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Knockdown effect of sweet basil (*Ocimum basilicum*) essential oil and a mixed formulation on *Aedes aegypti* adults

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Abstract
Mosquito-borne diseases such as dengue, yellow fever, chikungunya and Zika fever are among the leading cause of deaths world-wide. There is no effective vaccine and treatment against dengue, chikungunya and Zika fever. Thus they are prevented through mosquito control and personal protection from mosquito bites. The development of resistance of mosquitoes to most synthetic insecticides and repellents calls for the search for alternatives from plant origin. The purpose of the study was to determine efficacy of sweet basil (*Ocimum basilicum)* on *Aedes aegypti* adults. The test substances formulated into a jelly were applied on the rabbit skin and tested with starved *Aedes aegypti* adult females in a mosquito cup. Synergized crude oleoresin extract of pyrethrum and Vaseline pure petroleum jelly included as a positive and negative control respectively. The Kd50 was estimated to be 369 ppm and 0.027ppm for *Ocimum* oil and pyrethrum extract respectively. 1000ppm of Pyrethrum extract was comparable to 30000 ppm of *Ocimum* oil ($P > 0.05$). The combination of *Ocimum* and *Eucalyptus* oil led to a more superior product with knockdown effect. *Ocimum basilicum* can be formulated with other plant compounds and applied in mosquito control.

Keywords: *Aedes aegypti*, pyrethrins, PBO, *Ocimum basilicum, Eucalyptus citriodora*

1. Introduction
Aedes aegypti female mosquitoes are the vectors for the pathogens of different arboviral diseases such as dengue and dengue haemorrhagic fever, yellow fever, chikungunya fever [1] and Zika fever [2].
About 2.5 billion people are at risk for dengue, with approximately 50 million cases per year and about 500,000 cases of dengue hemorrhagic fever [3]. In 2013, dengue fever it caused an estimated 60 million symptomatic infections worldwide, with 18% admitted to hospital and about 13,600 deaths and its world wide cost is estimated at US$9 billion [4]. Moreover, Dengue infection of pregnant women may lead to miscarriage, low birth weight and premature birth [5].
Despite an effective vaccine, yellow fever causes an estimated 200,000 cases, including 30,000 deaths annually and over 90% of the cases are in Africa [6]. Zika fever epidemics have been reported in African and Asian countries. It is associated with Guillain–Barre syndrome and congenital anomalies such microcephaly and chorioretinal scarring [7].
There is neither treatment nor effective vaccine against Dengue, Chikungunya and Zika fever [7, 8]. Moreover, several side effects have been reported on yellow fever vaccine such as an infection of the nervous system which can lead to meningoencephalitis [9]. Moreover, fetal infection has been documented if administered during pregnancy that increases the risks of arbotion [10].Thus mosquito control and personal protection from mosquito bites is the only effective way of controlling the spread of these diseases. However most synthetic insecticidal and repellent formulations such as DEET and permethrin are not only unsafe to humans but also have effects on the environment [11, 12]. Frequent use of insecticides for mosquito control has also led to the development of resistance and undesirable effects on non-target organisms [13]. Moreover, long term exposure of new born babies and children to pyrethoids is associated with neurological effects [14].This calls for the search of an effective natural product from the plant origin that contain safe, biodegradable and eco-friendly chemicals.
Plants have been used for centuries for insect control. The insecticidal activity is due to production of secondary compounds as a result of coevolution of plants with insects. These include essential oils alkaloids, quinones, glycosides, and flavonoids [15].
Sweet basil (*Ocimum basilicum* L.) is an aromatic herb that is used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics [16].Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, warts, worms, kidney malfunctions and inflammation, hypertension and as a contraceptive [17] and as insect repellent [18]. The purpose of the study was to determine the mosquito knockdown effect of *Ocimum basilicum.*

2. Materials and Methods
2.1Test Mosquitoes
Aedes aegypti mosquitoes were reared in the Insectary at School of Biological Sciences, University of Nairobi. The colony has been bred continuously over the since 1984 without exposure to insecticides, repellents and pathogens.
The eggs were then floated in plastic rearing trays, half filled with tap water. The emerged larva was fed daily with 100mg of active dry yeast and the water was refreshed every two

days. The emerged pupae were collected using a Pasteur pipette and transferred in a container, three-quarters full of water that was inserted in a wooden cage. The emerged adults were continuously provided with 10% sugar solution in a feeding tube.

The emerged females were given access to blood meal from the blood vessels of the rabbit ears *ad libitum*. The colony was maintained at 25±2 ºC, 80±10% relative humidity and 14h:10h (light:dark) photoperiod. 3-7 day old, adult females were starved by being provided with only water for 24 hours prior to tests.

2.2 Collection and extraction of the plants

Ocimum basilicum whole plant was collected from Aberdare ranges. The plant was uprooted and transported in polythene bags. The voucher specimen was then deposited in the herbarium of the school of Biological sciences, University of Nairobi. Fresh leaves were picked from the plant, washed with clean tap water and shed-dried to a constant weight. Fully dried materials were pulverized into fine powder by use of a hammer mill until the powder passed through 1mm mesh sieve [19]. 1 kg of the plant material was soaked in hexane for four days in a plastic bucket and the mixture was constantly stirred with a wooden rod over that period. It was then decanted leaving soluble hexane fraction. The fraction was evaporated in a Rotary Evaporator ® at 60 ºC. The crude extract obtained was dried, precipitated and crystals of camphor were removed. The resultant oil extract was stored in a plastic container before being stored in a cupboard at room temperature until investigations.

Crude oleoresin extract consisting of 25% w/w of pyrethrins and Piperonyl butoxide (PBO) was kindly donated by Pyrethrum Board of Kenya. Eucalyptus oil was donated by BIOP Company Limited ready for formulation

2.3 Formulation of the test extracts

The test extracts were formulated using Vaseline Pure Petroleum Jelly® of Uniliver Kenya Limited. Different concentrations of *Eucalyptus* and *Ocimum* essential oil were volumentaly diluted with the melted jelly at 80º C and stirred continuously using a stirring rod until it was fully melted before being transferred using well-labeled hypodermic syringes into test tubes in a rack partially immersed in the water bath, arranged and labelled according to prepared concentration. The test tubes containing the test material was shaken and poured into respective labelled containers and then left to stand until they solidify. A combination consisting 3% of each of the test extracts in a ratio 1:1 was then prepared based on the previous results [20]. Similarly, crude oleoresin extract of pyrethrum was sucked from its container using an appropriate syringe and a needle before being transferred into a test tube. It was then synergised with PBO in a ratio of PBO to pyrethrum as 4:1(v:v) and the resultant stock solution was serially diluted in melted jelly to an appropriate concentration. It was then stored at room temperature away from sunlight until investigated for repellent activity.

2.4 Determination of the knockdown effects of the test extracts

The knockdown effect was performed on the rabbit skin as the host of *Aedes aegypti*. 50 female mosquitoes was aspirated from the mosquito cage and introduced into mosquito cup [20]. Different concentrations the extracts and a combination were applied on the shaven areas in the rabbit skin. The plastic container containing mosquitoes was the inverted on the test areas of a restrained rabbit for an hour and observations were made. All tests were done during the day at room temperature. Vaseline pure petroleum jelly and synergized crude oleoresin extract of pyrethrum served as negative and positive controls respectively.

The effect of the test formulations was noted and the number that was paralyzed was counted and calculated according to the following formulae;

$$\text{Knocked down (\%)} = \frac{\text{Number of adults knocked down per unit time}}{\text{Number of adults released}} \times 100$$ [21].

2.5 Data analysis

Data analysis was done by t tests, probit and one way ANOVA using Ms Excel, Graph pad prism and SPSS version 16 for windows. A *p* value of less than 0.05 was considered to indicate statistical significance.

3. Results & Discussion

Table 1: Knockdown effect *Ocimum basilicum oil*, *Chrysanthemum cinerariefolium* extract and a mixed formulation of essential oils on *Aedes aegypti* adults

Ocimum basilicum		***Chrysanthemum cinerariefolium* +PBO**		***Ocimum basilicum* +*Eucalyptus citriodora***	**Pure petroleum Jelly**	**P value**
Concentration (ppm)	% Knock down	Concentration (ppm)	% Knock down	% Knock down	% Knock down	
10	43±0.577	10	88±0.816	100±000	0.000	< 0.05
100	64±1.826	100	95±1.29	100±000	0.000	< 0.05
1000	78.5±.957	1000	100±000	100±000	0.000	< 0.05
10000	82.5±957	10000	100±000	100±000	0.000	< 0.05
20000	93±1.291	20000	100±000	100±000	0.000	< 0.05
30000	100±000	30000	100±000	100±000	0.000	< 0.05

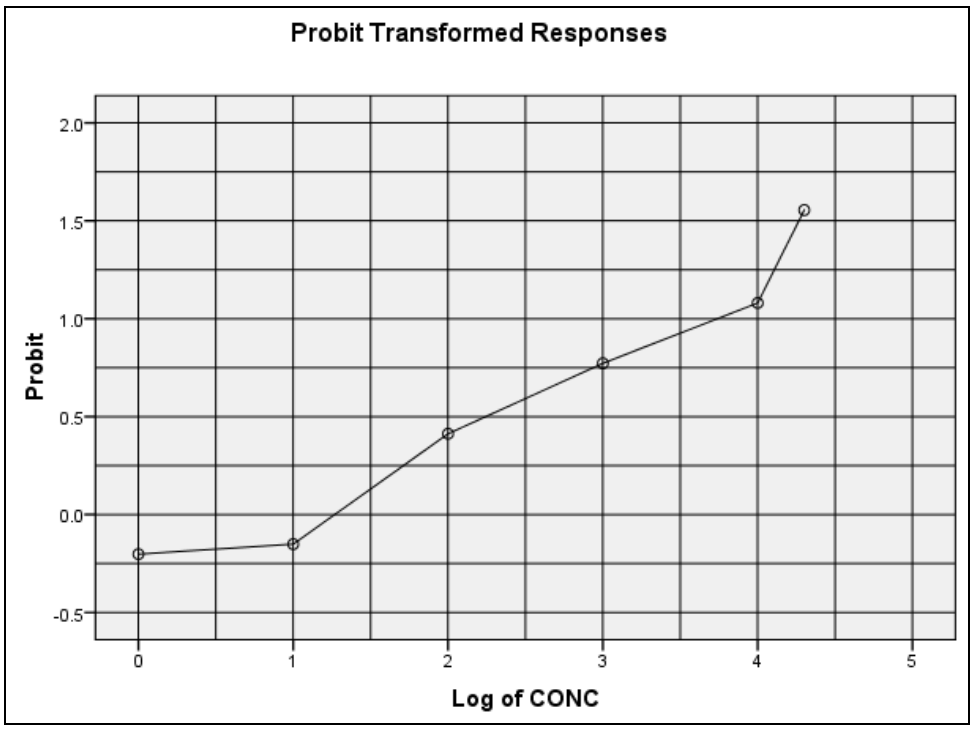

Fig 1: Knockdown effect of *Ocimum basilicum* oil on *Aedes aegypti* adults

At 30000ppm the oil all the tested mosquitoes was paralyzed within three minutes of the test. The activity was comparable to that of Pyrethrum (*Chrysanthemum cinerariefolium*) at 1000 ppm ($P < 0.0001$). The Kd 50 was of the extract estimated to be 369 ppm as compared to 0.027 ppm of crude oleoresin extract of pyrethrum. No such effect was seen in pure petroleum Jelly. The activity of pyrethrum extract was higher than of *Ocimum basilicum* oil ($t=2.252$)

The knockdown effect of *Ocimum basilicum* is most likely due to linalool and estragole which is present in the oil and has been tested to have knockdown effect on tephritid fruit fly [22] but the mode of action is unknown. The activity of *Chrysanthemum cinerariefolium* was due to pyrethrins that act by targeting the central and peripheral nervous system where they disrupt signal transmission along the nerve axon. They act by binding to the sodium channel causing it to remain open, whereby the nerve continues to be stimulated uncontrollably causing tremors. PBO stabilizes the extracts by acting on cytochrome p 450 enzymes. This allows pyrethrum to be effective with less active ingredient than would otherwise be required [23] besides inhibiting detoxification [24]. Though *Chrysanthemum cinerariefolium* extract is effective at low concentration than *Ocimum basilicum* oil pyrethrins are break down in the presence of sunlight thus limiting its application.

The combination of *Ocimum basilicum* and *Eucalyptus citriodora* oils lead to a superior product that paralyzed them within one minute after application. This activity is due to the synergy of their chemical components. The component responsible for the activity of *Eucalyptus citriodora* was most likely to be associated with the presence of its major components, such as citronellal, geraniol and β citronellol that have proven to have insecticidal activity against *Myzus persicae* and *Frankliniella schultzei* nymphs [25]. The activity of the combination of the oils was similar 1000 ppm of crude oleoresin extract of pyrethrum ($P > 0.05$) but significantly better on the concentration lower than 1000 ppm ($P < 0.001$). The knock down activity of *Ocimum basilicum* oil support the insecticidal effect of the extract as reported earlier [26].

4. Conclusion

The results validate its traditional application *Ocimum basilicum* in insect control. *Ocimum basilicum* can be upscaled, synergized with other essential oils and integrated into for mosquito control programmes. However, further research in the field conditions and in other mosquito species is necessary.

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