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EFFICACY OF VECTOBAC DT AND CULINEXCOMBI AGAINST MOSQUITO LARVAE IN UNUSED SWIMMING POOLS IN MALINDI, KENYA

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ABSTRACT. The efficacy and persistence of 2 bacterial larvicides, Vectobac-DT (*Bacillus thuringiensis israelensis* [Bti]) and CulinexCombi (Bti and *Bacillus sphaericus* [Bs]), were tested against *Anopheles gambiae* and *Culex quinquefasciatus* in temporarily unused swimming pools with rainwater in Malindi, Kenya. Pre- and posttreatment larval densities were recorded by sampling with the standard WHO dipping technique for 8 consecutive days. The larvicides were applied to the pools with a knapsack sprayer. The data showed that Vectobac-DT was highly effective against early instars of *An. gambiae* with 89% reduction within 24 h but not as effective against the early stages of *Cx. quinquefasciatus* with reduction of only 46%. CulinexCombi resulted in high mortalities to early instars of both species with over 97% reduction within 24 h, but showed a drastic reduction 48 h after application. Both Vectobac-DT and CulinexCombi were highly effective against late instars of both species, whereby Vectobac-DT persisted much longer than CulinexCombi. *Anopheles gambiae* was found to be more susceptible to both larvicides than *Cx. quinquefasciatus*. By their high efficacy and good persistence against mosquito larvae, both Vectobac-DT and CulinexCombi can be recommended for use in integrated mosquito control programs.

KEY WORDS Vectobac-DT, CulinexCombi, *Anopheles gambiae*, *Culex quinquefasciatus*

INTRODUCTION

Mosquitoes continue to be of increasing importance as vectors of major human infectious diseases such as malaria and filariasis. Malaria is a serious health problem in many tropical and subtropical countries, infecting between 300 and 500 million people annually, and is the leading cause of infant and child mortality in sub-Saharan Africa (WHO 1995, WHO/UNICEF 2003). Control of mosquito-borne diseases such as malaria has been facing serious challenges with the evolution and rapid spread of resistance to the common antimalarial drugs, coupled with the widespread insecticide resistance in the main mosquito vectors (Chandre et al. 1999, Hemingway and Ranson 2000, Phillips 2001, Mittal 2003).

Integrated vector control has received low priority over the past decades. However, more emphasis is currently being laid on vector control with the awareness that reduction of transmission is more important and of higher efficacy than previously thought. Measures for vector control include environmental management, chemical and biological control, as well as personal protection (Walker 2002). Following the continuous formation of larval habitats due to inadequate drainage systems, there is renewed interest in larval control with the availability of bacterial larvicides and insect growth regulators, which are effective mosquito control agents (WHO 1999). The advantages of these control agents over chemical control approaches include their effectiveness and safety to humans, nontargeted wildlife, cost effectiveness, and a reduced risk of resistance development (WHO 1999, Walker 2002, Mittal 2003, Sharma et al. 2003).

Bacillus thuringiesis israelensis (Bti) and *Bacillus sphaericus* (Bs) are potent bacterial larvicides that have been successfully used for control of mosquito larvae (Balaraman et al. 1983, Ansari et al. 1995, Biswas et al. 1997, Kumar et al. 1998). These 2 species of bacteria have been widely demonstrated to be effective larvicides against several mosquito species (Walker 2002, Mittal 2003, Russell et al. 2003). Whereas Bti activity does not last longer than a week in the field (Fillinger et al. 2003, Russell et al. 2003, Sharma et al. 2003), Bs (serotype H-5a5b) formulations have shown the capacity to persist and recycle in the field (Walker 2002, Fillinger et al. 2003, Fillinger and Lindsay 2006). The recycling capacity of Bs explains the longer duration of its larvicidal activity.

The efficacy of the bacterial toxins against mosquito larvae may be influenced by environmental conditions such as the concentration of suspended solids, water temperature and water depth, ionic content of the water, larval density, solar radiation, flow regime, and vegetative cover (Walker 2002). The toxin is short lived and degraded rapidly by UV light in aquatic environments. Its effectiveness is more related to particle concentrations on the upper layer of the water,

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and depth is not very critical (Mulla et al. 1990). Bti generally requires relatively clean water to be effective, whereas Bs can be used successfully in wastewater with high organic pollution. Despite its high efficacy and longer residual activity, the intensive use of *B. sphaericus* against mosquitoes has resulted in high levels of resistance (Mittal 2003). So far there are no documented cases of Bti resistance.

The objectives of this study were to assess the efficacy and residual activity of Vectobac-DT (Bti) and CulinexCombi (Bti+Bs) against *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say in unused swimming pools in Malindi, Kenya. The overall objective of the study was to establish a solution that will most likely address the problem of mosquito breeding in the many unattended swimming pools during the off-peak tourism season. During the tourism season (November to March) the pools are filled with tap water, regularly cleaned and chlorinated, and are thus unsuitable for mosquito breeding. However, during the off-peak season (April to July) the pools remain unused and unattended to. This period coincides with the long rains, and hence the pools constantly accumulate rain water and become important larval habitats for mosquitoes. The study was carried out within the frame work of a large integrated vector control project covering an area of 16 km^2, to collect baseline data for the routine off-peak tourism season management of swimming pools.

MATERIALS AND METHODS

Study area

The study was conducted in the coastal town of Malindi, which is the tenth largest town in Kenya and a major tourist destination. Malindi is approximately 108 km north of Mombasa and has a population of 80,000 people living within the municipality. Malindi District along the Kenyan coast serves as a seasonal tourist destination.

Mean daily minimum and maximum temperatures are 22 and 30°C, respectively, with RH 65%, as reported by the Malindi Meteorological Station located southwest of town at the Malindi Airport. Malindi is comprised of commercial, agricultural, and residential areas, with patches of vegetation and forest within and around the urban center. Altitudes range from sea level to approximately 50 m in the periurban areas west of town. Coastal Kenya has 2 distinct wet seasons, April to June and October to November. Precipitation varies from 75 to 1,200 mm per year throughout the coastal plain. Tourism, fishing, and trading are the major economic activities in this area, although many urban residents also engage in small-scale urban farming for personal consumption and sale.

Study design

The efficacy of the larvicides was studied in rain water collecting in temporarily unused swimming pools located within private residences in the up-market area of Malindi during the off-peak tourism season. A total of 12 swimming pools were selected, which were infested with larvae of *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say. Four pools were treated with Vectobac-DT (Bti only), and 4 were treated with CulinexCombi (Bti + Bs). Four pools served as untreated control to follow the natural fluctuation in the larval abundance.

Each of the Vectobac-DT tablets contains 1.3 $\times$ 10^6 International Toxic Units (ITUs) of Bti. The CulinexCombi tablets contain the following active ingredients: 1.0 $\times$ 10^6 ITUs of Bti and 2.5 $\times$ 10^4 ITUs of Bs. Both products are free of viable spores and contain only the bacterial toxins as active ingredients. The field concentration based on calibration trials was set at 2,000 liters per tablet. This corresponds to 650 ITUs Bti per liter for Vectobac-DT, and 520 ITUs Bti plus 1.25 ITUs of Bs in the case of CulinexCombi.

To determine the required dosage of the larvicides, the water volume of all the test pools was measured, and dosage was based on the water volume in the pool. Larvicide suspensions were then evenly applied to the water surface of the pools with manual knapsack sprayers (Hardi Kenya, Nairobi). This first day of treatment represented day 0 of the treatment application.

Larval sampling

Pretreatment and posttreatment larval densities for all the pools were obtained by sampling with the standard dipping technique (WHO 1975). In every pool 10 dips of 350 ml each were taken at random. A separate set of dipper and sieves were used for each treatment and control. In every dip all larvae were recorded according to species and instar. Posttreatment sampling was done daily for 8 consecutive days.

The percentage reduction in the larval densities was calculated using Mulla's formula (Mulla 1971) as follows:

$$\% \text{ reduction} = 100 - (C1/T1 \times T2/C2) \times 100,$$

where $C1$ and $C2$ are the counts in control pools before and after treatment and $T1$ and $T2$ are the counts in treated pools before and after treatment.

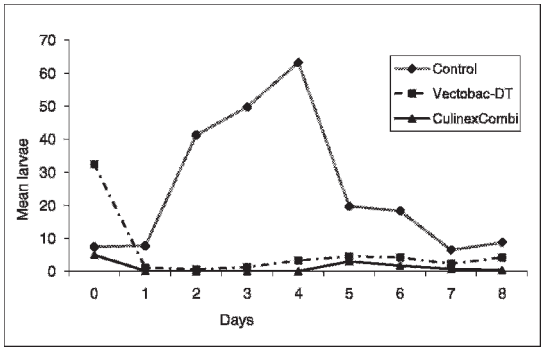

Fig. 1. Population dynamics of *An. gambiae* in control and treatment pools following the application of Vectobac-DT and CulinexCombi.

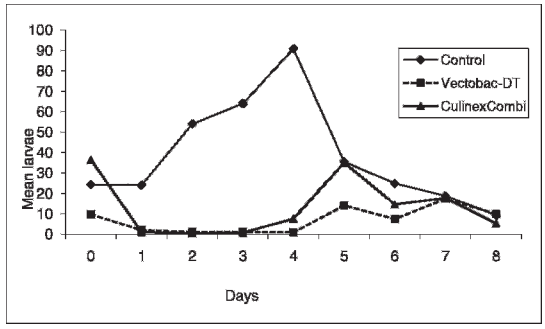

Fig. 2. Population dynamics of *Cx. quinquefasciatus* in control and treatment pools following the application of Vectobac-DT and CulinexCombi.

RESULTS

Population dynamics of *An. gambiae* and *Cx. quinquefasciatus* in unattended swimming pools

The strong dynamic of the population of *An. gambiae* in the control pools as compared to the treated pools over the 8-day trial period is shown in Fig. 1. In the control pools, there was a sharp increase in the population of late stages of *An. gambiae* within the first 4 days followed by a sudden decrease in the fifth day. This was in contrast with the treatment pools where population dropped drastically in 1-day posttreatment and remained substantially lower over the 8-day trial period.

The natural population flow of *Cx. quinquefasciatus* in the control pools as compared to the treated pools over the 8-day trial period is shown in Fig. 2. Like in the case with *An. gambiae*, population dynamics of late stages of *Cx. quinquefasciatus* followed a similar trend. As an example, the efficacy of Vectobac-DT and CulinexCombi is demonstrated showing that the *Anopheles* and *Culex* population dynamic could be broken and kept at a very low level.

Efficacy of Vectobac-DT and CulinexCombi against *Anopheles gambiae*

Vectobac-DT was effective against early instars of *An. gambiae* with a reduction of 89% of the larval population within 24 h. This effect lasted up to day 4 after application (Table 1). The larvicidal impact decreased on the fifth and sixth days, when reduction in larval density dropped to 41% and 16%, respectively. Vectobac DT was very effective against late instars, reducing the population by 97% within 24 h of posttreatment (Table 1). The reduction even increased gradually to 99% within 72 h. Larvicidal impact generally remained high up to day 8 posttreatment with Vectobac DT.

CulinexCombi resulted in very high mortalities to early instars of *An. gambiae* 24 h after application with a reduction in larval density of 99%. However, large variations were recorded during the following days of the experiment (Table 2).

CulinexCombi greatly reduced larval density of late instars of *An. gambiae* with 97.2% at 24 h posttreatment. The efficacy increased to 100% from 72 to 96 h. The effect was temporarily reduced to 77.2% by day 5 but later again increased gradually to 92.7% by the eighth day posttreatment (Table 2).

Efficacy of Vectobac-DT and CulinexCombi against *Culex quinquefasciatus*

Vectobac-DT was not effective against early instar *Cx. quinquefasciatus* since the reduction was only 46% within 24 h and dropped sharply to 23% within 72 h after application (Table 1). At day 4 a slight rebounce to 61% was noted. The larvicidal impact of Vectobac-DT was on the late instars of *Cx. quinquefasciatus*. The reduction of the larval population was above 97% for the first 3 days and remained at 94% on day 4 (Table 1). On day 5 the activity dropped to 34% and remained relatively low up to day 8.

Initial efficacy of CulinexCombi against early instars of *Cx. quinquefasciatus* was good with a reduction of 83% at 24 h. However, for the remaining 7 days CulinexCombi did not show any relevant effect (Table 2). CulinexCombi had a high larvicidal impact on late instars of *Cx. quinquefasciatus*. Reduction of the larval population was at 81% within 24 h posttreatment and increased to 95% within 48 h, reaching a maximum of 98% within 96 h. The effect of CulinexCombi was lost at day 4 for the remaining period of the experiment (Table 2).

DISCUSSION

Unused swimming pools that accumulate rainwater proved to be ideal sites to collect baseline data for large-scale treatments with Bti

Table 1. The effect of Vectobac-DT against the larvae of *An. gambiae* and *Cx. quinquefasciatus*.

		Days posttreatment							
		1	2	3	4	5	6	7	8
Species	Instars[1]	% reduction of larval proportion							
An. gambiae	Early	89	88	63	78	42	17	63	73
	Late	97	98	99	99	95	95	92	89
Cx. quinquefasciatus	Early	46	30	23	61	43	10	14	36
	Late	97	100	99	94	34	61	37	62

[1] Early instars = 1st and 2nd instars; late instars = 3rd and 4th instars.

or a combination of Bti/Bs. The swimming pools that were selected are transformed during the off-peak tourism season to micro-ecosystems offering ideal habitats to the aquatic fauna including mosquitoes such as *An. gambiae* and *Cx. quinquefasciatus*. These rainwater-catching swimming pools are exposed to the whole array of environmental factors, such as water quality and sunlight, which are also typical for other mosquito breeding places.

The high numbers of immature stages of the two mosquito species under investigation indicate that swimming pools in the residential area of Malindi represent dominant sources of *An. gambiae* and *Cx. quinquefasciatus*. The results of the control pools demonstrate that there is a steady supply with young instars from eggs, which are not affected by the larvicides. The observed fluctuations implied by larval population have been reported in other studies (Mulla et al. 1999).

The data demonstrate the field efficacy of Vectobac DT and CulinexCombi against *An. gambiae*, which is the main target vector within the integrated malaria vector control program in Malindi. The efficacy of both larvicides was readily demonstrated by a high initial reduction in larval population of the late instars. Overall Vectobac DT and CulinexCombi were equally effective against the 3rd and 4th instars of both species 24 h posttreatment, yielding more than 95% mortality.

Likewise, the efficacy of both biolarvicides against the *Cx. quinquefasciatus* population was satisfactory at least over a period of 96 h. Both larvicides showed a higher activity against 3rd and 4th instars of *An. gambiae* and *Cx. quinquefasciatus* than against the early larval stages. The consequences are that interventions have to be focused on 3rd and 4th instar larvae. This is especially important when breeding sites are retreated.

Both biolarvicides displayed 100% control within 4 days, and differences in persistence became evident at the fifth day. The activity against *Cx. quinquefasciatus* dropped considerably, while the efficacy against *An. gambiae* changed only slightly. This indicates that *An. gambiae* is more susceptible to Vectobac-DT and CulinexCombi than *Cx. quinquefasciatus*. This is consistent with studies in western Kenya (Fillinger et al. 2003, Fillinger and Lindsay 2006) where *An. gambiae* was found to be highly susceptible to both Bti and Bs.

Vectobac-DT seems to persist longer than CulinexCombi. This, however, is not in contradiction with the recycling capacity of *B. sphaericus*, present in CulinexCombi since the experiment with the swimming pools was not laid out to demonstrate the recycling potential of *B. sphaericus*. Highly polluted water and a continuous pressure by *Cx. quinquefasciatus* is required to obtain persistence through recycling. Within the large-scale field study in Malindi there are many highly polluted breeding sites of *Cx. quinquefasciatus* where the application of CulinexCombi will be ideal.

It can be concluded that Vectobac DT and CulinexCombi are highly suitable as larviciding tools in large-scale integrated field operations for

Table 2. The effect of CulinexCombi against the larvae of *An. gambiae* and *Cx. quinquefasciatus*.

		Days posttreatment							
		1	2	3	4	5	6	7	8
Species	Instars[1]	% reduction of larval proportion							
An. gambiae	Early	99	57	12	94	−114	72	8	66
	Late	97	100	100	100	77	86	84	93
Cx. quinquefasciatus	Early	84	−176	−28	68	−114	−19	−124	5
	Late	81	95	96	98	2	25	−130	−166

[1] Early instars = 1st and 2nd instars; late instars = 3rd and 4th instars.

vector control. The study recommends the integration and use of these microbials in mosquito control programs.

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