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Assessment of Larvicidal Properties of Some Plant Extracts against Third Instar Larvae of Mosquito Species

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Abstract:
The larvicidal properties of methanolic and ethanolic leaves and root extract of Balanites aegyptiaca, Calotropis procera and Eucalyptus globulus against the third instar larvae of mosquito was investigated. 20 batches of larvae were exposed for 24hrs to concentrations of 2,4,6,8 and 10ppm respectively. Larval mortality was observed in all the concentrations at various degrees with the exception of the control treatment. Methanolic leaf extract of E. globulus at 2ppm show the least mortality rate of 31.67% and an LC50 value of 6.39ppm, while B. aegyptiaca leaf exhibit the highest mortality of 68.33% with LC50 value of 6.03ppm. The percentage mortality of ethanolic extract of B. aegyptiaca was highest with 73.33% and LC50 value of 5.70ppm while E. globulus leaf was least with 36.67% and LC50 value of 6.03ppm. The methanolic root extract of B. aegyptiaca also shows the highest mortality rate of 75% and an LC50 value of 5.65ppm, E. globulus account for the least mortality rate of 55% and LC50 value of 6.92. Mortality rate of ethanolic root extract of B. aegyptiaca was highest with a value of 78.33% and LC50 value of 5.49ppm while E. globulus root exhibits the least rate with 65% and LC50 value of 6.03ppm. This study reveals that ethanolic extract B. aegyptiaca root is more potent as a larvicide as compared to Calotropis procera and Eucalyptus globulus.

Keywords: *Balanites aegyptiaca, calotropis procera, eucalyptus globulus, larvicidal properties*

1. Introduction

Mosquitoes are the vectors of a number of human and zoonotic disease pathogen affecting human and animal hosts, including those that cause malaria, filariasis, Japanese encephalitis (JE), dengue and yellow fevers. In view of the fact that mosquitoes develop genetic resistance to synthetic insecticides and even to bio pesticides such as Bacillus sphaericus the application of easily degradable botanicals for the control of mosquitoes is recommended. Mosquitoes are cosmopolitan and transmit a wide range of diseases (Mullen and Durden, 2009). Continued and repeated use of conventional mosquitocides such as organophosphorus (op) and carbmate insecticides, insect growth regulators and bacterial larvicides has often resulted in the widespread development of resistance and has undesirable effects on non-target organisms(Rozendaal,1997; WHO,2006) In particular, the use of conventional synthetic insecticides for the control of horticultural and veterinary pest has accelerated these adverse effects, these includes public concern for the environmental effects of insecticides, groundwater contamination, human health effects and undesirable effects on non-target organisms (Rozendaal,1997 ;Cooper,1991). Botanical biocides are relatively harmless to non-target organisms and present little risk to users and consumers (Satti et al., 2004)

Despite recent effort at malaria control, the disease transmitted by female anopheles mosquitoes still kills up to 800,000 worldwide, with over 90% of the death still in sub-Saharan Africa (WHO, 2010). More than 90% of the Nigerian population is at risk of stable malaria transmission with huge economic loss (FMOH,2010).

Balanites aegyptiaca Del, also known as “desert date” in English, a member of the family zygophyllaceae, is one of the most common wild plant species of the dry land areas of Africa and south Asia (Hall and Walker, 1991). The tree can grow to 6-10 meters in heights, is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture. The tree has thick, tough glossy leaves, spiny branches, a double root system and produces date-like fruits. The plants grow extensively even when neglected (Mohammed and Spiess, 2000). It can successfully grow in a marginal sand dome with saline and sewage water (Bishnu and Zee, 2005). Various parts of Balanites tree have been used for folk medicine in many regions of Africa and Asia (Hall and Walker, 1991; Iwu, 1993; Newinger, 1996). A literature survey has revealed antifeedent, antidiabetic, molluscicide, antihelminthic, and contraceptive activities of various Balanites extracts, [Ibrahim, 1992; Roa et al., 1997). Most of

the studies reported the active compounds to be saponins. Saponins are amphiphilic glycosidic compounds and a hydrophilic sugar chain. Saponins are freely soluble in both organic solvents and water (Bishnu and Zee, 2005).

Calotropis procera belong to the family Asclepiadaceae which is commonly known as giant swallow wort, milkweed, which grows in tropical region (Grace, 2006). This plant is a native of Asia and Africa (Rahman and Wilcock, 1991). It height is 2 to 4 meters but it may sometimes reach 6 meters (Grace, 2006). Milkweed is adapted to hot and dry climates and it can tolerate drought but prefers location that have 150 – 100mm rainfall (Ghias et al., 2012). Calotropis procera is able to grow in a wide range of soil such as alkaline and saline soils, but it prefers sandy soils. Parts of this plant, especially the root and bark are applied for the treatment of variety of illness including, malaria and snake bite (Ghasemi et al., 2012)]. It has been reported that leaves extracts of this plant have great nematicidal effect (Ghasemi et al., 2012). The latex of C. procera plants has important indigenous medicinal uses because of its purgative, antisyphilitic and antiodontalgic action (Ghias et al., 2012). Satti et al.(2004) also reveal that Asclepiadaceae was among the important flora families with several bioactive components.

Eucalyptus globulus belong to the family mrytaceae, commonly referred to as Tasmanian Blue Gum. It is one of the most widely planted genera, fast growing evergreen tree, bearing pendant leaves, native to Tasmania and south-east Australia (Akolade et al., 2012). This species is the most widely introduced and has been established in many countries including Nigeria (Akolade et al., 2012). Apart from its extensive use in the pulp industry, it also produces an Oleum eucalyptus that is extracted on commercial scale in many countries as raw materials in perfumery, cosmetics, food, beverages, aromatherapy and phytotherapy (Akolade et al., 2012). Eucalyptus globules leaves have potent action against Culex guinquefasciatus and Culex tritaeniorhynchus [Monza et al., 1994). The aim of this study is therefore; to assess the larvicidal properties of some plant leaves and roots extracts against third instar larvae of mosquito species.

2. Materials and Methods

2.1. Collection of Plant Materials

The leaves and roots of Balanites aegyptiaca (Balanitaceae) were collected from and around Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. The plant parts were authenticated by the department of Plant Science of the institution. Voucher specimens were deposited at the herbarium of the department.

2.2. Preparation of the Stock Solution and Test Concentration

The leaves and roots of the plant material was shade dried, pulverized and sieved to get a fine powder from which the extract was prepared. Methanol and ethanol extract of the plant were obtained by taking 200mg of the powdered leaves and roots in a separate container and added 20ml of the solvent. The cap vial was screwed and shaken vigorously to dissolve or disperse the material in the solvent. The mixture was then filtered through Whatman filter paper and the filtrate was evaporated under reduced pressure for 24hrs on a water bath to obtain the crude extract. The stock solution was serially diluted in ethanol and methanol separately (2ml solution to 18ml solvent). Test concentration was obtained by adding 0.1 – 10ml of the appropriate dilution to 200ml distilled water(WHO, 2005).

2.3. Collection and Rearing of Larvae

Larvae of mosquitoes were collected from any available stagnant water within Moddibo Adama University of Technology and transported to the laboratory of the institution. Larvae was reared in a plastic bowl containing tap water and covered by fine nylon mesh. The larvae were feed with food containing mixture of cabin biscuit and dried yeast until 3rd and 4th instar larvae was reached (WHO, 2005; Adeleke et al., 2008).

2.4. Larvicidal Bioassay

The larvicidal activity of the plants crude extracts was assessed according to method recommended by WHO (WHO, 2005), with slight modifications. 20 third and fourth instar larvae was transferred by means of a 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. 0.2ml of the stock was then added to 200ml in the cups to obtain the desired 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 1ml of the solvent was added. Larval food was then added to each test cup. After 24 hours 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 will be corrected according to Abbott's formula Corrected Mortality (%) = X 100. Where X= percentage survived in the untreated control and Y percentage survival in the treated sample (Abbot, 1925).

2.5. Data Analysis

LC50 value was calculated from a log dosage – probit mortality regression line at 95%Cl of upper confidence limit (UCL) and lower confidence level (LCL). Using a computer software programme, standard deviation of the mean LC50 values were calculated. Result with P< 0.05was considered statistically significant. Percentage mortality = $\frac{number\ of\ dead\ larvae}{number\ of\ introduced}$ x 100

3. Results

3.1. Larvicidal Properties of Methanolic Leaf Extract on Third Instar Larvae of Mosquito.

The percentage mortality of twenty 3rd instar larvae of mosquito species exposed for 24hrs to different concentrations of methanolic leaf extract of the three plant species and the larvicidal properties of the plants are shown on table 1. Eucalyptus leaves has 31.67% as the lowest mortality at 2ppm and the highest percentage mortality of 55% on the third instar larvae of mosquito. In Balanites leaf extract, the highest mortality rate of 68.33% was recorded in 10 ppm and the least mortality of 38.33% was in 2 ppm concentration. Similarly, highest mortality of 61.67% was recorded for 10 ppm concentration of Calotropis leaf extract whiles the least concentration at 2 ppm was 36.67% on third instar larvae. The LC50 values for Eucalyptus, Balanites and Calotropis were 6.39, 6.03 and 6.31ppm respectively.

3.2. Larvicidal Properties of Ethanolic Leaf Extracts on Third Instar Larvae of Mosquito.

Table 2 present the percentage mortality of third instar larvae of mosquito species which were exposed to different concentrations of ethanolic extract of Eucalyptus, Balanites and Calotropis leaves in part per million for 24 hours. The mortality rate was found to be highest with a value of 73.33% at 10ppm concentration for Balanites and lowest with a value of 36.67% for Eucalyptus at 2ppm concentration. The LC50 values for Eucalyptus, Balanites and Calotropis are 6.03, 5.70 and 5.75 respectively. No mortality was recorded in the control treatment.

3.3. Larvicidal Properties of Methanolic Root Extracts on Third Instar Larvae of Mosquito.

The percentage mortality and larvicidal properties of methanol root extract on the third instar larvae of Mosquito species are presented in Table 3. The mortality rate for the root extract of the three plant species range from the lowest value of 26.67% to the highest of 75%. Balanites root extract exhibited the highest mortality rate of 75% at 10ppm, while Eucalyptus shows the lowest mortality rate of 26.67% at 2ppm concentration. Balanites achieved the LC50 at the lowest value of 5.65ppm as compared to Eucalyptus and Calotropis. No mortality was recorded for the control

3.4. Larvicidal Properties of Ethanolic Root Extract on Third Instar Larvae of Mosquito.

Table 4 shows the mortality rate and larvicidal properties of all the root extracts of the three plant species (Eucalyptus, Balanites and Calotropis). At 2ppm concentration, Balanites and Calotropis show 35% mortality rate while Eucalyptus root extract was 31.67% mortality rate. The mortality rate ranges between 31.67% and 68.33% for all the three plants' root extract. Balanites root extract recorded the highest mortality rate followed by Calotropis and Eucalyptus root extract with the corresponding values of 78.33, 66.67 and 65% respectively. The LC50 values for Eucalyptus, Balanites and Calotropis are 6.03, 5.49 and 5.75ppm respectively.

Plants	0	2	Concentration in ppm 4	6	8	10	LC50
Eu Leave	0±0.00[a]	31.67±0.89[a]	45.00± 0.05[a]	48.33±0.64[a]	51.67±0.89[a]	55.00±0.64[a]	6.39
Ba Leave	0±0.00[a]	38.33±0.77[b]	41.67±0.75[b]	45.00±1.00[a]	50.00±0.66[a]	68.33±0.10[b]	6.03
Cal Leave	0±0.00[a]	36.67±0.89[b]	41.67±0.64[b]	51.67±0.97[b]	60.00±1.22[b]	61.67±1.53[c]	6.31

Table 1: Percentage Mortality of 3rd Instar Larvae of Mosquito species (20 larvae) exposed for 24 hours to Different Concentrations of Methanol Leaf Extracts in Part Per Million (ppm) of Three Different Plant Species.

Key:
Eu = *Eucalyptus globulus*
Ba = *Balanites aegyptiaca*
Ca = *Calotropis procera*
Values with the same superscript on the same column are not significantly different from each other and vice versa at $p<0.05$. Values are mean of 3 replicates.

plant	0	2	Concentration in ppm 4	6	8	10	LC50
Eu Leave	0±0.00[a]	36.67±0.89[a]	46.67±0.77[a]	50.00±1.00[a]	53.33±0.89[a]	66.67±0.09[a]	6.03
Ba Leave	0±0.00[a]	43.33±1.09[b]	46.67±0.89[a]	50.00±1.00[a]	55.00±0.00[a]	73.33±0.00[b]	5.70
Cal Leave	0±0.00[a]	43.33±1.89[b]	50.00±1.00[b]	51.67±0.77[a]	55.00±0.00[a]	65.00±1.58[a]	5.75

Table 2: Percentage mortality of 3rd Instar Larvae of mosquito (20 larvae) exposed for 24 hours to Different Concentrations of Ethanol Leaf Extracts in Part Per Millions of Three Different Plant Species.

Concentration (ppm)	0	2	4	6	8	10	LC50
Eu root	0.00±0.00[a]	26.67±1.89[a]	33.33±0.89[a]	41.67±0.77[a]	46.67±0.64[a]	55.00±0.00[a]	6.92
Ba root	0.00±0.00[a]	33.33±0.89[b]	38.33±0.41[b]	51.67±1.28[b]	56.67±1.28[b]	75.00±0.41[b]	5.65
Cal root	0.00±0.00[a]	31.67±0.88[b]	36.67±0.77[a]	51.67±1.64[b]	61.67±0.41[c]	61.67±0.66[c]	5.75

Table 3: Percentage mortality of 3rd Instar Larvae of mosquito (20 larvae) exposed for 24 hours to Different Concentrations of Methanol Root Extracts in Part Per Million.

plant	0	2	4	6	8	10	LC50
Eu root	0±0.00 [a]	31.67±0.89 [a]	40.00±0.01 [a]	50.00±0.02 [a]	53.33±0.67 [a]	65.00±1.00 [a]	6.03
Ba root	0±0.00 [a]	35.00±0.00 [b]	50.00±0.10 [b]	53.33±1.64 [b]	60.00±0.66 [b]	78.33±1.56 [b]	5.49
Cal root	0±0.00 [a]	35.00±0.00 [b]	38.33±1.64 [a]	56.67±0.88 [c]	58.33±0.77 [b]	66.67±0.77 [a]	5.75

Table 4: Percentage mortality of 3rd Instar Larvae of Mosquito (20 larvae) Exposed for 24 hours to Different Concentrations of Ethanol Root Extracts in Part Per Millions.

4. Discussion

Use of larvicidals against mosquitoes is an old method of control (Fillinger et al., 2004) and has of late been brought back on the market due to need of alternatives from harmful sprays (Bagavan et al., 2009; Kamaraj et al., 2009). Along the same line, the revival of research on plant-based pesticides over the last few decades responds to recognition of a need to replace harmful, non-selective and environmentally unfriendly synthetic insecticides and pesticides some of which have already been internationally banned. In this study, the methanol and ethanol leaf and root extract of three plant species, Eucalyptus globulus, Balanites aegyptiaca and Calotropis procera were found generally to have some larvicidal properties against mosquito larvae at various concentrations in part per millions as shown in Table 1 to 4. At the highest concentration rate of 10 ppm, the larvicidal activity was found to be higher against the third instar larvae. This is in accordance with the report of Senthilnathan (2007), that the higher the leaf extract of Eucalyptus tereticornis oil with increased doses on Anopheles stephensi, the greater the larvicidal effect. He also observed that first and second instar larvae were most susceptible to all treatments.

In this present study, Table 1 showed that the methanol leaf extracts of Balanites resulted in maximum activity on third instar larvae of mosquito with the mortality rate of 68.33%. The LC50 was 6.03ppm for Balanites plant which indicated that it is more potent than Calotropis methanol leaf extract and Eucalyptus methanol leaf extract LC50 which are 6.31 and 6.39 ppm respectively. Table 2 showed a similar trend in larvicidal activity of Balanites causing the highest rate of mortality to the third instar larvae of mosquito recording the maximum value of 73.33% mortality compared to Calotropis and Eucalyptus ethanol leaf extract with the respective values of 65 and 66.67% mortality rate. This corresponds with the LC50 value of Balanites, Calotropis and Eucalyptus which are 5.70, 5.75 and 6.03 ppm respectively. This shows that the higher the mortality rate, the lower the LC50 value. It also means that at lower concentration of Balanites extract, 50% mortality was recorded at 5.70 ppm compared to 5.75 and 6.03 ppm. In both Table 1 and 2, the ethanol leaf extract of Balanites was recorded to be of highest larvicidal potency than the methanol leaf extract of Calotropis and Eucalyptus plant which is in agreement with the findings of James et al. (2007), who reported that ethanol extraction is an effective method for processing plant extracts. The ethanolic extract of Balanites leaf causes higher mortality of mosquito larvae than the other plants leaf extract.

Similarly, in the methanol and ethanol root extract of the three plant species, the ethanol root extract of Balanites displayed more larvicidal activity as it account for 68.33% mortality rate which is the highest as compared to Calotropis and Eucalyptus root extract with 66.67 and 65% mortality respectively (Table 3 and 4). The lowest LC50 of 5.65 ppm was recorded in Balanites root extract while Eucalyptus root extract recorded the highest LC50 of 6.92 ppm. This indicates that, Balanites is more potent as larvicide than Eucalyptus and Calotropis root extract (Table 3). The effects of the ethanol and methanol extracts of Balanites, Eucalyptus and Calotropis on the mortality of the third instar larvae as presented in Tables 3 to 4 clearly showed that the ethanol extract of Balanites can be a good larvicides to synthetic larvicides which are not environmentally friendly. The LC50 values recorded for Balanites, Eucalyptus and Calotropis root extract are 5.49, 6.03, and 5.75 ppm respectively. The lowest value indicated more larvicidal activity as found in Balanites root extract than 6.03 ppm in Eucalyptus root extract

5. Conclusion

The results of this study showed that the ethanolic extract of Balanites aegyptiaca root at 10ppm concentration induced the best significantly mortality rate of mosquito larvae, LC50 was achieved at the lowest concentration as compared to the roots and leaves of Calotropis procera and Eucalyptus globulus methanolic extract under laboratory condition. The properties of the mentioned extract were attributed to potent secondary metabolites in the plants. The extract should therefore, be assessed under field conditions for a wider larval control utilization.

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