Original Research Article

Larvicidal efficacy of six medicinal plants on *Anopheles gambiae* s.l mosquito

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Abstract

The larvicidal efficacy of some medicinal plants (*Ocimum gratissimum*, *Chromoleana odorata*, *Terminalia catappa*, *Carica papaya*, *Vernonia amygdalina* and *Cymbopogon citrates*) against the larvae of *Anopheles gambiae* mosquitoes was investigated in acute bioassay. Ten healthy laboratory bred larvae were tested using five different concentrations (200, 400, 600, 800 and 1000ppm) and a simultaneous control at 24, 48 and 72 hours of exposure. The results showed that all the plant extracts were seen to be effective at less than 1% (1000ppm), thus possessing good to moderate effect against the larvae; *C. citrates* has been the most effective with 451.8ppm followed very closely by *O. gratissimum* with 458ppm, *C. papaya* with 510.2ppm, *V. amygdalina* with 582.3ppm, *T. catappa* with 620.9ppm, the least was *C. odorata* with 744ppm at the end of the exposure time. The efficacy of the extracts as seen from this study can be used as potential substitutes of synthetic larvicides and local environment due to their availability. The effects of the extracts were seen to be largely independent on external factors, hence the need for further study on the characterization and profiling of the active ingredients, their effect against non-target species and the best time of application is highly recommended as they fit in as alternative larvicides in reducing *Anopheles* menace infestation.

Keywords: Medicinal plants, LC$_{50}$, Extracts potency, *Anopheles* larva

INTRODUCTION

In tropical and subtropical regions, mosquitoes serve as vectors of life threatening diseases such as malaria, lymphatic filariasis, dengue, yellow fever and various encephalitis, etc (Tehri and Singh, 2015). Anopheline mosquitoes have been the only vectors of malaria to man and members of the *Anopheles gambiae* complex are the principal and most efficient vectors in tropical Africa. Malaria results in 214 million new cases and 438,000 deaths annually in the world with Africa accounting for 88% of the occurrence and death (WHO, 2015) The most profitable time of controlling them is as soon as the rain commences when the population are trying to establish and peak, (CDC, 2015). An understanding of their biology, itrates and ecology is very essential in developing control strategies to these diseases, (CDC, 2010; Godfray, 2013).

Control of mosquitoes has been carried out by the use of physical manipulation, genetic, biological and chemical methods, the latter has made by use of synthetic chemicals which has resulted in adverse effects on the environment and human lives due to biomagnifications, resistance buildup. Plant sources are an important weapon in the storehouse of mosquito control (Ghosh et al., 2012) with multiple activities such as anti-feedants, insecticides, repellants, growth inhibitors, oviposition deterrents, etc. (Tehri and Singh, 2015). These groups provide an advantage over synthetic insecticides as they are less toxic, less prone to development of insect

The plants used in this study are known for their traditional and medicinal uses, and these include Ocimum gratissimum, Chromolaena odorata, Terminalia catappa, Carica papaya, Vernonia amygdalina and Cymbopogon itrates against Anopheles gambiae s.l larvae.

**METHODOLOGY**

**Study Area**

This study was carried out at the Animal and Environmental Biology postgraduate laboratory, Animal house and the Pharmacognosy laboratory, University of Benin, Benin City, Nigeria.

**Plant Collection, Preparation and Extraction**

Leaves of the plants were collected from within the University and Evbuomore community; the leaves were rinsed, shade dried, pulverized and extracted by maceration.

**Concentrate Preparation**

Stock solution (10,000ppm) were prepared by dissolving 1000g in 100ml distilled water, the test concentration (200ppm, 400ppm, 600ppm, 800ppm and 1000ppm) were prepared by serially diluting the stock according to WHO protocol (WHO, 2005).

**Mosquito Collection and Rearing**

Larvae were collected from rain pools within the University, transported to the Laboratory, separated by instars into rearing pans, fed with yeast, stabilized to produce adult which were fed with 10%w/v sugar solution and bloodfed with guinea pig; the rearing environment was 27±5°C and 75-85%, the F2 generation were used for the assay.

**Larval Assay**

Assay was carried out according to WHO, 2005 with slight modification, 10 healthy L3/L4 larvae were placed in each test bowl containing 100ml of distilled water with the appropriate concentration, mortality was observed in 24, 48 and 72 hours, respectively, the setup was made up of four replicates and a simultaneous control which contained 1ml of solvent and 100ml of water.

**Data Analysis**

Data were analyzed using Graphpad Prism and SPSS, version 21. Values less than 5% (P<0.05) were considered to be statistically significant.

**RESULT AND DISCUSSION**

The result of the efficacy of O. gratissimum, Ch. odorata, T. catappa, Ca. papaya, V. amygdalina and Cy. citratus methanol extracts against An. gambiae larva for the different exposure and the regression equation for the plots is shown below in Table 1 while the figure 1 shows the variation of these efficacy, Figures 2-7 shows the regression plot of each plant extract against An. gambiae larvae. In this study, lethal concentration values were calculated at three time periods (24hrs, 48hrs and 72hrs respectively). At the end of the exposure period, it was observed that the efficacy was in the order Cy. citratus< O. gratissimum< Ca. papaya< V. amygdalina< T. catappa< Ch. odorata; which were within acceptable limits for consideration of an extract as a larvicide according to WHO (2005) standards. These plants have been investigated by several researchers and the findings agreed with their reports. Mgbebera, (2010) reported the comparative effect of Cy. citratus, O.gratissimum and A. indica ethanol extracts on Ae. aegypti larvae and observed that A. indica was most effective followed by O. gratissimum and Cy. citratus at LC50 values of 8.32mg/ml, 19.50mg/ml and 34.67mg/ml respectively. Chukwura and Iheuwumere, (2013) reported that O. gratissimum was the second most potent extract when compared with other extracts which is similar to this study, with a lower LC50 value of 52ppm against An. gambiae larvae, this may be due to the solvent and extraction methodology used. Ofoegbu et al., (2013) reported LC50 values of 60.9mg/ml and 73.6mg/ml for ethanol and methanol extracts respectively, Pratheeba et al., (2015) reported that the chloroform, acetone and hexane extracts produced LC50 values of 280ppm, 305ppm and 354ppm respectively which is lower and more potent than this study. Unachukwu et al., (2016) reported LC50 value of 50mg/ml of O. gratissimum against An.gambiae. Rajmohan and Loganikumar, (2011) reported an LC50 value of 101.49ppm on Ae. aegypti larvae, Lee Marvin et al., (2012) reported that Ch. odorata LC50 values were not derived because the mortality was less than 50% at the
<table>
<thead>
<tr>
<th>Extract</th>
<th>Time</th>
<th>LC₅₀</th>
<th>LCI</th>
<th>UCL</th>
<th>Regression Equation</th>
<th>χ²</th>
<th>p-Value</th>
<th>r-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. gratissimum</td>
<td>24hrs</td>
<td>782.4</td>
<td>616.4</td>
<td>993.1</td>
<td>Y=-4.524+0.0682X</td>
<td>6.576</td>
<td>0.001</td>
<td>0.9496</td>
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<td></td>
<td>48hrs</td>
<td>600.6</td>
<td>408.2</td>
<td>883.8</td>
<td>Y=-3.333+0.0875X</td>
<td>7.638</td>
<td>0.0007</td>
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<td></td>
<td>72hrs</td>
<td>458</td>
<td>259.2</td>
<td>809.5</td>
<td>Y=2.262+0.09214X</td>
<td>6.177</td>
<td>0.0002</td>
<td>0.9750</td>
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<tr>
<td>Ch. odorata</td>
<td>24hrs</td>
<td>2381</td>
<td>1285</td>
<td>4153</td>
<td>Y=-0.357+0.0257X</td>
<td>1.742</td>
<td>0.0002</td>
<td>0.9744</td>
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<tr>
<td></td>
<td>48hrs</td>
<td>1531</td>
<td>1073</td>
<td>2186</td>
<td>Y=1.071+0.03786X</td>
<td>1.524</td>
<td>&lt;0.0001</td>
<td>0.9908</td>
</tr>
<tr>
<td></td>
<td>72hrs</td>
<td>744</td>
<td>521.8</td>
<td>1061</td>
<td>Y=0.8333+0.006X</td>
<td>3.979</td>
<td>0.0002</td>
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<tr>
<td>T. catappa</td>
<td>24hrs</td>
<td>1334</td>
<td>1003</td>
<td>1774</td>
<td>Y=-2.5+0.0400X</td>
<td>2.739</td>
<td>0.0003</td>
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<tr>
<td></td>
<td>48hrs</td>
<td>706.6</td>
<td>558.5</td>
<td>894.1</td>
<td>Y=1.071+0.0728X</td>
<td>1.524</td>
<td>&lt;0.0001</td>
<td>0.9975</td>
</tr>
<tr>
<td></td>
<td>72hrs</td>
<td>620.9</td>
<td>549.1</td>
<td>702</td>
<td>Y=1.071+0.0728X</td>
<td>1.524</td>
<td>&lt;0.0001</td>
<td>0.9975</td>
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<td>Ca. papaya</td>
<td>24hrs</td>
<td>982.2</td>
<td>750.6</td>
<td>1285</td>
<td>Y=-2.024+0.0532X</td>
<td>4.271</td>
<td>0.0005</td>
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<tr>
<td></td>
<td>48hrs</td>
<td>766.6</td>
<td>558.5</td>
<td>894.1</td>
<td>Y=2.738+0.0629X</td>
<td>6.473</td>
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<td></td>
<td>72hrs</td>
<td>510.2</td>
<td>382.3</td>
<td>680.7</td>
<td>Y=3.095+0.679X</td>
<td>6.894</td>
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<tr>
<td>V. amygdalina</td>
<td>24hrs</td>
<td>1864</td>
<td>1318</td>
<td>2638</td>
<td>Y=-1.310+0.0268X</td>
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<td>0.0003</td>
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<tr>
<td></td>
<td>48hrs</td>
<td>1597</td>
<td>1009</td>
<td>2528</td>
<td>Y=1.667+0.0375X</td>
<td>2.282</td>
<td>0.0002</td>
<td>0.9793</td>
</tr>
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<td></td>
<td>72hrs</td>
<td>582.3</td>
<td>395.1</td>
<td>858.1</td>
<td>Y=0.008+0.0775X</td>
<td>6.971</td>
<td>0.0006</td>
<td>0.9613</td>
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<tr>
<td>Cy. citratus</td>
<td>24hrs</td>
<td>1057</td>
<td>820.9</td>
<td>1362</td>
<td>Y=-3.095+0.0504X</td>
<td>3.712</td>
<td>0.0003</td>
<td>0.9699</td>
</tr>
<tr>
<td></td>
<td>48hrs</td>
<td>794.5</td>
<td>756.4</td>
<td>834.6</td>
<td>Y=1.429+0.0628X</td>
<td>2.334</td>
<td>&lt;0.0001</td>
<td>0.9922</td>
</tr>
<tr>
<td></td>
<td>72hrs</td>
<td>451.8</td>
<td>316.7</td>
<td>644.5</td>
<td>Y=3.571+0.0879X</td>
<td>4.236</td>
<td>&lt;0.0001</td>
<td>0.9869</td>
</tr>
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</table>

**Figure 1.** Lethal concentration values of *O. gratissimum*, *Ch. odorata*, *T. catappa*, *Ca. papaya*, *V. amygdalina* and *Cy. citratus* extracts at the different exposure time against *An. gambiae* larvae
Figure 2. Regression plot of *O. gratissimum*

Figure 3. Regression plot of *C. odorata*

Figure 4. Regression plot of *T. catappa*

Figure 5. Regression plot of *Ca. papaya*

Figure 6. Regression plot of *V. amygdalina*

Figure 7. Regression plot of *C. citratus*

NB: *p*<0.05 are significantly different, *p*<0.001 are highly significantly different, while *p*<0.0001 are very highly significantly different.
end of the exposure time, Sukkhthankar, (2014) reported that LC$_{50}$ values for Cx. quinquefasciatus, Ae. aegypti and An. stephensi were 43ppm, 138ppm and 1613ppm with An. stephensi values higher than this present finding, and could be attributed to the solvent used and the susceptibility of the vector species tested. Umar, (2015) reported LC$_{50}$ values of 0.15%, 0.11%, 0.07% and 0.02% of aqueous extract of Ca. papaya against Cx. quinquefasciatus larvae. Ramanibai et al., (2014) reported LC$_{50}$ value of 80.56ppm of acetone extract of Ca. papaya resulting in 61.6% mortality. Sesanti et al., (2014) reported values of 422.311ppm with ethanol extracts of Ca.papaya on An.gambiae larvae. Rudi et al., (2013), reported LC$_{50}$ values of 43.073ppm- 55.347ppm of on eight Anopheles species collected in South East Minahassas using three solvents for extraction. The difference between this present result and those from reported findings may be caused by the use of different raw material such as the solvent, plant varieties, method of extraction, mosquito species, as observed in reports of Sesantie et al., (2014); Hayatie et al., (2015) using seed producing species when compared to the non seed producing species of Ca. papaya used in this study. Reports on V. amygdalina against Mosquito vectors are rare especially with Anopheles species, while those for T. catappa have majorly been on Aedes species. Hence, more research on their efficacy especially on malaria species, characterization and profiling of the active ingredients is required.

CONCLUSION: Please provide

REFERENCES

Dawuda KD (2016). Mosquito Larvicide Prospects of Terminalia catappa (L) and Tamarindus indica (L) Seed Extracts in Laboratory and Field Bioassays. MSC Thesis, Ahmadu Bello University, Zaria, Nigeria. Department of Biological Sciences.

