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Efficacy Trial of Camouflage ProVector Nettys With Entobac D Against *Aedes aegypti*

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Abstract: *Aedes aegypti is an important vector of several arboviruses having significant impact on human populations on several continents. Increasing prevalence of pesticide resistance is of great concern to public health officials as is concern of environmental impact of pesticide spraying. A preliminary trial of a new pesticide, composed of a mix of Bacillus thuringiensis israelensis and deltamethrin pesticides combined with an attractant bait are discussed. A camouflage system is discussed which may have military application in protecting military and sportsmen, such as campers and fisherman.*
Keywords: *resistance, mosquito control, Bacillus thuringiensis israelensis, deltamethrin*

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I. Introduction

Vector-borne diseases account for more than 17% of all infectious diseases globally, with billions of people at risk from vector-borne diseases, VBD, (WHO 2017). The yellow fever mosquito, *Aedes aegypti* has killed tens of millions of people throughout history through the transmission of several arboviruses, including yellow fever virus, Chikungunya, dengue viruses. *Ae. aegypti* is now considered to be the primary vector of Zika virus outside of Africa. Populations of *Ae. aegypti* have become resistant to pesticides in several countries and regions within countries (Rodriguez et al 2001). Deployed military forces often require protection from mosquitoes and mosquito-borne diseases. With military units having limited time to test mosquito populations for pesticide resistance in the area of deployment, having a pesticide within the logistics chain, able to effectively kill resistant mosquitoes provides a solution that has public health and military readiness impact. The current study was conducted to get a baseline of susceptibility of non-resistant *Ae. aegypti* to Entobac D, a new pesticide developed to rapidly knockdown mosquitoes, including pesticide resistant mosquitoes applied to two types of camouflage patterns.

II. Methods

Entobac D consists of a honey attractant formulation (Mosquito Attractant Bait, MAB™) with active ingredients compose of *Bacillus thuringiensis israelensis* (Bti) and deltamethrin. Bti has been used for over 60 years as a larvacide but recently has demonstrated lethality against adult *Ae. aegypti* and other adult mosquito species (Alongkat et al. 2016; Yalwala et al. 2016). A white card, 15 x 15 cm with 200 ul MAB applied the surface was used as the control; 200 ul of Entobac D were applied to the Universal and Woodland camouflage cards (15 x 15 cm) (Figure 1). Thirty adult *Aedes aegypti* were placed into each of three cages. Cards were placed in the bottom center of each cage, and a cotton ball with distilled water was placed at the top of each cage to serve as a source of water, and replenished *ad libidum*. Dead mosquitoes were counted each 24 hours for 168 hours. Nettys can be hung indoors or a colored or camouflage paper can be placed within empty plastic water bottles with Entobac D placed in the cap with holes (Super Netty) for outdoor use (Figure 2).

III. Results And Discussion

There was a significant reduction of *Ae. aegypti* within 24 hours in both the Woodland and Universal Camouflage patterns with Entobac D over Control (Chi-square= 25.71; $p \leq 0.05$ and Chi-Square 30.0; $p \leq 0.05$) respectively but no significant difference between the Camouflage patterns ($p \geq 0.05$) (Figure 3). There was 100% fatality within164 hours in both Camouflage patterns with Entobac D over Control with 17% fatality, a significant difference (Chi-square=42.86; $p \leq 0.05$). 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. When using Entobac with Bti alone there was a significant reduction in adult *Ae. aegypti* within 7 days under laboratory conditions (Alongkot et al. 2016). The Vector Control

specialist should determine whether rapid knockdown of adults, or increase auto-dissemination of Bti to larval habitats is required when making a decision whether to use Entobac (Bti) or Entobac D (Bti plus deltamethrin). In a field study of Entobac (Bti) there was a significant reduction of mosquitoes in Honduras (Kollars et al. 2016).

Arboviruses, such as Zika, dengue and Chikungunya are rapidly spreading globally and are causing increased disease burden (Kraemer et al. 2015). Multiple populations with resistance to various pesticides are popping up in different geographical areas within countries (Ishak et al. 2015). Mixing pesticides to repel or kill mosquitoes is an important step forward in combating pesticide resistant mosquitoes. For example, using a mix of organophosphates with repellents on bed nets and military clothing are effective in protection personnel (Pennetier et al. 2015). Mixing synergists, such as PBO can be effective increasing lethality of pyrethroid in resistant *Ae. aegypti* and could be useful where continuous application of deltamethrin is necessary (Vijayan et al. 2007). Many of these technologies are also used in the sporting industry, such as hiking, camping, fishing and hunting. The present study is the first to demonstrate the effectiveness of combining Bti with deltamethrin in killing adult *Ae. aegypti*. Additional field trials demonstrating the effectiveness of Entobac D in the ProVector Pesticide System are forthcoming.

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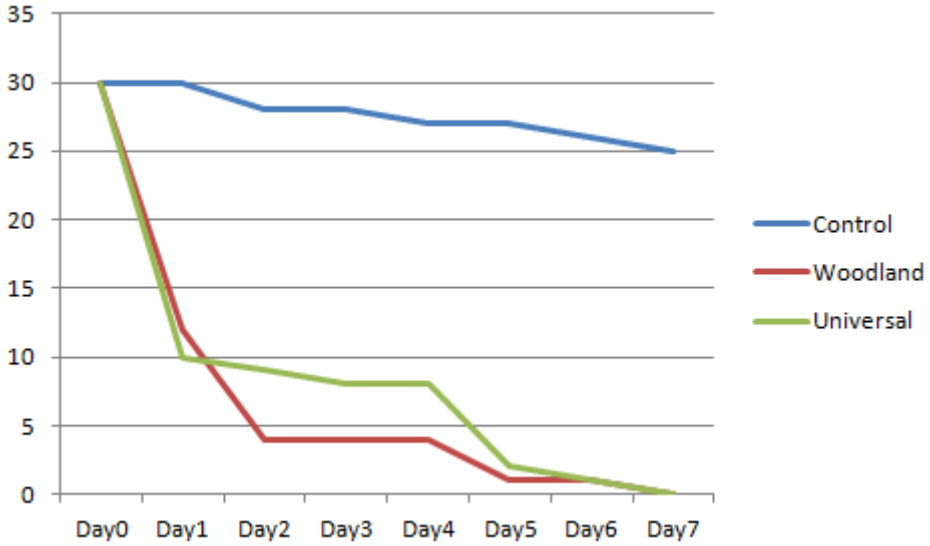
Figure 1. Woodland (left) and Universal (right) Camouflage Patterns used in the study.

Figure 2. Colored and Camouflage Super Nettys with Entobac D are being tested for outdoor control of mosquitoes.

Figure 3. There was a rapid and significant reduction ($p \leq 0.05$) of *Aedes aegypti* imbibing on Entobac D on both camouflage patterns.



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