EFFICACY OF THE ESSENTIAL OILS OF AFRICAN BLUE BASIL (*Ocimum kilimandscharicum* GUERKE) AS A PYRETHRUM SYNERGIST

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ABSTRACT

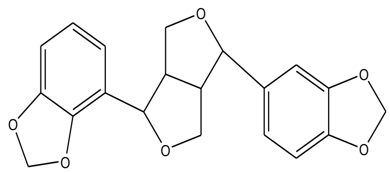
Concern on quality and safety of life in managing pests has shifted steadily from the use of conventional chemicals towards alternative botanical insecticides that are target-specific, biodegradable and environmentally safe. The history of insecticidal synergists originated with attempts to enhance the potency of pyrethrins. This discovery initiated the use of insecticide synergists, piperonylbutoxide (PBO), which is obtained from Safrole a main component of sassafras. *Ocimum kilimandscharicum* of the family Lamiaceae, commonly known as African Blue Basil and 'okita' in native language, is a perennial, under shrub with simple ovate-oblong leaves. The essential oil of *kilimandscharicum* has carminative, stimulant, antipyretic, anti-fungal and anti-bacterial properties. Literature review has revealed that the chemical composition of the essential oil has several economic importance, among them increases potency of pyrethrum. The study was to investigate the synergism of pyrethrins with essential oils from *O. kilimandscharicum.* Specific objectives were to determine the activity of pyrethrins and the crude extracts (essential oil of *O. kilimandscharicum)* on 4th instar larvae and adult of *Anopheles gambiae*. Also to compare the efficacy of pyrethrins combined with essential oils against conventional pyrethrum synergist; Piperonyl butoxide (PBO). In light of this communication, this article presents essential oil composition from the leaves of *O. kilimandscharicum*, collected from the Kipkaren in Nandi County, Kenya, possessed an active ingredient that could be used as pyrethrum synergist. In this study, essential oils from *O. kilimandscharicum* leaves were extracted by hydro-distillation using Clevenger type apparatus for 4 hours. Crude extracts were used with pyrethrins to conduct bioassays for larvicidal activity against 4th instar larvae and adult of *An. gambiae*. Mortalities were recorded after 1, 3, 6, 9, 12 and 24 hour's exposure. Bioassay data was evaluated by regression and probit analysis and used to determine the lethal doses (LC_{50} and LC_{90}) for the synergist mixtures. The results showed that LC_{50} and LC_{90} values for *O. kilimandscharicum* with pyrethrins were 0.00167 and 0.0076mg/ml respectively. The components of the essential oils of *O. kilimandscharicum* was separated and identified by GC-MS. The analysis resulted to nineteen compounds in which the most efficacious was most probably methyleugenol. The findings of this research could enable investigation of the active compound against *An. gambiae* and further exploration for large scale production (synthesis) of the synergists for commercial application. Since biological products are biodegradable as opposed to convectional pyrethrum synergist, piperonylbutoxide (PBO) currently being used by the Pyrethrum Board of Kenya.

Keywords*:* Lamiaceae, Essential oil composition, Synergist

INTRODUCTION

The history of insecticidal synergists originated with attempts to enhance the potency of pyrethrins (Philogene, 1993). This discovery initiated the use of insecticide synergists in search for better compounds. The discovery of *methylenedioxyphenyl* synergist started with realization that, the synergistic activity of sesame oil was due to the *sesamin* (**1)** and *sesamolin* (**2)** components. Synthesis and testing of related compounds led to sulfoxide, propylisome, tropital and piperonylbuoxide (**3**). *Propynylphosphonates* and certain amides such as MGK264 *(N-Octylbicycloheptenedicarboximide)* (**4**) were also effective. The shifting of compound for effectiveness, economics and toxicology led only to two major synergists for practical use, piperonylbutoxide and MGK264 (Casida & Quistad, 1995). Safrole (**5**) a main component of sassafras oil obtained from *Sassafras albiduma* plant of Lauraceae family is known for its synergist activity. Safrole (**5**) is the precursor for piperonylbutoxide, a synergist commonly used with pyrethrums (Dewick, 2002).

Many plant species including pyrethrum, *Artemisia* and essential oils from plants 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 synergistic or additive effects which may have better effects on the mosquitoes.



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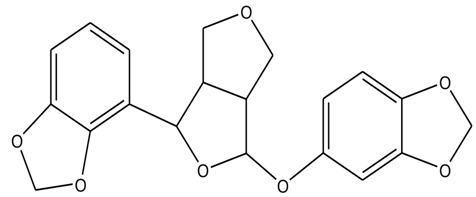

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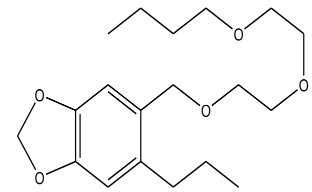

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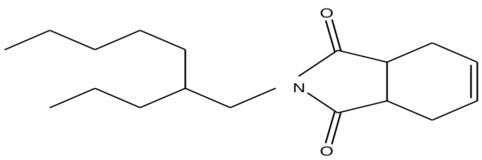

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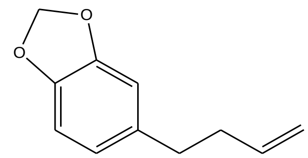

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Figure 1 Structures of insecticidal synergists;

Piperonylbutoxide (**3**), 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 (**3**) has determined that it can pose a distinct health risk when it becomes airborne (Kumar *et al.,* 2002). In addition, when used as a synergist or 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 leaves of higher plants. A synergist which is cheap, readily available and environmentally friendly is needed and many botanicals are readily available. Piperonylbutoxide is the most commonly used synergist for pyrethrum and pyrethroids and has a unique mode of action **(**Casida, 1970). Insects have in-built, complex systems that always attack an insecticide once it enters the insect body. Mixed Function Oxidases (MFO's) is one of the defense mechanisms. MFO's work by binding with the insecticide active site thereby renders it ineffective. When PBO is present in an insecticide, it binds with the MFO's, thus making the insecticide available to do its job (Metcalf *et al.,* 2002).

The genus Ocimum in the family Lamiaceae has tropical distribution with nearly two-third of the 160 species reported from West Africa and the remaining one-third from Asia and America. India is represented by nine species of Ocimum, mainly confined to tropical and peninsular regions. *O. kilimandscharicum* commonly known as African Blue Basil and '*okita'* in native language, is a perennial, under shrub with simple ovate-oblong leaves.Flowers are light purplish or white. Seeds are oblong, black to brown. *O. kilimandscharicum* is an exotic West African species. The plant has carminative, stimulant, antipyretic, anti-fungal and anti-bacterial properties Camphor is a major component of essential oil of *O. kilimandscharicum*.

The biological activity of camphor against the beetles, *Sitophilusgranarius, S. zeamais, Triboliumcastaneum* and *Prostephanustruncatus,* has been evaluated by Obeng-Ofori *et al.,* (1998) and found camphor to be effective when used in contact toxicity, grain treatment and repellency assays. According to their experimental findings, camphor applied either topically, impregnated on filter papers or whole wheat and maize grains was highly toxic to all the four species. nother study done, also reported that essential oils from *O. kilimandscharicum* and *O. suave* have remarkable knockdown effects (30-50%) when used as repellent against *Anopheles arabiensis, An. gambiae* and *Culexquinquefasciatus.* (Seyoum *et al.,* 2006).

MATERIALS AND METHODS

Collection of Plant Specimens

The plant specimens were obtained from Kipkaren in Nandi County, which is situated in the western part of Rift Valley Province. It borders Kakamega to the north-west, Uasin Gishu district to the north-east, Kericho district to the South-East, Kisumu district to the South-East, and Vihiga district to the west. The plant was identified by a plant taxonomist in the Department of Botany, University of Eldoret.

Preparation of extracts
The fresh plant leaves of the *O. kilimandscharicum* (1000 g) was subjected to hydro-distillation using Clevenger type apparatus for 4 hours. The oil was collected and dried over anhydrous sodium sulphate and stored in sealed vials at -4°C until analysis. The oil yield was 0.4% v/w.

Bioassay
Bioassay on 4th instar larvae and adult *An. gambiae* were conducted according to WHO guidelines. Methods for testing larvicidal action of the crude extracts were slightly modified from those of World Health Organization (WHO, 1996).

Gas chromatography-mass spectrometry*:*
The composition of the essential oils was determined using an Agilent 7890A Gas Chromatography – Mass Spectrometry instrument from Government chemist-Nairobi. Helium at 25cm/sec (0.73ml/min) was used as a carrier gas, and hydrogen was used for the flame. The GC conditions used were as follows: capillary column; fused silica (polydimethylsiloxane, 0.25 μm film thickness); temperature program: 70 °C for 8 min, 75 – 230 °C for 3 min, 230 – 240 °C for 5 min, 270 °C 5 min; carrier gas, Helium at 5 bar, linear velocity of 25cm/sec (0.73ml/min); injection port splitless at 250 °C; injection volume, 0.1 μL. The MS conditions were as follows: ionization EI at 70 eV; m/z range, 30-300 °C; scan rate 1 sec-1; ionization chamber at 180 °C; and transfer line at 280°C.

Identification of the components:
The identification of the essential oil constituents was based on a comparison of their retention times, and these constituents were further identified and authenticated using their MS library search (NIST and WILEY), and by comparison with MS literature data (Adams, 2007)

RESULTS
Larvicidal bioassay
Results presented in Table 2 shows that larval mortality varied greatly as varied concentrations of crude extracts of essential oil were used. The results on effects of the synergized mixture on the knockdown and mortality rates on 3-4 days old adult *An. gambiae* were shown in Table 4. Chemical composition and retention times of the essential oil of leaves of *O. kilimandscharicum* were presented in Table 3. The constituents listed in order of their elution decreasing order on the column.

Table 2. Different concentrations of the essential oil on 4th instar larvae compared with pyrethrins during a period of 1hr till 24hours

Tests	Concentration	Number Exposed	Number Dead			Mean	% Mort
			Rep.1	Rep.2	Rep.3		
Dist. Water (N-control)	0	20	0	0	0	0	0
Pyrethrins	0.03	20	20	20	20	20	100
Pyrethrins	0.02	20	14	15	13	14	70
Pyrethrins	0.01	20	11	10	12	11	55
Pyrethrins	0.005	20	5	6	7	6	30
Pyrethrins with A	0.03	20	20	20	20	20	100
Pyrethrins with A	0.02	20	19	18	17	18	90
Pyrethrins with A	0.01	20	15	16	16	15.6	78
Pyrethrins with A	0.005	20	11	10	12	11	55

KEY: A = *O. kilimandscharicum*

DISCUSSION
Bioassay
The essential oils from the extract showed different activities, with *O. kilimandscharicum* being more potent than the pyrethrines alone for all treatment periods and concentrations as shown in Table 1. Results on comparative effect of different concentrations of the synergistic mixture on mortality of adult mosquitoes were presented in Table 2. The table showed that combination of pyrethrins with *Ocimum* was much more effective on mosquito kill than pyrethrins alone thus comparison by an alternative method was desirable. Bioassay data was evaluated by regression and probit analysis and used to determine the lethal doses (LC_{50} and LC_{90}) for the synergist's mixtures. The results showed that LC_{50} and LC_{90} values for *O. kilimandscharicum* were 0.00167 and 0.0076 mg/ml.

Table 4. Knockdown and Mortality rates for different concentrations of the synergized mixtures/solutions on Adult An. *gambiae* in the first 3minutes till 24hours

Test	Conc.	No.		KD		Mean%			Mort	Rep3	Mean	%
mixture	mg/ml	Exp	Rep1	Rep2	Rep3			Rep1	Rexp2			
Py	0.1	20	14	15	15	14.7	73	20	20	20	20	100
	0.01	20	10	9	12	10.3	51	20	20	20	20	100
	0.001	20	6	5	8	6.3	32	20	20	20	20	100
Py/A	0.1	20	14	15	14	14.3	71	20	20	20	20	100
	0.01	20	14	12	14	13.3	66	20	20	20	20	100
	0.001	20	10	11	9	10	50	20	20	20	20	100
C	0	20	0	0	0	0	0	0	0	0	0	0
	0.1	20	20	20	20	20	20	20	20	20	20	100
Py/D	0.01	20	20	19	20	20	20	20	20	20	20	100
	0.001	20	18	20	20	19.3	96	20	20	2 0	20	100

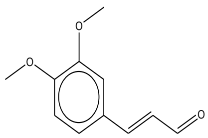
KEY: Py - Pyrethtru; C - Distilled water (negative control); Py/A - Py/*ocimum*; Py/D - Py/PBO (positive)

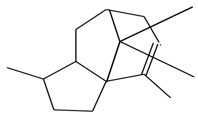
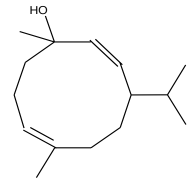
Table 3: Chemical composition of essential oil as found in *Ocimum kilimandscharicum*

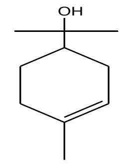
Essential oils Composition	RT in min
propenal, 3-(3,4-dimetho xyphenyl) (**6**)	16.518
α –Pathchoulene (**7**)	14.461
Germacrene D-4-ol (**8**)	14.365
α Terpineol (**9**)	14.149
Muurolene-ɣ(**10)**	13.473
(-)-Germacrene D **(11**)	13.039
Methyl Eugenol (**12**)	12.604
Eugenol (**13**)	12.504
Alfa-Copaene (**14**)	11.805
o-Menth-8- ene,4-isopropylidene-1-vinyl (**15**)	11.253
Linalool (**16**)	11.055
Estragol (**17**)	9.265
Terpinenol -4-ol (**18**)	8.985
ß-Ocimene (**19**)	7.393
ß-pinene (**20**)	7.171
Cyclohexene, 1-methyl-4-(1-methylidene) (**21**)	6.874
Cadinene δ (**22**)	6.678

GC MS Analysis

The separation and identification of the compounds in *O. Kilimandscharicum* resulted into seventeen compounds. Among them with the functional group *methylenedioxyphenyl* were; 2-propenal, 3-(3,4-dimetho xyphenyl) (**6**), Methyl Eugenol (**12**), Eugenol (**13**) and Estragol (**21**). Another different study by Isman (2006) and Tripathi *et al*., (2009) also indicated that some compounds found in the essential oil, included anethole (**23**), elemicine **(24**), estragole (**17**), methyl eugenols (**12**), methylene dioxy Compounds in (Figure 1), apiole (**25**), and myristicine (**26**). A similar study by Bhasin, M., (2012) showed that the essential oil obtained by hydro-distillation of the leaves of *Ocimum kilimandscharicum* Guerke, was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Forty-one constituents were identified. Chemical composition of the essential oil of *O. kilimandscharicum* were reported from India where the major constituents were linalool (**16**), (41.94-58.85%), methyleugenol **(12)** (17.0-15.82%) and apiole (**29**) (10.18-6.38%).





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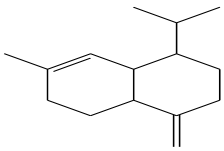
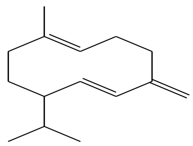
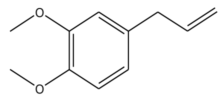

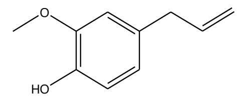

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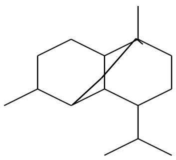
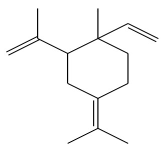
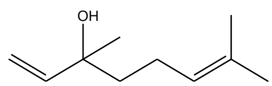

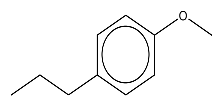

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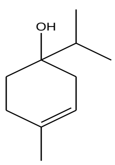
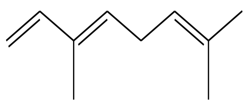
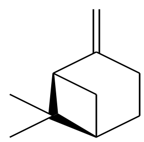
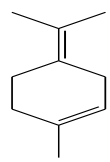
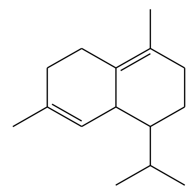
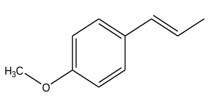
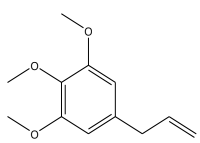


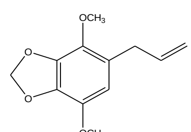





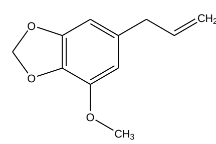
18 **19** **20** **21** **22**

Figure 2. Structures for the oil constituents of *O. kilimandscharicum* oil









23 **24** **25** **26**

A comparison of a similar study by Joshi, R.K., (2013) showed that above compounds were the most common constituents of *O. kilimandscharicum.*

CONCLUSION

A comparison of activities of pyrethrins and the crude extracts (essential oil) on 4th instar larvae of *A. gambiae*, showed that *O. kilimandscharicum* was effective for all treatment periods. An increase in concentration of the synergists caused a significant ($P<0.05$) increase in mortalities of larvae.

A comparison of the efficacy of pyrethrins combined with crude extracts (synergists) against Conventional pyrethrum synergist PBO, showed that PBO was still the most potent although non- biodegradable or synthetic. However, the use of biological synergists is inevitable since are available and environmentally friendly hence would promoting green economy.

Analysis and characterization of the more potent synergist, *Ocimum kilimandscharicum* resulted to nineteen constituents with majority being monoterpenes (C10) and mainly phenylpropane. which are potential sources of synergists for pyrethrins. Structures that bear a functional group *methylenedioxyphenyl*, basic framework of chemical structure within which likelihood of the compound with synergistic activity is predicted.

RECOMMENDATIONS

The findings of this research would enable further exploration for large scale production of botanicals synergists for commercial application.

Focus should be on the development of synergists that would give the most economical control of each intended stage of species, since synergist was observed to be more effective on adult stage than the larval stage.

There is a need to find out the most economical way for oil extraction since distillation was rather an expensive and a long process.

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