International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2022; 9(6): 140-143

www.dipterajournal.com
Received: 21-08-2022
Accepted: 25-09-2022

Amit Umesh Sirdesai
Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh, India

Suryaji Jadhav
Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh, India

Prasun Bandyopadhyay
Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh, India

Corresponding Author:
Amit Umesh Sirdesai
Dabur Research and Development Centre, Dabur India Limited, Ghaziabad, Uttar Pradesh, India

Comparative bio-efficacy evaluation against Aedes aegypti mosquitoes

Amit Umesh Sirdesai, Suryaji Jadhav and Prasun Bandyopadhyay

DOI: https://doi.org/10.22271/23487941.2022.v9.i6b.650

Abstract
Dengue is a mosquito-borne disease and according to WHO, dengue fever is one of the top ten global threats. The primary vectors that transmit Dengue are Aedes aegypti mosquitoes and, to a lesser extent, Ae. Albopictus. Dengue can be transmitted from person to person even with a single bite from infected mosquito, hence protection of one single mosquito bite is of utmost importance. Mosquito population inside houses is commonly controlled by spraying the mosquito repellents. The allethrins are the synthetic compounds commonly used in mosquito repellents. There are also many plant-based repellents developed, however, the efficacy of the plant based mosquito repellents is still not fully validated. The present study was carried out to assess the comparative bio-efficacy of the test item Aerosol spray containing d-trans allethrin (0.25%) along with control marketed product spray containing essential oils. The test product containing d-trans allethrin (0.25%) exhibited high efficacy against Aedes aegypti mosquitoes when tested in Peet Grady Chamber with exposure period of 60 minutes under laboratory conditions.

Keywords: Dengue, malaria, mosquito repellent, *Aedes aegypti*, d- trans-allethrin, essential oils

1. Introduction
Mosquito-borne diseases are a major cause of concern all over the world. Especially the regions with warm and wet environments are affected by diseases caused by mosquitoes. Dengue is a mosquito-borne disease that has spread in the tropical areas of the world and according to WHO, dengue fever is one of the top ten global threats-its also the most rapidly spreading (https://www.worldmosquitoprogram.org/en/learn/mosquito-borne-diseases/dengue) [1]. It is estimated that dengue causes 40,000 deaths every year [2]. The primary vectors that transmit Dengue are *Aedes aegypti* mosquitoes and, to a lesser extent, *Ae. albopictus*. *Aedes aegypti* species of mosquitoes is considered to be highly invasive and can carry several kinds of pathogens which can be transmitted to humans. These mosquitoes are commonly found in and around homes and bite during the day. Conventionally, an efficient strategy to prevent these mosquito-borne diseases is to prevent mosquito bites and control mosquito breeding. Until a vaccine is developed for dengue, the main protection from these vectors can be had by using ways of preventing mosquito bites. Dengue can be transmitted to a person from even a single bite from infected mosquito, hence, bite protection is important. Other ways of prevention are to use mosquito repellents and wearing protective clothing.
Several approaches utilized for preventing mosquito bites are usage of mosquito nets, medicated nets, mosquito traps, electric mosquito zippers, mosquito repellents, liquid vaporizers, insect killer sprays etc. Insect repellent (also commonly called "bug spray") is a substance applied to skin, clothing, or other surfaces to discourage insects (and arthropods in general) from landing or climbing on that surface [3]. The most common of insecticide compounds are d-allethrin, prallethrin, transfluthrin, and diethyl toluamide (DEET) [4, 5]. The allethrins are a group of related synthetic compounds used in insecticides. They are classified as pyrethroids, i.e. synthetic versions of pyrethrin, a chemical with insecticidal properties found naturally in Chrysanthemum flowers. They are commonly used in ultra-low volume sprays for outdoor mosquito control. Pyrethroids exert their insecticidal effect on the voltage-gated sodium channel (VGSC) located on the membrane of neurons.

When pyrethroids bind an open channel, they prevent its closure, thus prolonging the action potential and resulting in the insect's rapid paralysis, known as "knockdown" or kdr, and death [6]. Further, when these chemical particles are sprayed into the air, they may behave in the following ways: (i) remaining suspended in the air; (ii) adhering to a floor or a wall; (iii) after adhering to the floor/wall, vaporizing and/or diffusing again, or (iv) decomposing and disappearing due to light etc. Alternatively, there are also many plant-based repellents developed, however, the efficacy of the plant based mosquito repellents is still not fully validated. Many plants contain essential oil which can repel insects [7]. The plants prevent attack from plant eating insects by releasing compounds that repel these plant eating insects. Some of the potent natural repellent that have been studied are *Corymbia citriodora*, Citronella, plant based essential oils such as Patchouli oil, pine oil, thyme oil etc. However, most of these essential oils are highly volatile and this contributes to their poor longevity as mosquito repellents [8]. The modern pyrethroids that are the mainstay of the current malaria elimination program that is making excellent progress [9], are synthetic analogues based on the chemical structure of pyrethrins, discovered in the pyrethrum daisy, *Tanacetum cinerariifolium* from the Dalmation region and *Tanacetum coccineum* of Persian origin. Pyrethrins are highly effective insecticides, that are relatively harmless to mammals [10] and moreover, there are not many studies on plant-based mosquito repellents to establish their efficacy and safety.

Accordingly, a study was carried out to assess the comparative bio-efficacy of the test item Aerosol containing d- trans allethrin and control marketed Herbal Anti-mosquito mosquito spray containing essential oils. The present study highlights the potency of the test item Aerosol containing d-allethrin against *Aedes aegypti* mosquitoes when compared with an anti-mosquito spray containing herbal essential oils. The present study demonstrates that test product containing d-trans allethrin (0.25%) is an effective mode of preventing mosquito borne diseases specifically diseases caused by *Aedes aegypti* mosquitoes.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals/Reagent

Isopropyl alcohol (IPA)
Unscented liquid soap
10% sucrose solution

2.1.2. Equipment/Consumable: Peet Grady Chamber, Environmental Chamber, Weighing balance, Stopwatch, Digital Thermohygrometer, Recovery Jar, Paint brush, Aspirator, PPE (Personal Protective Equipment)

2.1.3. Products under evaluation

Table 1: Products under evaluation

Test	Odomos Flying Insect Killer (FIK) AEROSOL spray (d-trans Allethrin 0.25%)
Control	Marketed Good Knight Naturals Neem Anti Mosquito Room Spray (with Eucalyptus oil, Dill Oil, Patchouli oil, Neem Oil)

2.2. Details of Test System

Table 2: Details of Test System

Test Species	Non-blood fed, adult female mosquitoes of *Aedes aegypti*
Age of mosquitoes	2-5 days old
Source	Insectary, Ross Lifescience Pvt. Ltd.

2.3 Details of Test Design

Table 3: Details of Test Design

No. of Treatments	- Treatment with Test Aerosol sample - Treatment with Control Naturals Spray sample - Untreated Control
No. of Replicate	3 no
No. of insect per replicate	100

2.4 Details of Test Conditions

Table 4: Details of Test Conditions

Temperature (Maximum and Minimum)	28.6-26.7 ºC
Relative humidity (Maximum and Minimum)	82-69%

2.5 Method

2.5.1. Peet-Grady Chamber and Decontamination Check

The Peet-Grady Chamber (5.832m^3 = Length 1.8m x Width 1.8m x Height 1.8m) was used for this study. The Peet-Grady Chamber was designed as per WHO standards. The chamber has smooth internal wall panels made of glass materials to ensure easy cleaning of insecticide or solvent residues. A tight-fitting entrance door was fixed on one of the side walls of the chamber. The chamber was well lit with appropriate light system, as well as an exhaust fan in the ceiling to remove insecticide vapor after each test. Observation was done from all the four sides. One window was provided on one of the side walls of the chamber for easy introduction of insects. Before each exposure, the study chamber was cleaned with diluted liquid soap solution and then with a mixture of 10% IPA in water. Any devices used in a chamber was also wiped with 10% IPA in water. About 20 mosquitoes (through the port provided in the chamber) were introduced in the chamber for 30 minutes. No knockdown was observed during the exposure and the study was conducted.

2.5.2 Study Procedure
The study was conducted in Peet Grady Chamber against free flying non-blood fed female *Aedes aegypti* mosquitoes. Both test and control sample were tested with three replications. After contamination check 100 numbers of free flying mosquitoes were released inside the chamber.
The aerosol and spray were primed by pressing it in fume hood/out of the test area for 2-3 times before testing. After priming, pre weight of the Aerosol and spray were recorded. 0.65 0.10 g of the formulated product was sprayed for aerosol and as per label claim for spray, in a single application towards the center of the chamber. The knock down observations were recorded 2-minute interval upto 20 minutes and thereafter the observations were recorded at 5 minutes interval till 60 minutes.
At the end of experiment, the knocked mosquitoes were collected in recovery jar with 10% sugar solution to observe mortality at 24 hours of exposure.

2.5.3 Analysis
The KT_{50} and KT_{90} (time required to knock down 50% and 90% of the test population was estimated) values were pooled for analysis. KT_{50} and KT_{90} values were found by excel graphical calculation.

3. Results
The bio-efficacy of the test and control products was evaluated against *Aedes aegypti* mosquitoes through the present study. The summary of results is presented in Table 5.

Table 5: Bioefficacy study of aerosol and spray samples in Peet Grady Chamber against *Aedes aegypti*

Sr. No.	Product Information	Test Parameters	Replication			Average
			R-1	R-2	R-3	
1.	Test Product	KT_{50} (min.)	7.54	7.76	7.46	7.59
		KT_{90} (Min.)	12.63	13.91	13.14	13.23
		% Mortality after 24 hrs.	100.00	100.00	100.00	100.00
		Weight Loss (gm)	0.69	0.63	0.67	0.66
2.	Control product	KT_{50} (min.)	128.64	134.82	159.14	140.86
		KT_{90} (Min.)	210.19	229.47	270.25	236.64
		% Mortality after 24 hrs.	22.00	26.00	20.00	22.67
		Weight Loss (gm)	0.64	0.66	0.67	0.66

The results for the KT_{50} value indicated that the test product containing d-trans Allethrin (0.25%) exhibited average KT_{50} as 7.59 minutes while the average KT_{90} was 13.23 minutes against *Aedes aegypti*. On the other hand, comparative control product with essential oils exhibited an average KT_{50} value of 140.86 minutes and an average KT_{90} value of 236.64 minutes.
It was also found that the test product resulted in a 100% mortality after 24 hours with an average weight loss of 0.66g whereas the comparative control product with essential oils with an average weight loss of 0.66g resulted in only 22.67% mortality after 24 hours.

4. Discussion
An important way of controlling mosquito population inside houses is by way of spraying the insecticide/mosquito repellents. The allethrins are a group of related synthetic compounds commonly used in mosquito repellents. They are classified as pyrethroids, i.e. synthetic versions of pyrethrin, a chemical with insecticidal properties found naturally in Chrysanthemum flowers. The test product for present study containing d-trans allethrin, which is widely used effective synthetic mosquito repellent. This bio-efficacy was compared with marketed control product containing essential oils was assessed in the present study.
It was found that the test item containing d-trans allethrin showed a mean value of 100% mortality after 24 hours, an average KT_{50} value of 7.59 minutes, an average KT_{90} value of 13.23 minutes with an average weight loss of 0.66g indicating the high efficacy against *Aedes aegypti* mosquitoes when tested in Peet Grady Chamber. On the other hand, the control product was less efficacious than the test item as the KT_{50} value was obtained as 128.64 minutes while the mortality rate observed for the comparative test item was 22.67% after 24 hours. Thus, it could be inferred that the comparative control product was much less effective in controlling the population of mosquitoes in the Peet Grady Chamber. It has been seen that the d-allethrin aerosol formulations are highly effective against *Aedes aegypti* mosquito species [11] as the droplet of the insecticide effectively contacts the mosquito's body and is inhaled via respiratory system of mosquitoes [12]. Thus, allethrin's which are synthetic versions of pyrethrin, a chemical with insecticidal properties found naturally in Chrysanthemum flowers found to be significantly effective in controlling *Aedes aegypti* mosquitoes as compared to blend of essential oil spray which are Herbal alternatives.
The present study demonstrates that the test product, Odomos Flying Insect Killer Aerosol containing d-trans allethrin (0.25%), is an effective mode of preventing mosquito borne diseases specifically diseases caused by *Aedes aegypti* mosquitoes. It is also concluded that synthetic molecules d-trans Allethrin is far superior in providing efficacy as compared to the herbal essential oils when it comes to faster mosquito bite protection.

5. Conclusion
It is concluded from the study that the test product containing d-trans allethrin was found to be highly effective when compared to the control product containing essential oils when tested in Peet Grady chamber as per WHO protocol. The results of the study revealed that the test product containing d-trans allethrin (0.25%) was extremely effective by giving 100% Mortality rate after 24 hours and a mean KT_{50} value of 7.59 minutes and KT_{90} value of 13.23minutes with a weight loss of 0.66g against *Aedes aegypti* mosquitoes when compared with the comparative control product which showed comparatively lesser percentage Mortality rate after 24 hours of 22.67% and a mean KT_{50} value of 140.86 minutes and KT_{90} value of 236.64 minutes with an average weight loss of 0.66g under the study conditions.

6. Acknowledgements: Authors are grateful to Ross Lifescience Pvt. Ltd., for helping to conduct the experiment.

7. Conflicts of interest: The authors declare no conflict of interest.

8. References

1. https://www.worldmosquitoprogram.org/en/learn/mosquito-borne-diseases/dengue
2. World Health Organization. Vector-borne diseases. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases
3. https://en.wikipedia.org/wiki/Insect_repellent
4. Hendri J, Kusnandar AJ, Astuti EP. Identification of the bioactive compound and the use of antimosquito insecticide as well as the Dengue vector resistance to organophosphate in the three Dengue endemic cities in Banten Province (in Indonesian: Identifikasi Jenis Bahan Aktif dan Penggunaan Insektisida Antinyamuk serta Kerentanan Vektor DBD terhadap Organofosfat pada Tiga Kota Endemis DBD di Provinsi Banten). Aspirator. 2016;8(2):77-86. [Google Scholar]
5. Prasetyowati H, Astuti EP, Ruliansyah A. Household insecticide use in controlling Aedes aegypti population in the Dengue endemic areas, East Jakarta (in Indoensian: Penggunaan Insektisida Rumah Tangga dalam Pengendalian Populasi Aedes aegypti di Daerah Endemis Demam Berdarah Dengue (DBD) di Jakarta Timur). Aspirator. 2016;8(1):29-36. [Google Scholar]
6. Bowman NM, Akialis K, Cave G, Barrera R, Apperson CS, Meshnick SR. Pyrethroid insecticides maintain repellent effect on knock-down resistant populations of *Aedes aegypti* mosquitoes. PLoS ONE. 2018;13(5):e0196410.
7. Pant M, Sharma S, Dubey S, Naik SN, Patanjali PK. Utilization of biodiesel by-products for mosquito control. J Biosci. Bioeng. 2016;121(3):299-302.
8. Marta Ferreira Maia, Sarah J Moore. Plant-based insect repellents: a review of their efficacy, development and testing. Malaria Journal. 2011;10 (Suppl 1):S11.
9. Steketee RW, Campbell CC. Impact of national malaria control scale-up programmes in Africa: magnitude and attribution of effects. Malar J. 2010;9:299.
10. Cloyd RA. Natural indeed: are natural insecticides safer better than conventional insecticides? Illinois Pesticide Review; c2004. p. 17. [http://www.pesticidesafety.uiuc.edu/newsletter/html/v17n304.pdf].
11. Sayono S, Puji LM, Wulandari M, Didik S. Effect of D-Allethrin Aerosol and Coil to the Mortality of Mosquitoes. J Arthropod-Borne Dis. 2019;13(3):259-267.
12. Pemba D, Kadangwe C. Mosquito Control Aerosols' Efficacy Based on Pyrethroids Constituents. In: Insecticides-Advances in Integrated Pest Management. Edited by Dr Farzana Perveen; c2012. ISBN:978-953-307-780-2, InTech, Available at: https://bit.ly/340MJMR