IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 7 Ver. III (July 2016), PP 55-57
www.iosrjournals.org

A Plant Volatile Infused Honey Bait for Control of *Aedes aegypti* Larvae and Adults

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Abstract:*Pesticide resistance by mosquitoes is of growing concern as mosquito-borne diseases are spreading globally, seemingly unchecked. Besides a new formulation of Bacillus thuringiensis israelensis, no new pesticides have been deployed to combat adult mosquitoes in several decades. Fortunately new research by several scientific groups have successfully investigated volatile plant oils, long known to have repellent and insecticide qualities. We discuss a new plant volatile infused honey bait that can be used in water to control larvae or used as a spray or placed in bait applicators to control adult Aedes aegypti, an important vector of several arboviruses, including dengue, Chikungunya, yellow fever and Zika. The volatile infused honey bait was effective in killing larvae and adult mosquitoes within 24 hours. The efficacy of the toxic volatiles was prolonged in the toxic honey bait, even after one month of environmental exposure.*

I. Introduction

The increasing rate of multi-pesticide resistance in medically important mosquitoes is of great concern to public health officials and mosquito control programs across the globe. The development of pyrethroid resistance by mosquito vectors poses a significant risk to hundreds of millions of people. If additional means of practical and effective mosquito control are not developedthere may be a resurgence of malaria across Africa of pyrethroid resistant anopheline mosquitoes (Constant et al. 2012). Resistance to organophosphate, carbamate and pyrethroid pesticides in *Aedes aegypti* and *Culex quinquifasciatus* populations on several continents has also been identified (Mazzarri&Goergiout, 1995; Tantely et al. 2010). Pesticide resistancewill directly impact the effectiveness of Integrated Mosquito Management(IMM) programs seeking to mitigate the risk of mosquito-borne diseases in human populations, e.g. malaria, filariasis, Chikungunya, dengue, and Zika viruses.

In 2010, the International Vector Control Consortium (IVCC) stated, "There has been no new public health insecticide for around 30 years and new insecticides are desperately needed." The IVCC hasrecently identified and will take to candidate selection, nine novel chemical classes which have shown significant activity against adult mosquitoes (IVCC, 2016). During this time, several other new pesticides against adult and larval mosquitoes have been developed and tested. The first formulation of *Bacillus thuringiensis israelensis* (Bti) active against adult mosquito species which vector malaria, arboviruses and microfilaria has been deployedin several countries during the past several years (Greenemeir, 2007; Yalwala et al. 2016). There have also been great strides forward in testing and utilizing plant volatile extractsto control adult and larval mosquitoes. In this study,we describe the effectiveness of a multi-plant oil toxic bait that can be used to control both adult and larval *Ae. aegypti*.

II. Methods and Results

Ecobait™ contains atoxic honey bait pad with 1.5% eugenol, 0.25% clove oil and 0.25% thyme oilwithultramine blue as a dye-marker. Three replicates each of 25 control larvae and 25 test larvae in 100 ml deionized water, with test containing the toxic bait at a concentration of 200 ppm.Fish flakes were provided as food in control and tests *ad libidum*. Live and dead mosquito larvae were counted after 24 hours post exposure in control and test containers. Three replicates, each with 10% sucrosebait pads and 25 adult were placed in the control cages. Three replicates of Ecobait™ bait pads (2% plant volatile as above) were placed in testcages containing 25 adult mosquitoes each.Cotton balls with deionized water were added to control and test cages respectively. Ingestion of the toxic bait by adult mosquitoes was identified by observing blue-dyed bait within the mosquitoes.Live and dead adult mosquitoes were counted after 48 hours.Tests were repeated after one month of environmental exposure of the EcoBait™ bait pads to determine if efficacy of volatiles in the toxic bait was retained.

III. Discussion

The World Health Organization Pesticide Evaluation Scheme (WHOPES) was set up in 1960 in order to promote and coordinate the testing and evaluation of pesticides for public health. It functions in participation

with representatives of governments and pesticide manufacturers (WHOPES, 2016). WHOPES has a four-phase evaluation and testing program for studying the safety, efficacy, acceptability, and development of quality control specifications. The two primary objectives of WHOPES are to; 1) facilitate the search for alternative pesticides and application methods that are cost-effective and safe, and 2) develop and promote policies, strategies and guidelines for the selective and judicious application of pesticides for public health use.

In the United States, the Environmental Protection Agency (EPA) is the regulatory agency for pesticides (EPA, 2016). The EPA has determined that "minimum risk pesticides" (MRP) pose no or little risk to the environment or human health and has exempted them from the requirement to be registered under the Federal Insecticide, Fungicide, and Rodenticide Act.The EPA exempted these products to lower the cost and regulatory burden of the public and businesses for the MRP. The active ingredients in EcoBait™(eugenol, clove and thyme oil) all fall within the MRP. Another condition the EPA has implemented for MRP is the inert ingredients must be listed as minimum risk tolerance exemptions and commonly consumed food commodities (EPA, 2016); only these approved 25b matched inerts are used within EcoBait™. Kehinde et al. (2014) stated, "In the recent event of widespread of mosquito resistance to chemical insecticides, the use of plant extracts and essential oils are gaining prominence as alternative ways of controlling the insect vectors."

Several studies on the efficacy of various plant volatile oils on mosquito larvae have been conducted. Eugenol, geraniol, linalool, L-menthol and terpeniole were found to be larvicidal to various stages of larval*Ae.aegypti* (Knioa et al. 2008; Waliwitiya et al. 2009; Barbosa et al. 2012; Pandy et al. 2013). Eugenol is a common plant compound and effective in repelling important mosquitoes within *Aedes*, *Anopheles*, and *Culex* genera which transmit viruses, malaria, and filarial parasites (Maia & Moore, 2011; Krishnappa et al. 2012). Eugenol has also proven to be both ovicidal, larvicidal and adulticidal, but with varying concentrations depending on life stage and species (Bhat and Kempraj, 2009). Careful evaluation of the efficacy of volatile oils must be conducted as even within a species there is variation between field and laboratory raised strains, such as in *Ae. aegypti* (Barbosa et al. 2012). In a laboratory study conducted by the U.S. Army, eugenol combined with an attractive sugar bait (ASB) solution proved lethal to adult*Ae. aegypti*, *Cu. quinquefasciatus*, and *An. quadrimaculatus* in the laboratory. In the field, this same groupdescribed a>70% reduction for *Ae. atlanticus*, *Ae. infirmatus*, and *Cu. nigripalpus* and a >50% reduction for *An. crucians*, *Uranotaenia sapphirina*, *Culiseta melanura*, and *Cx. erraticus*, three weeks after the toxic ASB spray application. Non-target feeding of six insect orders, Hymenoptera, Lepidoptera, Coleoptera, Diptera, Hemiptera, and Orthoptera was not significant.They found a variation in the effectiveness of eugenol as a toxin as well as repellent between adult mosquito species.In a second study in Florida, the use of eugenol with a toxic bait significantly reduced *Ae. albopictus* in tire sites. *Aedes albopictus* populations were significantly reduced with ATSB-eugenol applications applied directly to non-flowering vegetation and as bait stations compared with non-attractive sugar baits and control (Revay et al. 2014). However, spraying of pesticides in the environment can be more expensive and less eco-friendly than when target specific applicators are used.

A major challenge to the use of plant volatiles as insecticides can be their loss of efficacy due to rapid oxidation, often within a few hours. The prolonged activity of plant volatiles in EcoBait™ greatly enhances the practicality and efficacy of this reduced risk pesticide for the control of mosquitoes and mosquito-borne diseases.

Another factor that reduces the effectiveness of volatile toxic baits is rainy weather and uv radiation, causing the bait to be washed away or degraded. This increases labor and material costs because of the need for reapplication of the toxic bait. Using the ProVector™ System with Entobac™ pads and EcoBait™pads is effective in controlling adult mosquitoes even during heavy rains because the pesticide is protected from rain and direct sunlight. In fact, the adult mosquitoes often transport the Entobac pesticide back to larval breeding sites, whereby the larvae are subsequently killed. In several countries, mosquito attractant applicators have been used to deliver Entobac (Bti) and Entobac D (Bti plus deltamethrin) impregnated on mosquito attractant bait pads to significantly reduce mosquito populations. For example, the mosquito population in two cities in western Kenya were significantly reduced when the ProVector™Flower with Bti was distributed within housing compounds (Yalwala et al. 2016). The ProVector™Tube with Entobac™D significantly reduced mosquitoes near a port city in Honduras within one month and greatly reduced the amount and number of pesticide application needed to reduce mosquito populations at a hotel beach resort in the Dominican Republic (Kollars et al. 2016). In a preliminary study in Georgia, the ProVector™Tube with EcoBait™significantly reduced the mosquito population within 24 hours and has been effective in controlling mosquitoes for one month. In addition to efficacy as an adulticide, the EcoBait™pad waseffective in reducing mosquito larvae in indoor and outdoor containers. Further testing of plant volatiles combined with other eco-safe and pesticide resistant reducing formulations is warranted to prove efficacy across mosquito species and among pesticide resistant strains. Another advantage of using a bait applicator to attract and deliver toxins to mosquitoes is the reduction of costs in material, reduced labor and lower quantity of pesticides released into the environment.The efficacy and safety of volatile oils for use as pesticides for adult and larval mosquitoes should be a priority area of

interest for WHO, USAID, Bill and Melinda Gates Foundation, and the Samaritan's Purse. These types of organizations are able to bring to bear the appropriate funding to further expand research into safe and cost effective alternatives necessary in protecting human and animal health and reducing the risk of vector-borne diseases transmitted by pesticide resistant mosquitoes.

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Table 1: There was a significant difference between control and test larvae and adults ($p<0.05$). There was not a significant difference between 24hr test and 24hr test using bait pad after 30 day environmental exposure.

Experiment	Mortality/Survival after 24 Hours	Mortality/Survival after 24 Hours (30 Day)Post environmental exposure of toxic bait pad
Control Larvae	1/75	0/75
Test Larvae	75/0	75/0
	Morality/Survival after 48 Hours	Mortality/Survival after 48 Hours (30 Day)Post environmental exposure of toxic bait pad
Control Adults	0/75	1/74
Test Adults	73/2	72/3