International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Variation in the Performance of Larvae Capturing Traps/Substrate during Larviciding on Domasi River in Zomba, Malawi

R.B. Mbewe [a*], D. Pemba [b], T. Mzilahowa [c]

[a] *Polytechinic, University of Malawi, P/Bag 303 Chichiri Blantyre 3.*

[b] *Chancellor College, University of Malawi, P.O Box 280 Zomba.*

[c] *College of Medicine, University of Malawi, P/Bag 360 Chichiri Blantyre 3.*

Abstract

Research was carried out on performance of larvae capturing traps during larviciding on Domasi River in Zomba. The study was aimed at assessing the impact of Bacillus thuringiensis israelensis (Bti) on black fly larvae colonization of different traps/substrates. Data on black fly larval density on the substrates were collected before and after Bti application. The monitoring was done using three different types of substrates: nylon strips, rocks and debris. The river was divided into three strata and breeding sites were determined. On each stratum all three types of traps/substrates were placed on purposively sampled breeding sites. Bti was applied to the river and its effect on substrate colonization determined. Data analysis included descriptive summaries, correlations, regression and analysis of variance using Microsoft excel and SPSS version 12. The results showed statistically significant differences in larval colonization of substrates. Substrates were capturing different densities before and after Bti application. Rock and Debris captured relatively high densities in both situations though the densities were much high before application. Nylon string had lower colonization. This study indicates that the performance of the different substrates at capturing larval density is affected by Bti due to its killing effect hence a consideration has to be made as to which substrate to use in larval monitoring.

Keywords: Bacillus Thuringiensis Israelensis (BTI); Larviciding; Substsrate; Black-fly; Larva monitoring; river.

---

*Corresponding author.

1. Introduction

Black flies have been a concern in many countries of the world because of Onchocerciasis (River blindness) the disease which they vector. Onchocerciasis is an infection caused by the parasite *Onchocerca volvulus* (a nematode worm), spread by the bite of an infected black fly. The symptoms of the disease include skin rash, eye lesions, and/or subcutaneous bumps under the skin. The most serious manifestation consists of lesions in the eye that can progress to blindness [1]. By 1994, an estimated 18-40 million people were affected worldwide, with about 270 000 having lost their sight [2].

In Africa, the species responsible for transmitting the disease are various sibling species belonging to *Simulium damnosum* species complex and *S.nevai*. Of the people living with Onchocerciasis, about 99% are in Africa and the disease is endemic in more than 25 nations located in broad band across the central parts of Africa [1]. In the entire world there are about 1200- 3000 known simuliid species arranged in 38 genera [3]. In 1988 about 170 species of *Simulium* in the Afrotropical Region [4]. In Malawi 24 species have been identified, on Zomba Plateau in particular there are about 16 known species. *Simulium zombaense* and members of the *Simulium damnosum* complex occur naturally on the plateau, and all are potential vectors of *Onchocerca volvulus* [5].

Different countries of the world have put in place different programs to control the flies. These programs include larviciding and chemotherapy. Larviciding has been carried out in America, Europe, and West Africa with some success [6,7]. Chemotherapy and Ivermectin drug has been used for treatment of Onchocerciasis. Other preventive measures of the disease include avoidance of infective bites by using insecticides such as DEET, and wearing long sleeve shirts and pants [1]. There is no vaccine against the disease yet.

Reports on black fly control trials in Zomba Malawi showed Black fly (*Simulium* spp.) outbreak around Malosa and Domasi areas in May 2006 [5]. There was an increased Oncho-dermal skin condition as a result of intense fly bites having 88% prevalence rate by April 2007 [8]. The consultation by the Ministry of Health (MoH) led to the initiation of larviciding programme to control the flies. *Bacillus thuringiensis israelensis* (*Bti)* was chosen in the control programme because it is target specific hence poses no threat to other aquatic life and also because *Bti* is environmentally friendly and biodegrades quickly in the environment [8].

According to author [8], Domasi River is one of the major sources of Onchocerciasis vector and was prioritized as far as *S. damnosum* ecology and bionomics research for control is concerned. Therefore, the larviciding of the river makes it necessary to assess the performance of the substrates used in larval monitoring upon *Bti* application. Different substrates or traps are used in monitoring densities of larvae in the rivers. These include trailing vegetation/ debri, rocks, strings and plastic boards. The study therefore, focused on these substrates and assessed their differences in performance.

2. Materials and Methods

The study was conducted in Zomba District located in southern Malawi.

The 2008 Population and Housing Census [9], indicates that the District has a total population of 591,903 that is about 5% of the national population. The population density is estimated at more than 209 persons per square kilometer and is higher in the urban area than the rural. Domasi River which is about 20 kilometers to the north of Zomba city was the focal point of the study.

The upper part of Domasi river has characteristic features of breeding places for black flies. These include swift flowing waters over stones and boulders. The study was conducted along this river because it is one of the rivers with the highest risk of Onchocerciasis vector sources.

Data on black fly larval population was collected before *Bti* application to provide baseline data. Thereafter data on larval population was continuously collected for a period of 5 months (July to November 2008) on a weekly basis.

Sampling was done using stratification. The stratum was based on altitude of the breeding sites. The stretch of the river with breeding sites was arbitrarily divided into three strata: upper (900 - 1000 m above sea level), middle (800 – 900 m above sea level) and lower section (700 – 800 m above sea level). This was done to ensure that the monitoring covers the entire sampled stretch of the river having breeding sites taking into account the inherent environmental conditions that affect species density and composition. On each of the sampled breeding sites three points were purposively sampled. The lower stratum had four breeding sites of which two breeding sites were sampled and three points were determined on each site. The middle stratum had six breeding sites of which three breeding sites were sampled and three points were determined on each. The upper stratum had two breeding sites and both were sampled with three points determined on each. On each sampled point traps/substrate in replicates of three were set. A total of 27 samples were collected on each monitoring visit and twenty monitoring visits were made on each site and a total of 540 samples were collected for analysis.

Table 1: Showing river strata, number of breeding sites and number of substrate per sampled site

Stratum	**Breeding site**	**Sampled site**	**Type of Substrate/Trap**		
			Nylon	**Rock**	**Debris**
Upper	2	2	3	3	3
Middle	6	3	3	3	3
Lower	4	2	3	3	3

Quantitative information on the population density of larvae was obtained. The three types of traps/substrates were set on the sampled sites to capture larvae. The traps used were nylon strips (threads) rocks and debris (trailing vegetation). However plastic boards were not used because they were being removed by people catching fish in the river every time they were set. The colonized substrates were compared to a scale of 2-10 to determine larval density according to the method of author [10]. The number of larvae per $16cm^3$ was put into classes as shown in table 2.

Table 2: Scale of Larval density

Class	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Number of Larvae per $16cm^3$	0	1(1-2)	3(3-4)	6(5-9)	16(10-22)	36(23-58)	88(59-120)	202(121-310)	500(311-800)	1050(>800)

The two sets of data obtained were used to determine changes in the larval density after the application of *Bti*. Trend in the larval population change were also observed over time during the period of larviciding.

Since temperature and flow rate of the river are site specific conditions affecting both larval occurrence and *Bti* performance [11], they were also monitored.

Weekly temperatures were recorded using two mercury thermometers. Both water and air temperatures were recorded as these affect the action of *Bti* and the activity of black fly such as feeding.

Flow rate was calculated by multiplying the stream cross-sectional area (in square meters) by the flow velocity (in meters/second) [12].

The flow rate was determined at the time of *Bti* application. Although flow rate varied throughout the study period, flow rate was determined twice corresponding to the number of *Bti* application. This was because *Bti* application among other factors like temperature depends on the flow rate in terms of the dosage to be applied.

2.1 Bti Application

Upon determining temperature and flow rate, *Bti* was applied. The application of *Bti* was done using backpack sprayers (Agricultural Knapsack (Backpack) Sprayer / Hand Sprayer (3wbs-20b)). The required amount of *Bti* which was determined to be double dosage of 0.72- 1.44 liters per cumec and was applied for ten minutes. This contained 0.05-2.5 ppm of *Bti* [12]. Two applications of *Bti* were made: 7th August and the second or follow up application on 4^{th} September 2008. Four applications were initially planned to be carried out for the entire study period however only 2 applications were actually done due to logistical problems.

2.2 Data Analysis

Data on the numbers of the adult flies and the larval densities were entered and analyzed using Microsoft Excel and SPSS. Data analysis included descriptive summaries, correlations and regression and Analysis of Variance (ANOVA). Correlations and regression analysis was done to investigate relationship between larvae density and factors such as water temperature, air temperature, river flow, *Bti* dose, altitude, and stratum. The analysis also assessed the relationship between type of trap and overall larval density as well as the effect of *Bti* on the overall larval density adjusted for type of trap. ANOVA was performed to quantify differences in density by trap.

3. Results

The activities of black fly larvae in terms of feeding and development to a large extent depend on temperature.

The average air temperature before application was 20 ºC and an average of 26ºC was recorded after *Bti* application showing a 26% increase in temperature. There was an increase in water temperature as well from an average of 16ºC before application to 20ºC after the application. Over the five month period of the study there was a significant increase in temperature (P= <0.001) as the hot season ensued.

However, correlation and regression analysis showed no significant effect of temperature, river flow, *Bti* dose, altitude, and stratum

3.1 *Comparison of substrate performance*

Table 3: Mean density on substrates before and after *Bti* application.

	Mean larva density		
Type of substrate	**Before application**	**After application**	**p-value (within substrate)**
String	5.55 ± 2.8	3.23 ± 2.9	**0.422**
Rock	8.07 ± 1.2	4.00 ± 2.8	
Debris	7.64 ± 1.3	5.38 ± 2.9	
Board	*	2.88 ± 0.76	-
p-value (across substrate)	**0.002**	**<0.000**	

* board not used before application

Density was observed to be high on rock followed by debris and lastly string before *Bti* application. ANOVA showed that the differences in the densities across the substrates were statistically significant.

After *Bti* application the larva density was high on debris followed by rock, string and lastly board. ANOVA showed that the differences in the densities across the substrates were also statistically significant.

On overall, debris was colonized more than the other substrates. These differences in substrate performance were statistically significant.

However, comparing the performance within the substrates both before and after application it was observed that there were no statistically significant differences.

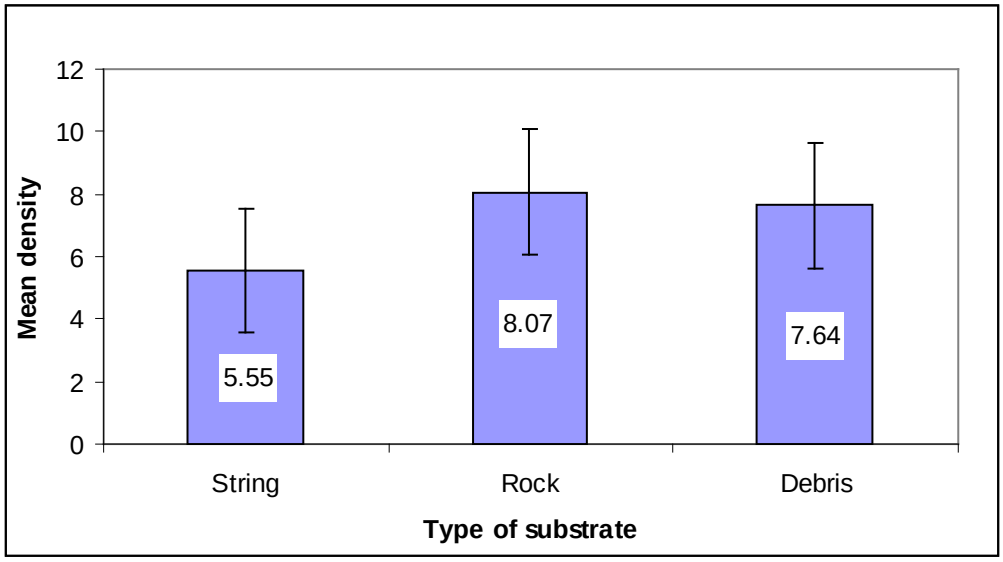

Figure 1: Differences in larva density on substrates before *Bti* application.

Density was high on debris followed by rock and lastly string.

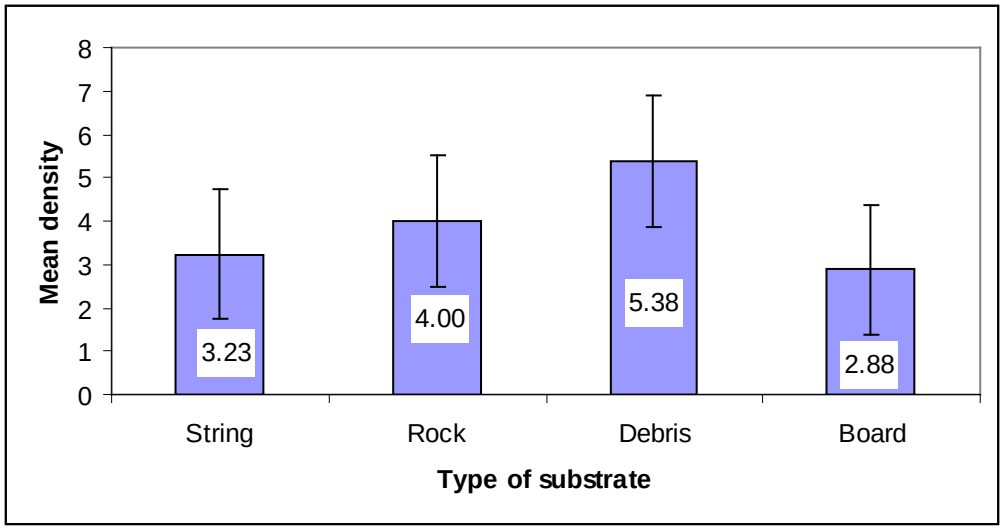

Figure 2: Differences in larva density on substrates after *Bti* application.

3.2 Variation of Larval substrate performance before Bti application

Analysis of Variance (ANOVA) on the three substrates used to monitor larvae density was conducted to assess the differences of taps in capturing larvae. Table 4 shows the associated p-values of mean larval density on substrates before and after *Bti* application. Before *Bti* application there were significant differences in trap performance. There were significant differences in the mean larval densities between nylon string and rock, nylon string and debris but no significant differences in the mean larval densities between rock and debris.

Table 4: Variations in substrate performance before *Bti* application

(I) type trap vs. (j) type trap		Mean difference (I-J)	Std. Error	Sig.	95% Confidence interval	
					Lower bound	**Upper bound**
String	**Rock**	-2.521	0.729	0.003	-4.289	**-0.754**
Rock	**Debris**	0.429	0.791	0.851	-1.489	**2.345**
Debris	**String**	2.093	0.729	0.017	0.325	**3.860**

After *Bti* application (Table 5) there were some significant differences in the contribution of the substrates. There was no significant difference in the mean larval densities on nylon string and rock and on nylon string and board but there was significant differences between nylon string and debris, and rock and debris, rock and board and debris and board.

Table 5: Variations in substrate performance after *Bti* application

(I) type trap vs. (j) type trap		Mean difference (I-J)	Std. Error	Sig.	95% Confidence interval	
					Lower bound	**Upper bound**
String	**Rock**	-.865	.355	.072	-1.779	**0.050**
Rock	**Debris**	-1.182	.358	0.006	-2.106	**-.259**
Debris	**String**	2.047	.354	<0.001	1.132	**2.962**
Board	**String**	-.7054	.5471	.570	-2.117	**.7060**
Rock	**Board**	1.570	.5494	.023	.1529	**2.987**
Debris	**Board**	2.752	.5494	<0.001	1.335	**4.169**

4. Discussion

Temperature significantly increased (P= <0.001) over the five month period of the study as the hot season ensued. However, correlation and regression analysis showed no significant effect of temperature, river flow, *Bti* dose, altitude, and stratum.Before *Bti* application, the larva density was high on rock followed by debris and lastly string and ANOVA showed that the differences across the substrates were statistically significant. However, there was no significant differences in the mean larval densities between rock and debris. This suggests that before *Bti* application where larval density was high, rock and debris were significantly consistent and more efficient in trapping larvae than string.

After *Bti* application the larva density was observed to be high on debris followed by rock, string and lastly board. ANOVA also showed statistically significant differences in the densities across the substrates.

However, there were some significant differences in the contribution of the substrates (Table 5). There was no significant difference in the mean larval densities on nylon string and rock and on nylon string and board but there was significant differences between nylon string and debris, and rock and debris, rock and board and debris and board. This suggests that after *Bti* application where larval density was low, nylon string and rock, and nylon string and board were performing almost uniformly with no significant differences. This further suggests that when the larval density is high in the river the contribution of nylon string as a substrate is low but increases as the larval densities decrease. Nothing much can be said on plastic board since it was not used before application and hence comparisons are not possible.

However there was no statistically significant differences in performance within substrate before and after *Bti* application (Table 3). The performance of the different substrates were seen not to be affected by the treatment hence no significant differences in themselves before and after application except for board

On overall, debris was colonized more than the other substrates. These differences in substrate performance were statistically significant.

The results on comparing the performances of the substrates also show that rock and debris which are natural substrates are good at capturing larvae both at low and high densities, while the nylon string which is an artificial substrate is good at capturing larvae at low density. Generally, at low densities, the traps perform differently with Debris > Rock ≥ String. When Board is considered the relationship is Debris > Rock > String ≥ Board. In this case the String and Board are equally efficient. Studies by [4] found the contribution of string to be insufficient despite it working successfully in other studies. The current study also shows that nylon string performed weakly as compared to debris and rock.

Overall, debris performed better at capturing larvae, followed by rock, nylon string and plastic board was least. These observations show that the substrates provided significant contribution in estimating larval density in the river. Author [13]concluded that these substrates are not perfect but are effective to some extent. The substrate differences reported here would explain some of the variation observed in the study.

5. Conclusions

The traps used for capturing larval density showed significant differences in the mean densities before and after application. Rock had more larval attachments before application followed by debris. However after application debris had more larval attachments followed by rock. Debris, a natural substrate, attracted more larvae attachments than the other traps on overall and proved to be a good trap in collecting information on larval density when densities are either low or high.

Statistically it was concluded that larval densities on the substrates were not affected by the application of *Bti* in the river and since, the performance of the substrate was already different before *Bti* application. However, there is need to consider type of substrate when monitoring larval densities during larviciding.

Natural substrates such as debris and rocks can be used for trapping larvae to monitor population densities before and after *Bti* application. Nylon string can successfully be used when the densities are low and hence recommended after *Bti* application.

References

[1] Centre for disease Control. Factsheet on Onchocerciasis. Internet: www.cdc.gov/ncidod/dpd/parasites/ onchocerciasis/factsht_onchocerciasis.pdf, May. 10, 2008 [Jun. 20, 2010]

[2] P. Courtright, K. Johnston, and L. Chitsulo.. A new focus of Onchocerciasis in Mwanza district. International Centre for eye health, Barthstreet, London ECIV9EL UK and International Eye Foundation – Malawi, 1994.

[3] D.S. Kettle. Medical and veterinary Entomology. Willingford, UK . 1990.

[4] T.M.T. Roberts. Biology of simuliid larvae of the Mulungusi Basin,Zomba plateau: Taxonomy and ecological notes. MSc. Thesis, University of Malawi, Zomba, 1988, pp 208-507.

[5] D. Pemba and C. Alezuyo. Zomba backfly outbreak report to the Ministry of Health, Lilongwe. Chancellor College. Zomba, 2006.

[6] World Bank. Internet: http://www.worldbank.org/afri/gper/partnerships.htm; Aug.11, 2008 [Jun. 10, 2010]

[7] 7African Programme for Oncocerciasis Control (APOC). Internet: http://www.apoc.bf/en/index.htm; April. 21, 2006 [June.10, 2010]

[8] D.F. Pemba. Larviciding of Black fly – Zomba, Southern Malawi. Report Submitted to Department of Environmental Affairs, Lilongwe, Malawi through the Ministry of Health (unpublished report). 2008.

[9] National Statistical Office (NSO). Housing and population census. Government of Malawi, Zomba. 2008.

[10] P.W. Palmer. A rapid method of estimating the abundance of immature blackflies (Diptera:Simulidae). Onderstepoort Journal of Veterinary Research vol.61, pp. 117-126. 1994

[11] Figueiró R, Araújo-Coutinho CJ, Gil azevedo1 LH et al. (2006) Spatial and temporal distribution of Black-flies (Diptera: Simuliidae) in the Itatiaia National Park, Brazil. Neotrop. Entomol. [On-line] 35 (4), pp. 542-550 Available:http://www.scielo.br/scielo.php? [Jun. 23, 2008]

[12] Technical Use Bulletin for VectoBac 12AS Mosquito and Blackfly Larvicide, 2003. VALENT BIOSCIENCES COORPERATION.

[13] R. Hutchnison. Simuliidae (Black flies). Internet: http://www.roberth.u-net.com/blackflies.htm; 2008 [Jul, 03 2010]