

# Mosquito Control Aerosols' Efficacy Based on Pyrethroids Constituents

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## 1. Introduction

Mosquitoes are found all over the world except in the Antarctica. Mosquitoes are two winged insects that belong to the insect order Diptera. Members of the genera *Aedes*, *Culex* and *Anopheles* are well known and responsible for bites in humans. Only Female mosquitoes bite. This is because they require blood to assist in vitellogenesis. Feeding behaviour of mosquitoes is either classified as zoophilic or anthropophilic. Mosquito species with zoophilic behaviour prefer feeding on animals. Mosquitoes with anthropophilic behaviour prefer feeding on human blood. (Fradin, 1998). Mosquitoes are vectors of many life threatening diseases in humans. They also transmit disease in animals such as dogs and horses. Diseases transmitted include malaria, dog heart worm, West Nile virus, and Eastern Equine Encephalitis (Pemba, 2008).

Malaria is Africa's major cause of mortality in those younger than 5 years of age and constitutes 10% of the continent's overall disease burden (Farenhorst et al, 2008). To reduce the ability of mosquitoes to transmit diseases requires well planned strategies and several methods have been developed (Pemba, 2008). The main stay of mosquito control are chemicals also known as insecticides which could be in form of insect growth regulators, biopesticides, genetically engineered biopesticides, repellents and attractants (WHO, 1996). Insecticide resistance is one of the biggest challenges in pest control (Diabate, 2002). Insecticide resistance has drastically affected mosquito control programs like insecticide-treated nets in Africa, indoor residual sprayings (Asia) and fogging sprays in the Caribbean (Bonnet et al, 2009).

Some factors contributing to resistance are genetic changes and metabolic changes in the vector/pest body. Genetic resistance comes about when the site usually targeted by an insecticide changes as a result of gene shuffling and metabolic resistance is a result of increased detoxification of a particular insecticide. In the Caribbean, where dengue is one of the major health problems; the vector *Aedes aegypti* has wide insecticide resistance developed. To establish efficacy of pyrethroid and organophosphate ultra-low volume space sprays studies were conducted in Martinique where *Aedes aegypti* has been shown to be resistant to conventional insecticides. Wild type population showed high levels of resistance to deltamethrin, organophosphate (naled), and pyrethrum. Simulated-field trials showed

that this resistance can strongly reduce the knock-down effect and mortality of deltamethrin and synergized pyrethrins. This finding has important implications for dengue vector control and emphasizes the need to develop innovative strategies to maintain effective control of resistant in *Ae. aegypti* populations (Marcombe et al,2009).

Studies in aerosol efficacy have focused on the atomisation and charge properties of the particles. At low concentrations of 1.57 g kg<sup>-1</sup> of bioallethrin and 0.29 g kg<sup>-1</sup> of bioresmethrin, charged aerosols achieve a significant reduction in KDT<sub>50</sub> by up to 50%. In one study by Khadri, bioefficacy of three pyrethroid aerosols on mosquitoes was tested in simulated room conditions. Each aerosol product was tested based on the insecticide manufacturers' recommended dosages. All the aerosols induced complete or very high mortality. Insecticide droplet analysis indicated variable uniformity of the droplets was produced. The aerosol insecticides were effective against mosquitoes provided they were used in accordance with the manufacturers' recommendations (Khadri et al 2009).

Resistance is overcome by changing insecticide, as long as the insecticides do not work the same way(mode of action), are not similar in formulation so as not similarly detoxified. As a way of resistance management rotational use of insecticides or combinations are adopted. One method that relies very much on insecticide combination for improved efficacy as well as resistance management is in insecticide aerosol formulations. The back bone of space sprays formulation are pyrethroids.

Pyrethroids are mostly used because of their low volatility, high insecticidal potency, and low toxicity to mammals under normal conditions of use (Cakir et al, 2008). Pyrethroids are synthetic chemicals very similar in structure to naturally occurring pyrethrins, but are often more toxic to insects, as well as to mammals, and last longer in the environment than pyrethrins. More than 1,000 synthetic pyrethroids have been developed, but only a few of them are currently used.

Pyrethroids are synthetic esters derived from the naturally-occurring pyrethrins. One exception to the axiom that all pyrethroids are esters of carboxylic acids is noteworthy. There is a group of oxime ethers that exhibits insecticidal activity similar in nature to the pyrethrins and pyrethroid esters (Davies 1985). Little data exist regarding these compounds, and no commercial products have been produced. Commercially available pyrethroids include allethrin, bifenthrin, bioresmethrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate (fenvalerate), flucythrinate, flumethrin, fluvalinate, fenpropathrin, permethrin, phenothrin, resmethrin, tefluthrin, tetramethrin, and tralomethrin.

Most commercial pyrethroids are not one single molecule; rather, they are several molecules with the same chemical formula that have their atoms joined together in the same sequence, but have a different arrangement of the atoms in space. Such compounds are called stereoisomers. If the stereoisomers are not mirror images of one another, they are called diastereomers and have different physical properties like boiling point, melting point, and solubility. If they are non-superimposable mirror images of each other, they are called enantiomers and properties like boiling point, melting point, and solubility are identical. However, both diastereomers and enantiomers can have different insecticidal properties and different toxicities. Some pyrethroids are composed of as many as eight different stereoisomers. Pyrethrins and pyrethroids are often combined commercially with other chemicals called synergists, which enhance the insecticidal activity of the pyrethrins and pyrethroids. The synergists prevent enzymes from breaking down the pyrethrins and

pyrethroids, thus increasing their toxicity. Studies have shown that detoxication of xenobiotics such as synthetic pyrethroids is catalyzed by monooxygenases (MO) and nonspecific esterases (NE). Piperonyl butoxide (PBO) and MGK-264 are known inhibitors of insect detoxication systems. Both synergists are used in various insecticide compositions, mainly in aerosol cans. Best synergism occur in a mixture of PBO or MGK-264 with pyrethrins. Metabolic resistance to insecticides easily overcome by use of synergists. Several registered insecticide compositions include the synergist PBO which is to improve the Efficacy of the insecticide (Eremina2002). The great success of pyrethroids is also related to their strong efficacy at low dose, fast killing effect and relative low cost of production. (Bonnet et al, 2009). Household synthetic pyrethroids used as space sprays contain isomers such as permethrin, d-tetramethrin, esbiothrin and deltamethrin either alone or in combination with other synthetic pyrethroids (Rapeeporn et al, 2005). To come up with a recommendation on which aerosol combination is most effective has not been a primary focus of scientists. This has made it difficult for buyers to make a decision when buying since so many aerosols are on the market.

## 2. Aerosol sprays

*What are aerosols:*. Aerosol spray is a type of dispensing system which creates an aerosol mist of liquid particles. This is used with a can or bottle that contains a liquid under pressure intended to be delivered to their biological targets. The liquid under pressure comes out as a mist. The pressure inside the container remain constant as the payload is delivered by an evaporating liquid to gas propellant. Outside the can, the droplets of propellant evaporate rapidly, leaving the needed fine particles or droplets in this case insecticide- floating in the air.

Aerosol insecticides are easier to use and deliver than other forms of insect killers as such does not need professionals to use. They also work faster as there is no need to wait for an insect to approach as with baited mode of insecticide delivery. The insecticide floats around like a gas filling the volume/space thus reaching and landing on everything including the target insect, that could be hiding in even tight spaces. Aerosol insecticides are available in containers designed to prevent waste. Some aerosol insecticides come in metered dose delivery design, usually in the range of 3,000 sprays. Each spray is measured to deliver just the right amount of insecticide required to work effectively. The other good aspect of aerosol insecticides is that very small quantities are used as compared to other forms of insecticide application. This is due to the nearly gas (mist) form of the insecticide. The very low amounts involved ensure sub lethal dosage to humans thus being very safe for humans.

*Challenges when using aerosols:* One of the most common challenge in aerosol insecticide usage is that since the insecticide is delivered in a mixture of a propellant gas and insecticide mist, these two forms easily get into respiratory system, eyes of people using them, in some instances causing adverse immunological responses reactions as well as, sneezing etc. Another challenge is that the insecticide's effectiveness depends on several conditions and one such condition is dosage of application. With aerosol it is very difficult to determine the right amount to be dispersed at a particular time as this amount depend very much on the volume of space in which the insecticide is to work and the susceptibility of the target organisms. All this has an effect on the efficacy of the insecticide. Metered aerosol cans to an

extent have helped to overcome the dosing quantity delivery determination challenge, and secondly making aerosols insecticide from a mixture of several types of insecticides including synergies is intended to overcome the susceptibility challenge.

### 2.1 Aerosol sprays composition

Even though the chemical composition of the aerosol sprays that are commercially sold is more or less the same, the amounts and types of pyrethroid isomers making up the aerosols differ from one brand to another hence affecting the efficacy. The formulated product contains many inert ingredients that can increase the toxicity of the product when compared to the technical-grade material. By law, the active ingredient must be identified by name on the pesticide label and its percentage must be provided. Non-active ingredients (sometimes called inert ingredients) do not need to be identified by name on a pesticide label, only the percentage of non-active ingredients must be specified, so it is often difficult to determine what other chemicals are included in the final formulated product (ATSDR, 2003).

All the aerosols tested in this study had the following isomers as part of their composition: imiprothrin, pralletrin and allethrin. The main difference among the aerosols was the presence of a pyrethroid that was not present in the others (Table 1). These included D-Phenothrin, a synthetic pyrethroid with high lethal activity against household insect pests, Tetramethrin, the second generation of synthetic pyrethroid, is a contact insecticide with a rapid knockdown action on flies (WHO, 2004). Manufacturer information showed that D-phenothrin is usually added at a higher rate (twice) that of tetramethrin which instead is combined with a synergist piperonyl butoxide. Piperonyl butoxide inhibits cytochrome P450 and esterase which are detoxification enzymes in insects. This inhibition paves way for elevated concentrations of active insecticide in the target animal for a longer period thus being lethal (Moores, et al. 2009).

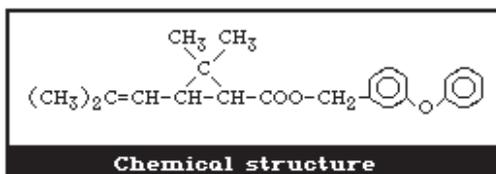
Aerosol constituent	Aerosol A	Aerosol B	Comments
Prallethrinin	0.4g/kg	0.34g/kg	
Imiprothrin	0.92g/kg	10g/kg	
D- phenothrin	0.92g/kg	-	
Tetramethrin	-	0.4g/kg	
Piperonyl butoxide	-	Present	Synergy : Cytochrome P450 and esterase inhibitor

Table 1. Aerosol Composition as identified from package label.

Details of particular components of interest:

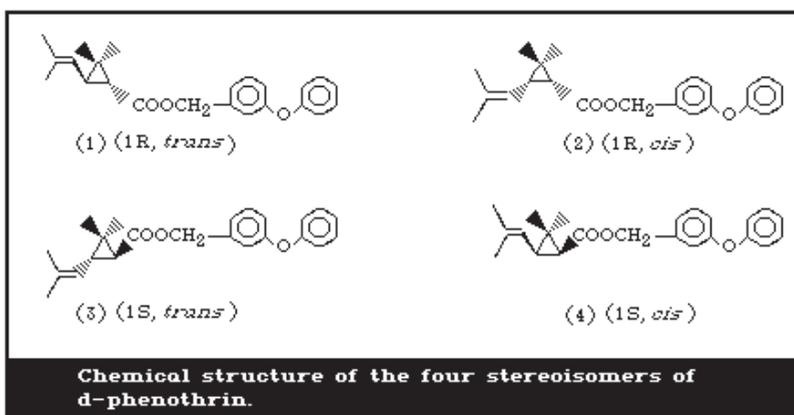
Phenothrin

Molecular formula:  $C_{23}H_{26}O_3$



Racemic phenothrin was first synthesized by Itaya in 1969. It is prepared by esterifying (1*R*, *cis*,*trans*)-2,2-dimethyl-3-(2,2-dimethylvinyl) cyclopropanecarboxylic acid (chrysanthemic acid) with 3-phenoxybenzyl alcohol (Fujimoto et al., 1973).

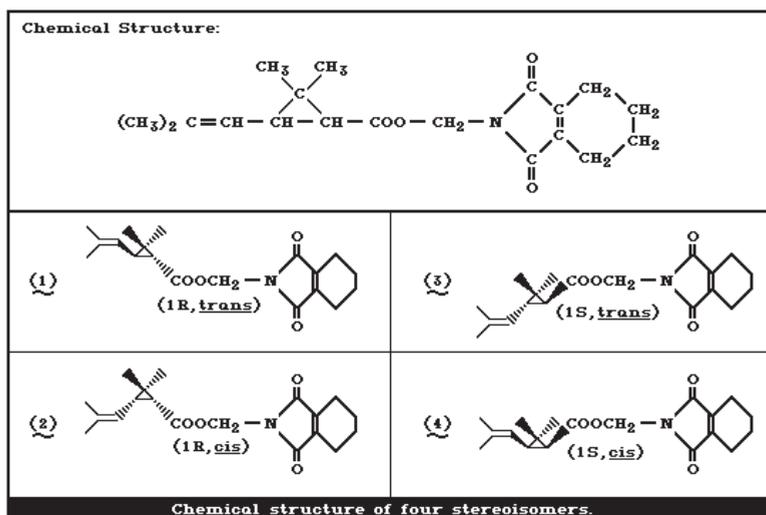
Phenothrin is thus a mixture of four stereoisomers. The *cis*:*trans* isomer ratio is 1:4 and the optical ratio of 1*R*:1*S* is 1:1 (racemic). The four isomers are present in the approximate ratio of 4:1:4:1 .. d-Phenothrin is a mixture of 2 isomers, 1*R*,*cis*,*trans* (i.e., the *cis*:*trans* ratio being 1:4). The technical grade is 92.5-94.5% pure. The major impurities found in seven d-phenothrin preparations (average purity, 94.0%) are ethyl chrysanthemate (2.31%), 3-phenoxy-6-bromobenzyl *cis*,*trans*-chrysanthemate (0.66%), 3-phenoxytoluene (0.43%), and 4-phenoxybenzyl *cis*,*trans*-chrysanthemate (0.39%) (Miyamoto et al., 1984).



Registry of Toxic Effects of Chemical Substances (RTECS) (1981-82 edition).

Tetramethrin Molecular formula:  $C_{19}H_{25}NO_4$

Chemical structure:



Tetramethrin was first synthesized in 1964 by Katoet. It is prepared by the esterification of (1*R*,*cis*,*trans*)-2,2-dimethyl-3-(2,2-dimethylvinyl)-cyclo-propanecarboxylic acid (chrysanthemic acid) with 3,4,5,6-tetrahydrophthalimidomethyl alcohol.

It is a mixture of four stereoisomers. The [1*R*,*trans*] isomer is the most active biologically of the isomers, followed by the [1*R*,*cis*] isomer. Registry of Toxic Effects of Chemical Substances (RTECS) (1981-82 edition).

### Piperonyl butoxide

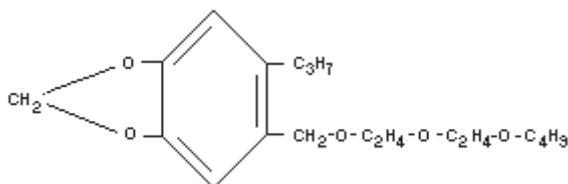
Chemical name: 3,4-methylenedioxy-6-propylbenzyl n-butyl diethyleneglycol ether

Synonyms

Alpha-[2-(2-*n*-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene or 6-(propylpiperonyl)-butyl carbityl ether or (3,4-methylenedioxy-6-propylbenzyl) (butyl diethylene glycolether) ether.

Empirical formula: C<sub>19</sub>H<sub>30</sub>O<sub>5</sub> (molecular weight 338)

Structural formula



Piperonyl butoxide is derived from piperic acid. Its synergistic activity is believed to be due to the presence of the methylenedioxy group in the molecular structure. Negherbon (1959). Piperonyl butoxide cannot be used as an insecticide alone. It is an effective synergist to pyrethrins and allethrin. The synergy effect is so pronounced that the resulting kill of insects is much greater than that which can be produced by pyrethrins alone. (FAO Meeting Report No. PL/1965/10/1 & WHO/Food Add./27.65)

This study looked at constituent isomeric influence on efficacy for two brands of commonly used aerosols in Southern Africa. Aerosol testing records for Southern Africa are hard to find, thus may indicate lack of aerosol insecticide testing in this region. Similar studies in other parts of the world have been carried out in simulated home environment, which to a larger extent affect the results as insecticide dispersal is affected by air circulation which is restricted by presence of furniture and other household items. Such studies are useful for assessing impact in home usage but not when focus is on the impact of the constituent isomers. To make it more important and acceptable this study used the peet glad chamber which ensures that there is no restricted air circulation, hence insecticide dispersal being uniform and unrestricted. The use of wild type mosquitoes collected from houses ensured that testing was conducted on vector population existing in the peoples' houses not the susceptible laboratory strain, hence more realistic situation.

### 2.2 Objectives

Even though the chemical composition of the aerosol sprays that are commercially sold is more or less the same, the amounts and types of pyrethroid isomers making up the aerosols differ from one brand to another, affecting the efficacy. The main objective of this study was

to compare knockdown as indicator of efficacy basing influenced by isomeric composition based on container label.

### 2.2.1 Methodology

Testing was done on space sprays (aerosol insecticides) that are readily available on the markets and mostly used in households. The testing followed World Health Organisation Pesticide Evaluation Scheme (WHOPES) standards as per requirement in aerosol testing. Testing was done in a Peet-grady chamber.

*Culex pipiens* mosquitoes were collected using an aspirator from random houses and were kept in a cage where they were fed with a sucrose solution for them to stay alive. The F1 generation from these mosquitoes was used in the study.

Adult mosquitoes were reared in constant temperature incubator. A plastic cup with a relatively moist filter paper was placed in the cage so that the mosquitoes had a favourable place to lay their eggs. Once the eggs were laid the filter paper was removed and placed in a container filled with distilled water so that they float off it. When they hatched the larvae was fed yeast. The larvae were kept in water troughs at 27°C in an incubator. Pupae were placed in dishes so that adults emerged in the cages where they were placed.

### Knockdown/Efficacy Tests

Standardized mosquito rearing and testing are essential to ensure the reliability and reproducibility of data. This is generally 27 ± 20C temperatures, relative humidity (RH) 80 ± 10% and photoperiod 12:12 hours (light: dark). WHOOPES's set procedures for aerosols testing in a Peet-grady chamber were followed. Space sprays were identified using letters not names. A common method used to determine the efficacy of a space spray uses the log probit analysis.

A total of 50 sugar fed 2-5 day old mosquitoes were placed in a small cage and placed in the chamber where there was a window for observation. Immediately before the test an automatic dispenser was shaken and aerosol was sprayed away from the chamber in a fume hood, for 3-5 seconds. Thereafter, 0.65±0.10g of the formulated product was sprayed, in a single application towards the centre, directly from the aerosol can. The number of mosquitoes knocked down was recorded every 10 minute intervals for a total of 60 minutes using a hand counter. The knocked down and all remaining mosquitoes were carefully transferred into separate clean holding cups. Mosquitoes were provided with 10% sugar solution on cotton wool and held for 24 hours at 27 ± 20C and 80 % ± 10% RH. The mortality was recorded 24 hours after exposure. The efficacy of a product is usually assessed using the minimum of three replicates and a control.

### 2.2.2 Results and discussion

Single blinding was used in this study in that the applicants did not know which brands of the aerosol they were administering. All cans were painted white. The space sprays that were used in this test were just identified as A and B. Aerosols A and B are the two most commonly sold on Malawian markets. A common method that is used to determine the efficacy of a space spray is known as a log probit analysis. This method describes the relationship between the time that has passed for the insecticide to induce knockdown in mosquitoes and the mortality at a prescribed dosage of the space sprays. It is a statistically derived average time interval during which 50% or 90% of a given population may be expected to die following acute administration of a chemical or physical agent (radiation) at a given concentration under a defined set of conditions(IUPAC, 1997).

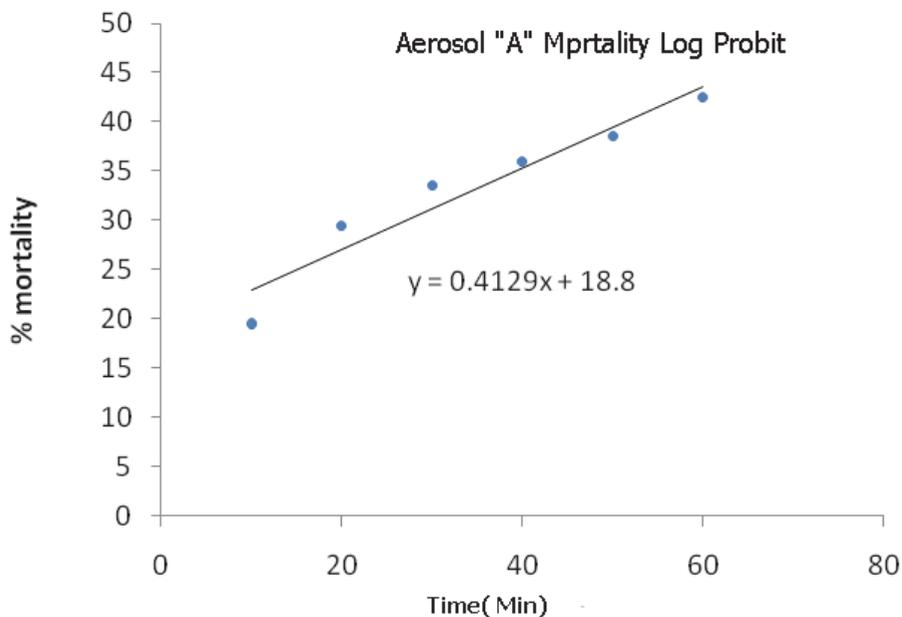


Fig. 1. Mortality Log probit after exposure to aerosol A.

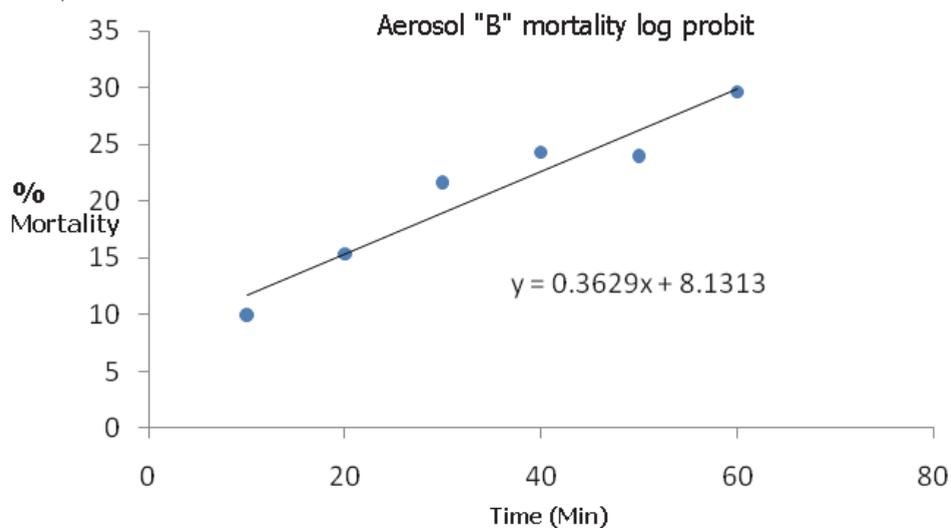


Fig. 2. Mortality Log probit after exposure to aerosol B.

Space spray	LT <sub>50</sub> (P=0.05)	LT <sub>90</sub> (P=0.05)
B	115.6 (109.956927-121.243073)*	226.15 (220.506927-231.793073)*
A	75.73(69.315463-82.144537)*	172.81(166.395463-179.224537)*

Table 2. Values are statistically significant LT<sub>50</sub> and LT<sub>90</sub>.

After 24 hrs the percent mortality for B was at 89% and that of A was 99%.

Using the log probit equations, the tabulation shows that LT<sub>50</sub> for aerosol A is 75.73 minutes while that of aerosol B is 115.6 minutes. This means A and B takes these respective durations to kill half the population of the mosquitoes that were used per test. At 90% mortality of the mosquito population tested aerosol A again displays better efficacy in knocking down mosquitoes. The LT<sub>90</sub> for A is 172.81 minutes while that for B is 226.15 minutes, t-test statistically significant ( $P < 0.05$ ). The mosquitoes have shown a marked ability to withstand aerosol B unlike A. This could indicate a possible resistance or lower efficacy of the insecticide. In this case resistance would not be a possibility for the following reasons. The active ingredients in A and B have similar mode of action and belong to the same class of insecticide, thus if there was resistance to B as indicated by large LT values, cross resistance should also have been noted in A. The point of importance in this study is that there is not metabolic resistance as such the poor efficacy of B is not a result of metabolic resistance. The presence of a synergy piperonyl butoxide should have helped overcome any cytochrome p450 or esterase mediated metabolic resistance.

The isomeric composition of A includes the synthetic pyrethroids *Prallethrinin*, *Imiprothrin* and *D-phenothrin*. B is composed of the synthetic pyrethroids similar to those in A, *tetramethrin* and the synergist *piperonyl butoxide* however there is no *D-phenothrin*. The two space sprays are different in that at least each has a pyrethroid that is not used in the other. *D-Phenothrin* is a synthetic pyrethroid with high lethal activity against household insect pests. *Tetramethrin*, is a second generation of synthetic pyrethroids, is a contact insecticide with a rapid knockdown action on flies (WHO, 2004). According to container labels *D-phenothrin* is added at 0.92g/kg whereas *tetramethrin* is added at 0.4g/kg per 300ml of the product. *Prallethrin* in A is added at 0.4g/kg while in B it is added at 0.34g/kg. *Imiprothrin* is at 0.92g/kg in A and in B it is at 10g/kg.

The chemical compositions show that *D-phenothrin* is An important pyrethroid isomer since it is the component that differentiates A from B. It is added at a higher concentration than its corresponding isomer, *tetramethrin* and as previously stated it is very lethal to mosquitoes. All these factors must contribute to the lower LT values for A. Another important point is that all constituent pyrethroids except *imiprothrin* are added at a higher concentration in A than in B, supporting the higher efficacy that has been exhibited by A. While it has been concluded that the use of *piperonyl butoxide* and *tetramethrin* with synthetic pyrethroid insecticides provides the best results for the control of house flies, it does not seem to be quite an effective combination in the case of mosquitoes as Cakir indicated (Cakir et al, 2008) as also indicated in this study.

Another factor of interest is the addition of *imiprothrin* at a very higher rate than other components 10g/kg in B as compared to A's 0.92g/kg. The first one being that *imiprothrin*

is not important isomer and can be left out. The second possibility is that the manufacturer of aerosol B realizes that the constituents used were not that efficacy and decided to provide a very higher proportion of this particular component to make up for the low efficacy which would be in effective at low dosage. The addition of a synergy to slow down insecticide metabolism support the idea that components of Product B are of less efficacy or the dosage of the potent component provided is *under* dose as such needed to be enhanced(boosted) by other components.

Aerosol A has three active ingredients, while B has four active ingredients. Yet B has a lower efficacy rate as compared to A. Having more pyrethroid constituents or active ingredients should not be taken as a sign of a better insecticide. It could as in this case act as pointer of weakness. Another point of interest could be that a product label indicating more components could be indicative of weakness in the components and more are added to shore up the product performance.

The presence of a synergy where resistance is not reported should also act as a red flag on the efficacy of the insecticide's active components efficacy.

### 3. Conclusion

The study has confirmed what WHO indicates on pesticide database that pyrethroid isomer D-phenothrin is very potent when applied to dipteran members. Fast acting knockdown pyrethroid like tetramethrin need to be encouraged but the dosage should be at optimum recommended not lower dosages as in product B as this may easily result in the built up of resistance. The fact that a synergy is in cooperated when such low dosages are used should not be used as a reason that resistance may not arise. As the study has shown the product still remain inefficacy. Country regulatory bodies should set a minimum concentration at which every isomer/component should be added in aerosol insecticides, below which such products should not be.

### 4. Acknowledgment

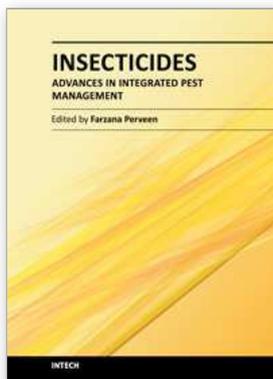
Our gratitude to Twalibu Tandwe, Raphael Kondwani and Martin Chiumia for their support in mosquito collection, rearing and data collection in the laboratory. Special mention goes to The Entomology section of the Department of Biological Sciences of Chancellor College of the University of Malawi and for allowing use of the insectary and equipment.

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## **Insecticides - Advances in Integrated Pest Management**

Edited by Dr. Farzana Perveen

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Hard cover, 708 pages

**Publisher** InTech

**Published online** 05, January, 2012

**Published in print edition** January, 2012

This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through *Bacillus sphaericus* and *B. thuringiensis*, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Dylo Pemba and Chifundo Kadangwe (2012). Mosquito Control Aerosols' Efficacy Based on Pyrethroids Constituents, *Insecticides - Advances in Integrated Pest Management*, Dr. Farzana Perveen (Ed.), ISBN: 978-953-307-780-2, InTech, Available from: <http://www.intechopen.com/books/insecticides-advances-in-integrated-pest-management/mosquito-control-aerosols-efficacy-based-on-pyrethroids-constituents>

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