Toxicity of Smoke from Pelletized Leaf Powder and Essential Oil of *Hyptis suaveolens* (L.) Poit (Wild Spikenard) Against *Anopheles gambiae* (Giles, 1902) (Diptera; Culicidae)

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Abstract

Mosquitoes are the most important single group of insect vectors of the causative parasites of several diseases including malaria, dengue and filariasis for which cheap, safe, locally available and environmentally benign control measures are being sought. This study aimed at determining the toxicity of smoke from pelletized leaf powder and essential oil of *Hyptis suaveolens* (L.) against third instar larvae and 0-2 day old adult *Anopheles gambiae* respectively in the laboratory. Test larvae were exposed to various concentrations ranging from 0ppm (control) through 50 to 500ppm of leaf essential oil at ambient laboratory temperature ($25\pm 1^{\circ}\text{C}$) in standard bioassay for 96 hrs using Complete Randomized Block Design. Larval mortality was recorded at 24, 48, 72 and 96 hours of exposure. For exposure to pellets, 0-2 day old adult test insects were exposed to smoke from smoldering pellets having concentrations: 0% (Control), 20, 40, 60, 80 and 100% in glass fumigation chamber where the test smoke and insects were contained. Data from quantal responses were subjected to Probit analyses for determination of lethal concentration values. Results showed that essential oil and smoke from smoldering pellet were toxic to both larvae and adults at all concentrations respectively. The computed 24 hr- LC_{50} of test essential oil against larvae is 1448 ppm; 48 hr- LC_{50} 551.47 ppm, 72 hrs LC_{50} is 187.43 ppm and 96 hours LC_{50} is 142.10 ppm, respectively. The insecticidal pellet LC_{50} at 30, 60, 90 and 120 minutes values were 107.79%, 69.56%, 31.27% and 22.15%, respectively. The results show that *H. suaveolens* could be exploited as a plant based insecticide for the control of *Anopheles* mosquitoes and suggests the need for further work including field studies.

Keywords: *Anopheles gambiae*, *Hyptis suaveolens*, Quantal response, Probit analysis, Biopesticide,

INTRODUCTION

There has been increased interest in exploring a wide diversity of plant natural products for use in the control of insect pests by researchers. Studies carried out have also focused on the

determination of insecticidal potency and bioactivity of locally available plant species used as various formulations including powders, solvent (including ethanol, methanol, Petroleum ether) extracts, fixed oils, essential oils of leaves, stem bark, roots, fruits, seeds and so of the candidate plant species (Denloye *et al.*, 2009; Oyewole, *et al.*, 2010). Many of the

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plant species that form the focused of explorations have leveraged on the availability and uses of the plants by local communities (Ibidapo, 2005; Denloye, *et al.*, 2009). Exploiting these local plant species may offer reliable alternatives to the synthetic insecticides widely acclaimed to contain noxious compounds that are toxic against humans and non-target species generally, expensive, not readily available and requiring complex technical know-how for their correct application. It is widely speculated that many local plants are yet to be explored for their insecticidal potency.

Among the plethora of plant species which are used against insects that have received little attention with attendant dearth of reports is the Wild Spikenad, *Hyptis suaveolens* L. (Poi) 1806. Like many plants of the world, *H. suaveolens* may have a rich store of phytochemicals that could be used in future control programs against mosquitoes and other insect pests of public health importance. Its leaf and branches are traditionally used as insect repellents (Lobani *et al.*, 2011).

Mosquitoes have earned prominence over centuries for their nuisance value and vectorial capabilities in the transmission of deadly diseases including malaria, dengue fever, yellow fever, chikungunya, encephalitis, filariasis, and Zika Virus Disease (Fradin and Day, 2002). In Nigeria, Anopheles mosquitoes have become the focus of control strategies to break the transmission cycle of Malaria, just as it is globally done in the fight against the disease. The six primary vectors of malaria in Nigeria are *Anopheles culicifacies*, *An. stephensi*, *An. fluviatilis*, *An. minimus*, *An. girus*, and *An. gambiae* (Amajoh *et al.*, 2002). These species are responsible for transmission in specific ecotypes, and of these; *Anopheles culicifacies* is squarely responsible for transmission of about 60-70% of the malaria in rural plains and peri-urban areas.

Mosquito control have often focused on the

use of synthetic insecticides which are mostly complex toxic compounds with adverse impacts on non-target species; such as pesticide poisoning, bioaccumulation in fatty tissues and so on (National Resource Council, 1983; Kutz *et al.*, 1991) the need for technical expertise and equipment for effective application and so on. These aftermaths of synthetic insecticides use against mosquitoes have become worrisome and therefore provoked the need for the use of environmentally safe, cheap, locally available and easy-to-use agents to achieve the control of these vectors. This need has generated interests in searching inwards for locally available plant materials to exploit as possible sources of the desired agents of the control of these mosquitoes and tackle the malaria scourge. There is a dearth of studies on the mosquitocidal activity of *H. suaveolens*, and the few available reports indicate palpable information gap that need to be closed for dependable conclusions on the possible insecticidal uses of *H. suaveolens*. Ivoke *et al.* (2009) assayed *H. suaveolens* against freshly laid eggs and larval instars of *An. gambiae* complex and reported that the test extracts significantly reduced egg viability and that they respectively demonstrated significant larval toxicity. Arivoli *et al.* (2011) evaluated *H. suaveolens* extracts for larvicidal, adult emergence inhibition and ovicidal activity against *An. stephensi* and two other species of mosquitoes (*Culex* and *Aedes*). The study showed that *H. suaveolens* extracts had effective bioactivity against the insects and that *An. stephensi* was the least susceptible of the three test mosquito species. Also, Ayangae-Kaa *et al.* (2015) reported that the dried leaf extract of *H. suaveolens* showed toxicity against the eggs of *An. gambiae* than it did to either the larvae or pupae, and thus emphasizing the ovicidal efficacy of *H. suaveolens* extract.

The foregoing highlights indicate that gaps exist in *H. suaveolens* insecticidal studies for example none of the studies has reported on the chemical constituents of *H. suaveolens* tested and

no study has shown the efficacy of smoke from pellets of the dried leaves in Nigeria, but an earlier report by Denloye *et al* (2006) documenting the efficacy of smoke from the dried leaves of the Baobab tree (*Adansonia digitata*) had indicated the need to explore the use of smoke from pellets as insect control strategy. The present study was therefore, aimed at determining the toxicity of leaf essential oil vapour and pelletized dried leaves of *H. suaveolens* against adults or larvae of *An. gambiae* under ambient laboratory conditions.

MATERIALS AND METHODS

Preparation of Test Plant Materials

Leaves of *Hyptis suaveolens* were obtained from Badagry area of Lagos State. The plant sample was identified at the herbarium of the Botany Department, University of Lagos by Dr Nodza with voucher number of LUH 8176. The leaves were plucked, rinsed under running tap water and air-dried on the laboratory bench at temperature of $27 \pm 1^\circ\text{C}$ and 75% relative humidity for 10 days. The dried leaves were pulverized using Sonik® SB 1212 model electric blender and stored in air-tight container until when needed.

Extraction of Test Essential oil

Essential oil was extracted from 500 g pulverized *Hyptis suaveolens* leaves by hydrodistillation for 4-6 hours in a Clavenger apparatus (Kasali *et al.*, 2006). The oil was collected over hexane and stored in the refrigerator at 4°C using amber airtight glass vials to prevent evaporation and ensure photo stability.

Preparation of Pellets

Insecticidal pellets were prepared from the test leaf powder blended with pulverized wood shavings using Renew® a common commercial laundry starch following the method of

Tawatsin *et al* (2002). Pellets were prepared by mixing 0.2 g *H. suaveolens* leaf powder with 0.8 g of pulverized wood shaving and 2 mls of Renew® starch as a binder to make 20% concentration. The mixture was stirred for two minutes and moulded into 1.0 g pellet. The same procedure was used for all other test pellet concentrations namely 40, 60, 80 and 100%. The control was prepared with 1.0 g of pulverized wood shaving and starch only.

GC-MS Analysis of *H. suaveolens* for Chemical Constituents

The chemical constituents of test *H. suaveolens* essential oil was determined by means of Gas chromatograph/mass spectroscopy (GC/MS) following standard procedure previously described by Denloye *et al* (2009).

Test Insects

Anopheles gambiae sl eggs obtained from the insectary of Nigeria Institute of Medical Research (NIMR), Yaba, Lagos were incubated in the Pest Management Laboratory at Education Trust Fund (ETF) Building, Lagos State University where they hatched into larvae and subsequently used to raise a fresh stock culture of the test insect. The eggs were incubated in opaque glass cabinets and upon hatching they were maintained on plankton collected from with plankton net from the University Fish Hatchery. Adult males and females that emerged were maintained on 10% glucose solution and blood meal respectively from human volunteers who were given prophylactic antimalaria treatment two weeks before the experiments. The volunteers also gave their written consent before the commencement of the experiment.

Toxicity of *Hyptis suaveolens* pellet smoke against adult test Mosquitoes

Twenty (0-2 day old) adult *An. gambiae* sl were introduced into experimental laboratory glass chambered (40×40×40 cm) square cages. A

20% concentration pellet was ignited with lighter and mounted on a hanger inside the cage which was then covered to avoid the smoke escaping. The number of mosquitoes knocked down or dead was recorded at 30, 60, 90 and 120min of exposure. Mosquitoes found on the cage floor unable to fly but moving their legs, antennae or any body part were counted as knocked down. Similarly, mosquitoes that were knocked down and unable to move any body part upon gentle prodding of the abdomen with a fine brush brittle were counted as dead and used for quantal response computations. The same procedure was used to set up similar experiments using 40, 60, 80 or 100% pellets, respectively. In each case control was set up using pellets without *H. suaveolens* leaf powder. All experiments and control were made with four replicates, respectively.

Toxicity of *Hyptis suaveolens* essential oil against *An. gambiae* larvae

Stock solution of *H. suaveolens* essential oil was prepared with 1ml of the oil with 0.3 mls of hexane in 100 mls of water. The hexane in the mixture served as diluent to enhance miscibility of the essential oil in water. Serial dilutions of the stock solution were prepared to obtain 10, 50, 100, 200, 300, 400 and 500 ppm for larvicidal bioassay following the procedure of WHO (2005) For larvicidal assay 20 third instar larvae were introduced into each disposable transparent cup containing 10, 100, 200, 300, 400 and 500 ppm of *H. suaveolens* essential oil. The control set up contained only distilled water and hexane in proportion 1000 ml to 0.3 ml, each experiment and control was replicated three times and arranged in Completely Randomized Block Design. Mortality was recorded after 24, 48, 72 and 96 hrs, respectively. Larvae were considered dead when they floated, drowned or failed to move any part of their body with gentle swirling of the exposure cup.

Data Analyses

Analyses of dose response data on mortality of test mosquitoes was carried out using Environmental Protection Agency (EPA) Probit Analysis computer software following correction for mortality as a result of natural occurrence in control set up (Abbot, 1925, Finney, 1971). These analyses were used to compute Lethal Concentrations (LC) values and associated Confidence Limits (CL).

RESULTS

Acute toxicity of *Hyptis suaveolens* against Third Instar larvae of *Anopheles gambiae*

The computed Lethal Concentration (LC) values for the toxicity of *H. suaveolens* against *An. gambiae* are presented in Table 1. The essential oil extract was toxic to the larvae from concentrations 100 to 500 ppm but not toxic at 50 ppm as there was no mortality recorded at the lowest concentration tested.

Toxicity of *Hyptis suaveolens* against adult *Anopheles gambiae* sl

Toxicity test showed that *H. suaveolens* pellet smoke was toxic against adult *Anopheles gambiae*. The computed LC_{50} values of the smouldering pellet against the test insect at 30 mins exposure time was 107.79ppm while it was 22.15ppm at 120 mins. Comparison between the LC_{50} values at 30 and 60 mins exposure gave no significant difference as shown by overlap in the computed Confidence Limits (Table 2).

Chemical Constituents of Test *Hyptis suaveolens* leaf Essential Oil

Gas Chromatography Mass Spectroscopy (GC-MS) analysis gave chromatograph which revealed peaks depicting 13 major compounds in the test essential oil (Figure 1). These peaks

Table 1: Toxicity of *Hyptis suaveolens* leaf essential oil against third Instar Larvae of *Anopheles gambiae*.

Exposure Time (hr)	LC ₅₀ (ppm)	95% Confidence Limits	Slope	Degree of Freedom	Chi-square
24	1448.27	565.09-1580	0.899±0.652	3	3.16
48	551.47	NE	1.064±0.654	3	6.87
72	187.43	16.61-454.18	1.052±0.652	3	1.23
96	142.10	57.28-213.63	1.387±0.662	3	1.23

ppm = Parts per million

NE = Not Estimable, slope not significantly different from 0.

SE = Standard Error

Table 2. Toxicity of *Hyptis suaveolens* pellets smoke against adult *An. gambiae*

Exposure Time (mins)	LC ₅₀ (ppm)	95% Confidence limits	Slope ± SE	Degree of Freedom	Chi-square
30	107.79	74.56-396.41	1.85±0.62	3	0.138
60	69.56	49.92-127.03	1.81±0.57	3	1.321
90	31.27	14.46-42.43	2.07±0.55	3	1.323
120	22.15	10.48-30.72	2.44±0.61	3	1.966

SE = Standard Error

showed that Phenanthrene (27.094%) gave the highest percent occurrence followed by Caryophyllene with 15.102%, Hydrophenanthrene (10.658%), Santolina trienee (9.201%), Carophyllene (8.78%) occurrence while Bicyclo(6.1.0) nonane had 0.907% the lowest occurrence by volume (Table 3).

Fig. 1: Chromatograph from GC/MS Analysis of leaf essential oil of *Hyptis suaveolens*

Table 3: Chemical Constituents of the leaf Essential oil of *Hyptis suaveolens* with their percent composition

SN	Compounds	% Composition
1	Oxime-,methoxy-phenyl-	2.87
2.	Alpha.-cubebene	3.215
3.	Caryophyllene	15.102
4.	Santolinatrienee	9.201
5.	Bicyclo(6.1.0) nonane, 9-(1-methyle	0.907
6.	Bergamotol,Z-alpha-trans-	4.894
7.	3.beta.,17 dihydroxyestr-4-e	3.344
8.	Hydrophenantherene	10.658
9.	Phenanthrene	27.094
10.	4"a-Methyl-8"-methylidene-decahy	5.317
11.	Diazoprogerone	2.878
12.	Caryophyllene	8.878
13.	Heptadeca-1,9-dien-4,6-diyn	5.643

DISCUSSION

Hyptis suaveolens extracts have been shown to have effect on target insects at various stages of their development as demonstrated by Ayange-Kaa *et al* (2015) who worked on effect of dried leaves of *Hyptis suaveolens* on egg, larvae, pupa and adult mosquito and discovered that the eggs showed highest mortality to *Hyptis suaveolens* extracts. The work is equally in line Bobbo *et al* (2016) who assessed the larvicidal activity *H. suaveolens* and *Balanites aegyptiaca* leaves and root extract against mosquito species and discovered that both plants were toxic to mosquito species at all concentrations. The plant equally showed high toxicity to *Anopheles stephensi* and *Culex quinquefasciatus* in hexane extract as shown by Arvoli and Tennyson (2011). The same way this present study demonstrated the toxicity of *H. suaovelens* essential oil and pellets against third instar larvae and 0-2 day old adult *An. gambiae* have been demonstrated in this study.

The result showed that *H. suaovelens* was effective against the test mosquito species in line with reports received from rural dwellers in Badagry area of Lagos State as opposed to mere adherence to traditional practice or folklore. This throws more light on the study reported by Egunyomi *et al.* (2010) that studied 10 popularly used mosquito repellents in Ibadan, Nigeria.

The present study agrees with findings of Conti *et al* (2012) who worked on larvicidal and repellent activity of *H. suaveolens* essential oil against *Aedes albopictus* and discovered that essential oil of *H. suaveolens* had a significant repellent activity and could confer 16 to 135 minutes protection against biting mosquitoes with differences in repellency rates being a function of concentration and time of observation. While larvae mortality range is between 93.33 and 98.33%. same efficacy was also reported by Murugesan *et al.* (2015) reported that leaf extracts of *H. suaveolens* to be highly effective to larvae of *Culex quinquefasciatus*. Also, Govindaraja *et al.* (2012) reported strong larvicidal effect of *H. suavoelens* against the same species. The foregoing therefore support the findings in this study.

Equally effect of pellets and plant based coils have shown to be similar in their mode of action; this have been demonstrated by Tawatsin *et al* (2002); since pellets and coils are among the most common household insecticide products used for personal protection against mosquitoes and other biting dipterans.

The larvicidal and repellency of *H. suavoelens* in this study may be due to the activity of the chemical constituents of the test plant species. The chemical constituents obtained by GC/MS analysis in this study are comparable to those reported by earlier workers such as Benelli *et al* (2012) who reported 50 compounds found in *H. suavoelens* from

Burkina Faso and Chatri *et al* (2014) that also reported 50 compounds from Indonesia. The differences in number of compounds may be due to ecotype variations since the plant materials used in this study were not sourced from the same locations as the earlier studies by Syamasundar *et al* (2012) who showed variations in volatile oil composition of different wild collections of *Hyptis suaveolens* from Western Ghats of India and discovered that the essential oils showed considerable significant differences in their compositions due to areas of collection. The same was also observed by Lohani *et al* (2011) who worked on variations in essential oil composition and biological activity of *H. suaveolens* from two sites; Raniphokari and Mohand area of India. It is therefore imperative for further studies on the chemical analysis of the plant's oil extract, insecticidal activities and its mode of action.

In conclusion, this study makes obvious the potential of *H. suaveolens* as a mosquito control agent for rural dwellers of Badagry LGA, Lagos, Nigeria. Further studies be carried out to determine the constituent of *H. suaveolens* that possess mosquitocidal properties for further exploitation.

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