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EFFECTS OF SUBLETHAL DOSE OF *BACILLUS THURINGIENSIS* H-14 EXPOSURE ON *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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Abstract Sublethal effects were studied at the parental and offspring stages which has been exposed to *Bti* at the larval stage (parental) in comparison to the control untreated group. Biological aspects inclusive of blood-engorgement rate and fecundity (number of eggs produced) of the parental stage were studied. For the offsprings, study variables include offspring survival rate and developmental period (number of days required at every life stage). Late third/early fourth instar larvae (parental) were exposed to *Bti* at LC_{50} (16.75 ITU/L). Larvae that survived from treatment were further cultured to study the sublethal effects of *Bti* compared to the control untreated group. *Aedes albopictus* larva, pupa and adult survival rate was significantly reduced (p=0.05) either at the parental stage or at the offspring stage after treatment with *Bti* at a dosage of LC_{50} compared to control. Lower blood-engorgement rate was recorded by the treated group compared to control. *Aedes albopictus* treated with *Bti* at the sublethal dose (LC_{50}) in this study demonstrated lesser egg production compared to the control group. The offsprings took a longer time to develop from egg to adult. Reduction in the number of eggs produced and increased immature developmental period, are added advantages that makes *Bti* suitable for the control of mosquitoes.
Key Words *Bacillus thuringiensis* H-14, sublethal effects, dengue vectors.

INTRODUCTION

Research on the sublethal effects of either pesticides or microbial agents against vector pests is relatively a new study and less studied. *Aedes aegypti* found to be reduced in their egg laid after exposed to the sublethal doses of Abate® by Reyes-Villanueva *et al*. (1990). Similar results were documented for dieldirn (Duncan, 1963), d-phenothrin and d-allethrin (Liu et al., 1986). Many more research reported that insecticides reduced the fecundity of the affected insects, while others indicated contradictory finding. *Aedes aegypti* treated with sublethal dose of tetramethrin was reported to be increased in their egg production (Liu et al,. 1986). The sublethal effects of mosquito coil against *Ae. aegypti* and *Culex quinquefasciatus* have been demonstrated (Yap et al., 1996). *Culex quinquefasciatus* blood-engorgement activity have been showed to be decreased to less than 40% after exposed to mosquito coil with or without active ingredient (Yap et al., 1996). Sublethal effects of *Bti* against *Ae. aegypti* larvae on the development and adult size is first reported by Hare and Nasci (1986). In their study, the duration of larval development was increased whereas the adult body size was decreased .

Bacillus thuringiensis Var *israelensis* act as larvicide due to the toxin produced will be activate by the alkaline in the mid-gut of mosquito larvae and activated toxin will disrupt the mid-gut epithelia (Davidson and Sweeney, 1983; WHO, 1999). Since larval development was slowed by reduced food availability (Patrican and DeFoliart, 1985), it is possible that the sublethal dose of *Bti* may affect the nutrient assimilation of the exposed larvae and lead to the longer larval development period. Sublethal effects in longevity and fecundity of *Bti* against mosquitoes have been preliminary studied and reported (Merdan, 1982). The mechanism of *Bti* in killing the larvae of mosquitoes is by disrupting the mid-gut epithelia, making it possible for the sublethal dose exposure of *Bti* to affect the nutrient assimilation and prolong the larval development period (Hare and Nasci, 1986). Control of dengue vectors at larval stage is much easier when compared to adult stage since the movement area of larvae is limited in the water whereas adults show a wider flight range. Sublethal effects in prolonging the development period of dengue vector immature stage will increase the chances of *Bti* to kill the larvae. Sublethal effects information against *Aedes* mosquitoes are very important in the dengue vectors control program. The effects of *Bti* at sublethal dose (LC_{50}) to the survived larvae until the offspring were studied. The survival rate, blood-engorgement rate, development period and sex ratio after exposure to *Bti* at sublethal dose were also studied.

MATERIALS AND METHODS

Sublethal effects were studied at the adult stage which has been exposed to the *Bti* at larval stage and at the F1 generation (offspring) in comparison to the control untreated group. Biological aspects inclusive of blood-engorgement percentage, egg-laying percentage, fecundity (number of eggs produced) at the parental stage were studied. For the offspring study, variables inclusive of eggs hatchability percentage, percentage of emergence at every life stages (L1 to L4, pupae and adults) and growing period (number of days required at every life stages) were also carried out.

Twenty late third/early fourth larvae of laboratory cultured *Ae. albopictus* were treated with *Bti* at LC_{50} (16.75 ITU/L) for 24 hours at 200 ml of LC_{50} dose per cup. A total of twenty replicates were conducted. Control untreated larvae were also conducted under the similar conditions as comparison. Survived larvae were transferred to a clean container with seasoned untreated water for further sublethal effects observation.

Number of adults emerged from the survived larvae were observed and recorded until no further emergence occurred (for a maximum of 7 days). Adult female mosquitoes were provided blood feeding at the age of three to five days during the day. Blood-engorgement was provided by using immobilized white rats. The white rats were immobilized by using confined wire frame. The number of blood-engorged female was recorded a day after. The blood-engorged female mosquito was then transferred to a paper cup individually with wet cone-shaped filter paper placed in the middle of the paper cup. The top of the paper cup was cover with fine nylon mesh (mesh size 0.5 mm). Sucrose was provided through the nylon mesh by using cotton pad.

Fecundity. For the fecundity study, number of eggs produced by the blood fed female mosquito was counted daily until no further eggs were observed or for a maximum period of 7 days. The number of eggs was counted using dissecting microscope at the magnification of 40X. After counting the eggs, the filter paper with eggs was submerged into a tray with 1000 ml of seasoned water for the offspring hatchability study. The whole counting process was less than 6 hours.

Offspring longevity. Five of the filter papers with the number of egg closest to the mean value were used for the longevity and percentage of emergence study for the offspring. The filter papers with eggs were allowed to air dry for a period of less than 6 hours while counting the eggs before submerging into the water. A tray with 1000 ml of seasoned water for each batch of eggs was used for the hatching rate study. Number of larvae emerged were recorded daily and the larvae were transferred to another culture tray after they have emerged to the next instar. Mosquito larval food was provided at 0.2 g per tray daily. The mosquito larval food was made by using ground powder mixed from milk powder, beef liver, vitamin B and dog food at the ratio of 1:1:1:1.

The emerged pupae were kept in a cup covered by nylon mesh and provided with sucrose. Adult emergence and the sex ratio were observed and recorded. Eggs' hatchability percentage, percentage of emergence at every life stage (L1 to L4, pupa and adults), growing period (number of days required at every life stages) and adult sex ratio for the offspring were calculated. Control batches of mosquitoes without any treatment were also conducted.

Data Analysis. The following formula was used for the calculation of the offspring life development period:

$$\text{Offspring life development period} = \frac{\sum \text{(number of emerged offsprings) x (number of days after submersion of eggs in water)}}{\text{Total number of emerged offsprings}}$$

The sublethal effects were determined through the results obtained from the treated group compared to the control group and statistically analysed using non parametric two sample Kolmogorov-Smirnov Test ($p < 0.05$) using SPSS computer program.

RESULTS AND DISCUSSION

Late third or early fourth larvae were exposed to *Bti* at LC_{50} (16.75 ITU/L). Survived larvae were further cultured to study the sublethal effects of *Bti* compared to the control group. Tables 1 and 2 indicated the sublethal effects of *Bti* compared to control against *Ae. albopictus*. *Aedes albopictus* survival rate at different life stages after being treated with *Bacillus thuringiensis* H-14 at LC_{50} at larval stage (late third/early fourth) for 24 hours are shown in the Table 1, whereas fecundity, life development period and sex ratio of offsprings are shown in Table 2.

Table 1. *Aedes albopictus* survival rate, blood-engorgement rate and effects on the offspring after being treated with *Bacillus thuringiensis* H-14 at LC_{50} (16.75 ITU/L) at larval stage (late third/early fourth) for 24 hours.

	Percentage (%) ± SE*	
	Control	**Treated**
Parental larva survival rate[1]	94.25 ± 1.67a	53.00 ± 2.52b
Parental adult emergence rate[1]	92.50 ± 1.68a	45.00 ± 1.95b
Parental female rate	52.78 ± 0.75a	46.94 ± 1.68b
Parental female blood-engorgement rate	90.92 ± 1.83a	54.17 ± 5.15b
Offspring survival rate (Larva 1)[2]	93.56 ± 1.21a	78.38 ± 1.42b
Offspring survival rate (Larva 2)[2]	89.50 ± 1.07a	70.16 ± 2.03b
Offspring survival rate (Larva 3)[2]	83.11 ± 1.54a	65.73 ± 2.22b
Offspring survival rate (Larva 4)[2]	81.29 ± 1.89a	61.68 ± 1.06b
Offspring survival rate (Pupa)[2]	79.85 ± 1.94a	58.23 ± 1.29b
Offspring survival rate (adult)[2]	77.49 ± 2.69a	57.02 ± 1.45b
Offspring Female rate[2]	50.40 ± 1.13a	47.55 ± 2.94a
Offspring Male rate[2]	49.84 ± 0.98a	52.45 ± 2.94a

[1]Twenty larvae were treated in each replicate and a total of twenty replicates were carried out.
[2]The results indicated were sublethal effects on F1 and based on five replicates (five batches of eggs) which the numbers of eggs produced was closest to the fecundity result.
*Mean percentages followed by the same letters within the same rows are not significantly different ($P > 0.05$, non parametric two sample Kolmogorov-Smirnov Test)

Table 2. Fecundity, life development period (days) and sex ratio for *Aedes albopictus* after being treated with *Bacillus thuringiensis* H-14 at LC_{50} (16.75 ITU/L) at larval stage (late third/early fourth) for 24 hours.

	Mean ± SE*	
	Control	**Treated**
Fecundity[1]	108.33 ± 5.23a	81.45 ± 4.40b
Development (days)		
Eggs to larva 1	1.99 ± 0.05a	3.94 ± 0.43b
Eggs to larva 2	3.45 ± 0.06a	6.37 ± 0.44b
Eggs to larva 3	4.81 ± 0.09a	8.86 ± 0.42b
Eggs to larva 4	6.34 ± 0.22a	10.75 ± 0.55b
Eggs to pupa	8.57 ± 0.33a	13.24 ± 0.43b
Eggs to adult	11.17 ± 0.08a	15.23 ± 0.46b
Eggs to adult-female	11.20 ± 0.21a	15.44 ± 0.43b
Eggs to adult-male	11.16 ± 0.15a	15.11 ± 0.55b
Sex Ratio (female: male)	1.02 ± 0.04a	0.93 ± 0.11a

[1]Mean number of eggs produced by each female
*Mean number followed by the same letters within the same rows are not significantly different (P > 0.05, non parametric two sample Kolmogorov-Smirnov Test)

Aedes albopictus larvae survival rate was significantly reduced ($p < 0.05$) to 53.00 ± 2.52% compared to control 94.25 ± 1.67% (Table 1). For the adult emergence rate, 45.00 ± 1.95% and 92.50 ± 1.68% were recorded for treatment and control group respectively. However, lesser female rate was indicated for treated group based on the number of adult emerged compared to control, i.e. 46.94 ± 1.68% and 52.78 ± 0.75%, respectively. Lower blood-engorgement rate was recorded by the treated group with 54.17 ± 5.15% compared to control 90.92 ± 1.83% (Table 1).

Aedes albopictus treated with *Bti* showed significant reduction ($p < 0.5$) in the offspring survival rate compared to control untreated (Table 1). Offspring survival rate from egg to larva 1, egg to larva 2, egg to larva 3 and egg to larva 4 were significantly reduced compared to control, i.e. from 93.56 ± 1.21% to 78.38 ± 1.42%, 89.50 ± 1.07% to 70.16 ± 2.03%, 83.11 ± 1.54% to 65.73 ± 2.22% and 81.29 ± 1.89% to 61.68 ± 1.06%, respectively (Table 1). Meanwhile, offspring pupa and adult survival rate were also decreased from 79.85 ± 1.94% to 58.23 ± 1.29% and 77.49 ± 2.69% to 57.02 ± 1.45%, respectively (Table 1). Nevertheless, similar percentage of female emergence compared to control, i.e. 50.40 ± 1.13% to 47.55 ± 2.94%, respectively was recorded (Table 1).

Lesser eggs were produced after beng treated with *Bti* compared to control ($p < 0.05$). The number of eggs produced was decreased from 108.33 ± 5.23% to 81.45 ± 4.40% after being treated with *Bti* (Table 2). *Aedes albopictus* offspring treated with *Bti* significantly ($p < 0.5$) showed longer development period (number of days) compared to control (Table 2).

Development duration (days) from egg to larva 1, egg to larva 2, egg to larva 3 and egg to larva 4 were significantly increased compared to control, i.e. from 1.99 ± 0.05 to 3.94 ± 0.43 days, 3.45 ± 0.06 to 6.37 ± 0.44 days, 4.81 ± 0.09 to 8.86 ± 0.42 days and 6.34 ± 0.22 to 10.75 ± 0.55 days, respectively (Table 2). Meanwhile, the development periods for pupae and adults from egg were also increased from 8.57 ± 0.33 to 13.24 ± 0.43 days and 11.17 ± 0.08 to 15.23 ± 0.46 days, respectively. However, similar sex ratio (female to male) for the treated offsprings was obtained compared to control with 0.93 ± 0.11 and 1.02 ± 0.04, respectively (Table 2).

Aedes albopictus larva, pupa and adult survival rate was significantly reduced ($p < 0.05$) either at parental stage or at the offsprings after treated with *Bti* at the dosage of LC_{50} compared to control. Lower blood-engorgement rate was recorded by the treated group compared to control. Many researchers reported that insecticides reduce the fecundity of the affected insects. Fecundity of *Aedes aegypti* was found to be reduced after being exposed to the sublethal doses of Abate® (Reyes-Villanueva et al., 1990), dieldrin (Duncan 1963), d-phenothrin and d-allethrin (Liu et al., 1986). *Aedes albopictus* treated with *Bti* at the sublethal dose (LC_{50}) in this study produced lesser eggs when compared to the control group. By reducing in the number of eggs produced, *Bti* is one of the more effective mosquito control tool because it decreases the total population directly.

Bacillus thuringiensis Var *israelensis* can act as a larvicide because alkaline properties in the mid-gut of mosquito larvae will activate the toxin and disrupts the mid-gut epithelia (Davidson and Sweeney, 1983; WHO, 1999). Since larval development was slowed by reduced food availability (Patrican and DeFoliart, 1985), it is possible that the sublethal dose of *Bti* may affect the nutrient assimilation of the exposed larvae and lead to the longer larval development period. Sublethal effects of *Bti* against *Ae. aegypti* was is first reported where the duration of larval development was increased (Hare and Nasci, 1986). Longer life development period (number of days) was required by the offsprings to emerge to become an adult from egg in this study after *Bti* treatment. The mechanism of *Bti* in killing the larval of mosquitoes is disrupts the mid-gut epithelia, it is possible that the sublethal dose exposure to *Bti* may affect the nutrient assimilation and prolong the larval development period (Hare and Nasci, 1986). By prolong the immature stage development period, it will provide wider change (longer time) for the larva treatment especially by using *Bti*. Furthermore, larva treatment is one of the easier ways for vector mosquitoes control because their limited movement area in water whereas adult show wider flight range.

CONCLUSION

Aedes albopictus larva, pupa and adult survival rate was significantly reduced ($p < 0.05$) either at parental stage or at the offsprings after treated with *Bti* at the dosage of LC_{50} compared to control. Lower blood-engorgement rate was recorded by the treated group compared to control. *Aedes albopictus* treated with *Bti* at the sublethal dose (LC_{50}) in this study produced lesser eggs when compared to the control group. Longer life development period (number of day) was required by the offsprings to emerge to become an adult from egg in this study after *Bti* treatment. *Bacillus thuringiensis* H-14 did not significantly decrease the *Ae. albopictus* offspring sex ratio. By reducing in the number of eggs produced and increasing the immature development period, *Bti* is one of the more effective mosquito control tool.

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