ-endotoxin, being a glycoprotein which becomes activated to toxins by the digestive processes of particular insects which are therefore susceptible to the specific serotype (Forsberg 1976). A *Bti* reference standard powder (IPS-78) was produced by the Pasteur Institute, Paris and several experimental preparations are now available from commercial sources (WHO 1979a). This paper compared the larvicidal activity of water dispersible powder and concentrate formulations of *Bti* applied to *An. arabiensis* larvae.

MATERIALS AND METHODS

A strain of *Anopheles arabiensis* (SENN) from Sennar area, Sudan, susceptible to DDT (100% mortality on 4.0% DDT for 2 hr), resistant to dieldrin (100% survival on 4.0% DL for 2 hr) and susceptible to malathion (100% mortality on 5.0% malathion for 1 hr), was maintained at the London School of Hygiene and Tropical Medicine and used in all experiments. Larvae were reared on a diet of finely ground Farex® baby food and kept at a temperature of 25-26°C. Lots of 25 second instar larvae in 200 ml of freshly treated deionized water were used for each test, replicated 3-6 times at each concentration. All tests were conducted in clean 250 ml plastic tubs which were discarded after use. Mortality of treated and control larvae was counted after 24 and 48 hr continuous exposure to the treatments.

Three preparations of *Bti* were tested:

1. The Pasteur Institute IPS-78 reference standard water-dispersible powder (wdp) designated with an arbitrary titer of 1000 International Toxic Units (ITU) per mg.
2. An experimental wdp from Abbott Laboratories Ltd. Lot code number ABG-6108 (density 1.3g/ml).
3. A water-dispersible concentrate (wdc) "San 402-1" from Sandoz Ltd. (density 1.1g/ml).

For wdp use, a quantity of 200 mg was suspended in 5 ml of deionized water in a 17 × 1.5 cm test tube, and thoroughly agitated in a vortex mixer for 3 min. (de Barjac 1979). One ml of this suspension was immediately diluted to a volume of one liter in a conical flask to give a stock solution of 40 mg per liter or 40 ppm (w/v). This was then further diluted to give the required testing doses, taking care to shake the flask well first in order to overcome sedimentation which became noticeable within a few minutes.

The stock emulsion was prepared by diluting one ml of wdc (specific gravity 1.1) with deionized water to make a volume of one liter. This contained 154 mg of *Bti* per liter, or 154 ppm (w/v), as determined from the dry weight after evaporation of 1 ml wdc heated for 4 hr in a ventilated oven at 113°C; procedure recommended by Dr. H. D. Burges (personal communication). Further dilutions of the wdc were made as described for the wdp suspensions.

RESULTS

Figure 1 shows dose/response regression lines plotted from data for larval mortalities after exposure to concentrations ranging from 0.01 to 0.4 ppm for the wdp and 0.0385 to 1.54 ppm for the wdc. Results were computer analyzed using a maximum likelihood probit regression analysis program, weighting values according to the number of observations for each treatment concentration, to obtain estimates of the LC_{50} and LC_{90} values for the 3 preparations (Table 1). Standard deviations were only 0.001-0.003 ppm.

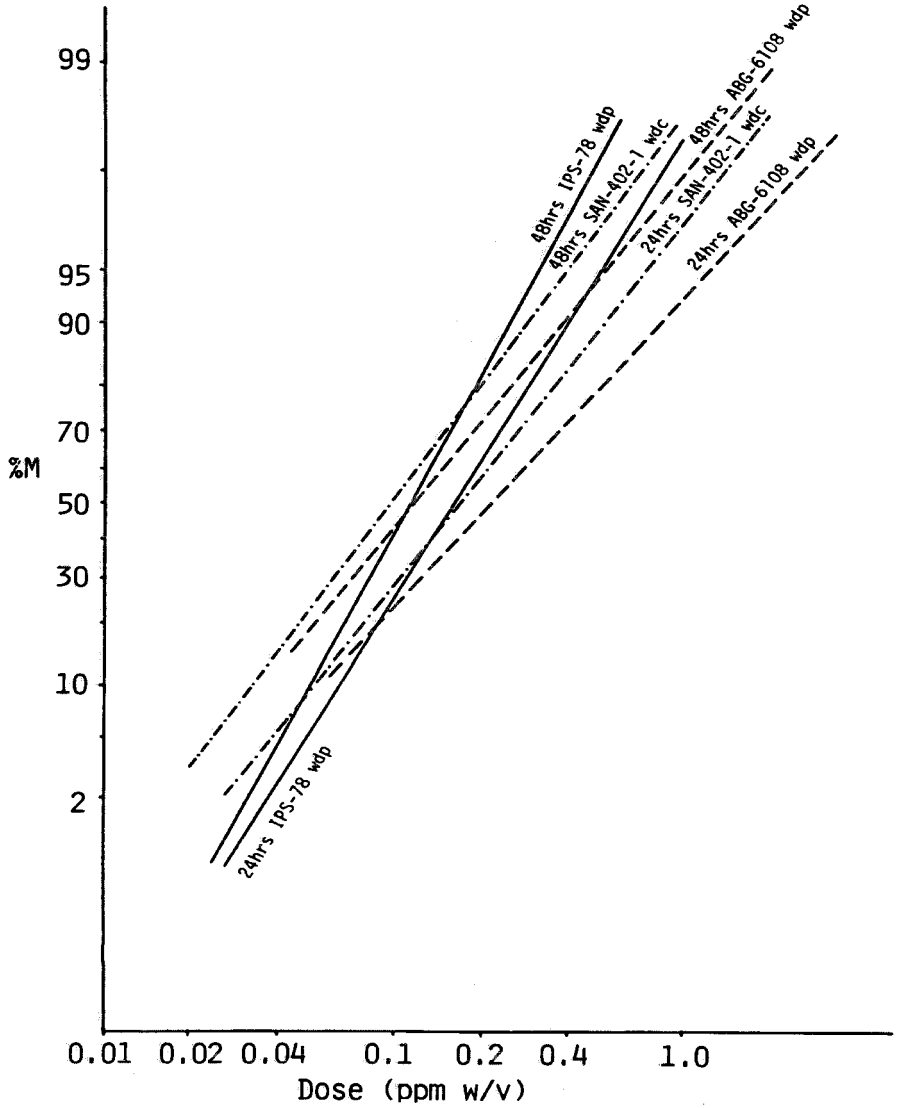

Fig. 1. Dose/mortality response of *Anopheles arabiensis* to 3 formulations of *Bacillus thuringiensis* serotype H-14.

Table 1. LC_{50} and LC_{90} values expressed as ppm (w/v) for *An. arabiensis* larvae exposed to *Bti* at the second instar.

Bti preparation	Exposure (hr)	LC_{50}	LC_{90}
1. wdp IPS-78	24	0.159	0.395
	48	0.113	0.249
2. wdp ABG-6108	24	0.211	0.838
	48	0.119	0.296
3. wdc San 402-1	24	0.163	0.510
	48	0.099	0.293

Continuous exposure for 48 hr as compared with 24 hr gave increased effectiveness, in terms of greater larval mortality, by factors of 1.4 to 2.8-fold.

At the LC_{50} level, the potency of the experimental preparations, expressed in terms of International Toxic Units (ITU), can be calculated by comparison with the standard IPS-78 wdp from the following formula given by WHO (1979b):

$$\frac{100 \times LC_{50} \text{ standard}}{LC_{50} \text{ tested preparation}}$$

Thus the titers of preparations tested were determined for 24 and 48 hr larval exposure as follows:

wdp ABG-6108 = 750 - 940 ITU/mg
wdc San 402-1 = 970 - 1140 ITU/mg

DISCUSSION

Comparing the water-dispersible powders with each other, the results show that the IPS-78 standard was more effective than the experimental preparation ABG-6108 against *An. arabiensis* larvae. This agrees with other comparative studies (e.g. Van Essen and Hembree 1980) and is enhanced by the steeper slope of dose/response lines for IPS-78 (Fig. 1). However, the relative activity (R.A. = LC_{50} IPS-78/LC_{50} ABG-6108) values of 0.75 and 0.94 with *An. arabiensis* were only about half as much as those reported by Van Essen and Hembree (1980) for *Ae. aegypti* (Linn.) as 0.36 at 24 hr and 0.42 at 48 hr exposure of larvae. This inconsistent relative activity of the two powders can be attributed to the difference between *An. arabiensis* and *Ae. aegypti* larvae in their intrinsic susceptibility to *Bti* (de Barjac 1978b, de Barjac and Coz 1979), in combination with differential ingestion rates arising from behavioral contrasts between surface-feeding anopheline larvae versus bottom-feeding culicine larvae; c.f. observations on differential intake and susceptibility to *Bacillus sphaericus* by *An. albimanus* (Wied.), *Cx. quinquefasciatus* Say and *Ae. aegypti* (Ramoska and Hopkins 1981).

The emulsion made from wdc San 402-1, which was found to have high ITU strength (see Results Section) had lower LC_{50} than for wdp ABG-6108 and similar LC_{50} to that for wdp IPS-78 at 24 hr exposure; but when *An. arabiensis* larvae were continuously exposed for 48 hr, the effectiveness of the wdc was greater than for either wdp. We take this to show that, for effective control of anopheline larvae, it is more efficacious to formulate *Bti* so that it persists in availability and becomes ingested readily by the larvae, rather than simply to apply higher dosages to the water containing larvae. The advantage of using an emulsion against anopheline larvae is presumably due to reduced rates of sedimentation, whereas the rapid sedimentation of wdp formulations may be beneficial for use against culicine larvae that feed by browsing on the substrate. We are therefore improving the formulation of *Bti* in order to optimise its availability to *Anopheles* larvae which feed mainly from the water surface.

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