THE EFFECT OF CLAY ON THE PERSISTENCE OF BTi TOXICITY AGAINST MOSQUITO LARVAE IN MOROGORO, TANZANIA

B.S. Kilonzo, S.S. Madoffe, S.M.S. Maliondo, B.M. Msanya, R. Mabagalla and D.H. Kitojo

Abstract:

Laboratory colonies of Anopheles gambiae and Culex quinquefasciatus were reared from parental larvae obtained from the Ifakara Health Research Centre and septic tanks in Morogoro Municipality respectively. Water ponds (1 M³) were constructed at selected sites and left open for mosquitoes to breed in freely. Various concentrations of Bti crystals were mixed with varying concentrations of clay and tested against third instar larvae of each species at various periods of time post-preparation. A total of 60 larvae (20 x 3) were exposed to each mixture in the laboratory, and each experiment was replicated four times. Mortality rates were recorded after 24h. exposure.

The observations revealed that for every concentration of Bti crystals used, the toxicity on both species of mosquitoes was less persistent in crystal/clay mixtures than in crystals alone. It was also shown that such persistence was much less in mixtures containing large concentrations of clay (5mg/ml) than in those containing low concentrations (0.05 - 0.5 mg/ml) of clay. It was further observed that the toxicity against Culex and Anopheles larvae was more persistent in mixtures containing higher concentrations of Bti crystals (0.12 - 0.3 μg/ml) than in those containing low concentrations (0.03 - 0.06 μg/ml) of the toxin. Furthermore, it was observed that C. quinquefasciatus larvae succumbed to Bti crystal/clay mixtures for longer periods than A. gambiae larvae.

It was generally concluded that clays have a negative impact on the persistence of Bti toxicity against mosquito larvae and that such impact is more significant in mixtures containing large concentrations of clay. It was also concluded that despite the environmental friendliness of Bti, its applicability by communities is limited in view of its short persistence when it is adsorbed to tropical soils which are found in most natural mosquito breeding sites.

Introduction

Mosquitoes are probably the most important and most widely distributed insect vectors of human diseases in Tanzania and elsewhere in the tropics. Of the many species found all over Tanzania, *Anopheles gambiae, A. funestus* and *Culex quinquefasciatus* are the major vectors of bancroftian filariasis while the former two species are the main vectors of human malaria (White and Magayuka, 1968, Magayuka and White, 1971;

Minjas and Kihamia, 1991; Kilama, 1995). *Aedes aegypti* has been demonstrated to be a potential vector of bancroftian filariasis (White and Munnis, 1971).

Many conventional measures for controlling both adult and larval stages of mosquitoes have been and are still being applied in many parts of the country. Most of such measures involve chemical methods despite their detrimental effects on the environment and the fact that their continuous application facilitates development of resistance among the target and non-target species. Recent investigations however, have shown that use of insecticide (chemical) - impregnated nets, a method whose application does not easily contaminate the environment, substantially reduces mosquito populations and malaria transmission (Kilama, 1991; Njunwa et al. 1997). However, the method is not, at the moment, applied by most communities who are at risk, probably due to high costs of the nets.

Biological methods including predators and pathogens such as larvivorous fish, and insecticidal bacteria are environmentally benign. However, such methods have been little applied in Tanzania. *Bacillus thuringiensis subspp. israelensis* (Bti) has been shown to be quite promising and widely applied in controlling Anopheles, and Simulium larvae elsewhere (Muller *et al.* 1982; Kilama, 1991; WHO, 1991; Margalit *et al.* 1995). Kilonzo *et al.* (2001) revealed that Bti crystals were effective against *A. gambiae* and *C. quinquefasciatus* in the laboratory and that clays have negative impacts on such effectiveness.

However, various observations in the field have shown that the Bti toxin is probably affected by various environmental and climatic factors including UV light, PH, heat and organic materials which consequently reduce its toxic effects (Leong *et al.* 1980; Ignoff *et al.* 1981; Schnell *et al.* 1984). Despite such impact, it was felt desirable to find out whether clays also affect the persistence of Bti toxicity against *Anopheles* and *Culex* larvae and thereby considers a possibility of coupling the toxin with clays prior to application in the targetted mosquito breeding sites. The purpose of this paper therefore is to report observations of persistence experiments carried out with laboratory-reared mosquito larvae, and Bti crystal/clay mixtures.

Materials and Methods

Stock and Experimental Mosquitoes:

Parental larvae (mixed instars) of *Culex quinquefasciatus* were collected from a septic tank in Morogoro Municipality and used to raise a stock colony of the insect in captivity. Parental larvae (second instar) of *Anopheles gambiae* were obtained from an insectary at the Ifakara Health Research and Development Centre, and similarly used for raising a stock colony in captivity. Each colony was kept in a separate room of the insectary where the mean temperature and relative humidity were maintained at 27 - 30^{0}C and 80 - 90% respectively. Larvae were fed on special fishmeal (Tetramin) obtained from Germany through the above Centre. Adult mosquitoes were fed on sugar solution adsorbed in clean cotton wool. Whenever oviposition was required in order to obtain larvae for experimental purposes or for expanding the stock colony, the relevant colony was starved for 24h and fed on a young guinea pig obtained from a well maintained laboratory colony, partly shaved on the abdomen and confined in a wire-mesh strainer. The animal was removed as soon as large numbers of female mosquitoes were fully-gorged. Occasionally however, *Anopheles* females were reluctant to feed on guinea pigs and hence voluntary hand-feeding was adopted.

Eggs were oviposited on wet pieces of filter paper placed in petri dishes containing small amounts of distilled water and placed in the cages. The filter papers with eggs were then transferred to clean plastic trays (40 x 25 x 12 cm) or round stainless-steel trays (27.5 x 4 cm) half filled with distilled water. Soon after hatching, larvae were regularly fed as described for parental larvae above.

Origin and preparation of stock and experimental materials

Mother stock suspensions (MSS) of *Bacillus thuringiensis israelensis* (Bti) crystals were isolated and supplied by Dr. Otorino of the CNR Instituto per la Genesis e-l' Ecologia del Suolo, Italy in a concentration of 4.339 mg/ml. The crystals (4.339 mg/ml MSS) were diluted so as to obtain an ESS of 30.025 µg/ml.

Stock suspension of standard clay was provided by the same supplier (Dr. Otorino), at a concentraion of 33.3 mg/ml. Known volumes of the clay were thoroughly mixed with known volumes of de-ionised water and Bti crystals to obtain various concentrations of Bti crystal/clay mixtures used in the experiments.

Preparation and tests with Bti crystals and Bti/clay mixtures

Three different volumes (1.5, 15 and 150 ml) of clay were thoroughly mixed with five different volumes (1, 2, 4, 5, 10 ml) of the 30.025 µg/ml ESS of Bti crystals and made up to 1000 ml with de-ionised water. Final concentrations of the mixtures were as follows:

Bti crystals (ml)	**1**	**2**	**4**	**5**	**10**
Amount of clay (ml)	1.5 15 150	1.5 15 150	1.5 15 150	1.5 15 150	1.5 15 150
Bti crystal concentration (µg/ml)	0.03	0.06	0.12	0.15	0.3
Clay concentration (mg/ml)	0.05 0.5 5.0	0.05 0.5 5.0	0.05 0.5 5.0	0.05 0.5 5.0	0.05 0.5 5.0

The mixtures, together with the various concentrations of Bti crystals alone, were kept at room temperature and tested against larvae of laboratory-reared *A. gambiae and C. quinquefasciatus* larvae at 7 days intervals for 63 and 84 days respectively.

Equal volumes (150 ml) of the mixture containing 0.03 µg/ml Bti crystals and 0.05 mg/ml clay were dispensed into three clean plastic cups. A similar procedure was repeated with the same concentration of Bti crystals alone and mixtures containing the same concentration of Bti and various concentrations of clay (0.05, 0.5 & 5.0 mg/ml). Similarly, such tests were carried out with mixtures of 0.06, 0.12, 0.15 and 0.3 µg/ml Bti and 0.05, 0.5 and 5.0 mg/ml clay, and similar concentrations of Bti crystals alone. De-ionized water was used as controls.

Batches of 20 late third instar larvae of the experimental mosquitoes (same age and size) were pipetted from the rearing trays into small beakers containing distilled water. They were then poured into a fine tea siever and transferred to the above plastic cups containing either Bti crystals alone, Bti crystal/clay mixtures or de-ionized water (control). Mortality rates were recorded after 24h exposure. Moribund larvae were considered as dead.

Results and Discussions

Pure Bti crystals with concentrations ranging from 0.03 to 0.3 µg/ml killed over 50% of *Culex* larvae for up to 42 - 56 days post - preparation (Table 1). When the same concentrations of the crystals were coupled with 0.05 mg/ml of clay, they killed over 50% of the same species of larvae for up to 7 - 28 days only. However, mixtures of similar concentrations of Bti crystals with 5.0 mg/ml of clay failed to kill 50% of the larvae on day 7 after their preparation.

In fact such mixtures were totally ineffective against *Culex* larvae after 28 days of their preparation. These results suggest that the clay, which was used, has a negative impact on the persistence of larvicidal effects of Bti crystals against *Culex,* and that such impact is more significant in large concentrations of clay. The results also show that persistence of larvicidal effect is longer in high concentrations of Bti crystals (56 days for 0.3 µg/ml) than in low concentrations (0.03 - 0.15 µg/ml) Tables 1 - 3.

A similar pattern was observed for *Anopheles* larvae. However, mixtures containing low concentrations of Bti (0.03 - 0.06 µg/ml) did not kill the larvae. Mixtures containing high concentrations (0.12 - 0.3 µg/ml) were effective for up to 14 days (Tables 4 - 6).

LT50 was only obtained with mixtures of 0.3 µg/ml of crystals with 0.05 - 0.5 mg/ml of clay at 7 day post-preparation.

The observations broadly suggest that Bti crystals alone are effective against larvae of *C. quinquefasciatus* and *A. gambiae* for longer periods (i.e. more persistent) than Bti crystals coupled with clay. The data also suggest that the persistence of such effectiveness is longer for *C. quinquefasciatus* than for *A. gambiae*, a fact which is probably attributable to the feeding habits of the two species. The clay-coupled Bti crystals tend to sink under the surface of the water/suspension in the test cups and hence more available to *Culex* larvae which feed at deeper sites of water bodies, than to *Anopheles* larvae which are mostly surface feeders. It was concluded from the current observations that the larvicidal effect of Bti crystals alone is more persistent than in crystals/clay mixtures, and that Bti crystals/clay mixtures are less effective on larvae of *A. gambiae* than those of *C. queinquefasciatus*, a fact which is probably attributable to the feeding habits of the two species.

Acknowledgments

Our sincere gratitudes are due to the European Union (EU) for funding a project on "The Impact and fate of pesticidal proteins from *Bacillus thuringiensis spp.* in tropical soils and waters as influenced by interactions with clays - MICROBE - (Project No. INCO no ERBICI8 CT 970135) under which this study was carried out. We also wish to thank the Sokoine University of Agriculture Administration for permission to conduct the studies, and all our Project collaborators, especially Prof. G.G. Ristori, Dr. O.L. Pantani and Dr. A. Fereres for provision of various materials and for useful contributions during our annual and other project meetings. Our final appreciations are due to the support staff of the MICROBE project for rearing our stock and experimental mosquitoes, the Ifakara Health Research and Development Centre for provision of our parental larvae of *Anopheles gambiae* and special larval food, Mr. R.S. Shemdoe for computing and analysing our data, and Mrs. T. Sembua for typing the manuscript of this paper.

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