ORIGINAL PAPER

Impacts of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* insect larvicides on mosquito larval densities in Lusaka, Zambia

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ABSTRACT

The study assessed the impact of bio-larvicides- *Bacillus thuringiensis* var. *israelensis* (Bti) and *B. sphaericus* (Bs) on anopheline mosquito larval densities in four selected areas of Lusaka urban district. Larval densities were determined using a standard WHO protocol at each study area prior to and after larviciding. Ninety percent (90%) of the collected mosquito larvae and pupae were preserved in 70% ethanol, while 10% were reared to adults for species identification. Prior to larviciding, the largest number of mosquito larvae collected was culicines. Among the anophelines, *Anopheles coustani* Laveran (13.5%) (n = 111) and *An. squamosus* Theobald (9.5%) (n = 78) were identified from all the study areas with *An. rufipes* Gough (1.1%) (n = 9) collected from one study area only. None of the major malaria vector species reported for Zambia were identified. No mosquito larvae were found in freshwater bodies following the larviciding exercise. Possible reasons for the absence of known major malaria vectors could be the re-introduction of effective vector control and loss of suitable breeding grounds. The study highlights the potential of larviciding using Bti and Bs for malaria vector control and its integration with indoor residual spraying and insecticide treated nets.

INTRODUCTION

Malaria is a leading cause of morbidity and mortality world-wide, accounting for more than one million deaths annually, mostly in tropical countries of Africa and Asia.[1-3] In Zambia, the disease accounts for about 4.3 million clinical cases with an average of 6,000 deaths annually.[4,5] Malaria control involve an integrated approach using effective treatment with Artemisinin-based Combination Therapy (Artemether/Lumefantrine) and vector control.[6] Presently the frontline vector control interventions are insecticide treated bed nets (ITNs) and indoor residual spraying (IRS).[7-10]

Despite significant impacts rendered by ITNs and IRS in operational settings, these interventions are undermined by the development of insecticide resistance in malaria vectors[7] and difficulties to achieve high coverage areas.[11] As such, community-based larval source management using larviciding was recently introduced as a complementary tool within the context of the Integrated Vector Management (IVM) strategy.[12,9]

The biolarvicides *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* are live biotoxin-producing strains of bacteria belonging to the *Bacillus* group that have been used to eradicate larval stages of mosquitoes, particularly where malaria, filariasis or certain arboviruses are present.[13-16] Elimination of anopheline mosquito larvae from their aquatic habitats reduces adult mosquito vector densities and consequently reducing the incidences of the malaria in the affected communities.[17]

The National Larviciding Programme (NLP) was initiated in 2010 through a bilateral agreement between the Cuban and Zambian governments. This study reports on the impacts of the Bti. and Bs. larvicides on mosquito larval densities in selected areas of Lusaka urban district.

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MATERIALS AND METHODS

Study Areas

This study was conducted for a period of one year in four randomly selected areas of Lusaka urban district in Zambia (Latitude, 15-16° S; Longitude, 28-30° E) with an altitude of 1280 m above sea level.[18] Ibex hill/Kalikiliki area; (Latitude, 15° 24.707' S; Longitude, 28° 22.296' E); Venta/Manzi valley area (Latitude 15° 22.564' S; Longitude, 28° 24.148' E); Chamba valley area (Latitude, 15° 21.587' S; Longitude, 28° 20.01' E) and; Chelstone area (Latitude, 15° 21.924' S; Longitude, 28° 23.836' E). The climatic conditions in the study areas include three seasons: warm and wet season (November-April), cool and dry season (May-August), and a hot and dry season (September-October). In winter, temperatures fall as low as 4°C and during summer the temperatures rises to as high as 38°C. The presences of dams, marshes, ponds and streams in the study areas provide ideal conditions for mosquito breeding throughout the year.

Product Application

Large mosquito breeding sites with high submergent, emergent and surrounding vegetation were aerial sprayed with Bs. larvicide on 20, 21, 23 and 24 June 2011 using a fixed-wing, single-engine aircraft. Smaller and more accessible water bodies were sprayed with the larvicide Bti. using Hudson X-pert pressure spray pumps. The recommended dose of 5 ml of larvicide per square meter of surface of active mosquito breeding site was applied according to the manual larvicide application specifications.[19] For aerial sprays, the recommended dosage of 15 liters of larvicide per hectare was used. Both aerial and manual larvicide applications were done by a combined team of Cuban and Zambian technical personnel and trained community volunteers.

Mosquito Sampling

Pre-spraying sampling for mosquito larvae and pupae to collect baseline data was done monthly throughout the study period starting in August, 2010. Larviciding was conducted from 20th to 24th of June 2011, while post-spraying sampling began on the 27th of June 2011 until August, 2011.

Each identified mosquito habitat was visited on each sampling occasion. Three 12m^2 sampling spots were randomly selected and geo-referenced using a Geographical Positioning System (GPS). Ten scoops of a standard dipper (350 ml capacity) were made from breeding sites. Ninety percent (90%) of the collected mosquito larvae and pupae were preserved in screw-cap vials containing 70% ethanol, while 10% of the larvae and pupae were taken live to the laboratory at National Malaria Control Centre (NMCC) for rearing to adult stage for species identification. In the laboratory both preserved and live mosquito larvae and pupae collected were enumerated to determine their densities.

Mosquito Identification

Adult anopheline mosquitoes were identified morphologically using the computer software[20] and manual keys.[21, 22] No molecular mosquito species identification using Polymerase Chain Reaction (PCR) was conducted. The culicine mosquitoes were only of interest in the assessment of impacts of the biolarvicides on mosquito densities in the aquatic habitats.

Data Analysis

Frequencies of mosquito larvae and pupae of different species per scoop of the standard dipper (350 ml capacity) were used to estimate larval and pupae densities of the pre- and post-larviciding periods in the study areas and compared using ANOVA in Statistix version 9.0.

RESULTS

Three species of *Anopheles* mosquitoes were identified during the pre-spraying phase of the study; *Anopheles rufipes* Gough (9) in one study area (Chamba valley) and *Anopheles coustani* Laveran (111) and *Anopheles squamosus* Theobald (78) in all the four study areas (Table 1 and Figure 2). No *Anopheles gambiae* sensu lato or *Anopheles funestus* complex mosquito species were collected from all study areas. In addition, 321 culicine mosquitoes were also collected.

Table 1. Mosquitoes collected from the four study areas of Lusaka urban in June 2011

Mosquito Species	Study Areas			
	Ibex hill/ Kalikiliki	Venta/ Manzi	Chamba valley	Chelstone
Anopheles coustani	+	+	+	+
Anopheles squamosus	+	+	+	+
Anopheles rufipes	-	-	+	-
Culex spp.	+	+	+	+

Prior to larviciding, the larval habitat colonisation rates were in the order; Venta dam (33%), Chelstone-Zambia airways ponds (41%), Chamba valley quarry/ stream (44%) and highest in Ibex hills stream (50%) (Figure 1.). After larviciding, the habitat colonisation rates reduced to zero ($p < 0.05$) in all four study areas (Figure 1.).

Fig. 1: Pre-and Post-larviciding % Colonisation rates from study areas.

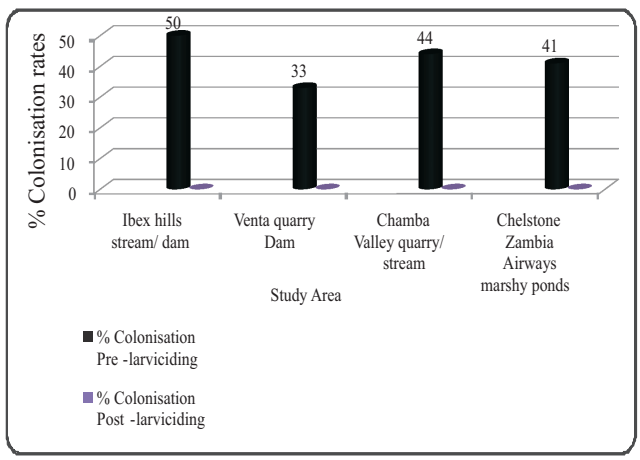

Fig. 2: Mosquito larvae species abundance

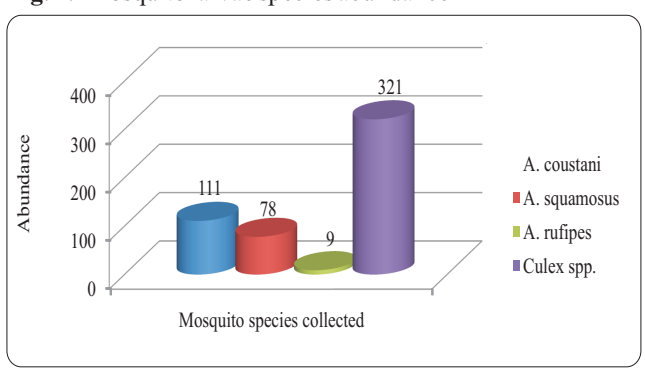

Fig. 3: Pre-larviciding Mosquito larvae densities in the study areas

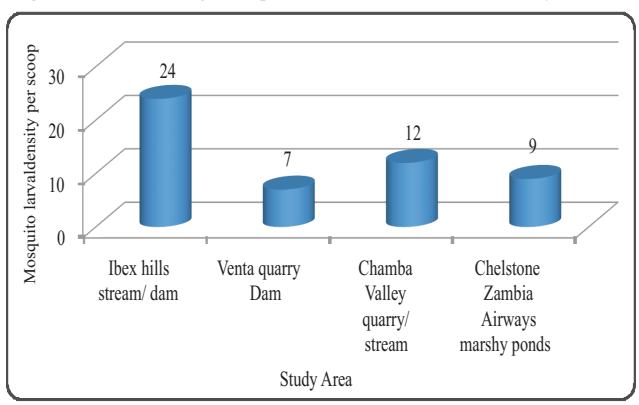

Figure 2 and 3 show the abundances and densities of mosquito larvae respectively, in the four study areas prior to spraying with the biolarvicides. Ibex hills and Venta sites showed the greatest and the lowest mosquito larval densities respectively. There were no mosquito larvae and pupae of any species found in the aquatic habitats following the spraying of the water bodies with Bti and Bs ($p < 0.05$).

Discussion

Earlier published data indicate that *Anopheles gambiae s.s* and *Anopheles arabiensis* constituted 10% of the total *Anopheles gambiae* complex sibling species collected in peri-urban Lusaka.[23] This study shows complete absence of these two primary malaria vector species in all the study areas of Lusaka urban district.

One possible reason for the absence of *An. gambiae* sensu lato and *An. funestus* sensu lato could be as a result of expansive deployment of the IRS programme since its re-introduction in 2003. In addition, rapid increase in the rate of urbanization of Lusaka district has eliminated suitable breeding sites for *Anopheles* mosquito vectors. For instance, Kalikiliki dam greatly reduced in size because of the encroachment of house construction during the study period. The situation could also be a function of the sampling methods employed in this study. In this regard, more robust sampling methods for both larvae and adult anopheline mosquitoes are required for species characterization.

Three anopheline mosquito species were identified during the pre- larviciding sampling period; *Anopheles coustani*, *An. squamosus* and *An. rufipes* (Table 1). The species are classified as secondary vectors of malaria and predominantly feed on animals (Zoophagic) and outdoors (exophagic).[21] While these results are consistent with the findings by NMCC, these species were found habouring malaria parasites in Tanzania, Congo and Zimbabwe [21,24] and are believed to be responsible for maintaining the transmission of malaria at low levels in most urban areas.[25] It is therefore cardinal to determine the significance of these secondary vectors in malaria transmission in Zambia.

During the pre-spraying period, habitat colonization rates and larval densities were scored at 33-50% and larval densities of 7-24 (Figure 1). Interestingly, the habitat colonization rates and larval densities declined sharply to zero in the post-spraying period with larvicides. The complete clearance of all mosquito larvae species demonstrate the high efficacy of Bti and Bs for the control

of both anopheline and culicines mosquitoes. It has been reported elsewhere in Sub-Sahara Africa that these biolarvicides tend to be very effective when sprayed on water bodies using aircraft and by hand, respectively.[26-28] Their larvicidal potency has been proven in both laboratory and field trials for control of mosquitoes and black flies.[29, 26, 30] These larvicides are species-specific in their targets, environmentally friendly[31-34] and are highly recommended by World Health Organization.[10, 35]

The problem of insecticide resistance has been reported in major malaria vector species to insecticide classes used in both IRS and ITNs in Africa and are well documented.[37] Both ITNs and IRS are associated with difficulties in achieving high coverage's[38] and hence LSM strategies would afford control as an insecticide resistance management option and opportunities to extend coverage of vector control programmes.[11,17,39,40] Integration of Larval Source Management (LSM) using Bti and Bs with the already existing vector control strategies such as ITNs and IRS would further enhance the impacts of the malaria vector control programme in Zambia.[36] The current findings also highlight the potential of community-based larval control programmes using Bti and Bs which are more likely sustainable than the highly technical IRS intervention. Studies have suggested that the costs of implementing a community based LSM programme equal those of implementing IRS and ITNs.[11]

While high coverage of IRS has a huge bearing on the absence of primary malaria vector species in Lusaka urban district, the use of Bti and Bs in Lusaka urban district afforded effective control for mosquitoes. Larviciding with biolarvicides will offer environmentally safe vector control alternative, serve as an insecticide resistance management tool and clear the residual malaria transmission currently occurring in urban parts of Zambia.

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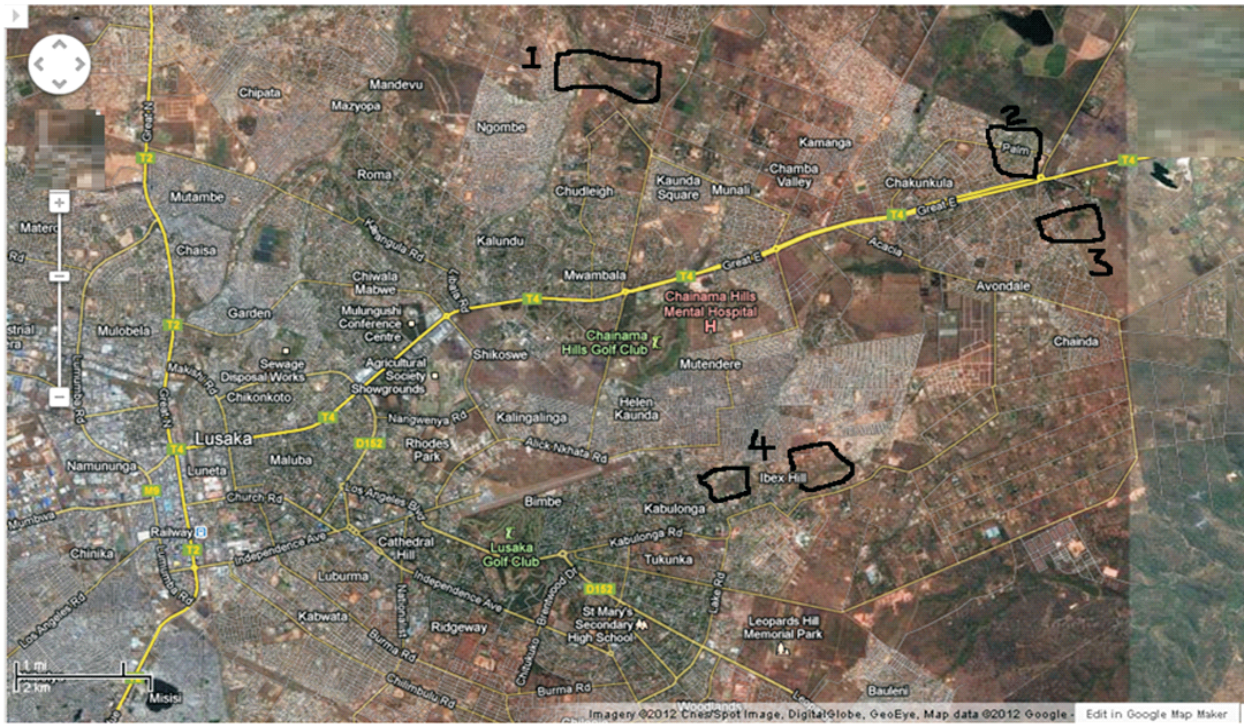
Appendix 1: Study areas in Lusaka urban district



Estimates of location of study areas; 1= Chamba valley study area and 2= Chelstone Zambia airways ponds study area, Venta area and Ibex hills/ Kalikiliki areas. (Source: Google Earth).