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*Full Length Research Paper*

# Evaluating the Larvicidal Efficacy of *Lepidagathis alopecuroides* and *Azadirachta* indica Against *Anopheles gambiae* and *Culex quinquefasciatus*

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**Comparative analysis of the larvicidal properties of aqueous extracts of leaves of *Lepidagathis alopecuroides* and *Azadirachta indica* (neem) was carried out on *Anopheles gambiae* and *Culex quinquefasciatus.* Assays showed that *L. alopecuroides* was more toxic to both larvae, while *C. quinquefasciatus* was more susceptible to extracts of both plants. For extracts from 500 mg of leaves in 1 L of water, the lethal time ($LT_{50}$) for *C. quinquefasciatus* and *A. gambiae* with *L. alopecuroides* extract was in the ratio 1:4.5, while it was 1:21.8 with neem extract. No mortality was recorded in *A. gambiae* exposed to neem at all the concentrations until the emergence of the adult. The results suggest that *L. alopecuroides* is more potent than neem and could be developed as a cheap, effective and renewable resource that could be incorporated into the Roll Back Malaria program in Nigeria and other countries.**

**Key words:** *Lepidagathis alopecuroides, Azadirachta indica, Culex quinquefasciatus, Anopheles gambiae,* larvicide.

## INTRODUCTION

Different strategies have been devised to reduce the prevalence of malaria and other mosquito-borne diseases in endemic regions of the world. Biological control at the larval stage of development of mosquitoes is one of the techniques which affords a cheap, easy to use, and environment friendly method of malaria control. Natural insecticides are less phytotoxic and do not accumulate chemical residues in flora, fauna and soil. Phytochemicals with mosquito larvicidal activity occur in the oils, leaves and roots of plants (Sharma et al., 1998; Ojewole and Shode, 2000; Sosan et al., 2001). An excellent review of the activity of neem, *Azadirachta indica,* and other plants with proven mosquito control potential has been made (Mittal and Subbarao, 2003).

The shrub *Lepidagathis alopecuroides* (Vahl) belonging to the family Acanthaceae possesses strong piscicidal activity against mudskipper, *Periophthalmus papillio* (Obomanu, unpublished data). Many piscicides also possess insecticidal properties. We now report our findings on the larvicidal activity of *Lepidagathis alopecuroides* and neem on *Culex quinquefasciatus,* and the malaria vector *Anopheles gambiae.* The use of crude aqueous plant extracts for the investigation was to ensure easy adaptability of the research findings by the local people.

## MATERIALS AND METHODS

### Collection of mosquito larvae

Larvae of *C. quinquefasciatus* and *A. gambiae* were collected from pools of water and gutters respectively around the Federal Housing Estate in Port Harcourt and stored in transparent buckets covered

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**Table 1.** Lethal concentrations (LCs) of *L. alopecuroides* on (a) *C. quinquefasciatus* (b) *A. gambiae* and (c) neem on *C. quinquefasciatus* (LC+95% confidence intervals).

| Time (min) | Lethal concentrations (LC) | | |
|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | $LC_{95}$ |
| **(a) *L. alopecuroides* on *C.quinquefaciatus*** | | | |
| 10 | 384.69(306.39-533.47 | 774.39(594.91-1398.44) | 884.87(667.46-1652.90) |
| 15 | 199.29(150.45-236.23) | 673.25(590.65-806.68) | 807.61(699.71-984.11) |
| 20 | -0.87(-370.49-73.84) | 168.68(110.47-256.15) | 216.75(166.08-388.56) |
| **(b) *L. alopecuroides* on *A. gambiae*** | | | |
| 30 | 409.77(375.78-453.94) | 831.08(732.03-985.17) | 950.46(830.27-1138.52) |
| 70 | 166.38(-24461.62-300.11) | 472.69(328.68-52935.98) | 557.52(383.9074830.00) |
| 110 | 26.00(-289.32-62.58) | 100.81(68.33-122.17) | 122.02(106.32-202.45) |
| **(c) Neem on *C.quinquefaciatus*** | | | |
| 105 | 1479.43(897.8033154.97) | 3120.56(1737.9279273.45) | 3585.80(1975.2392348.26) |
| 165 | 300.00(184.78-415.22) | 1569.77(1072.66-3764.16) | 1929.73(1294.19-4743.71) |
| 225 | 47.19(-9.09-69.94) | 127.44(113.67-150.87) | 150.19(132.83-189.45) |

with nets at room temperature (28 ± 2$^{O}$C) in the laboratory.

#### Extraction procedure

Weighed fresh leaves of *L. alopecuroides* (0.1, 0.2, 0.3, 0.4 and 0.5 g) were added to 400 ml of de-ionized water (pH 7.0) and ground in a sterile blender. After filtering, the residue was washed with more water (200 ml) and filtered. Each of the filtrates thus obtained from 100, 200, 300, 400 and 500 mg of the leaves was made up to 1 L to give the extracts Aa, Ba, Ca, Da, and Ea, respectively. The procedure was repeated with leaves of neem to give the corresponding extracts Ai, Bi, Ci, Di, and Ei.

#### Bioassay

Each test solution comprised thirty larvae of a particular mosquito in 300 ml of the plant extract in a beaker. Five replicates were set up for each concentration. Mortality was observed at 5 min intervals and recorded. Control larvae were kept in similar condition without treatment. Lethal time ($LT_{50}$) and lethal concentration were obtained by using Probit Analysis, probit model.

## RESULTS

The LC values ($LC_{50,\ 90,\ 95}$) of aqueous extracts of *L. alopecuroides* on *C. quinquefasciatus* and *A. gambiae* and also extracts of neem on *C. quinquesfasciatus* at different time intervals are given in Table 1. In each case, the toxicity of the extracts was found to increase with time and concentration. With increase in exposure time $LC_{50}$ of *L. alopecuroides* on *C. quinquesfasciatus* decreased from 384.69 mg/l (10 min) to -0.87 mg/l (20 min), while that of neem on the same organism decreased from 1479.43 mg/l (105 min) to 47.19 mg/l (225 min). A similar trend was observed for *L.alopecuroides* on *An. gambiae.* The larvicidal activities of *L. alopecuroides* and neem were in the order *Cu. quinquefasciatus* > *An. gambiae*. Thus *C. quinquefasciatus* was more susceptible to extracts of both plants.

In 25 min, extract Aa effected 100% mortality in *C. quinquefasciatus* but it took 4.5x as long to cause the same mortality level in *An. gambiae,* and 11.7x for extract Ai to effect 100% mortality in *C. quinquefasciatus* (Table 2). Extract Ea effected 100% mortality in *A. gambiae* at 4.5x the time required to effect similar mortality in *C. quinquefasciatus*, but extract Ei required 21.8x as long to effect 100% mortality in *C. quinquefasciatus* (Table 2). No mortality was recorded in the control for both larvae. Also *A. gambiae* exposed to all the concentrations of neem recorded 0% mortality until the emergence of the adult.

ANOVA indicated that the cumulative mortality of *u. quinquesfasciatus* and *A. gambiae* exposed to *L. alopecuroides* and neem was significant ($p < 0.05$) at the various exposure concentrations and times (Table 3). Interactions between time and concentration on cumulative mortality were also found to be significant ($p < 0.05$, Figures 1 – 3).

## DISCUSSION

Mortality of larvae of *C. quinquefasciatus* and *A. gambiae* exposed to the plant extracts increased with time of exposure and concentration of extracts as was also reported for larvae of *C. quinquefasciatus* and other mosquitoes exposed to extracts of plants such as *Nerium indicum* and *Euphorbia royleana* (Jang et al., 2002; Singh et al, 2002; Srivastava et al., 2003; Choochote et al.,

**Table 2.** Lethal time (LT) of (a) *C. quinquefasciatus* (b) *A. gambiae* exposed to *L. alopecuroides* and (c) *C. quinquefasciatus* exposed to neem (LT+95% confidence intervals).

| Weight (mg) of leaves extracted | Lethal Time (mins.) | | |
|---|---|---|---|
| | $LT_{50}$ | $LT_{90}$ | $LT_{95}$ |
| **(a) *C. quinquefasciatus* exposed to *L. alopecuroides*** | | | |
| 100 (Aa) | 15.44(13.03-1793) | 21.58(18.88-2739) | 23.33(20.23-3037) |
| 200 (Ba) | 12.02(-0.12-19.21) | 21.77(16.12-67.23) | 24.54(18.02-83.55) |
| 300 (Ca) | 11.07(-6.90-19.54) | 19.30(13.96-93.86) | 21.63(15.63-119.17) |
| 400 (Da) | 9.51(3.73-12.96) | 17.72(14.01-29.61) | 20.04(15.74-35.52) |
| 500 (Ea) | 6.98(0.53-9.93) | 15.75(12.57-24.24) | 18.24(14.49-29.79) |
| **(b) *A. gambiae* exposed to *L. alopecuroides*** | | | |
| 100 (Aa) | 69.07(50.34-87.36) | 114.48(94.25-165.69) | 127.35(103.82-190.77 |
| 200 (Ba) | 65.16(47.84-81.49) | 104.49(86.78-146.53) | 115.65(95.29-167.50) |
| 300 (Ca) | 55.07(31.71-73.63) | 98.69(78.2-152.76) | 111.06(88.10-179.26) |
| 400 (Da) | 49.75(39.68-59.04) | 70.22(60.59-91.80) | 76.02(65.14-102.46) |
| 500 (Ea) | 31.72(21.69-40.15) | 57.21(47.45-77.41) | 64.43(53.11-89.60) |
| **(c) *C. quinquefasciatus* exposed to neem** | | | |
| 100 (Ai) | 181.19 (165.81-196.86) | 248.59(226.96-288.18) | 267.69(242.04-316.32) |
| 200 (Bi) | 160.56(140.82-177.96) | 233.21(210.22-276.50) | 253.81(226.44-307.88) |
| 300 (Ci) | 147.05(134.36-158.53) | 200.17(186.10-221.00) | 215.23(198.95-240.53) |
| 400 (Di) | 162.05(143.12-179.27) | 214.13(194.44-250.50) | 228.89(206.36-273.32) |
| 500 (Ei) | 152.30(84.83-196.40) | 212.79(176.37-401.77) | 229.94(189.13-473.17) |

**Table 3.** Cumulative mortality of (a) *C. quinquefasciatus* exposed to *L. alopecuroides,* (b) *A. gambiae* exposed to *L. alopecuroides* and (c) *C. quinquefasciatus* exposed to neem.

| Mosquito spp | Weight (mg) of Leaves Extracted | | | | |
|---|---|---|---|---|---|
| | **100** | **200** | **300** | **400** | **500** |
| (a) | $51.25\pm39.34^{e}$ | $57.07\pm29.81^{d}$ | $65.33\pm32.60^{c}$ | $70.00\pm28.56^{b}$ | $78.67\pm23.53^{a}$ |
| (b) | $52.00\pm27.36^{e}$ | $54.00\pm27.49^{d}$ | $64.00\pm21.36^{c}$ | $80.00\pm19.67^{b}$ | $86.00\pm18.07^{a}$ |
| (c) | $46.00\pm35.92^{d}$ | $54.00\pm37.73^{c}$ | $62.00\pm37.89^{b}$ | $64.00\pm38.52^{b}$ | $70.00\pm39.29^{a}$ |
| **Time (min.)** | | | | | |
| | **5** | **10** | **15** | **20** | **25** |
| (a) | $26.00\pm14.07^{e}$ | $39.39\pm18.64^{d}$ | $62.00\pm16.70^{c}$ | $94.00\pm8.43^{b}$ | $100.00\pm0.00^{a}$ |
| | **30** | **50** | **70** | **90** | **120** |
| (b) | $38.00\pm16.64^{e}$ | $54.00\pm14.10^{d}$ | $68.00\pm20.14^{c}$ | $76.00\pm21.35^{b}$ | $100.00\pm0.00^{a}$ |
| | **90** | **120** | **180** | **210** | **240** |
| (c) | $8.00\pm4.29^{e}$ | $30.00\pm7.87^{d}$ | $72.00\pm18.38^{c}$ | $86.00\pm18.15^{b}$ | $100.00\pm0.00^{a}$ |

Mean in the same row with the same superscript are not significantly different at P>0.05.

2004). The time at which 100% mortality was recorded for the range of concentrations of exposure for larvae of the mosquitoes indicated that *L. alopecuroides* was far more potent than neem and hence could be effective in the control of these mosquitoes (Figures 1-3). Similar inferences can be drawn from the $LT_{50}$ values (Table 2). Moreover, the differential responses could be accounted for by the differences in the instar stage of the larvae, mosquito species and plant extracts. The instar stage of the larvae used in the study was not determined. Al-Sharook et al. (1991) recorded differences in the mortalities of various larval stages of *C. pipiens molestus* exposed to crude extracts of *Melia volkensii* and *M. azaderach* (Meliaceae) . Similar observations were made for *C. fatigans* treated with neem leaf extracts (Azmi et al., 1998) and the toxicity of myrrh from *Commiphora molmol* against *C. pipiens* and *Aedes caspius* (Massoud and Labib, 2000).

The range of the $LC_{50/90}$ values and ANOVA results on mortality for the different plant extracts on the larvae of

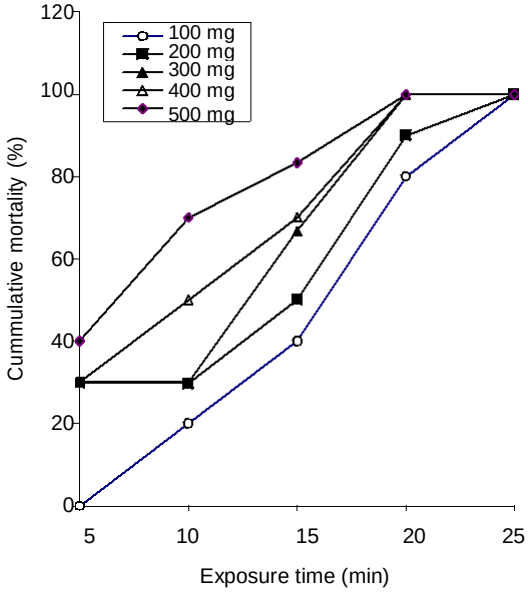


**Figure 1.** Cumulative mortality of *C. quinquefasciatus* exposed to *L. alopecuroides*.

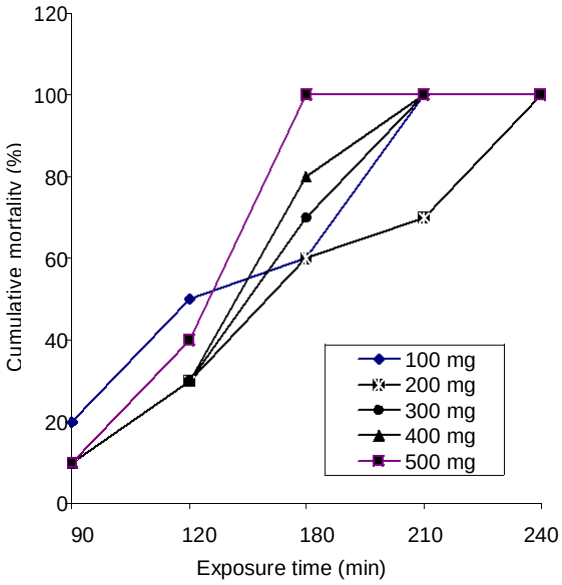


**Figure 3.** Cumulative mortality of *C. quinquefasciatus* exposed to neem

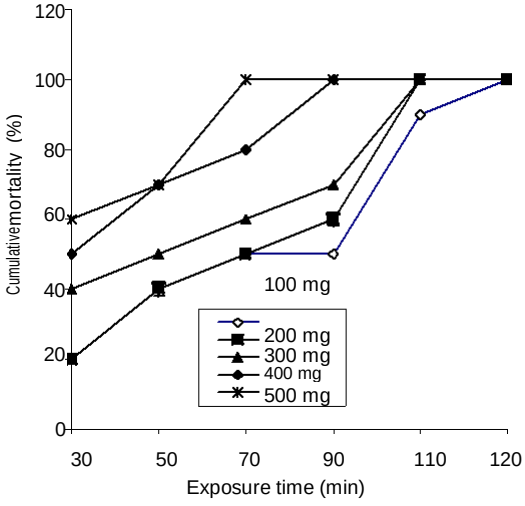


**Figure 2.** Cumulative mortality of *Anopheles gambiae* exposed to *L. alopecuroides*.

the mosquitoes might be due to the differential toxicity of the active ingredients. The neem tree, *A. indica*, a Meliaceae, is one of the most commonly studied plants for the control of mosquitoes (Mittal and Subbarao, 2003). It contains several biologically active principles, azadiracthtin being the predominant insecticide in the seeds, leaves and other parts (Mulla and Su, 1999). Azadirachtin produced 100% mortality in *Anopheles stephensi* at 1 ppm (Nathan et al., 2005). In *L. alopecuroides*, a screening study showed the presence of alkaloids, tannins, saponins and flavonoids, as well as antimicrobial activity (Obomanu et al., 2005). However, the active principles responsible for the toxicity against the mosquito larvae have not been identified. Nonetheless, the high potency may be due to the fact that one or more of the principles are more toxic than azadirachtin.

Studies have established that the activity of phytochemical compounds on target species varies with respect to plant parts from which they are extracted, solvent of extraction, geographical origin of the plant and photosensitivity of some of the compounds in the extract, among other factors (Sukumar et al., 1991). These factors, including responses in developmental stages of different mosquito species to sublethal concentrations of specified extracts of *L. alopecuroides* , particularly the effect on growth and reproduction, need to be evaluated.

The results from this study show that pools of water around human habitats can be inoculated with pounded *L. alopecuroides* leaves as a potent control against the Brancroftian filariasis vector *C. quinquefasciatus*, and more importantly, the malaria vector *A. gambiae*.

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