IOSR Journal Of Pharmacy **www.iosrphr.org**
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 6, Issue 2 (February 2016), PP. 01-03

Susceptibility of Adult *Aedes aegypti* and *Anopheles dirus* to Bait Containing *Bacillus thuringiensis israelensis*

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Abstract: The susceptibility of adult *Aedes aegypti* and *Anopheles dirus* to1.2% and 17.2% concentrations of *Bacillus thuringiensis israelensis* impregnated Mosquito Attractant Bait (MAB) was tested. There was a significant difference between the proportion of adult *Ae. aegypti* and *An. dirus* survival within 3 days when feeding on MAB control verses MAB with 17.2% Bti (Chi-square (df-1), 12.29; $p \leq 0.05$). Bti in baits may be a new tool for public health and vector control professionals as part of integrated pest management for control of adult mosquitoes.

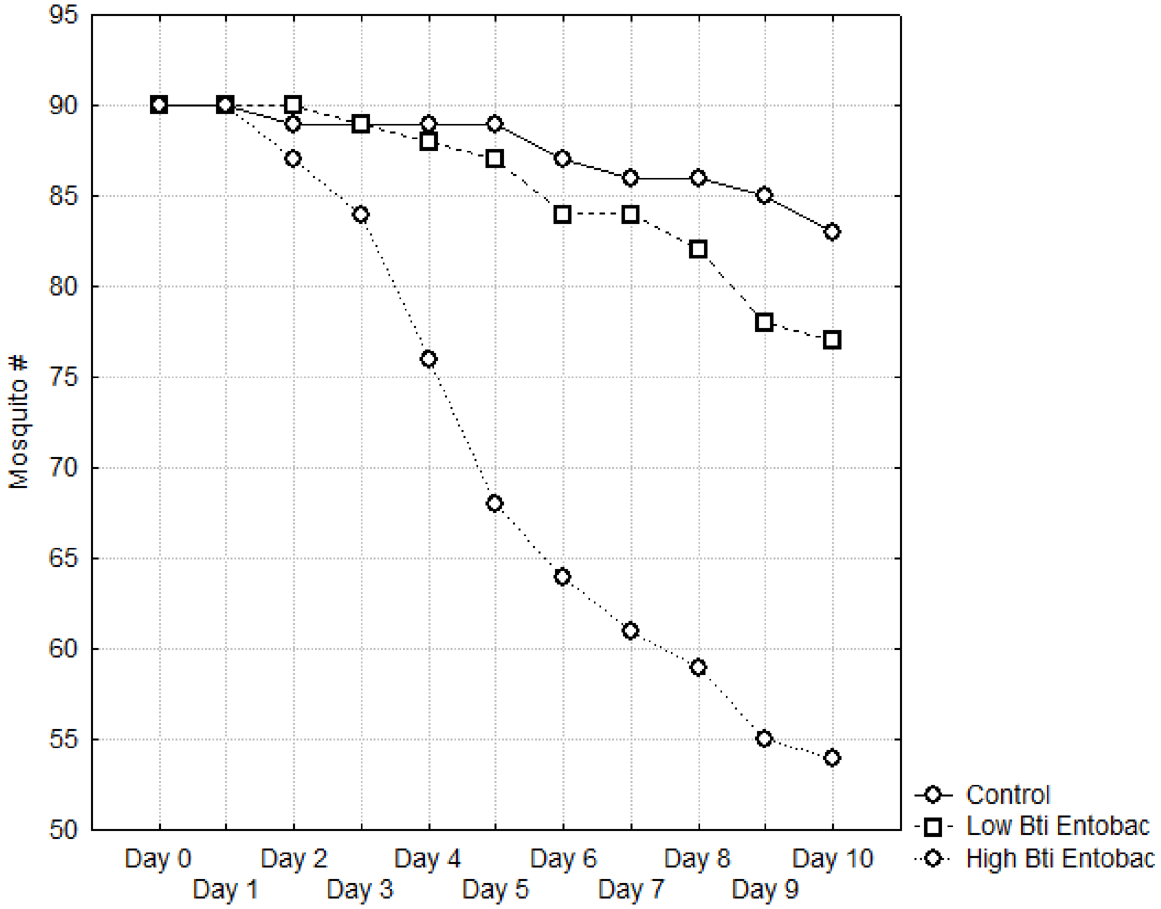
Key Words: *Aedes aegypti, Anopheles dirus, Bacillus thuringiensis israelensis, Bti*

Sugar baits containing pesticides sprayed on bushes or used in bait stations have been used effectively against adult mosquitoes (Müller and Schlein, 2006; Müller et al. 2008). These methods, however, may also affect non-target species and have not contained *Bacillus thuringiensis israelensis* (Bti). Use of a target specific pesticide has advantages of reducing pesticide contamination of the environment as well as reducing cost of controlling the pest species. *Bacillus thuringiensis israelensis*, a target species pesticide, was first discovered in Israel in 1976 (Margalit and Dean, 1985). Goldberg and Margalit (1977) described the activity of Bti against larval mosquitoes, and for over 60 years Bti has been used to reduce mosquito populations. There are numerous advantages to using Bti to reduce mosquito populations including its nontoxic properties to non-target organisms, e.g. humans, mammals, birds, fish, beneficial insects, plants and most aquatic organisms (EPA 1998). Another advantage of using Bti is the lack of development of pesticide resistance in mosquito populations; it has been called an ideal pesticide (Glare and O'Callaghan, 1998). However, little research has been conducted on the use of Bti as a mosquito adulticide. Klowden and Bull (1984) described variation in susceptibility among adult *Aedes aegypti*, *Anopheles freeborni* and *Culex quinquefasciatus* to Bti in sucrose solutions at varying concentrations. Zahira and Mulla (2005) found reduced surface tension affected mortality of adult *C. quinquefasciatus* caused by application of biopesticides to the water but suggested that mortality may also be due to imbibing water containing Bti. We describe the adulticidal activity of Entobac™ pesticide (MEVLABS, Inc.), composed of Bti in a nectar like Mosquito Attractant Bait (MAB™) against adult *Ae. aegypti* and *An. dirus* mosquitoes. Entobac™ bait based when used alone against larvae is >99% effective or it can be used against adult male and female mosquitoes when used within devices such as the ProVector Flower ™ and the Bugshield Tube ™.

Colony-raised *Ae. aegypti* and *An. dirus* adult mosquitoes were starved for 24 hours. Blind trials were conducted with three replicates of 30 mosquitoes from each species placed in separate test cages with Mosquito Attractant Bait (MAB™) bait pads as negative control and Entobac™ bait pads (MAB™ plus Bti) with concentrations of 1.2 % Bti and 17.2% Bti Entobac™ formulations. Each cage was provided supplemental distilled water in cotton balls ad libidum. Bait pads in polystyrene weighing boats were placed in the bottom of the respective cages. Live and dead mosquitoes were counted every 24 hours for 10 days. Chi-square analysis and Kruskal Wallace ANOVA were used to analyze data (Statistica, Statsoft, Inc.)

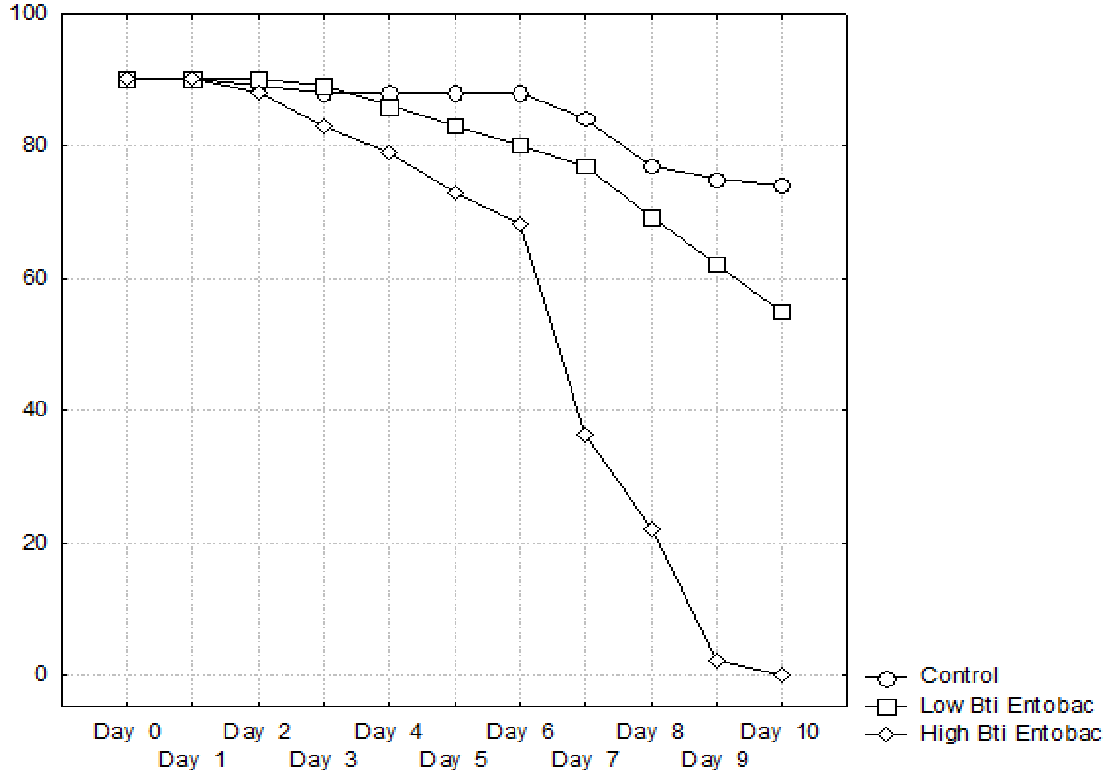
There was a significant reduction of survival of *Ae. aegypti* after 7 days of feeding on MAB control (mean reduction day 7=1.33, df=4, $p \leq 0.05$) Figure 1. There was a significant reduction of *Ae. aegypti* within 6 days using Entobac™ 1.2% (mean reduction day 6=2.00, df=4, $p \leq 0.05$). There was a significant reduction of survival of *Ae. aegypti* within 3 days when feeding on Entobac 17.2% Bti (mean day 3=2.0, df=4, $p \leq 0.05$). There was no significant difference between the proportions of adult *Ae. aegypti* survival during 10 days when feeding on MAB Control and Entobac 1.2% Bti (Chi-square (df-1), 2.03; $p \geq 0.05$). However, the median survival rate was significantly different within 9 days, Kruskal-Wallis ANOVA by Ranks Test: H (1, N=6) = 4.0, $p \leq 0.05$). There was a significant difference between the proportion of adult *Aedes aegypti* survival within 3 days when feeding on MAB control and Entobac 17.2% Bti (Chi-square (df-1), 12.29; $p \leq 0.05$). The median survival rate was significantly different within 4 days between Entobac 1.2% and Entobac 17.2%, Kruskal-Wallis ANOVA by Ranks Test: H (1, N=6) = 4.0, $p \leq 0.05$).

Figure 1. Survival of adult *Aedes aegypti* feeding on MAB control verses Entobac 1.2% Bti and Entobac 17.2% Bti



There was a significant reduction of survival of *An. dirus* feeding on MAB control within 7 days (mean=2.0, df=4, $p \leq 0.05$) Figure 2. There was a significant reduction of survival of *An. dirus* feeding on Entobac 1.2% Bti in 4 days (mean=1.33, df=4, $p \leq 0.05$). There was a significant reduction of survival of *An. dirus* within 3 days when feeding on Entobac with 17.2% Bti (mean=2.33, df=4, $p \leq 0.05$). There was a significant difference between the proportion of adult *An. dirus* survival within 6 days when feeding on MAB control and Entobac 1.2% Bti (Chi-square (df-1), 5.71; $p \leq 0.05$). The median survival rate was significantly different for adult An. dirus feeding on MAB and Entobac 1.2% within 5 days, Kruskal-Wallis ANOVA by Ranks Test: H (1, N=6) = 4.1, $p \leq 0.05$). There was a significant difference between the proportion of adult *An. dirus* survival within 3 days when feeding on MAB control and Entobac 17.2% Bti (Chi-square (df-1), 6.72; $p \leq 0.05$). The median survival rate was significantly different between MAB control and Entobac 17.2% within 3 days, Kruskal-Wallis ANOVA by Ranks Test: H (1, N=6) = 4.0, $p \leq 0.05$).

Figure 2. Survival of adult *Anopheles dirus* feeding on MAB control verses Entobac 1.2% Bti and Entobac 17.2% Bti



Entobac with Bti was effective in killing adult *Ae. aegypti* and *An. dirus* in the laboratory. However, *An. dirus* was more susceptible than *Ae. aegypti*, ~98% verses ~40% mortality, respectively after 8 days. Mortality was >95% within 24hrs for female and male *Aedes*, *Anopheles*, and *Culex* species which ingested Entobac D (with deltamethrin) mortality was >95% within 24hrs. There was a concentration effect of Bti on adult mosquitoes as found in other studies, Zahir and Mulla (2005). Older mosquitoes are more important epidemiologically because of their increased opportunity to acquire and transmit pathogens. Klowden and Bulla (1984) found older *Ae. aegypti* and *An. freeborni* were more susceptible than 4 day old females but adult *Ae. agypti* were less susceptible to *An. freeborni*. Bti is considered one of the safest larval pesticides. Bti baits may also be an effective choice for public health and vector control professionals as an additional tool for integrated pest management of adult mosquitoes. The views expressed in this publication are those of the authors and do not reflect the official policy of the United States Government.

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