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Effects of Steam Extract of Essential Oil of the Leaves of *Hyptis Suaveolens* L. Poit (Bush Mint) On *Anopheles* Mosquito.

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Abstract
Mosquitoes are important causes of human ailments that could lead to morbidity and mortality. The control of its infestation with synthetic chemical insecticides have been hazardous to man and the environment. A fresh leaf of Heptis suaveolens essential oil was extracted using steam extracting apparatus and a colourless liquid with a greenish tinge was gotten. The physicochemical property of the oil showed, low specific gravity at 25^0C to be 0.67, Saponification value of 191 mgKOH/g , Refractive index at 25^0C was determined to be 1.39, Iodine Value was found to be 114.5 and acid value of 3.30 mgKOH/g. The dour of the essential oil was poignant and an unstable liquid at room temperature. The phytochemical screening showed marked presence of tannin, saponins, alkaloids, rotenone, glycosides, steroids and others are sparingly present substances like the triterpenoids, Terpernoids and phenol. The exposed anopheles mosquito showed increase in mortality with increase in concentration in all metamorphosis stages. The LC_{50} for wild and laboratory mosquito was calculated to be 1.68mgl^{-1} and 1.35mg/ml respectively. While the LC_{50} of the metamorphosis stages of eggs, larva and pupa were recorded as 2.86, 1.75 and 3.65mg/ml respectively. The results of the effects of essential oil on all the life stages of anopheles mosquito showed that it is an important natural product that can effectively control mosquito infestation and associated diseases.

Key words: *Repellant, Mortality, metamorphosis, essential oil, Physicochemical properties and repulsive*

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I. INTRODUCTION

Mosquito infestation and its increasing resistance to different types of insecticides and repellent have been responsible for many human ailments leading to morbidity and mortality (Asekun *et al.,* 2013). Synthetic chemicals in use are becoming less effective aside their associated allergies, other disadvantages include high cost, availability issues in rural areas, and their unfriendly environmental sides among others (WHO, 1986; Chandre *et al.,* 1998; Penilla *et al*., 1998; Doliantis and Sinclair, 2002). *Anopheles stephensi* is one of the most common mosquitoes in Nigeria and the primary vector of malaria (Donald, 2004).

Plants are a rich store-house of phytochemical substances that can serve as precursors for the synthesis of useful drugs and other synthetic chemicals Egunyomi *et al.,* (2010).

Hyptis suaveolens originated from Central America and Tropical South of America (Gratz, 1997*).*The leaves of *H. suaveolens* have been reported in folklore medicine as a stimulant, as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in anti rheumatic, anti suporific baths, and anti fertility agents. Also, applied as an antiseptic in burns, wounds, and various skin complaints (WHO, 1986; George *et al.*, 2014).

Essential oil producing aromatic herbs including *Hyptis* genus has increasingly become a focus of intensive research because of their multiple diverse potential values (WHO.2009; Kaufmann and Briegel. 2004). This informed the need to look for natural sources that will be abundant and cheep to formulate a new anti mosquito agents that can repel or kill the mosquitoes with view to minimize the frequency of irritant incidence of mosquito bites and associated sicknesses (Kaufmann and Briegel 2004).

Insect pest management is facing the economic and ecological challenges worldwide. Due to the human and environmental hazards caused by most of the synthetic pesticidess, allergies and resistance of these pests is increasing hence, the need for the identification of novel effective insecticides to combat this problem (Shaalam *et al*., 2005; Wasserman *et al.*, 2004).

The traditional insecticidal effect of *H. suaveolens* on mosquitoes was observed by mosquitoes moving away from the plant's minty odor and also from the fumes of burning dry leaves of the plant (Jennifer, 2011; Arivoli, and Samuel, 2012). Traditional claim of burnt leaves of *H. Suaveolens* (bush mint), could be as effective as DEET for personal protection against mosquito bites abound (Fang. 2010 and WHO. 2011).

This research is aimed at extracting the essential oil of *H. suaveolens* and determining the LC_{50} of the extract with hope of determining the repellant properties of the plant.

II. MATERIALS AND METHODS

Sample was collected in Nasarawa town, Nasarawa state and taken to Raw Material and Research Development Centre (RMRDC) Idu Karmo, Abuja, where it was identified as *H. suaveolens* (L.) Poit

Two sets of Anopheles mosquitoes were collected (laboratory and wild mosquitoes). The laboratory mosquitoes were obtained by collecting larvae from breeding sites and taken to the laboratory and nurtured to adults in net covered cartons of 1235cm area while the wild mosquitoes were fetched using insect net in dark areas and taken to the laboratory. The samples were fed with wet cubed sugar and kept for the L_{50} and the repellant response (Shaalam, 2005)

Extraction of Essential Oil From the Fresh Leaves of *H. suaveolens*.

Fresh leaves of *H. suaveolens* was weighed (500g) and heated in water at 100^0c. Oil and water vapour evaporated and condensed in the condensation chamber and cooled. The essential oil was removed by density differentials with the condensed steam using a separating funnel leaving the pure oil residue. The essential oil was covered because of its volatility and kept for the mortality and repellant assay.

Pilot Study was done to Determined the Definitive concentrations of the *H. suaveolens e*ssential Oil for the definitive test.

Determination of Lethal Concentration (LC_{50}) of Eggs, Lava, Pupa and Adult Anopheles Mosquito

The steam extract of essential oil of *H. suaveolens* was exposed to adult laboratory, wild and the metamorphosis stages of Anopheles Mosquito. 100 mosquitoes were placed in 1225cm3 carton that all the sides were removed leaving 5cm at the edge to hold them in place. It was then covered with a net to allow for observation. The soaked essential oil of *H. suaveolens* were placed on all the sides and wet sugar cube was placed at the centre. Mosquito falling on the white paper without coming up are recorded per time. The number dead are then plotted on the mortality probit kill table to determine the LC_{50}. For the eggs, larva and pupa the extracts were introduced into different concentrations of the extract and observed for continuation of metamorphosis (Fang, 2010).

These were done to all the concentrations of the essential oil in three replications.

III. Results

Phytochemical Constituents of Leaves of *Hyptis suaveolens* (Bush Mint)Essential Oil.

Phytochemical Constituent	Status
Alkaloids	++
Steroid/Triterpenoids	+
Glycosides	++
Terpenoids	++
Phenol	++
Tannin	+
Saponin	++
Flavonoids	++

Key

+ = present in small amount

++ = Present in moderate amount

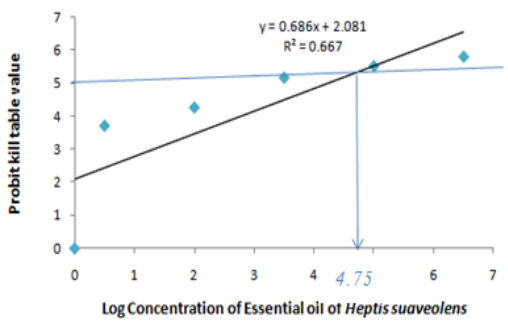


Figure.1 LC_{50} anopheles eggs exposed *H. suaveolens*

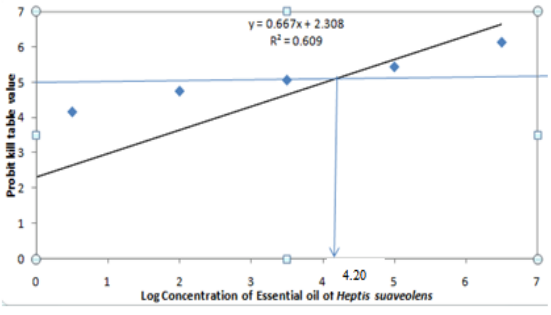
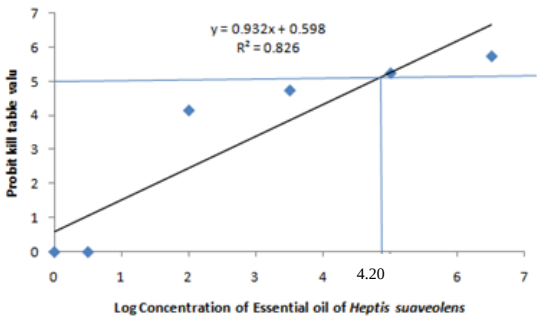


Figure.2. LC_{50} Larva of anopheles exposed to *H.suaveolens*



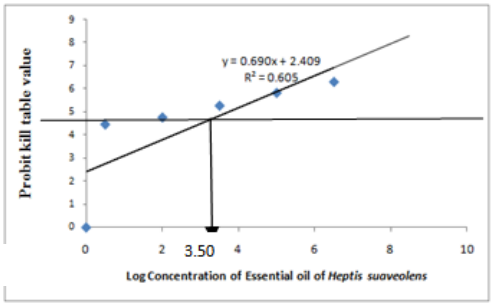


Figure.3 LC_{50} of Pupa Anopheles exposed to *H. suaveolens* Figure.4. Laboratory Adult mosquito exposed to *H.suaveolens*

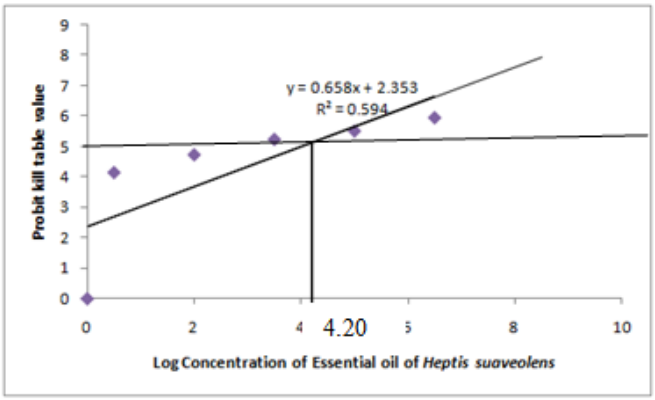


Figure.5. wild Adult mosquito exposed to *H.suaveolens*

IV. DISCUSSION

The physicochemical property of the oil showed, low specific gravity at 25^0C to be 0.67, Saponification value of 191 mgKOH/g , Refractive index at 25^0C was determined to be 1.39, Iodine Value was found to be 114.5 and acid value of 3.30 mgKOH/g. these values are similar to the findings of [14] on the bioassay of herbal mosquito repellent formulated from the essential oil of plants

The phytochemical screening showed marked presence of tannin, saponins, alkaloids, rotenone, glycosides, steroids and others are sparingly present substances like the triterpenoids, Terpernoids and phenol the result agrees with the works of [16] on evaluation of extracts & oils of insect repellant plants from Sweden and Guinea – Bissau.

The eggs of anopheles mosquito as represented in Figure1 was determine to have the LC_{50} of 4.83mgl^{-1} .With increase in concentrations of the essential oil of *H. suaveolens* the mortality of the eggs increases as the eggs fail to develop into lava. This finding is similar to the works of [15] on their work on Larvicidal activity of the essential oils of methanol extracts of Malaysian plants on *Aedes aegypti* even though the LC_{50} of the plants were lower. Furthermore, the findings of [9] and [13]on their separate works on Comparative efficacy of insect repellants against mosquito bite.

Figure.2 showed the larvicidal activity of the essential oil of *H. suaveolens* was determined at 4.20mg/ml. Mortality showed increases with increase in concentration. This agreed with the work of [1]in the research on the effect of dried leaves extract of *H. suaveolens* on various stages of mosquito development in Benue State, Nigeria

The pupa level of development in figure.3 showed the LC_{50} to be 4.85mg/ml this revealed a higher value compared to those of eggs and lava however, had a similar pattern of mortality increasing with increase in concentration of the essential oil of *H. suaveolens* this could be due the fragile state of these stages [2][13].

The effect of different concentrations of essential oil of *H. suaveolens* on the mortality of adult mosquito showed in figure 4 and 5. on laboratory hatched *Anopheles* mosquitoes and the wild mosquito were determined to be LC_{50} of 3.50mgml and 4.20mg/ml respectively. The higher concentration the essential oil on wild mosquito could be due to acquired resistance to other chemicals in the environment[12].

The vulnerability of the developmental stages showed that *H. suaveolens* can be targeted more effectively at this stages while the general low concentration of all the stages show that the essential oil of *H.*

suaveolens is a suitable anti mosquito infestation and a possible subtitude to environmentally hazardous synthetic aerosols.

V. CONCLUSION

The use of the steam extract of the essential oil of *Heptis suaveolens* is an effectice anti mosquito agent that can be used to control the insect's infestation and by extension control the may sicknesses associated by it.

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