

MEVLABS, Inc.

Developing Research in a Developing World™

Technical Paper - Entobac and Entobac D Pesticides and Devices for the Control of Mosquitoes and Flies

Thomas M. Kollars, Jr., PhD

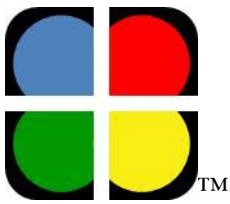
Introduction

There are approximately 3,000 mosquito species found globally. Mosquitoes are among the most efficient vectors of animal and human pathogens, causing many debilitating and often fatal diseases. Globally, malaria is the most prevalent vector-borne disease, with over 2.4 billion people around the world at risk of contracting this disease. Between 1 and 3 million deaths occur annually from malaria, most in children from sub-Saharan Africa. Every 45 seconds a person dies from malaria. Another mosquito-borne disease of particular concern to children's health is dengue virus, the second most important tropical disease with approximately 50 to 100 million cases of dengue fever and 500,000 cases of Dengue Hemorrhagic Fever (DHF) each year. DHF is fatal to approximately 40% of children that contract this painful and deadly virus. Yellow fever, Chikungunya, Rift Valley fever, West Nile virus, Japanese encephalitis, and filariasis, are regionally important mosquito-borne diseases that are particularly dangerous for children. Filth flies also pose a significant threat to public health in communities around the world. Diarrheal diseases are the number one killer of children in Africa. Flies account for about 30% of diarrheal diseases.

Among the tools mankind has used against mosquitoes and the deadly microbes they carry are drugs, vaccines, pesticides, and bed nets. The drugs developed for prophylaxis and treatment of malaria have had great success. However, drug resistance by the parasite is emerging in many parts of the world. Successful vaccines have been developed against some important mosquito-borne viruses, e.g. Japanese Encephalitis virus and Yellow Fever virus. After decades of trials no successful vaccines have been approved for use for either malaria or dengue virus. Pesticides have been used both outdoors and indoors to effectively kill mosquitoes but they are often expensive, kill other species besides mosquitoes, are ineffective where pesticide resistance has developed in mosquitoes, and meet resistance where people are concerned about harmful effects to health and the environment. Pesticide treated bed nets have proven to be quite successful in assisting mankind in our fight against malaria but have not been effective in protecting people from dengue which is carried by mosquitoes that bite mainly during the day. Problems associated with the use of pesticide impregnated bed nets include insufficient distribution resulting in unprotected family members, ineffectiveness of bed nets over time or when damaged, lack of beds for attachment, and resistance by some people to use bed nets due to their smell and health concerns of exposure to touching and breathing pesticides by themselves and their children. Always use insecticide treated bed nets and a complimentary mosquito control method such as ProVector™, BFT Bug Fighter™ for Mosquitoes and Flies, Super Netty™, Netty™ or indoor residual spray.

Entobac™ biopesticide for control of adult and larva mosquitoes

Entobac™ is a patented technological leap forward in mankind's fight against mosquitoes, malaria and other mosquito-borne diseases because it uses *Bacillus thuringiensis israelensis* (Bti) to kill both larva and adult mosquitoes. To kill larva mosquitoes, Entobac™ can be placed directly into larva breeding sites. To kill adult mosquitoes a bait pad is incorporated into the patented ProVector™ Flower device. The ProVector™ Flower and Entobac™ were developed by Dr. Tom Kollars at MEVLABS, Inc. to be an economical and environmentally safe "GREEN" method for killing both larva and adult mosquitoes. ProVector™ using Entobac™ is the first pesticide capable of killing both larva and adult mosquitoes with Bti biopesticide. Bti is virtually non-toxic according to the EPA and is World Health Organization



MEVLABS, Inc.

Developing Research in a Developing World™

WHOPEs recommended for mosquito larva control. Because the ProVector™ targets adult mosquitoes by luring them to feed on Entobac™, it is cost effective and environmentally safe. Indoor Residual

Spraying (IRS) is very effective in the spatial elimination of malaria by reducing adult mosquito populations. Like IRS, the ProVector™ with Entobac™ eliminates the adults however, Entobac™ is harmless to people and pets. Laboratory studies have also shown that Bti in Entobac™ can be delivered to larvae mosquitoes by adult mosquitoes as they die in breeding sites. While insecticide treated bed nets effectively protect people while they are sleeping, the ProVector™ can help the entire household (average of 7 people) 24 hours a day when they are not under the bed net. The ProVector™ Flower with Entobac™ attracts mosquitoes indoors from approximately 5 meters. We recommend the ProVector™ Flower be used in combination with bed nets and other integrated pest management strategies to provide maximum protection to individuals and families at risk of malaria and other mosquito-borne diseases. The Entobac™ Bait Pad is designed to last months in the ProVector™ Flower, depending on the number of mosquitoes that feed on the bait pad. Each home should have a minimum of one ProVector™ Flower with Entobac™ Bait Pad. Another major goal of our team is to have the plastic ProVector™ Flower manufactured in countries where it is used. The ProVector™ is designed for use indoors or outdoors when protected from rain and direct sunlight. There are two methods of delivery 1) Entobac™ as an adulticide/larvicide used in devices and 2) Entobac™ L is a larvicide in pellet form directly added to water.

The ProVector™ Flower imitates the look of flowers in order to trick the adult mosquitoes into ingesting the Entobac™ biopesticide which imitates flower nectar



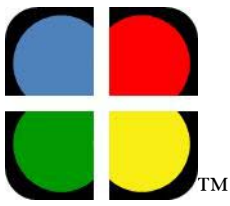
ProVector™ Flower with Entobac™ Bait Pad



ProVector™ Flower in a Kenyan Home

Entobac™ D formula for control of Flies and Mosquitoes:

Entobac™ D adds Deltamethrin pyrethroid to give the added benefit of killing filth flies. It rapidly kills mosquitoes and flies, but loses the advantage of the mosquitoes taking Bti to larval breeding sites. It is not used in water. Deltamethrin is widely used to treat bed nets and clothing. Entobac™ D targets adult mosquitoes in the ProVector device. Mosquitoes and Filth Flies are targeted when Entobac™ D is used in the BFT Bug Fighter™ for Mosquitoes and Flies, Super Netty™ or the Netty™. After ingesting Entobac™ D, adult mosquitoes and flies die within 24 hours.



MEVLABS, Inc.

Developing Research in a Developing World™

Super Netty™ and Netty™ device

The Super Netty™ and Netty™ are two devices that provide eco-friendly and low-cost mosquito and fly control. The Super Netty™ consists of a 6 inch square of multi-colored cardboard with a plug of Entobac™ D pesticide. The Netty™ device consists of a piece of paper with a color pattern on one side and Entobac™ D pesticide applied to the other side. The Super Netty™ is used only indoors. The Netty™ can be used indoors or outdoors. When used outdoors, the Netty™ must be rolled color side out and placed inside of a clear plastic bottle. The bottle is then hung upside down to protect the Netty™ from the weather. When used indoors, the Netty™ can be placed in a bottle or hung alone. The Super Netty™ lasts up to 1 to 2 months and the Netty™ lasts 1 to 2 weeks depending on mosquito and fly populations.



Super Netty™ with Entobac D plug



Netty™



Netty™ in plastic bottle outdoors

BFT Bug Fighter™ (Fly and Mosquito) control

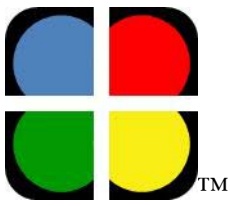
The BFT Bug Fighter™ for Flies and Mosquitoes uses the Entobac™ D Bait Pad for control of Flies and Mosquitoes. Mosquitoes and Flies feed through holes in the bottom of the tube. The Entobac™ D Bait Pad can last several months and must be replaced when bait is depleted. The BFT Bug Fighter™ can be used indoors or outdoors to safely deliver Bti and Deltamethrin pyrethroid to mosquitoes and flies.



BFT Bug Fighter™ for Flies and Mosquitoes



BFT Bug Fighter™ and ProVector™ are being used in State Orphanages and Retirement Homes in Honduras



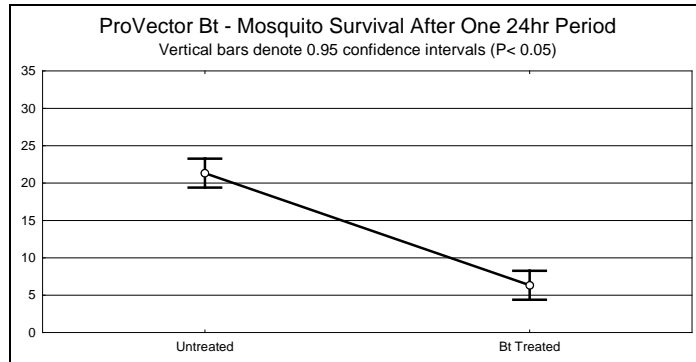
MEVLABS, Inc.

Developing Research in a Developing World™

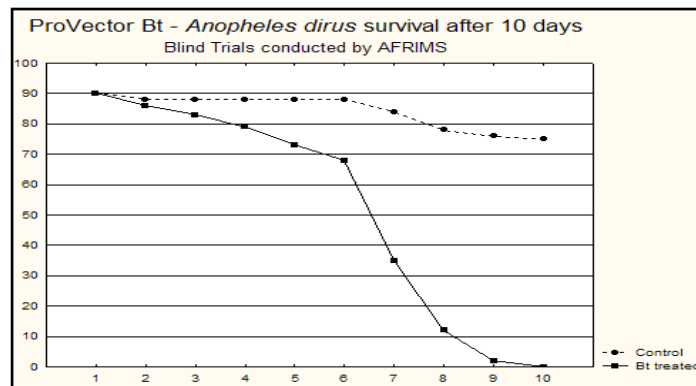
LABORATORY TRIALS

ENTOBAC™ used in ProVector™ and ENTOBAC D™

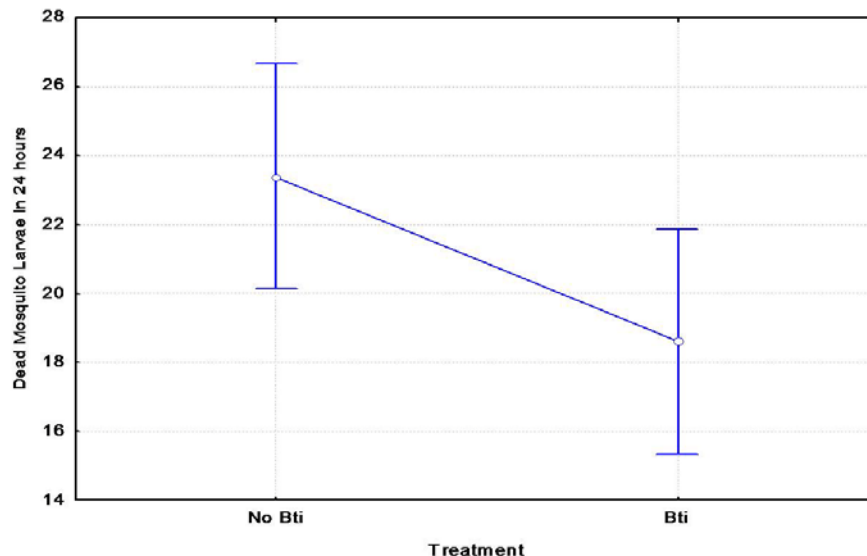
Efficacy Study 1. BDIDL-Georgia Southern University - The efficacy of Entobac™ against *Aedes aegypti* was 80% within 24 hours (graph on right). *Aedes* species transmit dengue virus around the world.

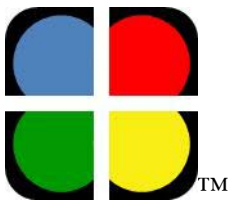


Efficacy Study 2. AFRIMS-Walter Reed Army Institute of Research (Thailand) - Scientists found a 100% reduction of malaria infected *Anopheles dirus* mosquitoes within 10 days. These results indicate a reduced mosquito population and an interruption of the malaria life cycle in the mosquito (American Mosquito Control Association Conference, 2009).



Efficacy Study 3. Adult mosquitoes killed by Entobac™ killed mosquito larvae (*Aedes aegypti*) compared to negative control ($p \leq 0.05$).



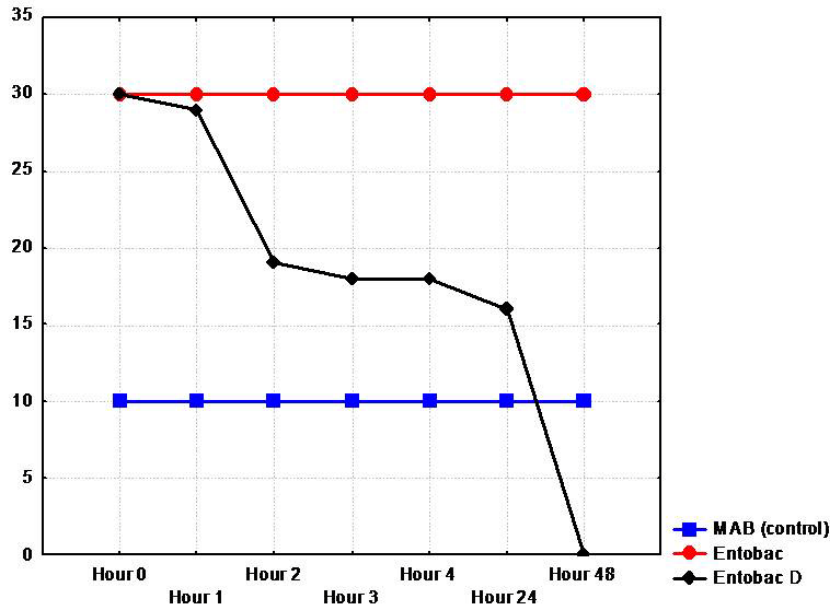


MEVLABS, Inc.

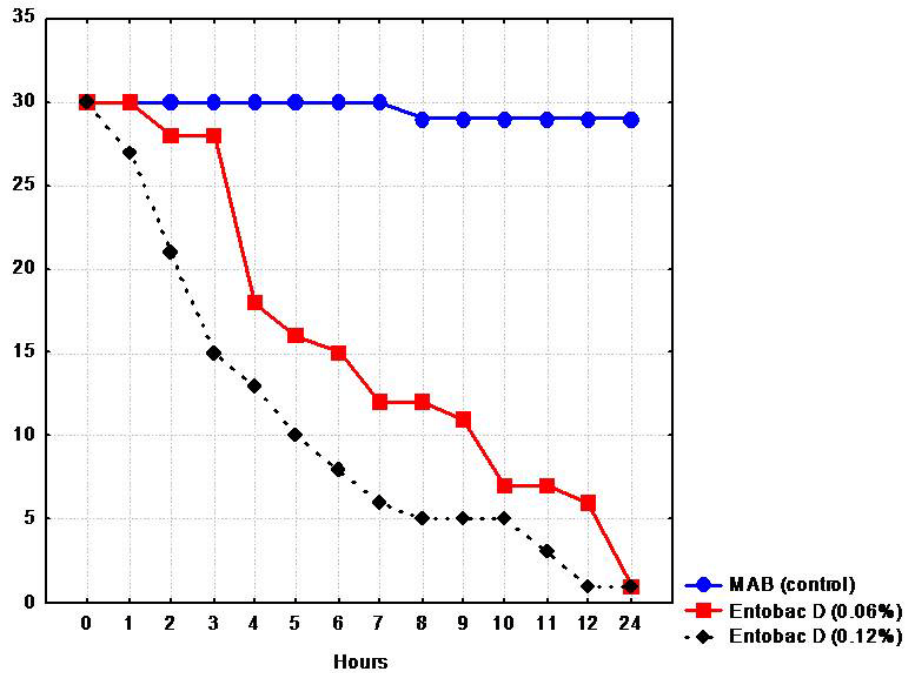
Developing Research in a Developing World™

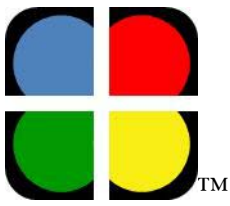
Efficacy Study 4. Comparison between Control, Entobac™ and Entobac™ D on survival of adult flies (*Musca domestica*) and adult Mosquitoes (*Aedes aegypti*); (Chi Square = 27.8, $p \leq 0.05$)

Number of House Flies



Number of Mosquitoes





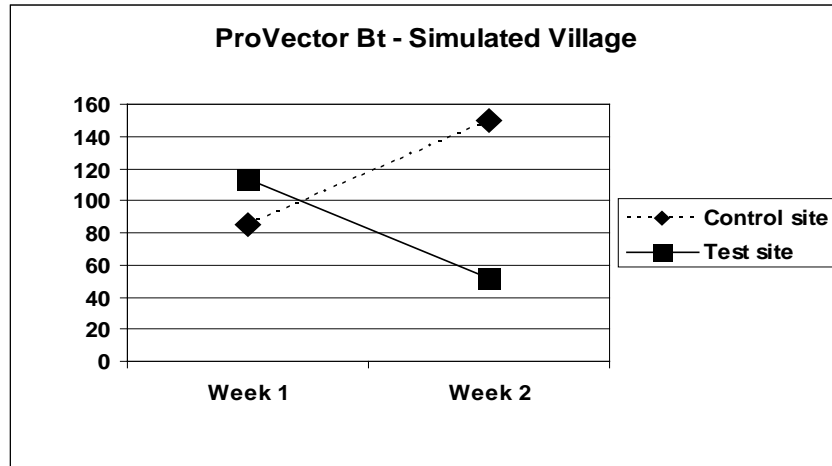
MEVLABS, Inc.

Developing Research in a Developing World™

FIELD TRIALS

ProVector™ with Entobac™ and Entobac™ D

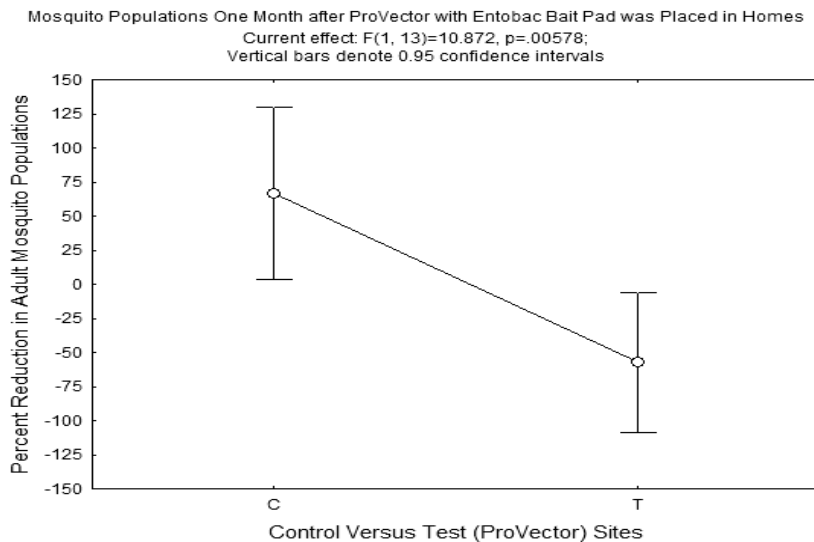
Efficacy Study 1: Georgia, USA. Within two weeks, the mosquito population at the site with ProVectors™ with Entobac™ Bait Pad (test site) was 3 times lower than the mosquito population at the site with no ProVectors™ (control site).

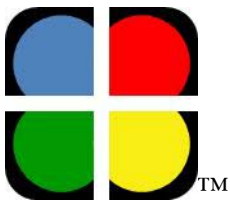


Efficacy Study 2: Kenya, Reduction in Mosquito Populations in Ahero and Nyalenda, Kisumu (2009)

(Independent Data Collected by US Army Medical Research Unit, Walter Reed Army Institute of Research and Kenya Medical Research Institute)

After one month, there was a significant reduction of mosquitoes in the 9 homes with ProVector over the 7 control homes (Chi-square=9.35, $p \leq 0.05$). Mosquitoes returned to normal populations once the bait pads were used up within 5 months. There was a significant difference between the mean percent growth of the ProVector in homes versus the control homes (ANOVA: $F(1,13)=10.9$, $p \leq 0.05$). The mosquito population was reduced by >60% outside homes with ProVectors in them. Whereas, the mosquito population grew by nearly 75% in the control sites. There was an approximately 135% lower number of mosquitoes around homes with the ProVector™ with Entobac™ than homes without the ProVector™ with Entobac™.



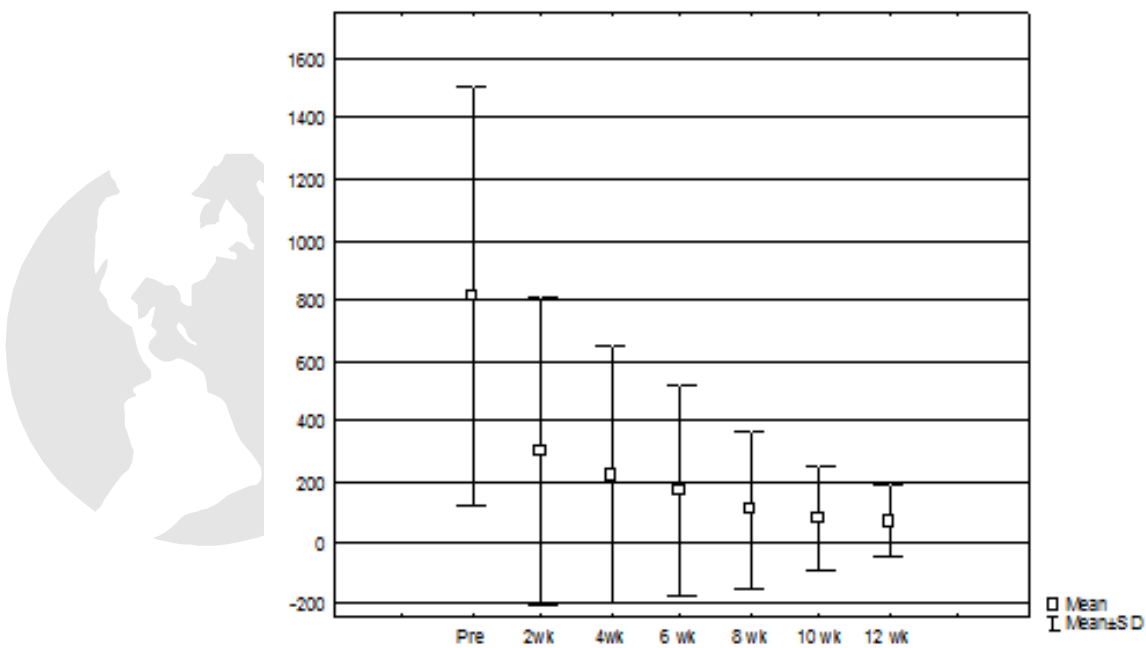


MEVLABS, Inc.

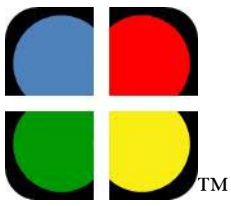
Developing Research in a Developing World™

Efficacy Study 3: Sierra Leone, Roll Back Malaria, WHO Sierra Leone, collected data from heads of family (2010). This study was conducted in collaboration with Roll Back Malaria, WHO and the Afro European Medical Research Network. Number of mosquitoes was estimated using Boolean Logic subset theory. There was a significant reduction of the estimated number of mosquitoes within 2 weeks. By the end of the study the reduction was from a mean of 800 mosquitoes to 70 mosquitoes counted during each 2 week period.

Estimated Mean Number of Mosquitoes before and after ProVector™ with Entobac™ Bait Pad was placed in Homes in Freetown, Sierra Leon (Data Independently Collected by Roll Back Malaria, World Health Organization).



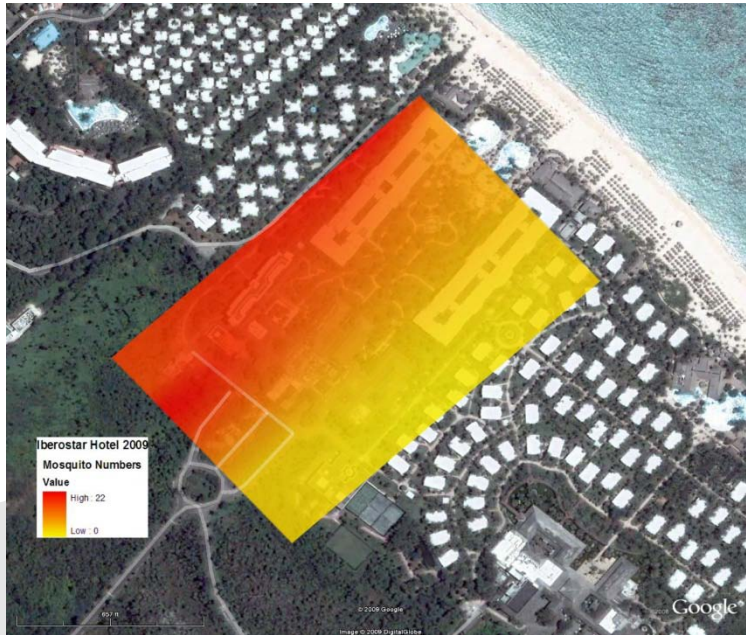
	Mean	Mean	t-value	df	p
Pre Estimate vs. 2wk estimate	807.93	304.133	3.580	75	0.01
Pre Estimate vs. 4wk estimate	807.93	224.646	4.334	74	0.01
Pre Estimate vs. 6 wk estimate	807.93	170.717	4.541	68	0.01
Pre Estimate vs. 8 wk estimate	807.93	107.336	4.827	64	0.01
Pre Estimate vs. 10 wk estimate	807.937	75.435	4.956	62	0.01
Pre Estimate vs. 12 wk estimate	807.937	70.811	4.573	58	0.01



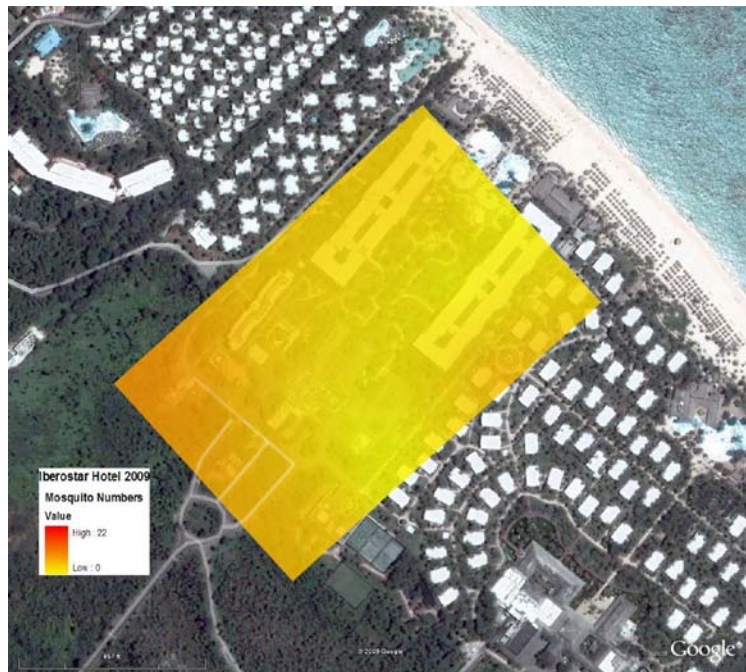
MEVLABS, Inc.

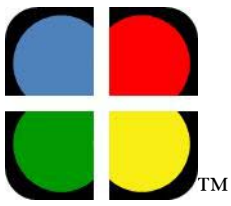
Developing Research in a Developing World™

Efficacy Study 4. Dominican Republic - Mosquito numbers were significantly reduced after one month of placing ProVector™ with Entobac™ on balconies in a hotel complex (below). Red indicates high and yellow indicates low numbers of mosquitoes in mosquito traps. Hotel staff and management also reported lower numbers of mosquito bites.



Spatial pattern of mosquitoes Before (TOP)
ProVector™ with Entobac™ was placed in hotel balconies and
After one month of use (BOTTOM).

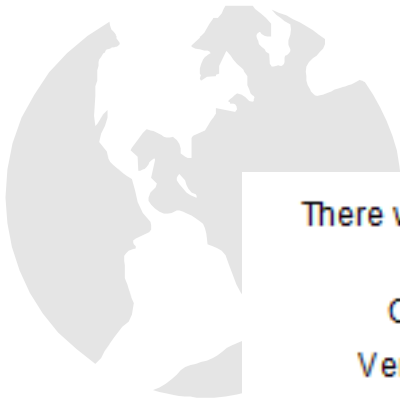




MEVLABS, Inc.

Developing Research in a Developing World™

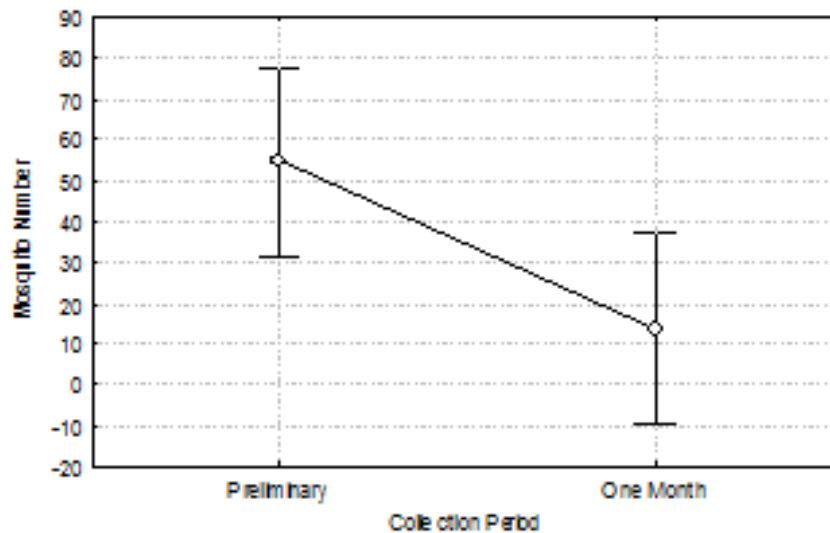
Efficacy Study 5. The Outdoor Prototype of ProVector™ with Entobac D quickly reduced mosquito populations within one month in southeast Georgia

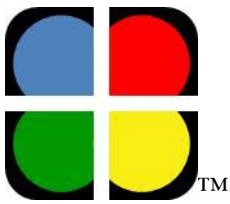


There was a significant reduction in mosquitoes at the Georgia test site within one month

Current effect: $F(1, 14)=7.0882, p=.01858$

Vertical bars denote 0.95 confidence intervals





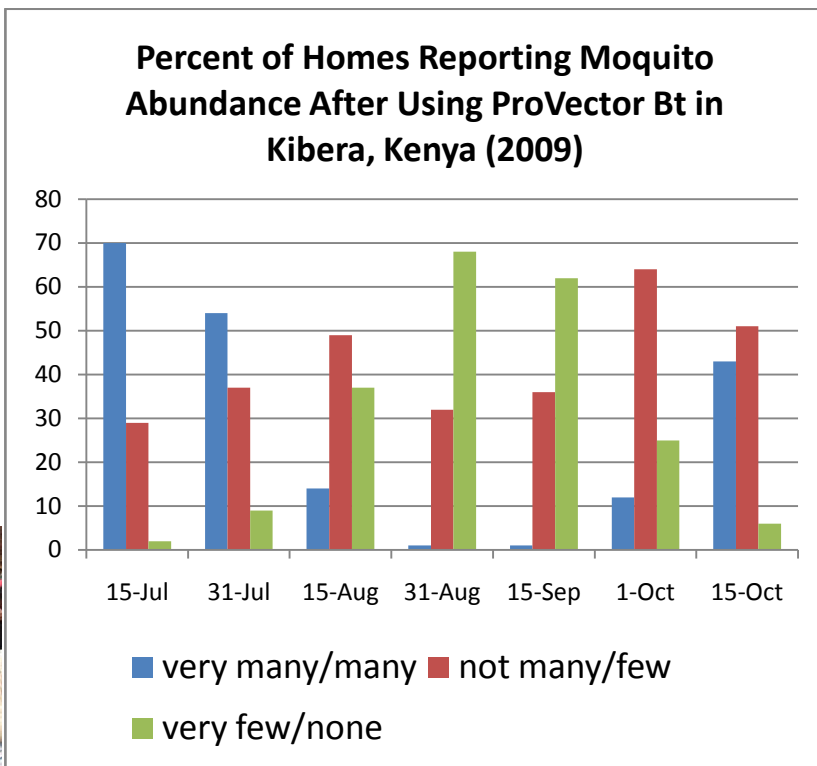
MEVLABS, Inc.

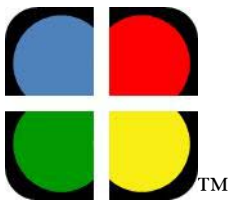
Developing Research in a Developing World™

VECTOR CONTROL COMMUNITY PROJECTS

Fundamental to community health is access to low cost, user friendly and environmentally safe methods of mosquito control. The Vector Control Community Project is a collaborative effort among ProVector, LLC, MEVLABS, Inc., the Afro-European Medical Research Network (AEMRN), and other governmental and non-governmental organizations around the world with the goal of reducing malaria, dengue, and other mosquito-borne diseases as well as diseases spread by flies. To date, over 60 Vector Control Community Projects have been established in more than 35 countries. Some of our partners include: Roll Back Malaria in Sierra Leone, Ministry of Health in Uganda, Children of the Nations (COTN) in the Dominican Republic, Malawi, Uganda, and Sierra Leone, and Riziki Kenya and Serving In Missions (SIM) in Kenya. Vector Control Community Projects introduce our new technology into communities and have resulted in positive feedback from public health workers, community leaders and the local populations. The information gathered has been used to improve the usability and effectiveness of our products. Through these projects, we have received positive feedback of successful and significant reduction in mosquito numbers, not only within the homes or buildings in which they were placed, but also in areas surrounding these communities. We have distributed over 5,000 ProVector devices around the world, helping approximately 35,000 family members and dozens of communities. For example, in Algodon, Dominican Republic, 200 ProVector Bts were disseminated by Children of the Nations (COTN) workers to every home in the village. COTN representatives later reported that when the rains came, “there were Mosquitoes everywhere, EXCEPT in the homes where the ProVector flowers have been placed”.

Community Project 1. Women with Infants Receiving Instruction from Dr. Kollars and Riziki team on the Use of ProVector (Photo) and Feedback (Graph) in Kibera Slums in Kenya

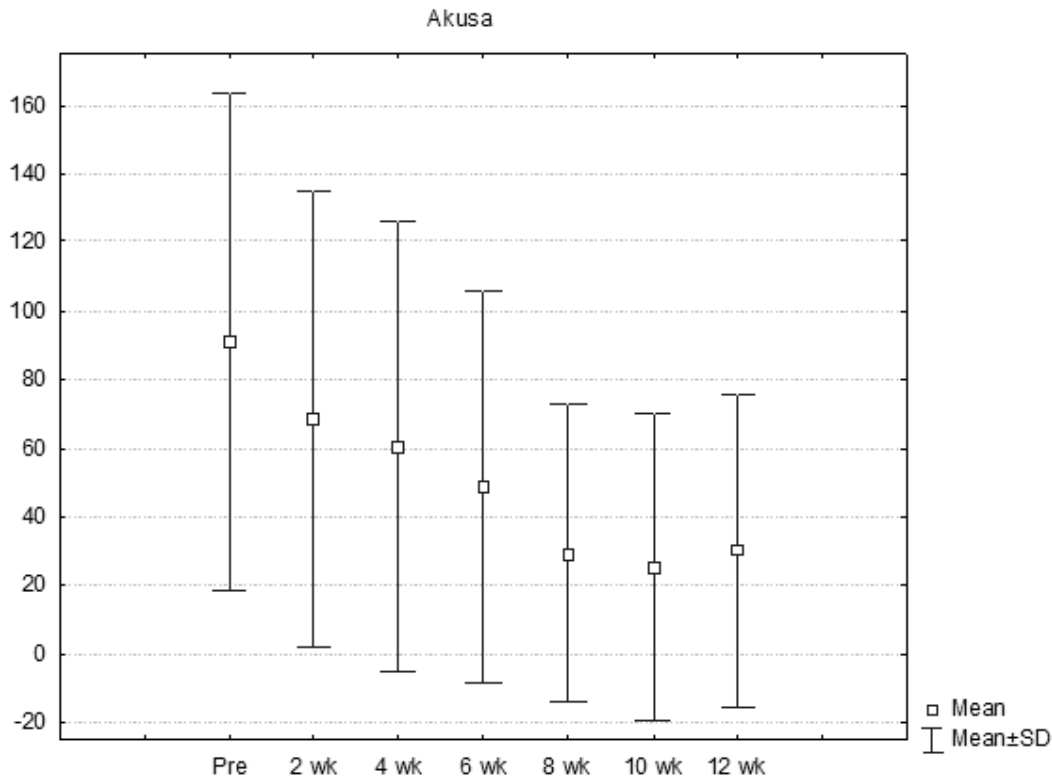




MEVLABS, Inc.

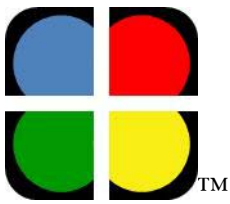
Developing Research in a Developing World™

Community Project 2. Estimated Mosquito Populations dropped significantly in Akuse Village located along the Volta River in Ghana (Data Collected Independently Mr. Alfred Clottey, Ministry of Agriculture)



T-test for Independent Samples (Ghana 2009) Note: Variables were treated as independent samples Include condition: v1="Akuse"

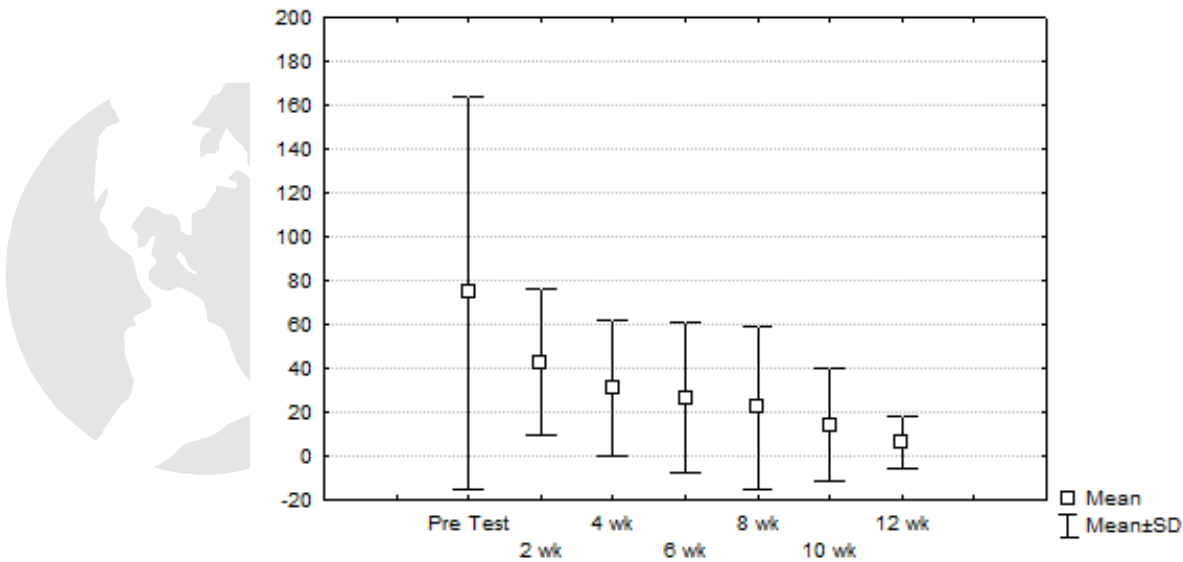
	Mean	Mean	t-value	df	p
Pre Estimate vs. 2 wk Estimate	91.26667	68.13333	0.912247	28	0.369427
Pre Estimate vs. 4 wk Estimate	91.26667	60.60000	1.214211	28	0.234809
Pre Estimate vs. 6 wk Estimate	91.26667	48.66667	1.791907	28	0.083962
Pre Estimate vs. 8 wk Estimate	91.26667	29.40000	2.841166	28	0.01
Pre Estimate vs. 10 wk Estimate	91.26667	25.33333	3.010144	28	0.01
Pre Estimate vs. 12 wk Estimate	91.26667	30.26667	2.762340	28	0.01



MEVLABS, Inc.
 Developing Research in a Developing World™

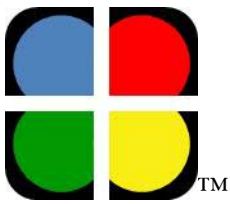
Community Project 3. Estimated Mosquito Populations dropped significantly in Eldoret Village, Kenya.

Estimated Mean Number of Mosquitoes before and after ProVector with 6 month bait pad was placed in homes in Eldoret, Kenya (Courtesy Revival Power Outreach, Switzerland, 2010)



T-test for Independent Samples (Eldoret Kenya) Note: Variables were treated as independent samples

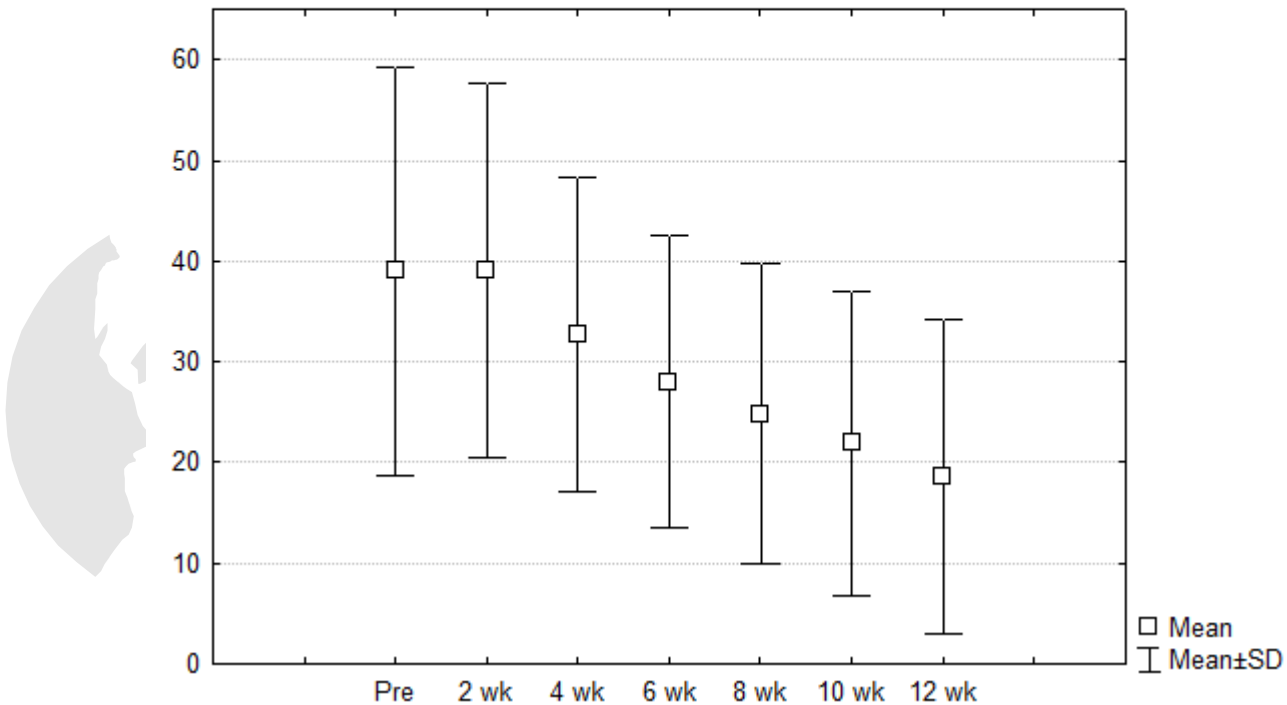
	Mean	Mean	t-value	df	p
Pre Test vs. 2 wk	74.54545	42.63636	2.220277	86	0.05
Pre Test vs. 4 wk	74.54545	31.00000	3.058479	86	0.01
Pre Test vs. 6 wk	74.54545	26.62791	3.290119	85	0.01
Pre Test vs. 8 wk	74.54545	22.25000	3.585900	86	0.01
Pre Test vs. 10 wk	74.54545	14.20455	4.313055	86	0.01
Pre Test vs. 12 wk	74.54545	5.90909	5.057206	86	0.01



MEVLABS, Inc.
 Developing Research in a Developing World™

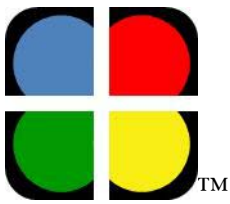
Community Project 4. Estimated Mosquito Populations dropped significantly in Nairobi, Kenya.

Estimated Mean Number of Mosquitoes before and after ProVector with 3 month bait pad was placed in homes in Nairobi, Kenya (Courtesy Methodist Church of Kenya, 2010)



T-test for Independent Samples (Nairobi Julius fuzzy) Note: Variables were treated as independent samples

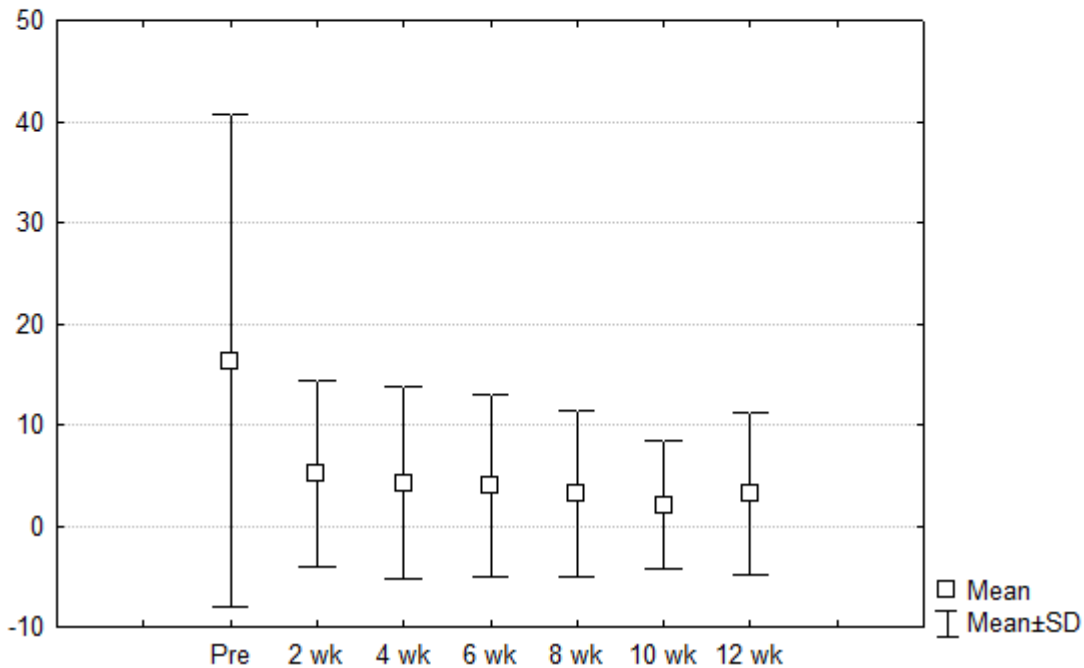
	Mean	Mean	t-value	df	p
Pre Test vs. 2 wk estimate	39.02564	39.07143	-0.010605	79	0.991566
Pre Test vs. 4 wk estimate	39.02564	32.76190	1.565560	79	0.121449
Pre Test vs. 6 wk estimate	39.02564	27.95238	2.844185	79	0.01
Pre Test vs. 8 wk estimate	39.02564	24.80952	3.612222	79	0.01
Pre Test vs. 10 wk estimate	39.02564	21.90476	4.337077	79	0.01
Pre Test vs. 12 wk estimate	39.02564	18.61905	5.098409	79	0.01



MEVLABS, Inc.
 Developing Research in a Developing World™

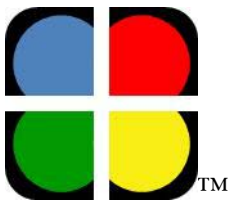
Community Project 5. Estimated Mosquito Populations dropped significantly in Shimu Village, Kenya.

**Estimated Mean Number of Mosquitoes before and after ProVector with 3 month bait pad was placed in homes and hospital ward in Shimu /Kitale, Kenya
 (Survey Data courtesy Revival Power Outreach and AEMRN, 2010)**



T-test for Independent Samples (Shima Kitale) Note: Variables were treated as independent samples

	Mean	Mean	t-value	df	p
Before Estimate vs. 2 wk estimate	16.33841	5.198864	5.662771	338	0.01
Before Estimate vs. 4 wk estimate	16.33841	4.289080	6.072487	336	0.01
Before Estimate vs. 6 wk estimate	16.33841	4.043103	6.232878	336	0.01
Before Estimate vs. 8 wk estimate	16.33841	3.272189	6.609907	331	0.01
Before Estimate vs. 10 wk estimate	16.33841	2.050000	7.201251	322	0.01
Before Estimate vs. 12 wk estimate	16.33841	3.157051	6.445665	318	0.01



MEVLABS, Inc.

Developing Research in a Developing World™

*Note on EPA approval and registration in various countries: The Entobac™ Bait Pads that are used with the ProVector™ devices contain either, 1) Entobac™, a formulation that contains *Bacillus thuringiensis israelensis* (Bti), which has been registered for use in the United States to kill larval mosquitoes but not registered for use to kill adult mosquitoes, or 2) Entobac™ D, a formulation that contains Bti and Deltamethrin. Deltamethrin is registered by the EPA for use in the United States but is not registered by the EPA for use in combination with Bti. The BFT Bug Fighter™, Super Netty™, and Netty™ devices contain Entobac™ D. Although these products are in the process of being registered by the EPA, they currently cannot be sold in the United States under the FIFRA as a pesticide to kill adult mosquitoes. They can be sold to U.S. and foreign organizations for use of these products overseas. Deltamethrin and Bti are approved and used in many countries to control mosquito populations; registration among countries may vary.

The World Health Organization, WHOPES lists both Deltamethrin and Bti for vector control (Pesticides and Their Application WHO/CDS/NTD/WHOPES/GCDPP/2006.1. MEVLABS, Inc. completed successful testing of these products with the WHO Roll Back Malaria Program in Sierra Leone to protect families from mosquitoes.

Ongoing projects - MEVLABS, Inc. has a Cooperative Research and Development Agreement with the U.S. Army, and ongoing projects with the Ministry of Health, Kenya, Ministry of Health, Uganda, Ministry of Health, Sierra Leone, Ministry of Health, Honduras. The Vector Control Community Projects includes dozens of NGO's such as Compassion International, Children of the Nations, Methodist Church Kenya, and the Afro European Medical and Research Network. The National Disaster Management Agency of Pakistan has approved the use of MEVLABS, Inc. vector control products. MEVLABS, Inc. products are approved for use in Nigeria and Nicaragua. Additional registrations are in development in several countries.

Products:

ProVector™, Entobac™, BFT Bug Fighter™, Super Netty™, and Netty™ products are trademarked and patented by MEVLABS, Inc., www.mevlabs.com.

Entobac™ and Entobac™ D Bait Pads, Entobac™ L, BFT Bug Fighter™, Super Netty™ and Netty™ are manufactured by ProVector LLC (EPA Establishment #: 086158-GA-001), www.provectorllc.com.

Information about Vector Control Community Projects can be found at www.vectorcontrolcommunity.com.