

COMPARATIVE ASSESSMENT OF TOXICITY OF *Hyptis suaveolens* AND *Balanites aegyptiaca* ROOT AND LEAVES METHANOL EXTRACTS AGAINST FOURTH INSTAR MOSQUITO LARVAE

¹Bobbo, A.A., ²Pukuma, M.S., ³Nganjiwa, J.I. and ⁴Daniel, L.

¹Department of Animal Health College of Agriculture Science and Technology, Jalingo, Taraba state, Nigeria.

²Department of Zoology, Modibbo Adama University, Yola, Adamawa State, Nigeria

³Department of Science. Laboratory. Technology, Ramat Polytechnic Maiduguri, Borno State, Nigeria

⁴Department of Public Health, Ministry of Health and Human Services, P.M.B 2070 Yola, Adamawa

Corresponding Author's Email Address: - bobbo_ahmed@yahoo.com

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ABSTRACT

The use of chemical insecticides to control mosquitoes result in environment hazards and development of resistance in vector species. This research work is about an alternative control method that is considered as safe to environment and non-target species and also bio-degradable. Hence, the toxicity effect of *Hyptis suaveolens* and *Balanites aegyptiaca* root and leaves methanol extracts against 4th instar mosquitoes larvae were investigated. Twenty group of fourth instar larvae were exposed to various concentrations of 2, 4, 6, 8 and 10ppm, mortality was observed and recorded after 24 hours and larvicidal bioassay was conducted according to WHO protocol. Results are mean of three replicates. Percentage mortality was calculated using Abbott's formula, while the L was determined by a log dosage probit mortality regression line and mean $\pm$ standard deviation was obtained, Significant decision was made at $p < 0.05$. The result revealed that, *Balanites aegyptiaca* methanol leaves extracts exhibited the highest mortality rate of 75.00% $\pm$ 1.00 at 10ppm and LC50 achieved at 1.69 $\pm$ 1.25ppm than the *Hyptis suaveolens* leaves with 55.00% $\pm$ 1.00 mortality and LC50 at 1.75 $\pm$ 0.15ppm, while *B. aegyptiaca* root possesses the highest mortality rate of 63.33% $\pm$ 1.15 compared to *H.suaveolens* root methanol extracts with 55.00% $\pm$ 1.00 as mortality. Findings revealed that mortality was concentration dependent and there were significant difference between dependent and responses at $p < 0.05$. Leaves extracts also showed better mortality rate compared to the root extracts. Both the experimental plants contained some potent of toxicity effect on larvae which might be suitable alternative to chemical larvicide.

Keyword- *Hyptis suaveolens*, *Balanites aegyptiaca*, larvicidal properties, mosquito

INTRODUCTION

Mosquitoes are vector for various disease including malaria, yellow fever, filariasis Japanese encephalitis and chikungunya. Among these mosquito borne diseases dengue fever dengue hemaorrhagic fever, yellow fever and chikungunya are endemic in Southeast Asia and Africa (Maillard *et al.*, 1993). These disease are transmitted by *Aedes aegypti* (Linn.). One of the methods available for controlling the mosquitoes is use of synthetic insecticides. Mosquitoes develop genetic resistance to synthetic insecticides (Wattel *et al.*, 1981) and even to biopesticides such as *Bacillus sphaericus* (Tabashnik, 1994). Synthetic insecticides also adversely affect the environment by contaminating air, water, and soil. There is an urgent need to find

alternatives to the synthetic insecticides which are more potent and low-cost.

Plants are a rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are ecofriendly (Sukumar *et al.*, 1991). Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemical may act as insecticides, antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants. Plant based pesticides are less

toxic, delay the development of resistance because of its new structure and easily biodegradable (Ignacimuthu, 2000). Plant based products do not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponine (Wiseman and Chapagain, 2005), steroids (Chowdhury et al., 2008; Ghosh et al., 2008), isoflavonoids (Joseph et al., 2004), essential oils (Cavakanti et al., 2004), alkaloids and tannins (Khanna and Kannabiran, 2007) have potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitos (Yang et al., 2004).

Balanites aegyptiaca (L.) Del., is a perennial tropical plant used in food preparations and herbal medicine, especially in Africa and some developing countries. It is also called desert date (English), adua (Hausa, Nigeria), tanni (Fulfulde, Nigeria) and heglig (Arabic). *B. aegyptiaca* belongs to the family *Balanitaceae*. The plant attains a height of more than 6 meters. It has a multiplicity of uses and almost every part of the plant is useful including, leaves, thorns, bark of root and fruit. *B. aegyptiaca* is used to treat so many illnesses including, diarrhoea, hemorrhoid, stomach aches, jaundice, yellow fever, syphilis and used as a laxative (Ojo et al., 2006). For instance, the fruit is used to treat liver disease and as a purgative and sucked by school children as a confectionary in some countries (Barley, 1962 and Croach, 1962). The bark is used in the treatment of syphilis, round worm infections and as a fish poison. The aqueous leaf extract and saponins isolated from its kernel cakes have anti-bacterial activity (Doughari et al., 2007) and potent larvicidal activity (Zarroug et al., 1988), respectively. Studies conducted elsewhere on some parts of the plant indicated the presence of many flavanoids, saponins and other important phytochemicals (Maksoud and Al-Hadidi, 1988). The alcoholic extract of the pulp and kernel contained sterols, terpenes and saponins as predominant compounds whereas tannins, alkaloids and resins were found in slightly small amount (Abdel-Rahi et al., 1986).

Hyptis suaveolens (L.) Poit is a potent medicinal herb commonly called as 'American mint' in English it found in West Africa especially Northern Nigeria, Philippines and Tropical America.

Hyptis suaveolens (L.Poit) commonly called bush mint, bush tea, pignut, is known as vilayati tulsi in hindi; konda thulasi in Telugu; bhustrena in Sanskrit; daddoya-tadaji in Hausa; efiri in Yoruba; nchuanwu in Ibo; tanmotswangi-eba in Nupe and kachu-kachugha in Fulfulde. Pharmacological properties of this plant includes antioxidant (Rajiv, 2010) antimicrobial (Satish et al., 2010), antibacterial (Kavitha and Satish, 2010), anticancer (Mudgai et al., 1997), antiinflammatory, antiseptic (Shenoy et al., 2009) and antimalarial (Ziegler et al., 2002). Phytochemical constituents of *Hyptis suaveolens* include alkaloids, terpenes, phenols, tannins and steroids (Gills, 1992). The plant gained increasing attention when it exhibited insecticidal properties (Peerzada, 1997). The plant showed feeding deterrent (Simmond and Blanney, 1992) and insect repellent activity (Oparaeke et al., 2002). Olotuah, (2013) pointed out that methanol extract of *Hyptis suaveolens* possessed insecticidal activity against *Sitophilus oryzae*, *Sitophilus zeamais* and *Callosobruchus maculatus*. In East and West Africa, plants of the *Hyptis* genus are commonly utilized against mosquitoes. In Guinea Bissau (West Africa), it was demonstrated that smoldering leaves of *Hyptis suaveolens* showed repellent activity beyond 80%, whereas for fresh leaves the repellent activity was greater than 70% (Palsson and Jackson, 1999). The plant also exhibits larvicidal activity against *Aedes aegypti* (Cavalcanti et al., 2004) and *Culex quinquefasciatus* (Okigbo et al., 2010). Further, the plant was also screened for larvicidal, adult emergence inhibition and ovicidal activity against vector mosquito's viz., *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Arivoli and Samuel, 2011).

METHODOLOGY

Two different plants were used for the study i.e. *Hyptis suaveolens* (bush tea) and *Balanites aegyptiaca* (Balanitaceae) root and leaves. The plant parts were collected in and around Jalingo metropolis, and were authenticated by a Taxonomist from the Department of Plant Science MAUTECH.

Collection and Rearing of Larvae

Larvae of mosquitoes were collected from available stagnant water within and around Jalingo metropolis using dipper and pipette as described (Adeleke et al., 2008) and was transported to the

biochemistry laboratory of the Modiddo Adama University, Yola. Larvae were reared in a rearing tray containing tap water and cover by fine nylon mesh. The larvae were feed with food containing mixture of cabin biscuit and yeast until the 4th instar larvae was reached.

Preparations of stock solution and test concentration

The leaves and roots of the plant were shaded dried, pulverized and sieved to get a fine powder from which the extract was prepared. Methanol extract of the plant were obtained by taking 200g of the powder leaves and root in a separate container and 200ml of the solvent was added. The cap vial was screwed and shaken vigorously to dissolved or dispersed the material in the solvent. The mixture was then be filtered through Whitman filter paper and the filtrate was then evaporated under reduced temperature at 50°C on a water bath dryness to obtain the crude extract. The stock solution was diluted separately (WHO, 2005).

Larvicidal Bioassay

Larvicidal bioassay was done according to a standard procedure provided by World Health Organization; Guideline for laboratory and field testing of mosquito larvicide (WHO, 2005). Twenty 4th instar larvae were transferred by means of strainer or droppers to small disposable test cups or vessels each containing 200ml of water. The depth of the water in the cups was maintained between 5cm and 10cm. Exactly 0.2ml of the stock was then added to 200ml in the cups to obtain the desire target dosage starting with the lowest concentration of 2ppm, 4ppm, 6ppm, 8ppm and 10ppm respectively. Three replicates for each concentration and equal number of control were simultaneously set up with tap water, to which 1 ml of the solvent added. Larval food was then added to each test cup. After 24hr exposure, larval mortality was then recorded. Moribund larvae were counted as dead larvae for calculating percentage mortality. The result was then recorded on the data recording forms. If the control mortality is between 5% and 20%, the mortalities of the treated groups were corrected according to Abbott's formula (Abbott, 1925)

Precautionary Measures to prevent larval Mortality

A broad surfaced rearing container was used with intermittent food supply, food was provided in a finely crushed and suspended form, while Scum formation was reduced by using a glass rod to regularly stir the water so as to enhance aeration.

Data Analysis

LC50 value was calculated from a log dosage, probit mortality regression line at 95% confidence level (CL) of upper confidence limit (UCL) and lower confidence level (LCL). Using Abbot's formula percentage mortality was calculated and the mean $\pm$ standard deviation presented. Analysis of variance (ANOVA) was used to determine the significant differences between treatments. Regression analysis was used to determine the relationship between concentrations and mortality rate. Results with $p < 0.05$ will be considered statistical significance.

RESULTS AND DISCUSSION

Toxicity effect of Methanol Leaf Extracts on 4th Instar Larvae of Mosquito

Figure 1 shows the percentage mortality of the 4th instar larvae of mosquito after 24hrs of Exposure to various concentrations of methanol extracts of the two plants. *Balanites aegyptiaca* leaves showed 26.67 $\pm$ 1.15, 43.33 $\pm$ 1.53, 48.33 $\pm$ 0.58, 56.33 $\pm$ 1.15 and 75.00 $\pm$ 1.00 percentage mortality when exposed to various concentrations of 2ppm, 4ppm, 6ppm, 8ppm and 10ppm respectively. The 10ppm revealed the highest mortality rate of 75.00% and at 2ppm 26.67% was recorded as mortality after 24hrs exposure. LC50 was achieved at 1.65PPM (Figure1). The *Hyptis suaveolens* recorded 30.00 $\pm$ 1.00, 35.00 $\pm$ 1.00, 38.33 $\pm$ 0.58, 46.67 $\pm$ 1.15 and 55.00 $\pm$ 1.73 as percentage mortality for same concentrations of 2ppm, 4ppm, 6ppm, 8ppm and 10ppm respectively. The lowest mortality of 30.00% at 2ppm recorded while the highest mortality rate of 55.00% at 10ppm recorded and LC50 was achieved at 1.75ppm respectively.

Toxicity effect of Methanol Root Extracts of 4th Instar larvae of Mosquito

Table 2 and (Figure2) Show the results of percentage mortality of Methanol root extracts of two experimental plants parts on the 4th instar larvae. *Balanites aegyptiaca* showed 30.00 $\pm$ 1.00,

43.33±0.58, 46.67±1.15, 48.33±0.58 and 63.33±1.15 as percentage mortality when exposed to various concentrations of 2ppm, 4ppm, 6ppm, 8ppm and 10ppm while *Hyptis suaveolens* possess 23.33±0.58, 38.33±0.58, 46.67±1.53, 53.33±0.58 and 55.00 as percentage mortality for same

concentration of 2ppm, 4ppm, 6ppm, 8ppm and 10ppm respectively *Balanites aegyptiaca* has the highest mortality of 63.33% at 10 ppm and LC50 was achieved at 1.70ppm and 23.33% at 2ppm was recorded.

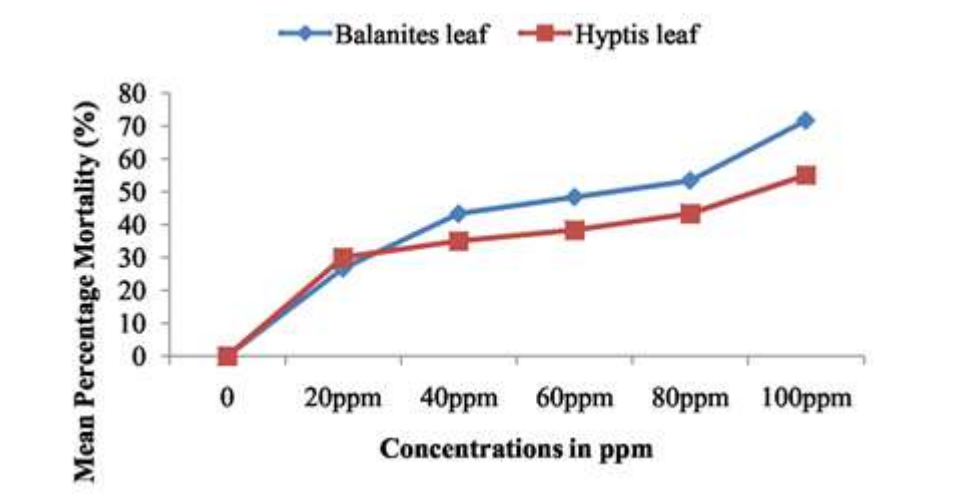


Figure. 1. Comparison of *Balanites* and *Hyptis* leaf Methanol extracts against the larvae of mosquito

Table 1. Percentage Mortality of twenty Mosquito Larvae (20) Exposed for 24 hours to Different Concentrations of Methanol Leaf Extracts in Parts Per Million (ppm).

Concentrations in ppm	0	2	4	6	8	10	LC50
Plant part							
Bal leaf	0±0.00 ^a	26.67±1.15 ^a	43.33±1.53 ^a	48.33±0.58 ^a	56.33±1.15 ^a	75.00±1.00 ^a	1.65±0.34
Hyp leaf	0±0.00 ^a	30.00±1.00 ^b	35.00±1.00 ^b	38.33±0.58 ^b	46.67±1.15 ^b	55.00±1.73 ^b	1.75±0.15

Bal = *Balanites aegyptiaca*, Hyp = *Hyptis suaveolens* Values with same superscript on the same column are not significantly different from each other

From the result above methanol leaves of *Hyptis suaveolens* and *Balanites aegyptiaca* were found to have some toxicity effects against mosquito larvae at various concentration in parts per million (ppm). The toxicity effects were found to be higher against the fourth instar larvae at the highest concentration rate of 10ppm. This is in accordance with the result Rawani et al., 2009 that the higher mortality rate was recorded with increase in concentration against mosquito

species. The result revealed that maximum toxicity effects of 75.00% mortality was observed in *B. aegyptiaca* methanol leaf extract at 10ppm, and LC50 was achieved at 1.65 ± 0.34ppm, while 55.00% was observed in *H. suaveolens* methanol leaf extracts and LC50 was achieved at 1.75 ± 0.15 ppm, this results showed that *B. aegyptiaca* was more active as a larvicide compared to *H. suaveolens*. Result showed significant difference between *B. aegyptiaca* and

H.suaveolens leaf. Finding is in agreement with (Adogoke *et al.*, 2013) who reported that methanol leaf extract exhibit high mortality rate on larvae, but this is contrary to the findings of

Murugesan *et al.*, 2015 who reported that aetone exhibited the highest larvicidal properties. This variation could be due to the method of phytochemical extraction and plant location

Table 2: Percentage Mortality of twenty Mosquito Larvae (20) Exposed for 24 hours to Different Concentration

Plant part	Concentrations in ppm						LC50
	0	2	4	6	8	10	
Bal root	0±0.00 ^a	30.00±1.00 a	43.33±0.58 a	46.67±1.15 a	48.33±0.58 b	63.33±1.15 a	1.70±0.11
Hyp root	0±0.00 a	23.33±0.58 ^b	38.33±0.58 a	46.67±1.53 a	53.33±0.58 a	55.00±1.73 a	1.75±0.15

Bal = *Balanites aegyptiaca*, Hyp = *Hyptis suaveolens*, Values with the superscript on the same column are not significantly different from each other

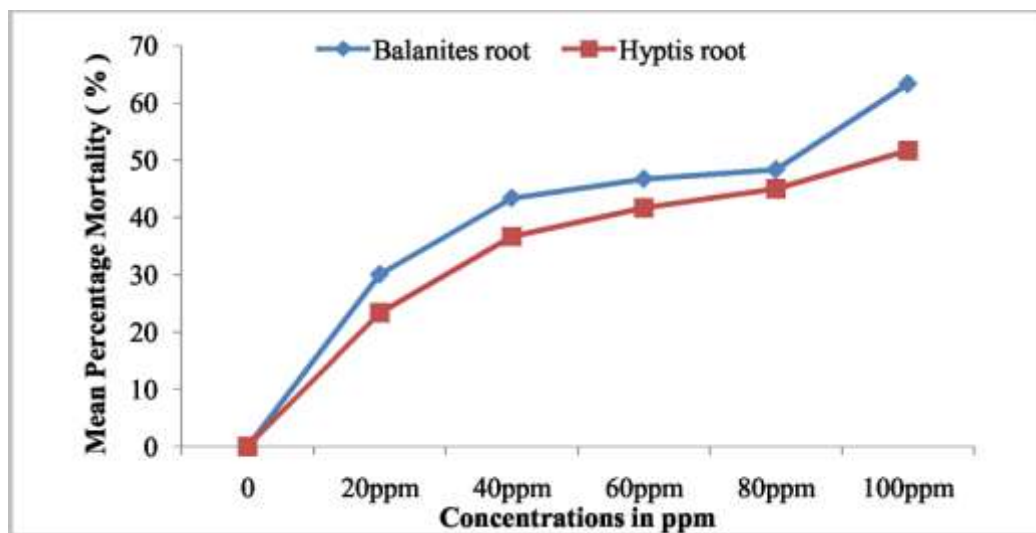


Fig. 2. Comparison of Balanites and Hyptis root Methanol extracts against the larvae of mosquito at different concentrations in ppm

Also in Table 2 and figure 2 above, *Balanite. aegyptiaca* methanol root extracts exhibited the highest mortality rate of $63.33 \pm 1.15\%$ at 10 ppm and LC50 was achieved at 1.70 ± 0.11 ppm, while *H.suaveolens* methanol root had the lowest mortality rate of $55.00 \pm 1.00\%$ with LC50 at 1.69 ± 1.25 . The results showed that, *B.aegyptiaca* methanol root exhibited the highest toxicity effect. These findings is in accord

with the findings of Nganjiwa *et al.*, 2015, who revealed that the highest mortality was recorded in *B.aegyptiaca* as compared to other experimental plants. Several studies have shown that tissue of *B. aegyptiaca* plant contain high amount of saponins , therefore the high rate of mortality observed in all tissues may be attributed to the presence of saponin compound (Liu and Nakanishi, 1982; Kamel *et al.*, 1991; Farid *et al.*,

2002). This is also agrees with the findings of (Wiesman *et al.*, (2006) that, saponins extracted from fruits of *B. aegyptiaca* shows 100% larvicidal activity against *Aedes agypti* mosquito larvae. Result shows no significant deference between the comparison of methanol root extracts $p < 0.05$ at 10ppm. In comparing the relative toxicity of Both the leaves and the root of the experimental plants parts revealed some

larvicidal tendencies at different concentration, however, *B. aegyptiaca* roots and leaves are considered more potent in larvicidal properties compared to *H. suaveolens* roots and leaves. In conclusion, the extracts of the two plant species offer potential tools that could supplement currently available control techniques for mosquito larvae.

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