A Comparison of Two Botanical Pyrethrum Synergists from *Ocimum kilimandscharicum* and *Tagetes minuta* in the Control of Malaria Vector the *Anopheles gambiae*

Rachel A. Ondigo
University of Eldoret, Kenya
orache01@gmail.com

Kefa Sum
Pyrethrum Board of Kenya
ksum@pyrethrum.co.ke

Paul K. Ndalut
University of Eldoret, Kenya
pkndalut@yahoo.com

Titus M. Muthoka
University of Eldoret, Kenya
scientifictito@gmail.com

Abstract

Mosquitoes are general vectors that carry disease-causing viruses and parasites from person to person. Some of these diseases can be life threatening such as dengue, malaria, rift valley fever, yellow fever among others. Vector control is by far one of the most successful methods for reducing the incidences of diseases. However, the emergence of wide spread insecticide resistance and the potential environmental concern associated with some synthetic insecticides has indicated that additional approaches to control the proliferation of mosquito population would be an urgent research area. Concern to quality and safety of life in controlling mosquito, has shifted steadily from the use of conventional chemicals toward alternative insecticides that are target-specific, biodegradable, environmentally safe, and botanicals in origin. Pyrethrins are natural plant compounds used in commercial vector control. They are usually formulated with synergist to improve quality of insecticide, increase its efficacy, fight resistance and make it cost effective; thus, necessitating the need to explore more on natural synergists. In this study, the essential oils from Ocimum kilimandscharicum and Tagetes minuta flowers were extracted by steam distillation. Crude extracts was used to conduct a bioassay for larvicidal and adulticidal activity against 4^{th} instars An. Gambiae larvae. For the bioassay, six concentrations (10ppm,20ppm 30ppm and 40ppm) each with 3 replicates, with a final total number of 60 larvae for each concentration. For each concentration of oil solution twenty 4th instars larvae were inserted. Mortalities were recorded after 1, 3,6, 9, 12 and 24hours exposure, during which no food was offered to the larvae. Each batch of replicates contained distilled water (negative control) pyrethrins (as positive control) and 1% DMSO at the same concentrations as the test solution as standards. Bioassays provided LC_{50} and LC90 values for the synergist mixtures. For O. kilim and scharicum the values were 0.0076 and 0.00167ml while for T. minuta it was 0.00361and 0.01644ml respectively. Data was evaluated by a regression and probit analysis. The active ingredients in the essential oils as separated and identified by GC-MS are with the author. This tests will enable author and other researchers identify the most active component of the synergist to enable any further large production be done in the future.

Key Words: Synergism, Essential Oil, Larvicidal, Adulticidal *An. gambiae,* Larvae

INTRODUCTION

Malaria is the world's greatest parasitic killer disease and remains endemic in 102 countries with more than half of the world's population at risk (Hay *et al.*, 2009). About 2.4 billion people are at risk next to tuberculosis, of the most deadly form of malaria, *Plasmodium falciparum* (Hay *et al.*, 2009).The Word Health Organization (WHO) estimates that each year 300-500 million cases of malaria occur and more than 1 million people die of the same. Malaria control depends on elimination of mosquito breeding places, personal protection against mosquito bites, and treatment of active cases (Brooks *et al*, 2004). Even the most efficacious programme, such as pyrethroid-treated bed nets, have proven difficult to implement on a sustainable basis for reasons of availability, acceptability, and cost (Muller *et al.,* 1999; Snow *et al.,* 1997).

Background of the Study

Mosquito serves as crucial vector for a number of arboviruses (arthropod-borne viruses) and parasites that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood feeding (Clements, 2011). These arthropods are responsible for inflammation/encephalitis, dengue, malaria, rift valley fever, yellow fever and others. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat (Wongsrichanalai *et al.,* 2007).

Vector control is by far the most successful methods for reducing incidences of mosquito borne diseases(Murray *et al.*, 2012).Vector control is achieved via copious methods but the prominent one is the use of pesticides. These are substances or mixture of substances used to prevent, destroy, repel, attract, sterilize, or mitigate pests (Khater, 2012). Synthetic pesticides are generally persistent in nature. The World Health Organization (WHO) estimates that 200,000 people are killed worldwide, every year, as a direct result of pesticide poisoning (Salako *et al.*, 2011). Moreover, the use of synthetic chemicals has been restricted because of their carcinogenicity, teratogenicity, high and acute residual toxicity, ability to create hormonal imbalance, spermatotoxicity, long degradation period, and hazards, upsetting the balance of nature through disruption of natural enemies, pollinators and other wild life (Salako result in toxic residues in food (Khater, 2012). Repetitive use of synthetic pesticides has resulted in pesticide residue *et al.*, 2011). Besides, extensive groundwater contamination, evolution of resistance and resurgence of treated populations, outbreaks of secondary pests, i.e. those normally kept under control by their natural enemies has also been observed (Dubey *et al.*, 2010). More than 500 insect and mite species are resistant to one or more insecticides. The emergence of widespread insecticide resistance and the potential environmental issues associated with some synthetic insecticides has indicated that additional approaches to control the proliferation of mosquito population would be an urgent priority research (Hemingway & Ranson, 2000).

The use of natural products is one of the best alternatives for mosquito control (Murray *et al.*, 2012). The search for herbal preparations that do not produce adverse effects on the non-target organisms and are biodegradable remains a top research issue for scientists associated with alternative vector control (Kroes & Walker, 2004). Many plant species are known to possess biological activity that is frequently assigned to the secondary metabolites. Among these, essential oils and their constituents have received considerable attention in the search for new biopesticides. Many of them have been found to possess an array of properties, including insecticidal activity, repellency, feeding deterrence, reproduction retardation and insect growth regulation against various mosquito species (Duke *et al.,* 2000).

The plant kingdom is recognized as the most efficient producer of chemical compounds, synthesizing many products that are used to defend plants against different pests (Kroes & Walker, 2004). Botanicals have been in nature for millions of years without any adversative effects on the ecosystem. The repellency of plant material has been exploited for thousands of years by man by hanging bruised plants in houses, a practice that is still in wide use throughout the developing countries (Duke *et al.,* 2000). Currently, numerous products of botanical origin, especially the secondary metabolites, have received considerable renewed attention as potentially bioactive agents used in insect vector management. However, there is a little other than anecdotal, traditional or cultural evidence on this topic (Raven *et al.,* 2005). Moreover, plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes which was first recorded in writings by ancient Greek, Roman and Indian scholars (Kroes & Walker, 2004).

Botanical pesticides are easily decomposed by a variety of microbes common in most soils and, as a result, they reduce environmental contamination (Khater, 2012). They also maintain the biological diversity of predators that are often killed by broad-spectrum synthetic pesticides (Salako *et al.,* 2011). As a form of allelopathy, some pesitcidal plants serve as control agents for pests and diseases (Ogunnika, 2007). The Greek natural philosopher Pliny the Elder (1‘st century AD) recorded all the known pest control methods in __Natural History‘‘ (Rose, 2001). The use of powdered chrysanthemum as an insecticide comes from Chinese record. The other natural products like pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirach comes from Chinese record (Shaalan *et al.,* 2005). The other natural products like pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, *d*-limonene, camphor and turpentine were -productive (Duke *et al.,* 2000).

The use of synergists in insecticidal is among some important phytochemical insecticides widely used in developed countries (Picollo *et al.,* 2000). The discovery of DDT‘s and the subsequent development of organochlorines, organophosphates and pyrethroids suppressed natural product research as the problem for insect control were thought be solved (Turusov *et al.,* 2002). However, high cost of synthetic pyrethroids, environment and food safety concerns, the unacceptability and toxicity of many organophosphates and organochlorines, and increasing insecticide resistance on a global scale argued for stimulated research towards potential botanicals (Shaalan *et al.,* 2005).

In light of the scanty data on insecticidal efficacy of the herbal medicine especially in the tropical regions where there are large forested land; the aim of this study was to understand the antimosquicidal efficacy of combination therapy using two plant species and determine possible synergistic relationships.

Pyrethrins have been used in many varieties of insecticide, fogging products and in some pet products for over 100 years but their formulation without synergists are ineffective and thus, most of the paralyzed insects normally recover after a while (Matthews, Bateman & Miller, 2014). Piperonylbutoxide (PBO), a synergist, is often used in combination with pyrethrins, making the mixture more effective by not allowing the insect's system to detoxify the pyrethrins. However, the latest information regarding toxicity of piperonylbutoxide has determined that it can pose a distinct health risk when it becomes airborne (Kumar *et al.,* 2002). Pregnant women when exposed to this substance during the third trimester leads to delayed mental development in young children (Romero *et al.,* 2009). A study found a significant association between piperonylbutoxide (PBO), a common additive in pyrethroid formulations, measured in personal air collected during the third trimester of pregnancy, and delayed mental development at 36 months was observed (Horton *et al.,* 2011). Piperonylbutoxide (PBO) is also suspected to be carcinogenic, mutagenic, and teratogenic (Mahadevan *et al.*, 2009). PBO also attributes to chronic toxicity where it changes liver and kidney in test animals at high doses (Wang *et al.,* 2012).

Chemical pesticides also induce development of pest resistance to applied agents and non-target environmental impacts (Stenersen, 2004). Integrated pest management programs have demonstrated that current levels of pesticide use in many circumstances are not necessary and, frequently, are even counter compositions should be advantageous in that, the more expensive toxicant can be replaced by less expensive synergists, while still retaining an effective level of insecticidal activity (Norrisn *et al.,* 2003). Piperonylbutoxide (PBO) as a synergist used as pyrethrum formulation is very costly, toxic and its continuous supply is not guaranteed (Kumar *et al.,* 2002). The cost of PBO is almost three times that of pyrethrins (Romero *et al.,* 2009). This research tries to identify a possible replacement of PBO from naturally occurring sources (natural oils and essential oils) from seeds and leaves of higher plants. A synergist which is cheap, readily available and environmentally friendly is needed and many botanicals are readily available. Efforts should be made to promote the use of easy accessible natural pyrethrum synergists and affordable traditional insect/mosquito repellent plants.

Many plant species including pyrethrum, *Artemisia* and essential oils such as *Ocimum* have been proved to have mosquicidal properties (Kimbaris *et al.*, 2012). The insecticidal action of these plants is based on the active ingredients produced by the plants. When these plant biocides are combined with pyrethrins they may have synergist or additive effects which may have an influence on the mosquitos. (Synergism improves quality of insecticide, enhances efficacy, delays resistance and makes it cost effective). However, there are no toxicity data or studies available that have so far been conducted on the efficacy of combined therapies of these plant biopesticides. Furthermore, efficacies of plant extracts are known to be affected by among other things such as location, amount of active compounds in the plants, extraction procedure and species of organism under study, which makes it very difficult to generalize the antimosquicidal properties of many plant species (Njoroge & Bussmann, 2006). Lack of such information has limited the use of these plants as insecticides and natural pyrethrum synergists in mosquito control, therefore enhances the problem of malaria. The aim of this study was to compare synergism of the two plant species and find out which one increases potency of pyrethrum.

MATERIALS AND METHODS

Collection of Plants Specimen

The plant specimens were obtained from Kipkaren in Nandi County, which is situated in the western part of Rift Valley Province. It borders Kakamega County to the north-west, Uasin Gishu County to the north-east, Kericho County to the south-east, Kisumu Countyto the south-East, and Vihiga County to the west. It lies within latitudes 0º and 0º34‘ North and longitudes 34º44‘ and 35º25‘ Eastand covers an area of 2873KM^2 with a total population of 631,357 as of the 2009 census (GOK, 2013).

Specimen pyrethrins (oils) samples were obtained from pyrethrum Board of Kenya factory, located in Nakuru. Fresh aerial parts of full-bloom *T. minuta* and and *O. kilimandscharicum*were collected and packed in black polythene to prevent further photosynthesis and acquisition of artifact. Also to prevent volatilization and leaf damage, so as to reach the laboratory undistorted for identification. Collected plant materials were taken to the University of Eldoret herbarium for identification.

Preparation of Extracts

Essential oils from both plant specimens were obtained by steam distillation of fresh plant leaves flowers. The solvent was removed by rotary evaporation under reduced pressure at a temperature below 45ºC. The solutions were concentrated under reduced pressure, below 45ºC, to dryness. The resulting crude PE was kept at -20ºC until testing for their synergistic property against Anopheles mosquito. After extractions of single species, the plant species were combined with pyrethrins in different proportions ready for larvicidal and adulticidal test (synergistic tests).

Experimental Sites

The experiment was conducted in the laboratories of CDC/KEMRI in Kisumu. KEMRI/CDC Field Research Station is located in Kisian area near Kisumu City on the KEMRI Centre for Global Health Research (CGHR) campus. The Field Research Station is located in an area of western Kenya where P. falciparum malaria and HIV are major public health problems.

Rearing of *Anopheles gambiaess*

The test mosquito specimen chosen was *Anopheles gambiae*. It is the principal mosquito vector of human malaria in tropical Africa, and in Western Kenya and together with *An. funestus,* the most abundant in the region (Ndiath *et al*., 2014). Mosquito eggs of *A. gambiae*s.s were collected from CDC/KEMRI Kisumu and hatched in spring water in trays at room temperature in CDC/KEMRI laboratories. The method of insect rearing and experimental techniques were generally the same as used by GlynneJonnes and Chadwick (1960). Adult mosquitoes were managed in mosquito netting cage using the methods used by Ghosh *et al.* (2012).

Test for Synergim

Larvicidal bioassay. Methods for testing larvicidal action of the crude extracts were slightly modified from those of World Health Organization (WHO, 1996). A stock solution was prepared by dissolving a known amount (20ml) of the crude extract in an appropriate solvent and stored in a refrigerator at 15ºC. Twenty healthy, late 3^{rd}-4^{th} instars larvae were introduced into each testing cup (sterilized plastic drinking cup of 150 ml capacity), which contained 100 ml of distilled water. A measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of four concentrations,(10ppm,20ppm 30ppm and 40ppm) each with 3 replicates, with a final total number of 80 larvae for each concentration.

Each batch of replicates contained distilled water (negative control), pyrethrins (as positive control) at the same concentrations as the test solution as standard in each case. Larvae Mortality was recorded after 1, 3, 6, 9, 12 and 24 hours exposure, during which no food was offered to the larvae. Control mortality was accounted for by the formula of Abbott (1925).

Bioassay on adult mosquitoes. The WHO cones were used to evaluate the synergistic activity of the compounds. Twenty adult *A.gambiae* 3-4 days old, were introduced to the test chamber its surfaces (filter paper) having been smeared with pretreated solutions. The test compounds and 0.2% dry weight of pyrethrins, in the ratios of 1:1, 1:2, 1:3, 1:4 and1:5 ratios of pyrethrins to potentially synergistic compounds were used until a suitable concentration was obtained. The knockdown and mortality rates were monitored for three minutes then 24hours.The knockdown mosquitoes were removed, provided with food and water while the percentage kill was determined after 24 hours. A solutions of pyperonylbutoxide and pyrethrins with same concentration as test solutions were used as standards in each case.

Determination of factor of synergism. The increase in toxicity of the synergized pyrethrins was expressed using factor of synergism (FOS) which is usually expressed as; according to Finney (1952)

$$FOS = \frac{Dose\ of\ pyrethrum\ required\ to\ kill\ \ 50\%\ of\ the\ insect\ (L_{50})}{Dose\ of\ the\ synergized\ mixture\ required\ to\ kill\ 50\%\ of\ insect (LD_{50})}$$

Data Analysis

The bioassay data on mortality were corrected for control group deaths using Abbott formula (1925) and subjected to regression and probittests (Finney, 1952).Values of the median lethal doses were read and fitted from probitgraphs.

RESULTS AND DISCUSSIONS

Table1. Effect of combined plant extracts with pyrethrins in ratio 1:1 at conc. Of 0.01mg/ml on larvae compared to pyrethrins alone

Tests solutions	Time(hrs)	Number Exposed	Number Dead		
			Expt.1	Expt.2	Expt.3
Pyrethrin (Control)	1	20	15	16	14
	3	20	18	19	18
	6	20	20	20	20
	9-24	20	20	20	20
Pyrethrins and *ocimum*	1	20	17	18	17
	3	20	20	20	20
	6	20	20	20	20
	9-24	20	20	20	20
pyrethrin and T. *minuta*	1	20	9	10	8
	3	20	17	17	16
	6	20	20	20	20
	9-24	20	20	20	20

Larval mortality increased as the essential oil concentration increased. The essential oils from the different plant species had different activities. The *O.kilimandscharicum* was more potent than the *T.minuta* for all treatment periods and concentrations as observed in Table 1.

For example, the highest dose caused 100% mortality after 3-hour incubation with oils of *O.kilimandscharicum*. The same dose caused 100% mortality after 6 hours with oils of *T.minuta*. Essential oils from both extracts had larvicidal activity until 9 hours of incubation. Thereafter, the activity remained constant between 9 and 12 hours until 24 hours of exposure of the larvae to the essential oils.

Table 2. Different concentrations of the synergistically active compounds compared with pyrethrins during a period of 1hr till 24hours

Tests	Concentration	Number Exposed	Number Dead		
			Expt.1	Expt.2	Expt.3
Dist. Water(N-control)	0	20	0	0	0
Pyrethrins	0.03	20	20	20	20
Pyrethrins	0.02	20	14	15	13
Pyrethrins	0.01	20	11	10	12
Pyrethrins	0.005	20	5	6	7
Pyrethrins with *ocimum*	0.03	20	20	20	20
Pyrethrins with *ocimum*	0.02	20	19	18	17

Pyrethrins with *ocimum*	0.01	20	15	16	16
Pyrethrins with*ocimum*	0.005	20	11	10	12
pyrethrin with *T.minuta*	0.03	20	20	20	20
pyrethrin with *T.minuta*	0.02	20	14	13	13
pyrethrin with *T.minuta*	0.01	20	10	11	11
pyrethrin with *T.minuta*	0.005	20	6	7	6

Table 2 shows that synergistic effect of *ocimum* was so much greater that of *T.minuta* that comparion by an alternative method was desirable. The table indicates LC50 at a 0.01ml while for *ocimum* occurs at 0.005ml.

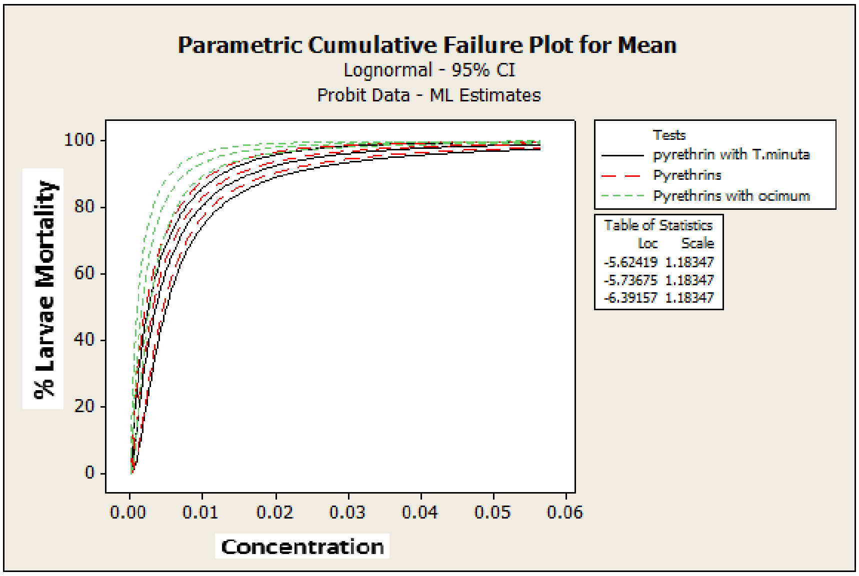

Figure 1. % larvae mortality against concentration of synergist

Table 3. Knockdown and Mortality rates for different concentrations ofthe synergized mixtures/solutions on Adult *An.gambiae*in the first 3minutes till 24hour

Test mixtures/sol n.	Conc .	No. Ex p	KDExp 1	KDExp 2	KDExp 3	MortExp 1	MortExp 2	MortExp 3
Pyrethrins	0.1	20	14	15	15	20	20	20
	0.01	20	10	9	12	20	20	20
	0.00 1	20	6	5	8	20	20	20
Pyrethrins with *ocimum*	0.1	20	14	15	14	20	20	20
	0.01	20	14	12	14	20	20	20
	0.00 1	20	10	11	9	20	20	20

pyrethrins with *T.minuta*	0.1	20	8	7	8	10	12	11
	0.01	20	5	6	6	12	13	12
	0.001	20	4	4	4	9	10	10
Dist.water(N -control)	0	20	0	0	0	0	0	0
pyrethrins with PBO	0.1	20	20	20	20	20	20	20
	0.01	20	20	19	20	20	20	20
	0.001	20	18	20	20	20	20	20

The WHO cones were used to compare the effect of the two synergists on the knockdown and mortality rates for 3-4 days old adult mosquitoes. The results of comparisons are given in Table 3 and show that *T.minuta* was less effective at all the pyrethrins to synergist concentrations tested. The mortality for mosquitoes after 24hours was also low. A comparison of pyrethrins plus *ocimum* at a concentration in the ratio 1.1with pyrethrins plus *T.minuta* at 1.5 showed that adding a large quantity of the less effective synergist does not compensate for its low activity.

Values of the median lethal dose read from these probit graphs are given in Figures 2.

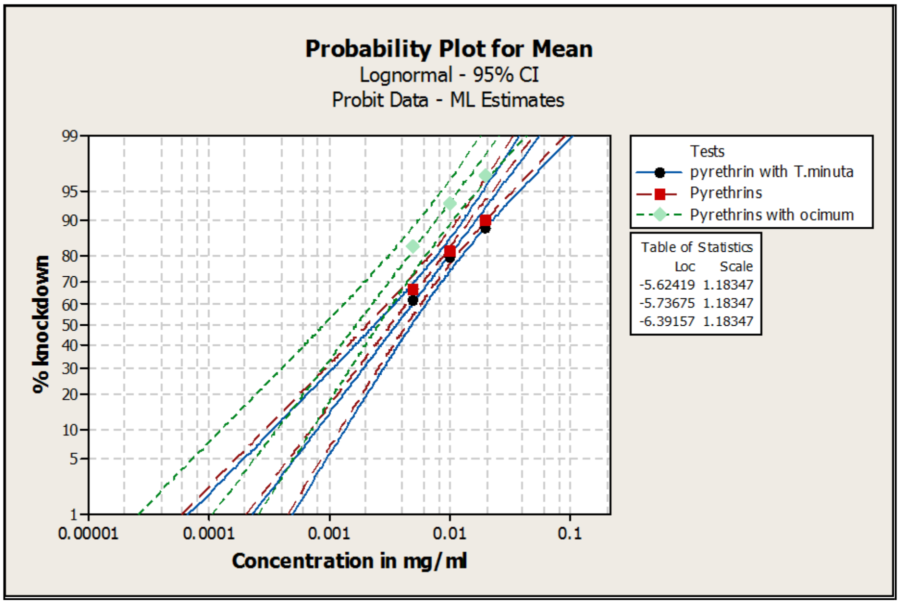

Figure 2. Comparisons of pyrethrins, pyrethrins plus *ocimum* and pyrethrins plus *T. minuta* .Three replicates of 20 adult mosquitoes at each dosage level. Knockdown during a period of 3minutes and 100% kill after 24 hours

This figure 2 represents regression/probit graphs showing of KD of mosquitoes over a time.The KD50 and KD90 values were 0.00167 and 0.00763ml respectively for the *O. kilimandscharicum* and 0.00361 and 0.01644 ml respectively for the *T.minuta*.

The increase in toxicity of the synergized pyrethrins was expressed using factor of synergism which is usually expressed as;

$$FOS = \frac{Dose\ of\ pyrethrum\ required\ to\ kill\ \ 50\%\ of\ the\ insect\ (L_{50})}{Dose\ of\ the\ synergized\ mixture\ required\ to\ kill\ 50\%\ of\ insect (LD_{50})}$$

Table 4 shows relative potency for the two synergists determined by the formula above,which also derived from Figure 2, the lower the R.P the more the lethal the synergist. O. *kilimandscharicum* had R.P of 0.532245againstT.minuta which had 0.896367.

Table 4: Relative Potency

Relative 95.0% Fiducial CI, taking (LC50) as the standard

Solutions	RatioPySyn	LarvaeLC50	Relative Potency	Adult LC50
Pyrethrins	---	0.0035	1.0	0.002701
Py/*ocimum*	1.1	0.0001	0.532245	0.001441
Py/ *T.minuta*	1.1	0.00035	0.8 96367	0.003020

CONCLUSIONS

The results show that *ocimum* varies greatly in its effect on different stages of mosquitoes. Thus it is a better synergist than *T.minuta* against larvae and adult stage. This wide variation in effectiveness emphasizes the importance of choosing the most suitable pyrethrum formulation for each individual application. A single formulation intended for all stages of insect may be convenient in manufacture but will not give the most economical control of each intended stage or species.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J.econ.Entomol*, *18*(2), 265-267.

Bilgin, M., & Birman, İ. (2010).Separation of propionic acid by diethyl carbonate or diethyl malonate or diethyl fumarate and the synergistic effect of phosphorus compounds and amines.*Fluid Phase Equilibria*, *292*(1), 13-19.

Clements, A. N. (2011). *The Biology of Mosquitoes: Viral, Arboviral and Bacterial Pathogens* (3). Cabi.

Dubey, N. K., Shukla, R., Kumar, A., Singh, P., &Prakash, B. (2010).Prospects of botanical pesticides in sustainable agriculture.*CurrSci*, *98*(4), 479-480.

Duke, S. O., Dayan, F. E., Romagni, J. G., &Rimando, A. M. (2000). Natural products as sources of herbicides: current status and future trends. *Weed Research-Oxford-*, *40*(1), 99-112.

Finney, D.J. (1952).*Probit Analysis*.Cambridge University Press.

Glynee, J.G.D., & Chadwick, P.R. (1960). A comparison of four pyrethrum synergists.*Pyrethrum Post* 5(3), 22-30

Ghosh, A., Chowdhury, N., & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *The Indian journal of medical research*, *135*(5), 581.

GOK.(2013). *Kenya Census 2009.* Retrieved from http://www.scribd.com/doc/36672705/Kenya-Census-2009

Hemingway, J., & Ranson, H. (2000).Insecticide resistance in insect vectors of human disease. *Annual review of entomology*, *45*(1), 371-391.

Horton, M. K., Rundle, A., Camann, D. E., Barr, D. B., Rauh, V. A., &Whyatt, R. M. (2011). Impact of prenatal exposure to piperonylbutoxide and permethrin on 36-month neurodevelopment. *Pediatrics*, *127*(3), e699-e706.

Keiser, J., Utzinger, J., De Castro, M. C., Smith, T. A., Tanner, M., & Singer, B. H. (2004).Urbanization in sub-saharan Africa and implication for malaria control. *The American journal of tropical medicine and hygiene*, *71*(2 suppl), 118-127.

Khater, H. F. (2012). Prospects of botanical biopesticides in insect pest management. *Pharmacologia*, *3*(12), 641-656.

Kimbaris, A. C., Koliopoulos, G., Michaelakis, A., & Konstantopoulou, M. A. (2012). Bioactivity of Dianthus caryophyllus, Lepidiumsativum, Pimpinellaanisum, and Illiciumverum essential oils and their major components against the West Nile vector Culexpipiens. *Parasitology research*, *111*(6), 2403-2410.

Kroes, R., & Walker, R. (2004). Safety issues of botanicals and botanical preparations in functional foods. *Toxicology*, *198*(1), 213-220.

Kumar, A., Valecha, N., Jain, T., & Dash, A. P. (2007). Burden of malaria in India: retrospective and prospective view. *The American journal of tropical medicine and hygiene*, *77*(6 Suppl), 69-78.

Kumar, S., Thomas, A., Sahgal, A., Verma, A., Samuel, T., & Pillai, M. K. K. (2002). Effect of the synergist, piperonylbutoxide, on the development of deltamethrin resistance in yellow fever mosquito, Aedesaegypti L.(Diptera: Culicidae). *Archives of insect biochemistry and physiology*, *50*(1), 1-8.

Mahadevan, U., Martin, C. F., Sandler, R. S., Kane, S. V., Dubinsky, M., Lewis, J. D., ...& Sands, B. E. (2009). 562 A Multi-Center National Prospective Study of Pregnancy and Neonatal Outcomes in Women with Inflammatory Bowel Disease Exposed to Immunomodulators and Biologic Therapy. *Gastroenterology*, *136*(5), A-88.

Matthews, G., Bateman, R., & Miller, P. (2014).*Pesticide application methods*. John Wiley & Sons. Murray,

C. J., Rosenfeld, L. C., Lim, S. S., Andrews, K. G., Foreman, K. J., Haring, D., ... & Lopez, A. D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *The Lancet*, *379*(9814), 413-431.

Ndiath, M. O., Mazenot, C., Sokhna, C., & Trape, J. F. (2014). How the Malaria Vector Anopheles gambiaeAdapts to the Use of Insecticide-Treated Nets by African Populations. *PloS one*, *9*(6), e97700.

Njoroge, G. N., & Bussmann, R. W. (2006). Journal of Ethnobiology and Ethnomedicine. *Journal of ethnobiology and ethnomedicine*, *2*, 8.

Norris, R. F., Caswell-Chen, E. P., &Kogan, M. (2003).*Concepts in integrated pest management* (p. 586). New Jersey: Prentice Hall.

Ogunnika, C. B. (2007, April). Medicinal plants: A Potential agroforestry components in Nigeria. In *Proceedings of the Humboldt Kellog/3rd Annual Conference of School of Agriculture and Agricultural Technology* (pp. 2-7).

Picollo, M. I., Vassena, C. V., Cueto, G. A. M., Vernetti, M., & Zerba, E. N. (2000). Resistance to insecticides and effect of synergists on permethrin toxicity in Pediculuscapitis (Anoplura: Pediculidae) from Buenos Aires. *Journal of Medical Entomology*, *37*(5), 721-725.

Raven, P. H., Evert, R. F., & Eichhorn, S. E. (2005). *Biology of plants*. Macmillan.

Romero, A., Potter, M. F., & Haynes, K. F. (2009).Evaluation of piperonylbutoxide as a deltamethrin synergist for pyrethroid-resistant bed bugs. *Journal of economic entomology*, *102*(6), 2310-2315.

Rose, J. (2001). *Herbs & Things: Jeanne Rose's Herbal*. Last Gasp.

Salako, A. A., Sholeye, O. O., & Dairo, O. O. (2011). Beyond pest control: A closer look at the health implication of pesticides usage. *Journal of Toxicology and Environmental Health Sciences*, *4*(2), 37-42.

Shaalan, E. A. S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H., & Mansour, A. H. (2005).A review of botanical phytochemicals with mosquitocidal potential. *Environment international*, *31*(8), 1149-1166.

Stenersen, J. (2004). *Chemical pesticides mode of action and toxicology*. CRC pres

Turusov, V., Rakitsky, V., & Tomatis, L. (2002). Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. *Environmental Health Perspectives*, *110*(2), 125.

Wang, J., Lu, J., Mook, R., Zhang, M., Zhao, S., Barak, L., ...& Chen, W. (2012). The Insecticide Synergist PiperonylButoxide Inhibits Hedgehog Signaling: Assessing Chemical Risks. *Toxicological Sciences*, kfs165.

WHO (1996). Methods for testing larvicidal action of the crude extracts.In Bagavan, A., Rahuman, A. A., Kamaraj, C., &Geetha, K. (2008).Larvicidal activity of saponin from Achyranthesaspera against Aedesaegypti and Culexquinquefasciatus (Diptera: Culicidae). *Parasitology research*, *103*(1), 223-229.

Wongsrichanalai, C., Barcus, M. J., Muth, S., Sutamihardja, A., &Wernsdorfer, W. H. (2007). A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *The American journal of tropical medicine and hygiene*, *77*(6 Suppl), 119-127.

World Health Organization. (2005). *The World health report: 2005: make every mother and child count.*

BIO-DATA

Rachel A. Odigo holds a Bachelor of Education Science and a Master of Science (Organic Chemistry). She is currently a Secondary School teacher at Chebisaas High School, Kenya. Her research interest is in Organic Chemistry especially anti-malarial studies.