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Comparative Larvicidal and Residual Activity of *Bacillus Thuringiensis*var. *Israelensis*and Temephos (1.0 % Sg) In Granules Against *Culex Pipiens Pallens*(Diptera: Culicidae) In Semi-Field Tests

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Abstract: *The study looked at comparative larvicidal activity and residual effects of granular formulations of Bacillusthuringiensis var. Israelensis (Bti); a bio pesticide and temephos (Abate); phosphorus based non-systemic pesticide. The study was carried out in semi-field station and used rainwater to simulate natural conditions. Mosquitoes of; Culexpipienspallens Coquillett; a member of the Culex pipiens complex were used. The third instar larvae were used to allow observations before pupation and adult emergence. The methods used were a modification of those used by Thavara et al [24] and Laws et al [20]. Abate applied at concentrations;1.0mg/l, 2.0mg/l and 4.0mg/l of 1% SG showed that the residual activity of abate is dependent on the concentration used in the treatment. Bti was applied at 0.4g/l, 0.8g/l and 1.6g/l. The study found that both formulations were effective, with Bti offering a short residual activity period of seven days to two weeks, while temephos gave prolonged residual period of up to two months. Between day one and day twenty five of the tests, there were no significant differences at $p > 0.05$ in the residual activity of the three concentration; 1.0 mg/l, 2.0 mg/l and 4.0 mg/l of Abate. After the day 25, however, there were significant differences between the residual activities of Abate at 1.0 mg/l and those of the 2.0 mg/l and 4.0 mg/l ($p < 0.05$) (Table 1). In Bti (Aquabac), the residual activity period of the three concentrations used in the tests differed significantly at $p < 0.05$ (Table 2). The findings about the larvicides can help choose the right larvicide to apply depending on the habitat, the population density, cost and length of the mosquito breeding season.*

Key words: *Larvicide, Temephos, Abate, Bti, Culex pipiens pallens, Residual activity*

I. Introduction

Application of larvicide involves introduction or manipulation of agents or organisms that suppress the mosquito populations[1]by killing the larvae at the breeding site. EPA describes a larvicide as chemical or toxin that kills the insect larvae[2]. Larvicides include biological insecticides like the microbial *Bacillusthuringiensis* and *B. sphaericus*. As people become increasingly aware of the side effects of chemical pesticides on the quality of their environment, microbial pesticides like those used in the control of mosquito larvae are getting more accepted [3]as human friendly ways of dealing with mosquito problems. Suppression of mosquito populations can be achieved by treating the breeding sites directly with microbial and chemical larvicides. During the malaria eradication campaigns in 1940s, larviciding played a major role in the eradication of *Anophelesgambiae*Giles in Brazil ([4], [5] and Egypt[6]. Larviciding works by reducing mosquito populations when the breeding sites are relatively few and easily identifiable for treatment[1]. It must be possible to treat enough of the breeding sites with a larvicide to have significant impact on the mosquito population and subsequent disease transmission. Mosquito species such as *Aedesaegypti* Linnaeus and An. *Stephensi* Liston, whose larvae are found in man-made water containers in urban areas, can be controlled using larvicides added directly into the water.

Larval control is viable in urban areas given the high density of humans needing protection and the limited number of breeding sites that can be easily identified in well planned cities. In certain rural areas like the jungles of Africa and Southeast Asia, where mosquitoes breed in small puddles of rain water spread over wide areas, larviciding may not be a viable vector control method. Other than predatory fish, bacterial pathogens including *Bacillusthuringiensisisraelensis* (Bti) and *B.sphaericus* (*Bs*) that attack and kill mosquito larvae are used [7]. The use of larvicides has several advantages in comparison with adulticides control. These include their effectiveness at relatively low doses, safety to humans and non-target organisms and their low cost of production in most cases. The larvicides face low risks of resistance development by the mosquito larvae [8].

Biological larvicides like the microbials are very specific in terms of which mosquito they control and the habitats in which they work [7]. Two species of the genus *Bacillus*;*B. thuringiensis* var. *israelensis* and *B. sphaericus* have demonstrated effective larvicidal activity against a wide range of mosquito species [1]. Since the discovery of the mosquito larvicidal activity of *Bti* spores, serotype H- 14 in 1977, different formulations of *Bti* has been made against the larvae of many species of mosquitoes. *Bti* has been used against *An. Albimanus*Wiedemann, *An. Sinensis*Wiedemann, *An. gambiae* Giles, *An. stephensi* Liston and *An. Arabiensis*Patton, all malaria parasite vectors in different parts of world ([9], [10].*Bacillusthuringiensis* var. *israelensis* causes lethal effect on the mosquito through toxins on the bacterial spores' coat. Most *Bti* formulations use dead spores so there no persistence or reproduction in the field after application.

Before the use of biological larvicides, man had already tried reducing the mosquito population by using chemical larvicides. The earliest chemical larvicide used was petroleum oil which when applied over the water surface smoothers and kills the mosquito larvae by suffocation[11]. Chemical larviciding is widely practiced in the United States to control nuisance-biting mosquitoes [12]. Before the 1940s, Paris green (copper acetoarsenite) was commonly used as a larvicide sprinkled in powder form on the water surface where it would be eaten by the mosquito larvae [13]. Paris green is no longer in use due to its high toxicity to vertebrates and other non-target organisms [14]. DDT was used as a larvicide in 1940s and 1950s but was replaced by other chemical larvicides in 1960s. Temephos, distributed under the trade name Abate, is a major chemical larvicide. Abate exhibits very low mammalian toxicity[12]. Abate has been used for control of malaria transmitting mosquitoes in India and Mauritius [15],[16]. The efficacy of chemical larvicides is influenced by the type of formulation used, the water quality in the application sites, and the susceptibility of the mosquito larvae to be controlled. The conventional fluid and wettable powder formulations give short larvicidal periods of one to two weeks especially in the tropics. This has been improved by application of slow-release granules and briquettes or microencapsulated forms ([13].

Larvicidal activity of both microbial and chemical larvicides tend to be longer in cooler, cleaner and less illuminated water ([17].Although temephos is not toxic to mammals [18], it is harmful to crabs, shrimps and zooplanktons and should not be applied in environmentally sensitive areas[12]. Microbial larvicides like *Bti* do notaccumulate in the environment or in the tissues of organisms and do not affect the non-target organisms [19].*Bacillusthuringiensis* and Abate can be applied to water used for drinking by humans and livestock [20]. Though resistance is not common, some concern about resistance developing in mosquito larvae towards Abate is growing [21]. Microbial larvicides like *Bti* and chemical Abate are not cheap for a low income household, and their use has so far been by the public health officials or the community under guidance and supervision of the local health administration.

This study compared the efficacy and residual using *Bacillus thuringiensis* var. *israelensis* and Abate in semi-field of setting. It has been established that Abate controls larvae for a considerable period of time when applied directly or in slow-release formulations into the breeding sites [20]. The efficacy of *Bti* has also been established [22]. This studycompared the residual activity of these two larvicides in semi-field setting to establish which of the two would give the best option in different conditions considering the cost of the larvicide involved.

II. Materials and Methods

Mosquitoes used

*Culexpipienspallens*Coquillett mosquitoes used in these tests were from a colony raised at the Kosin University Insectary for several years. The mosquitoes were reared and maintained at a temperature and relative humidity regime of 27 ± 1 ^{o}C and 75 ± 5% RH, respectively and a 13:11 light and dark photoperiod. The larvae were fed on a mixture of laboratory chow and brewer's yeast at a ratio of 2:1. The adults were fed and maintained on 10% sucrose solution presented using methods described by Gerberg*et al.*(1994)[23]. Third instar larvae were used in both the *Bti* and Abate tests. This was to ensure that the larvae did not pupate within the 24 hours of testing and yet they were visible for release in to the experimental tubs. For continuous supply of third instars, the breeding of *Cx. Pipienspallens* was carried out continuously in the Insectary. The tests were carried out in semi-field test sites at Kosin University. Twenty one hardened plastic tubs/troughs of about 500 liters in volume were used (Figs. 1 &2) for the tests carried outside in semi-field conditions.

Chemicals used data collection and analysis

Two larvicides, Abate 1% SG (T.J.C Chemical Co., Ltd, Jakarta, Indonesia) and Aquabac® 200 G (Becker Microbial Products Inc., 5786 Northwest 119th Dr., Coral Springs, Florida, USA) were compared for their larvicidal and residual effects. The active ingredient in Abate is temephos, a non-systemic organophosphorus insecticide. The temephos in the Abate was carried in sand granules to allow for slow-release of the active ingredient into the water during the test. The Aquabac® used consisted of 2.86% active ingredients of 7,000 ITU (International Toxic Units) of *Bacillusthuringiensis* Berliner var. *israelensis* (serotype H-14) and

97.14 % of solid materials used as a carrier for the active ingredient to increase the larvicidal activity period. Abate was tested at concentrations of 1.0 mg/l, 2.0 mg/l and 4.0 mg/l while Aquabac was tested at 0.4, 0.8 and 1.6 g/l and the tests replicated as indicated in Figure 1.1

Each of the 21 tubs were left to fill with 250 liters of rain water and left to settle for about a week before the start of the experiments. Each of the 21 tubs is fitted with overflow outlets to allow water to flow out if the volume increases beyond 250 liters. The three control tubs were not treated with any chemical. Nine tubes were use for tests on Abate; each 3 tubs for 1.0, 2.0, 4.0 mg/l concentrations. The other nine were used for tests on Aquabac; each 3 tubs for 0.4, 0.8, 1.6g/l concentrations. About 24 hours to the start of the experiments larval food was added to the water in all the tubs according to the recommendations of Gerberg et al. (1994) [23]. On day one of the tests, 25 *Cx. Pipienspallens* larvae at the third were introduced into each of the 21 tubs and left to feed for 24 hours. After 24, hours all the larvae were removed from each tube and the number of both the dead and the live larvae in each tub was counted and recorded. New batch of 25 larvae was then introduced into each of the tubs. New batches larvae were introduced on day 2, 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65. The site where these tests were carried out is completely exposed in field conditions. Therefore, water temperature was monitored daily for maximum and minimum temperatures by submerging a max/min thermometer. The amount of rainfall during the testing period was monitored by use of a rain gauge mounted on an exposed point within the testing site. Addition of rain water into the tubs would be suspected to influence the concentration of the larvicides within the tubs.

Introduction of new larvae into the treatment tubs was discontinued whenever mortality levels were observed to be very low indicating that the chemical concentration was no longer effective in killing larvae. The percentage mortality in each of the 21 tubs was calculated as ((25-no. of survivors)/25) x100 or as the (No. of dead /25) x 100. Correction for control mortality was not made as the mortality in the control was zero or less than 10% during the testing. The formulae used are similar to those used by Thavara et al. (2004, (2005)[24] and Law et al. (1968)[22].The percentage mortalities were statistically analyzed with t-tests at p=0.05 using SPSS 12.0 program (SPSS Inc. 2005) for Windows to find out if there were significant differences in the larval mortality among the various Abate and Aquabac treatments.

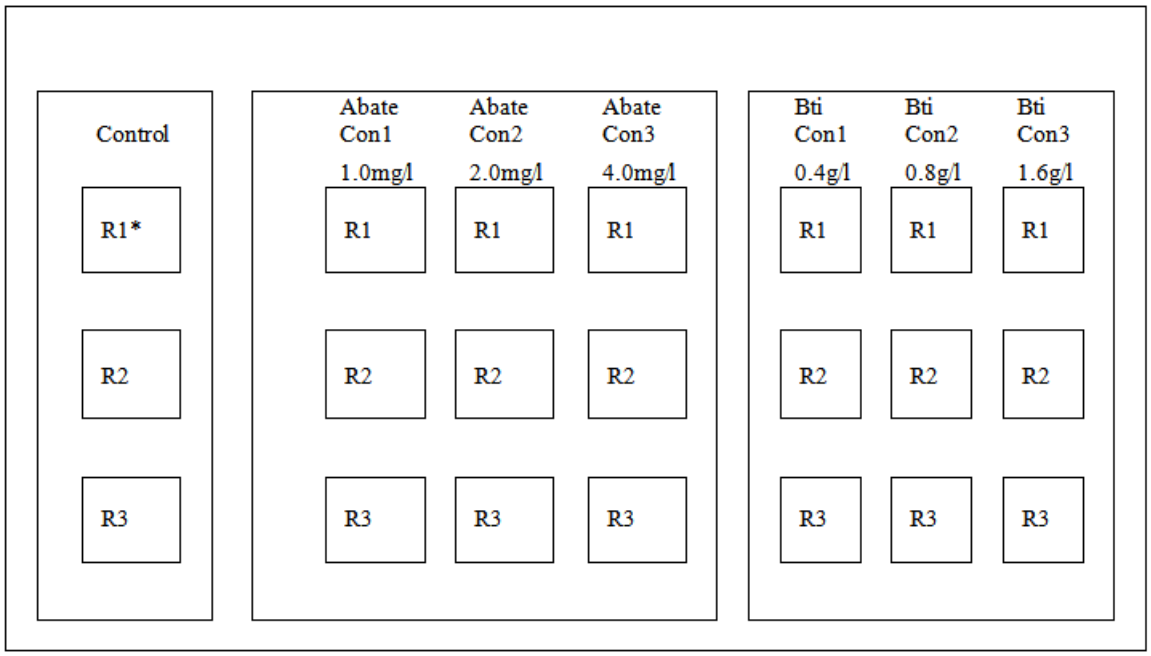

Fig 1. The experimental design layout of the water tubs at the semi-field sites.

*R1: Replicate 1, R2: Replicate, R3: Replicate 3.

III. Results

The results obtained with the two larvicides showed that they were effective against mosquito larvae and they gave mortality rates of 77% to 100%. The results are summarized in Tables 1 & 2; Figs.2 &3.

Table 1: Average percentage mortality of *Cx. Pipienspallens* in semi-field water tubs treated at 3 concentrations of TemephosAbate 1% SG in 3 replicates

		Concentration of Abate in the water					
Exposure Period Days	Mean No. of dead ± S.D in Control	1.0mg/l mean No. of dead ± S.D	1.0mg/l Avg % mortality ± S.D	2.0mg/l mean No. of dead ± S.D	2.0mg/l Avg % mortality ± S.D	4.0mg/l mean No. of dead ± S.D	4.0mg/l Avg % mortality ± S.D
1	00 ± 0.0	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
2	00 ± 0.0	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
3	00 ± 0.0	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
5	00 ± 0.0	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
7	00 ± 0.0	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
10	0.3 ± 0.6	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
15	0.3 ± 0.6	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
20	00 ± 0.0	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
25	1.0 ± 0.6	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
30	1.3 ± 1.6	21.7 ± 0.6	86.7 ± 2.3b	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
35	5.0 ± 1.0	18.3 ± 0.6	73.3 ± 2.3b	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
40	7.0 ± 1.0	15.7 ± 0.6	62.3 ± 2.3b	25.0 ± 0.0	100.0 ± 0.0a	25.0 ± 0.0	100.0 ± 0.0a
45	4.7 ± 0.6	14.3 ± 2.1	57.3 ± 8.3b	23.7 ± 0.6	94.7 ± 2.3b	25.0 ± 0.0	100.0 ± 0.0a
50		8.0 ± 1.0	32.0 ± 4.0b	22.0 ± 1.0	88.0 ± 4.0a	24.7 ± 0.6	98.7 ± 2.3a
55				23.0 ± 1.0	92.0 ± 4.0a	24.3 ± 0.6	97.3± 2.3a
60				22.3 ± 0.6	89.3 ± 2.3a	24.6 ± 0.6	98.7 ± 2.3a
65				20.3 ± 1.5	84.0 ± 6.9a	24.0 ± 1.0	96.0 ± 4.0a

*Means on the Same row followed by the same letter are not significantly different at 5% level of probability (Duncan's Multiple Range Test)

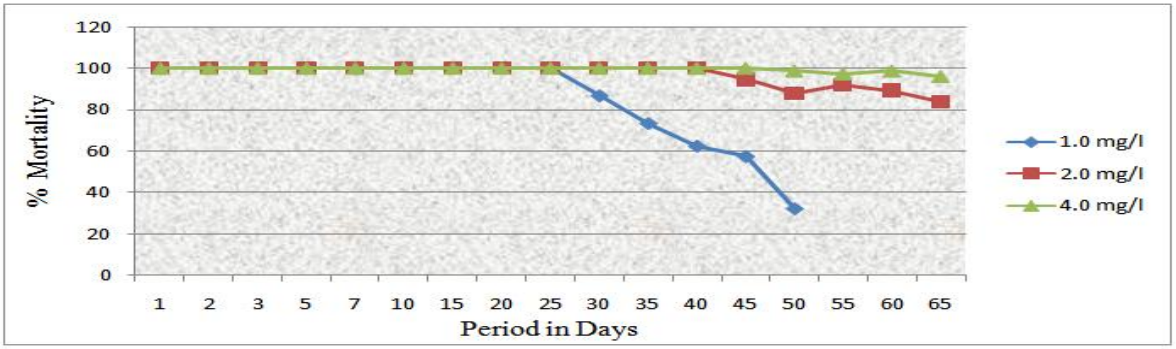

Fig.2: Average percentage mortality of *Culexpipienspallens* larvae in 3 concentrations (mg/l) of Abate 1% SG granules, 3 replicates,

Results of using 1.0mg/l, 2.0mg/l and 4.0mg/l of Abate 1% SG showed that the residual activity of abate is dependent on the concentration of the Abate used in the treatment. In the tubs treated with 1.0 mg/l, the dose usually recommended for use in water, the residual activity continued up day 25 with 100% mortality and then went down rapidly to below 50% by day 50 of the testing. In the tubs treated with 2.0mg/l of Abate, the residual activity continued up to day 60 and averaged between 80-100% larvae mortality. The tubs treated with 4.0mg/l of Abate had the highest residual activity with the mortality remaining between 90 to 100% larval mortality throughout the 65 days of the tests (Table 1 and Fig. 2). Abate gave very high mortalities of the larvae even at the lowest concentration of 1.0 mg/l.

Abate showed long residual activity periods which were probably enhanced by the fairly clean rain water in the experimental tubs and the slow-release carrier effect of the sand granules that carry the active ingredient. Between day one and day twenty five of the tests, there were no significant differences at $p > 0.05$ in the residual activity of the three concentration; 1.0 mg/l, 2.0 mg/l and 4.0 mg/l of Abate. After the day 25, however, there were significant differences between the residual activities of Abate at 1.0 mg/l and those of the 2.0 mg/l and 4.0 mg/l ($p < 0.05$). After day 25 and continuing to day 65, there were no significant differences between the residual activity of the 2.0 mg/l and 4.0 mg/l concentrations (Table 1 & Fig. 2).

In short lived water bodies like rain puddles around homes, mosquito populations can reduced by adding Abate of the 1.0 mg/l concentration since most of those puddles will dry up within two to three weeks. However, in temporary ponds that collect water after the rains and retain water for a month or longer, applying Abate at the 2.0 mg/l would keep the mosquito population under check. Using the higher concentration of 4.0 mg/l may only be considered in cases where the water stays in the environment for long as to allow for several breeding cycles of mosquitoes in the water. However, considering cost, it would necessary to use the lower concentration of 2.0 mg/l as it gives a prolonged residual activity period and could help cut on the cost. Abate chosen for use should take into consideration for how long the control is required among other factors.

Table 2. Average Percentage mortality of *Culexpipienspallens* in semi-field water tubs at 3 concentrations of *Bacillus thuringiensis* var. *israelensis*(Aquabac® 200G) granules in 3 replicates.

		Concentration of the Aquabac in the water					
		0.4g/l		0.8g/l		1.6g/l	
Period (Days)	average % Mortality ± S.D in Control	mean No. of dead ± S.D	Mean average % Mortality ± S.D	mean No. of dead ± S.D	Mean average % mortality ± S.D	mean No. of dead ± S.D	Mean average % mortality ± S.D
1	0.0 ± 0.0	19.3 ± 1.5	77.3 ± 4.0b*	21.7 ± 1.5	86.7 ± 6.1b	25.0 ± 00	100.0 ± 0.0a
2	0.0 ± 0.0	16.7 ± 1.2	66.7 ± 4.6b	19.7 ± 1.5	78.8 ± 6.1b	23.7 ± 0.6	94.7 ± 2.3a
3	0.0 ± 0.0	11.0 ± 1.0	25.3 ± 6.1c	18.0 ± 1.0	72.0 ± 4.0b	22.7 ± 0.6	90.7 ± 2.3a
5	0.0 ± 0.0	6.3 ± 1.5	25.3 ± 6.1c	16.7 ± 0.6	66.7 ± 2.3b	20.3 ± 0.6	81.3 ± 2.3a
7	0.0 ± 0.0	4.3 ± 1.2	17.7 ± 6.6c	7.7 ± 2.5	30.7 ± 0.1b	19.3 ± 0.6	77.3 ± 2.3a
10	0.3 ± 0.6			6.3 ± 1.5	25.3 ± 6.1b	14.0 ± 1.0	56.0 ± 4.0a
15	0.3 ± 0.6					10.3 ± 1.5	41.3 ± 6.1
20	0.0 ± 0.0					5.3 ± 0.6	21.3 ± 2.3

* Means on the same row followed by the same later are not significantly different at 5% level of probability (Duncan's Multiple Range Test).

Chart Area

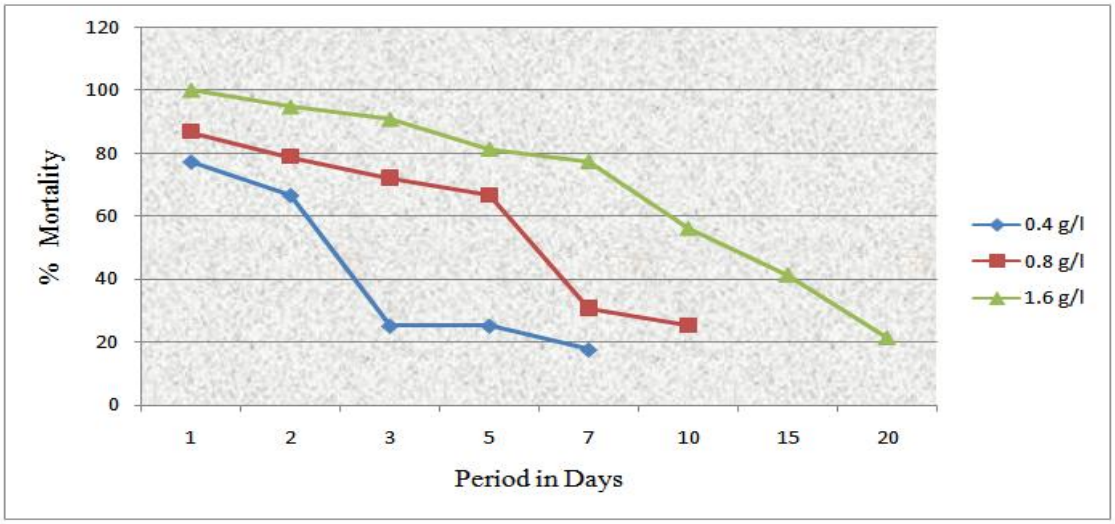

Figure 3: Average percentage mortality of *Culexpipienspallens*in 3 concentrations (g/l) of *Bti*, 3replicates.

In the tubs treated with Bti (Aquabac), high percentages of larvae mortality were observed within the first 24-48 hours after treatment (Table 2 & Fig. 3) and the mortality levels dropped down drastically with time indicating limited residual activity in the water tubs. In the tubs treated with 0.4 g/l of Aquabac, larval mortality dropped far below 50% within the first 3 days after treatment. In the tubs treated with 0.8 g/l of Aquabac, the mortality levels were 50% by the 5th day after treatment while in those tubs treated with 1.6g/l of Aquabac, the mortality levels were below 50% by the 13th day after treatment (Table 2 & Fig. 3). In Aquabac, the residual activity period of the three concentrations used in the tests differed significantly at $p < 0.05$ (Table 2). After 48 hours, the mortality levels in the 0.4 g/l concentration drastically reduced with many of the larvae surviving through the 24 hours of test (Fig. 3). Considering the short residual activity period of Aquabac it would be considered for control of mosquito population in short-lived water bodies and puddles that last for only a few days. Its application would be useful in areas where mosquitoes like those of the *Aedes*spp. diapause as eggs ready to hatch soon after water becomes available.

The results indicated that Abate granules, a non-microbial larvicide gave longer periods of residual activity compared to Aquabac granules, a microbial larvicide. While Aquabac granules had a residual activity that lasted for a period of less than 3 weeks at the highest concentration of 1.8g/l, Abate granules had a residual activity that lasted from 4 weeks at the lowest concentration of 1.0 mg/liter to over two months at the higher concentration of 4.0 mg/l. Abate granules were found to have better larvicidal efficacy and longer residual activity periods than Aquabac granules at all the concentrations used in these tests.

IV. Discussion

If a larvicide was to be chosen on the basis of longevity of its residual activity period within the mosquito breeding sites, Abate would be the larvicide of choice because of its prolonged residual activity after application. However, many factors are considered when choosing the larvicide to be applied in the particular breeding sites. Many factors including the density of the larvae in the breeding site, the cost of the control operation, the proximity to human habitation, the availability of skilled personnel, the length of the mosquito breeding period and the permanency of the water body in which the mosquitoes are breeding, are considered when choosing the larvicide to apply to a particular breeding site [24]. For water bodies that are temporary but remain in the environment for most of the mosquito breeding season, Abate granules would be more convenient as a low dose of 1.0 – 2.0 mg/l applied early in the breeding season will control mosquito larvae throughout the season. For those temporary bodies that last for a week to ten days, Aquabac would easily control the larvae if applied at the correct time. These tests were carried out in summer at period when water temperature in the test tubs ranged from 11^{o}C (lowest) and 33^{o}C (highest).

Rainfall, the source of water in which the mosquitoes bred was monitored during the whole period of the study. Therains that fell on certain days during the test period were considered not to have had any meaningful effect of the concentration of the larvicides added to the water in the tubs (Fig.4).

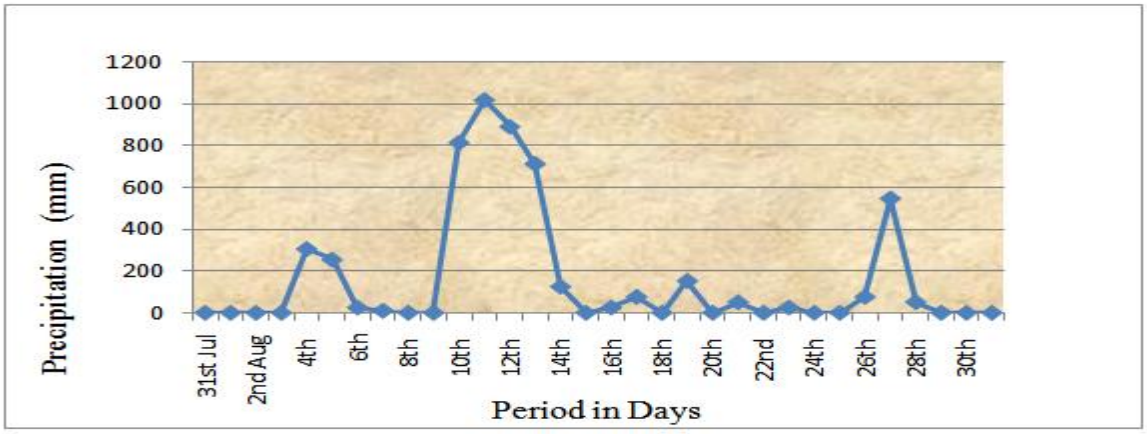

Figure 4: Precipitation (mm)during the test period of the study

For a long time now, *Bacillusthuringiensis* var. *israelensis* (*Bti*)has been identified as a biopesticide successful in the control of public health, agricultural and forest pests but it rarely persist for more than a month to give any degree of long term pest control [25]. Although the spores of *Bti* may survive for several years after application [26], there is a notable rapid decline in their population and toxicity. The persistence of *Bti* varies with habitat type, the density of the insect pest population, stability of climatic conditions and absence of ultraviolet radiation which destroys the insecticidal crystal proteins (ICP) of *Bti*. In well contained environmental conditions, the spores and crystals can be preserved for sufficient time to exceed the threshold dose needed for infection, killing larvae and completing growth cycle. The presence of *Bti* in the environment does not indicate any enhanced value in insect control as *Bti* rarely initiates epizootic unless it is initiated by external conditions such as insect overcrowding [27].

The factors that influence the persistence of introduced *Bti* toxins include the concentration used, the rate of consumption and inactivation by mosquito larvae and the rate of degradation by microbiota[28]. Effective use of *Bti* is subject to the two main issues with the use of microbials;the most suitable placement or application time as to when they will affect the most control and their persistence in the environment. Loss of persistence of biological activity is a result of the effect multiple environmental factors such as temperature, water, and sunlight. Due to*Bti* susceptibility to biodegradation and inactivation under field conditions, its commercial acceptability as an insecticide has been low [1].

In this study, these factors were effective as the tests were carried out in summer, a period of relatively high temperature and sunshine. Temperatures above 35^{o}C inhibit growth of *Bti* and the development of *Bti* insecticidal activity markedly decreases as the temperatures increases. *Bti* is completely inactivated as the temperatures approach 50^{o}C [29]. Though necessary for dilution and dispersal of *Bti*, water limits the persistence and subsequent field effectiveness. *Bacillusthuringiensis* spores survive longer if they are dry. It is estimated that *Bti* spores lose 18% of their stability each day if wet at 30^{o}C while there no loss in stability if kept dry [29]. Natural sunlight has the greatest effects on the persistence of *Bti* and this effect can be direct or indirect. This could explain the relatively short period of residual activity given by *Bti* in the current tests. The direct effects include deletions, cross-linking, strand breakages, and /or formation of labile sites on DNA. Indirect effects include; generation of highly reactive radicals such as peroxides, hydroxyls or oxygen by ultraviolet radiation which results in reduced field persistence[30].

Studies by Law et al. (1968)[20] showed that Abate has low toxicity for mammals and long term effectiveness against mosquitoes such as *Aedesaegypti* larvae in water. Review of the effectiveness of 1% Abate on sand granulesfound that it could be added to containers of water at a concentration of 1.0 PPM without any harmful effects on man and domestic animals. Granules of 1% Abate were shown to be effective on the larvae of *Aedesaegypti* for periods of 6-24 weeks[31]and that the effectiveness was affected by the density of the adult mosquitoes in the surrounding areas. The period of residual activity, obtained in the current studies agreed with those by the earlier researchers. In the current study, Abate granules even at the lower concentration of 1.0mg/l, was able to maintain high mortalities of *Culexpipienspallens* larva for a period of 3-4 weeks.

Limited information is available on the cost and economics of larviciding as a control measure for mosquitoes, a factor that is important especially in the poor countries. The expense involved while using Abate varies with the area to be treated, the quality of personnel, the method of treatment and subsequent surveillance efficiency [31]. In Southeast Asia and Africa, the practice of storing water for drinking and household use in earthen, cement and plastic containers is wide spread. These containers are the main breeding sites for mosquitoes like *Aedesaegypti*, the vector of hemorrhagic fever and *Culexspp*; the vectors of many arboviruses. In the developing countries poor construction of roads, drainage and sewage systems create surface water bodies in urban and peri-urban areas that offer breeding sites for *Culexpipiens* complex members. These mosquitoes can breed in both clean and organically polluted waters. Mosquito breeding in such water can be stopped by early treatment with Abate. This will kill larvae developing in the water for a period of over two months as shown in this study, and this will cover most of the active mosquito breeding period in these regions.

Abate can also control mosquito breeding in water stored for long term use such as rain water harvested in tanks around the homes. The long residual effectiveness of 1% SG formulation against mosquito larvae at the low concentration of 1.0 PPM is enhanced by the slow-release of the active ingredient by the sand granules that act as the carrier material. The uptake and the subsequent slow-release of some of the active ingredient back into the water by the walls of the water container further increases the period of residual activity by Abate 1% SG[31]. However, as was observed in the current study, after 3 to 4 weeks the efficacy of Abate at 1.0 PPM dropped and more larvae began to survive. In normal field conditions this would require that re-treatment be done. In field control programs, re-treatment should be done when the adult mosquitoes' density.

In Malaysia, *Aedesaegypti* and *Aedesalbopictus,* the two major vectors of dengue fever live close to human habitations and breed in artificial and natural containers which hold clean water. Such containers like flower pots, ant-traps, drums, earthen jars, concrete tanks, coconut shells and discarded vehicle tires are used by these species for breeding. Abate has been used to reduce mosquito populations by applying it to these breeding sites especially in water holding containers and has been found to be effective for at least 3 months [32].

It has been shown that when Abate granules are added to water in containers, not all the Abate is mobilized into the water immediately. It is hydrolyzed slowly until the highest concentration of Abate is reached within the water. This is why a small dosage of 0.5 PPM to 1.0 PPM is recommended[33]. Even when higher doses of Abate are used, the concentration of Abate in the water does not immediately increase appreciatively. When water is treated with Abate granules at a rate of 1.0 PPM, it means even the people who take water at the usual rate of 2 liters per day do not get more than 1 mg/day of abate. This level is still safe for the human body[20].

According to a study by[20]there was no significant change observed in either the plasma or red blood cells cholinesterase in people who used or drank water treated with abate granules. No congenital abnormalities were recorded among the people who used or drank water treated with Abate. However, complains about foul smell, bad taste and increase in turbidity were recorded among those who used the treated water. These negative factors caused some people to refuse to treat their water with Abate [34].

These same negative feelings, may be seen among the people when water is treated with abate to kill mosquito larvae but the benefit of being protected from the biting nuisance of mosquitoes and the diseases they transmit can be used to convince more people to accept Abate water treatment. However, *Bacillus thuringiensis* Var. i*sraelensis* does not produce foul smell in water, does not make the water turbid, and thus can be taken as a point of strength in convincing people to use *Bti* as a larvicide.

The choice of larvicide to control mosquito larvae in any area, should take into consideration the length of residual activity period provided by the larvicide, the length of the mosquito breeding season, density of the mosquito population and cost among many other factors. The cost factor is influenced not just by the cost of the chemical used, the personnel and surveillance but also by the cost of re-treatment in times of re-infection by larvae in the treated sites. The interplay of physical factors such as rainfall and temperature may prolong the mosquito breeding period. They may also provide water for breeding outside the general expected breeding season. This would require continuous surveillance which is considered as extra cost when choosing which larvicide to use. The acceptability of the larvicide by the local people should have to be considered when deciding which larvicide to use. Bti and Abate granules are efficient larvicides with varying residual larvicidal activity periods as shown by these studies.

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References

[1] Walker K. 2002. A review of the control methods for African malaria vectors.Office of health, Infectious diseases and Nutrition, bureau for Global Health, United states Agency for International Development (USAID).
[2]. EPA. 2000. United States Environmental Protection Agency; Prevention, Pesticides and toxic substances 7506C/May 2000/735-F-002
[3. Khetan KS. 2001. Microbial Pest Control. Marcel Dekker, Inc New York.
[4]. Soper FL, Wilson DB. 1943. Anopheles gambiae in Brazil 1930–1940. The Rockefeller Foundation, New York, Cited in: Gratz NG and Pal R. 1988. Malaria vector control: larviciding. Pp. 1213–1226, In: Malaria: *Principles and practice of malariology*. (Wernsdorfer WH, McGregor I, eds.), Churchill Livingstone, Edinburgh, U K.
[5]. Gratz NG, Pal R. 1988. Malaria vector control: larviciding. Pp. 1213–1226, In: Malaria: Principles and practice of malariology. Wernsdorfer WH, McGregor I, eds. Churchill Livingstone, Edinburgh, UK.
[6]. Shousha AT. 1948. Species eradication: the eradication of Anopheles gambiae from Upper Egypt. *Bull World Health Org* 1: 309 - 348.
[7]. Das PK, Amalraj DD. 1997. Biological control of malaria vectors. Indian *J Med Res* 106:174–197.
[8]. Yap HH. 1985. Biological control of mosquitoes, especially malaria vectors, Anopheles species. Southeast Asian *J Trop Med Pub Health* 16: 163 - 172.
[9]. Becker N, Margalit J. 1993. Use of Bacillus thuringiensis israelensis against mosquitoes and blackflies In: Bacillus thuringiensis, an environmental biopesticide: theory and practice. Entwistle PF, Cory JS, Bailey MJ, Higgs S (eds.), John Wiley and Sons, Chichester, United Kingdom.
[10]. Lacey LA, Lacey CM. 1990. The medical importance or Riceland mosquitoes and their control using alternatives to chemical insecticides.*J Am MosqContrAssoc* 6 (Suppl.):1-93.
[11]. Gratz NG, Pal R. 1988. Malaria vector control: larviciding. Pp. 1213–1226, In: Malaria: Principles and practice of malariology. Wernsdorfer WH, McGregor I, eds. Churchill Livingstone, Edinburgh, UK.
[12]. Florida Coordinating Council on Mosquito Control (FCCMC). 1997. Florida mosquito control: the state of the mission as defined by mosquito controllers, regulators, and environmental managers. University of Florida.
[13]. Rozendaal JA. 1997. Vector control: Methods for use by individuals and communities. World Health Organization, Geneva.
[14]. Coosemans M, Carnevale P. 1995. Malaria vector control: a critical review on chemical methods and insecticides. Ann Soc*Belge Med trop*. 75:13 - 31.
[15]. Kumar A, Sharma VP, Thavaselvam D, Sumodan PK. 1995. Control of Anopheles stephensi breeding in construction sites and abandoned overhead tanks with Bacillus thuringiensis var. israelensis. *J Am MosqContrAssoc* 11: 86 - 89.
[16]. Gopaul R. 1995. Entomological surveillance in Mauritius.*Sante* 5: 401 - 405.
[17]. Chavasse DC, Yap HH, (Eds). 1997. *Chemical methods for the control of vectors and pests of public health importance*. WHO/CTD/WHOPES/97.2. WHO, Geneva.
[18]. Ware GW. 1989. The Pesticide Book, 3rd edition. Thomson Publications, Fresno, California.
[19]. WHO [World Health Organization]. 1999. Bacillus thuringiensis. Environmental Health Criteria, No. 217. WHO, Geneva
[20]. Laws ER Jr, Sedlak VA, Miles JW, Joseph CR, Lacomba JR, Riviera AD. 1968. Field study on the safety of Abate for treating potable water and observations on the effectiveness of a control program involving both Abate and Malathion. *Bull WHO* 46:39 - 445.
[21]. Majori G, Sabatinelli G, Villani F, Petrarca V. 1986. Studies on insecticide susceptibility of *Anophelesgambiae* and *Culexquinquefasciatus* in the area of Ouagadougou, Burkina Faso (West Africa).*J Am Mosq Control Assoc* 2: 305 - 309.
[22]. WHO [World Health Organization]. 1982. Manual on environmental management for mosquito control with special emphasis on malaria vectors. WHO Offset Publication No. 66; Geneva.
[23]. Gerberg EJ, Barnard DR, and RA ward. 1994. Manual for mosquito rearing and experimental techniques. American mosquito control association, Lake Charles, LA
[24]. Thavara U, Tawatsin A, Ruthairal S, Morteza Z, Mulla MS. 2005. Sequential release and residual activity of temephos applied as sand granules to water storage jars for control of Aedes aegypti larvae (Diptera: Culicidae). J VectEcol 30(1): 62-72.

[25]. Pruett CJ, Burges HD, Wyborn CH. 1980. Effects of exposure to soil potency and spore viability of *Bacillusthuringiensis.J InvertebrPathol* 35: 168 - 174.
[26]. Addison JA. 1993. "Persistence and non-target effects of Bt in soil; a review". *Can J For Res* 23: 2329 - 2342.
[27]. Lambert B, Peferoen M. 1992. Insecticidal promise of *Bacillusthuringiensis.*Facts and mysteries about a successful bio pesticides*Bioscience* 42 (2): 112-122.
[28]. Tapp H, Stotzky G. 1995. Dot blot ELISA for monitoring the fate of insecticidal toxins from *Bacillusthuringiensis* in soil. *Appl Environ Microbiol* 61 (2): 602 - 609.
[29]. Ignoffo CM. 1992. Environmental factors affecting persistence of Entomopathogens. *Florida Entomol* 75 (4): 516 - 525.
[30]. Salama SM, Foda MS, Zaki FN, Khafallah A. 1983. Persistence of *Bacillusthuringiensis* Berliner spores in cotton cultivations. *Z AngewEntomol* 95: 321 - 326.
[31]. Bang YH, Pant CP. 1972. A field trial of Abate for the control of *Aedes aegypti* in Bangkok, Thailand.*Bulletin of the World Health Organization* 46: 416 – 425.
[32]. Lam SK. 1993. Strategies for dengue control in Malaysia. *J Trop Med* 35(4): 303 – 307.
[33]. WHO [World Health organization]. 1973. Safe use of pesticides. Twentieth report of the World Health Organization (WHO) expert committee on insecticides.*WldHlthtechnRep Ser* 513: 26.
[34]. Thavara U, Tawatsin A, Ruthairal S, Morteza Z, Mulla MS. 2004. Efficacy and longevity of a new formulation of temephos larvicide tested in village-scale trials against Aedes aegypti larvae in water storage containers. J Am MosqContrAssoc 20: 93 – 105