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# Comparative study on larvicidal potentials of *Cymbopogon citratus* stapf, *Ricinus communis* L. and *Allium sativum* L. on fourth instar larvae of *Anopheles* mosquitoes

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## Abstract

**Background:** The war against malaria in Africa especially Sub-Saharan Africa seems to be unending despite all efforts being invested on it for some decades. Mosquitoes have remained key transmitters of malaria. This study evaluated the larvicidal potentials of ethanolic leaf extracts of *Cymbopogon citratus*, *Ricinus communis* and *Allium sativum* on the fourth instar larvae of *Anopheles* mosquito.

**Results:** *Cymbopogon citratus* had the highest mortality of 78% (312) out of 400 larvae used in all the varied concentrations (100, 200, 300, 400 and 500 mg/l), and had the least $LC_{50}$ value of 54.08 mg/l. *Ricinus communis* and *Allium sativum* had mortalities of 59.75% (239) and 73.75% (295) with $LC_{50}$ of 141.25 mg/l and 81.096 mg/l, respectively. There was no mortality in the control. Temperature, pH and conductivity correlated positively with mortality ($p < 0.05$) while dissolved oxygen and total dissolved solids did not positively correlate with mortality ($p > 0.05$).

**Conclusions:** Ethanolic leaf extracts of *Cymbopogon citratus*, *A. sativum* and *R. communis* have larvicidal properties. However, *C. citratus* was most effective followed by *A. sativum* while *R. communis* was the least effective. The extracts of these plants can serve as replacements to synthetic insecticides because they are bio-degradable, ecologically friendly, safe for non-target organisms and do not lead to an aftermath problem and still have properties that can reduce pest populations.

**Keywords:** *Cymbopogon citratus* stapf, *Ricinus communis* L., *Allium sativum* L., Larvicidal potentials, *Anopheles* mosquitoes' larvae, Mortality

## Background

Means of controlling mosquitoes—the major transmitters of malaria have remained one of the major strategies of battling malaria. Chemical-based control measures, non-chemical-based control measures and biological control agents are currently major means of controlling malaria vector (Nwabor et al., 2017). For a very long time, chemical-based control measures have dominated over other strategies, but researchers have reported some environmental and health effects\implications of chemical-based insecticides which include disruption of the natural ecosystems, reemergence and increase of mosquito population, development of resistance in vectors, detrimental effects on non-targeted organisms and incidences of human health problems (Zhang et al., 2011); Nwabor et al., 2017). Therefore, there is need for alternatives with no or less adverse effects.

Plants are potential source of bioactive agents which can be used in chemotherapy and pest control (Nwabor

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et al., 2014a, 2014b). Thangamathi & Ananth, 2014 reported that phytochemicals may serve as appropriate alternatives to synthetic insecticides in future because they are relatively safe, economical and readily obtainable in most parts of the world.

In an effort to encourage and ensure ecological friendly\ environmental safety, many researchers have developed interest in producing alternative insecticides that will be bio-degradable and ecologically friendly. This can be achieved through the use of plant-based insecticides.

Larvicidal is a general term for killing immature insects by applying agents collectively called larvicides to control larvae and pupae stages of these insects (Connelly and Carlson, 2009). Larvicidal approach is a more proactive, pro-environment, target specific of controlling adult mosquitoes. The present research focused on determining and comparing the larvicidal potentials of *Cymbopogon citratus* (DC.) Stapf, *Ricinus communis* L. and *Allium sativum* L. on fourth instar larvae of *Anopheles* mosquitoes.

## Methods

### Study area

The study was carried out in Ezza North Local Government Area of Ebonyi State with coordinates latitude 6.322°N and longitude of 8.060°E. It is mainly a tableland and lies in the tropical rainforest belt of Nigeria. It is a rural settlement with 7 communities. The inhabitants are mainly farmers. It has two distinct seasons: dry season and the rainy season. The dry season has low relative humidity (45%) and high environmental temperature between 28 and 36 °C with little rainfall (November–March) while the rainy (wet) season (April–October) has high relative humidity (80%) and lower environmental temperatures of between 21 and 28 °C.

### Collection and identification of plant materials and preparation of plant extracts

The leaves of *Ricinus communis* (Castor Oil plant) and *C. citratus* (lemon grass) were collected from a farmland in Mgbabor Echara Ezza, North Local Government Area, Ebonyi State while *A. sativum* (garlic) was bought in a local market at the same location. Identification of *Cymbopogon citratus*, *Ricinus communis* L. and *Allium sativum* L. was conducted using identification keys of Clayton et al. (2016), Govaerts et al. (2014), Hanelt (1990), respectively.

These were air-dried at room temperature and pulverized into fine powder using a mortar and pestle. Ethanolic extract of the pulverized plants was prepared following the method of Redfern et al. (2014) using a Soxhlet extractor.

### Collection of mosquito larvae and determination of mortality of *Anopheles* larvae at varied concentrations

*Anopheles* mosquito larvae were collected from oviposition sites at the study area. Larvae were seen mostly in clear unpolluted rain water collections. Sampling was done between 7:00 A.M and 10:00 A.M using a standard capacity dipper with a long handle. The water collected was sieved using a sieving net to allow smaller larvae to pass through allowing only the required size to remain. *Anopheles* larvae lack a respiratory siphon and lie parallel to the surface of the water. The identification of the *Anopheles* species was done using Standard identification keys (Gillies & Coetzee, 1987; Le Goff et al., 2012).

Acute toxicity tests were carried out in groups. Each group consists of four replicates and each replicate consists of twenty larvae per beaker containing 250 ml of water. The duration of the experiment was 4 days (96 h), this applied for the various groups and the control. Percentage mortality was recorded from the four replicates after 24 h while dead larvae from each concentration were counted for the various plant extracts.

### Determination of physicochemical parameters of the plant extracts

The parameters were assessed and recorded on daily basis. The temperature of the water was measured using a mercury thermometer. The pH, Total Dissolved Solids and Conductivity were measured using Hanna meters. The dissolved oxygen level was determined using titration method.

### Analysis of data generated

Data obtained were analyzed using Statistical Package for Social Sciences version 23. The data obtained from larval mortality were subjected to probit analysis to calculate the $LC_{50}$ of the various plant extracts. Data generated from physicochemical parameters were analyzed using Pearson correlation and ANOVA. The relationship between the larval mortality and the physicochemical parameters of the plant extracts was determined. Chi-square test was also carried out to determine significant differences between mortality effects of the three plant extracts. At $P < 0.05$, values were seen to be statistically significant (Fig. 1).

## Results

### Comparison of the mean mortality values of ethanolic extracts of the three plants at varied concentrations

The highest mean mortality in each plant extract was observed in 500 mg/l concentration. *C. citratus* ranked highest with mean mortality value of 18.00 at 500 mg/l, while *A. sativum* and *R. communis* had mean mortality

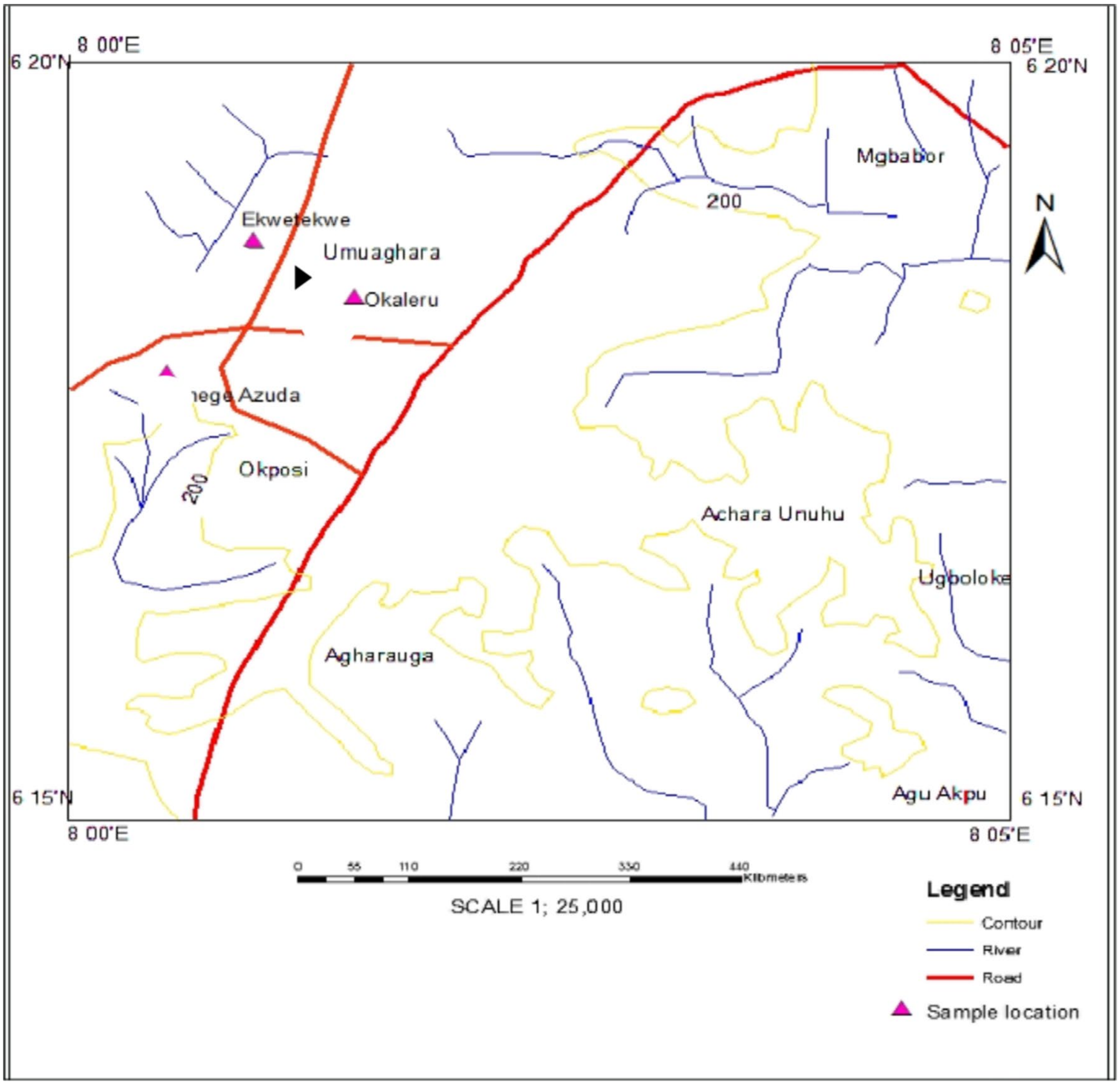


**Fig. 1** Map of Ezza North Showing the Study Locations

values of 17.25 and 15.00, respectively, at 500 mg/l. The least mean mortality value 9.25 was observed in *R. communis* at 100 mg/l. In all the concentrations, *C. citratus* had the highest mean mortality value of 18.00 at 500 mg/l. It was observed that the higher the concentration in mg/l of the extracts, the higher the mean mortality

**Table 1** Mean mortality values of ethanolic leaf extracts of *r. communis, a. sativum* and *c. citratus* at different concentrations

| Concentration (mg/l) | Mean ± Standard Deviation | | |
|---|---|---|---|
| | *Ricinus communis* | *Allium sativum* | *Cymbopogon citratus* |
| 0 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| 100 | 9.25 ± 11.15 | 11.00 ± 12.57 | 13.50 ± 11.62 |
| 200 | 10.50 ± 9.89 | 14.25 ± 12.09 | 14.00 ± 15.68 |
| 300 | 12.25 ± 14.48 | 15.25 ± 18.32 | 15.00 ± 18.57 |
| 400 | 12.75 ± 14.45 | 16.00 ± 22.20 | 17.50 ± 24.64 |
| 500 | 15.00 ± 22.76 | 17.25 ± 24.92 | 18.00 ± 28.74 |

values (Table 1). The lethal toxicity test of *R. communis* plant extract recorded $LC_{50}$ of 141.25 mg/l (Fig. 2), *A. sativum* recorded $LC_{50}$ of 81.096 mg/l (Fig. 3) while *C. citratus* recorded $LC_{50}$ of 54.08 mg/l (Fig. 4).

### Comparison of physicochemical parameters of ethanolic leaf extract of *Cymbopogon citratus*, *Ricinus communis and Allium sativum*

The mean temperatures of *R. communis*, *C. citratus* and *A. sativum* leaf extracts from day 1 to day 4 were (28.08–26.25 °C), (28.12–26.45 °C) and (28.20–26.45 °C), respectively. The highest pH (8.68–6.42) was observed in *R. communis* ethanolic leaf extract followed by *A. sativum* leaf extract (8.63–6.32), while the least pH (8.27–6.37) was recorded in *C. citratus* leaf extract. Reduction in dissolved oxygen was recorded from day 1 to day 4 as follows: *C. citratus* extract (2.90–2.35), *A. sativum* extract (3.62–2.08) and *R. communis* extract (3.12–2.22). From day 1 to day 4, increase in total dissolved solids was observed in *C. citratus* extract (392.33–420.17) mg/l, *A. sativum* extract (371.50–386.17) mg/l and *R. communis* (360–377.50) mg/l. Increase in conductivity was also observed in *C. citratus* extract (733.17–838.67) us/cm, *A. sativum* extract (753.17–874.17) us/cm and *R. communis* at (743.5–785.33) us/cm (Table 2).

Correlation between mortality and the physico-chemical variables was statistically significant for Temperature ($r=0.74$, $p<0.01$), pH ($r=0.62$, $p<0.01$) and conductivity ($r=-0.45$, $p<0.05$) while dissolved oxygen ($r=0.15$, $p>0.05$) and total dissolved solids ($r=-0.37$, $p>0.05$) were statistically non-significant in *R. communis* group (Table 3). Correlation between mortality and the physico-chemical variables was statistically significant for temperature ($r=0.71$, $p<0.01$), pH ($r=0.61$, $p<0.01$) and conductivity ($r=-0.45$, $p<0.05$) but was not significant for dissolved oxygen ($r=0.40$, $p>0.05$) and total dissolved solids ($r=-0.11$, $p>0.05$) in *A. sativum* group (Table 4). Furthermore, correlation between mortality and the physico-chemical variables was statistically significant for temperature ($r=0.70$, $p<0.01$), conductivity

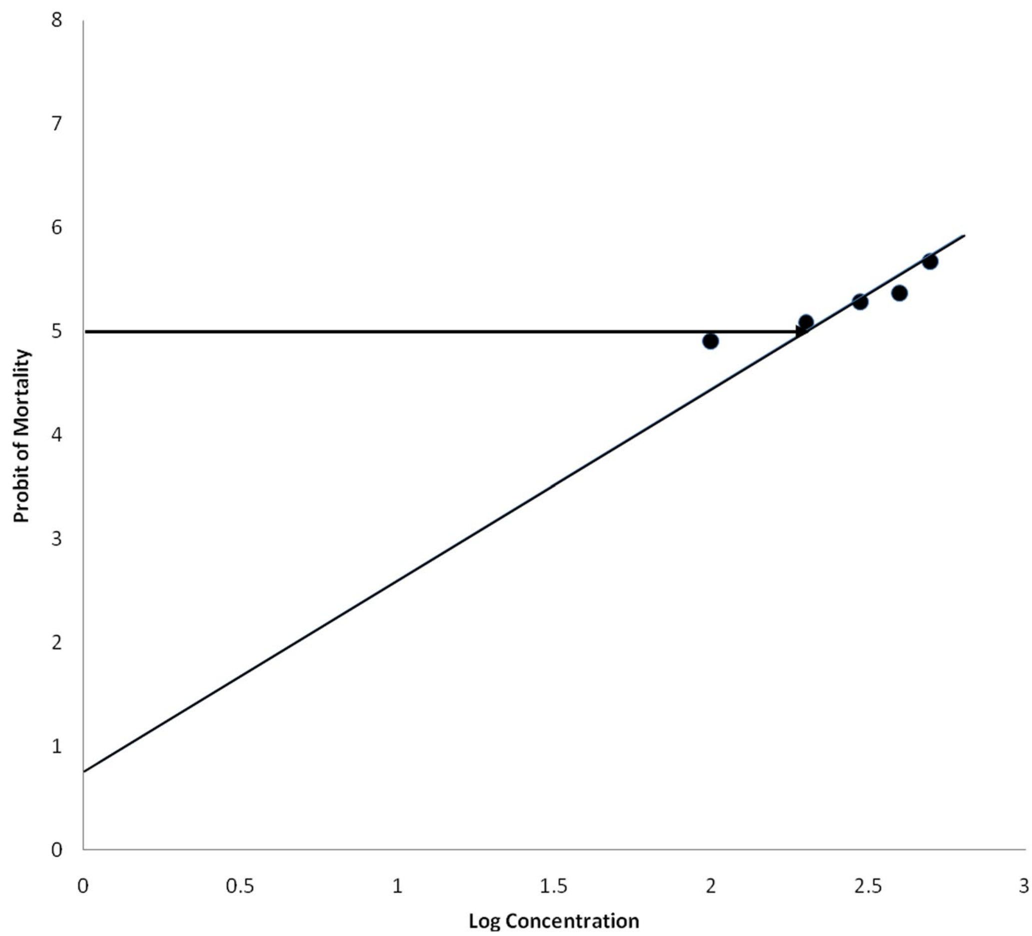


**Fig. 2** Graph of Probit of Mortality of *Anopheles* Larvae/Log of Concentration of Ethanolic Leaf Extract of *Ricinus communis* Showing the $LC_{50}$ at Lethal Toxicity

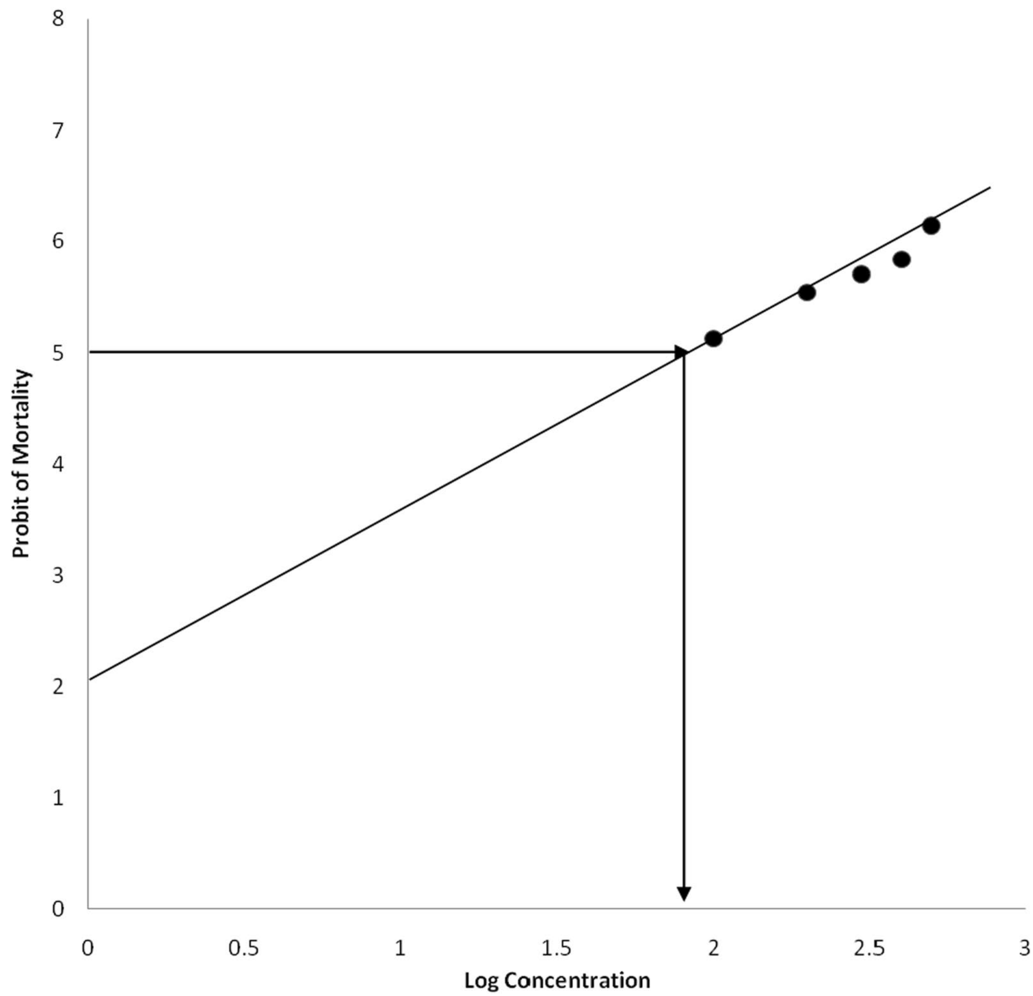


**Fig. 3** Graph of Probit of Mortality of *Anopheles larvae*/Log of Concentration of Ethanolic Leaf Extract of *Allium sativum* showing the $LC_{50}$ at Lethal Toxicity

($r = -0.54$, $p < 0.01$) and pH ($r = 0.51$, $p < 0.05$) but was not significant for dissolved oxygen ($r = 0.01$, $p > 0.05$) and total dissolved solids ($r = -0.15$, $p > 0.05$) in *C. citratus* group (Table 5).

## Discussion

*Cymbopogon citratus*, *Ricinus communis* L. and *Allium sativum* L., all had concentration dependent measurable larvicidal effects on *Anopheles* larvae. *Cymbopogon citratus* recorded the highest mortality. This finding consonants with Musa et al. (2015). Sluggishness in behavior and inability to come on surface observed on exposure to high concentrations also corroborates with Musa et al. (2015). Percentage mortality recorded by *A. sativum* in this present study agrees with Reyes et al. (2008). In an attempt to explain the difference in efficiency of extracts of selected plants used in the present study, we suggest that chemical composition and broad spectrum of biological activity for plant extracts can vary with plant age, the plant tissues used, geographical origin of plant, the species and age of a targeted organism maybe responsible. Findings of this study may imply that *C. citratus* extract exhibited proficient larvicidal potential and can be readily exploited as a preferred natural larvicide for the control of mosquito. This claim is supported with the empirical evidence that showed leaf extract of *C. citratus* to have stronger larvicidal effect than extracts of other plants.

The observed positive correlation of the leaf extracts pH with mortality implied that increase in pH led to an increase in mortality of *Anopheles* larvae. This corroborated with Adebote et al. (2008). The extracts temperature also correlated positively with mortality. This suggests that this parameter is an important factor for the development of *Anopheles* larvae. This is

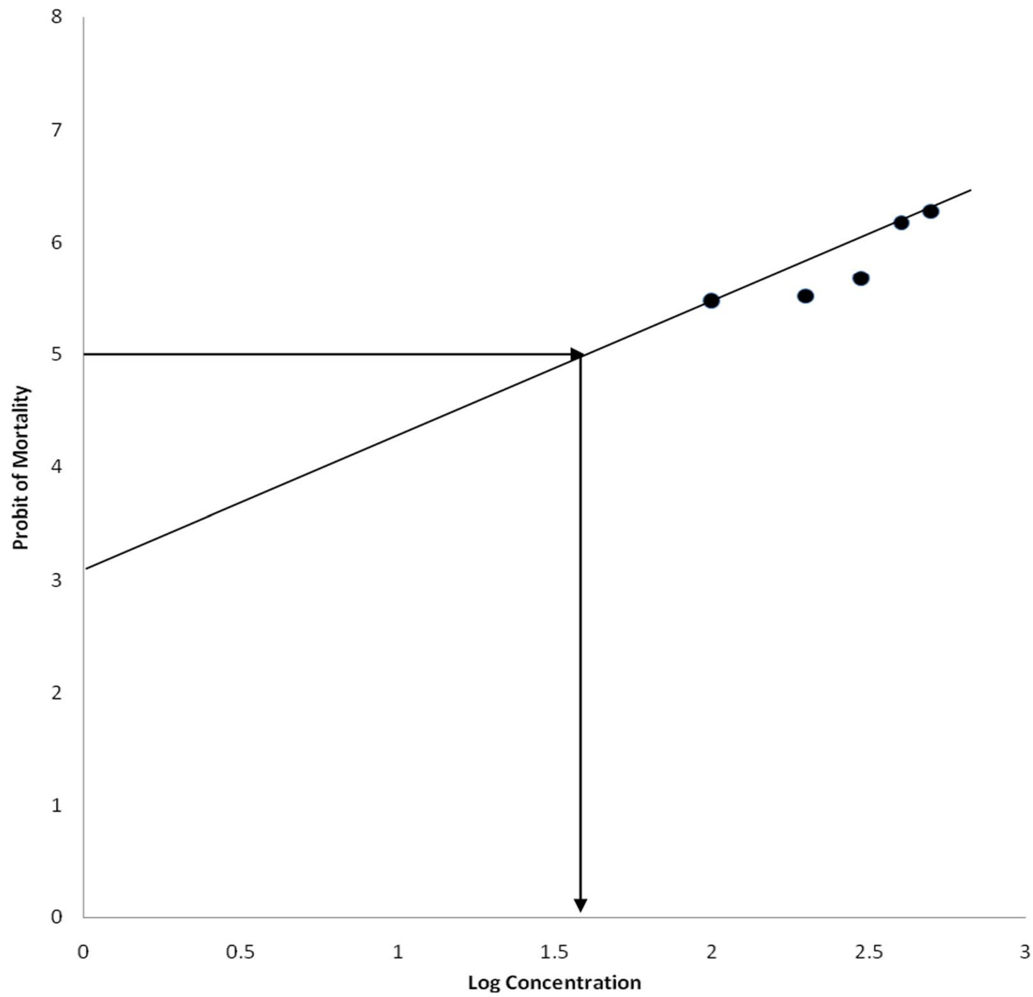


**Fig. 4** Graph of Probit of Mortality of *Anopheles larvae*/Log of Concentration of Ethanolic Leaf Extract of *Cymbopogon citratus* showing the $LC_{50}$ at Lethal Toxicity

**Table 2** Comparison of physicochemical parameters of ethanolic leaf extracts of *r. communis, a. sativum* and *c. citratus*

| Plant | Day | Parameters (Mean ± Standard deviation) | | | | |
|---|---|---|---|---|---|---|
| | | **Temperature** | **pH** | **Dissolved Oxygen** | **Total Dissolved Solids** | **Conductivity** |
| *R. communisis* | 1 | 28.08 ± 0.098 | 8.68 ± 0.04 | 3.12 ± 0.49 | 360.00 ± 4.94 | 743.50 ± 14.22 |
| | 2 | 26.80 ± 0.09 | 8.28 ± 0.075 | 2.58 ± 0.61 | 369.83 ± 13.31 | 759.67 ± 17.87 |
| | 3 | 27.17 ± 0.08 | 7.47 ± 0.44 | 2.38 ± 0.58 | 374.00 ± 19.09 | 787.83 ± 10.65 |
| | 4 | 26.25 ± 0.06 | 6.42 ± 0.26 | 2.22 ± 0.51 | 377.50 ± 26.74 | 785.33 ± 20.70 |
| *A. sativum* | 1 | 28.20 ± 0.09 | 8.63 ± 0.05 | 3.62 ± 0.46 | 371.50 ± 20.80 | 753.17 ± 24.05 |
| | 2 | 26.95 ± 0.06 | 8.15 ± 0.14 | 2.75 ± 0.73 | 375.83 ± 25.31 | 786.83 ± 29.87 |
| | 3 | 27.35 ± 0.06 | 7.60 ± 0.28 | 2.27 ± 0.61 | 379.00 ± 35.66 | 821.50 ± 34.58 |
| | 4 | 26.45 ± 0.08 | 6.32 ± 0.32 | 2.08 ± 0.61 | 386.17 ± 38.11 | 874.17 ± 40.43 |
| *C. citratus* | 1 | 28.12 ± 0.08 | 8.27 ± 0.12 | 2.90 ± 0.59 | 392.33 ± 11.89 | 733.17 ± 34.68 |
| | 2 | 26.90 ± 0.09 | 8.13 ± 0.14 | 2.65 ± 0.60 | 394.17 ± 16.63 | 763.50 ± 21.29 |
| | 3 | 27.47 ± 0.05 | 7.25 ± 0.26 | 2.50 ± 0.58 | 406.67 ± 20.89 | 791.50 ± 16.89 |
| | 4 | 26.45 ± 0.06 | 6.37 ± 0.26 | 2.35 ± 0.84 | 420.17 ± 32.99 | 838.67 ± 34.43 |

**Table 3** Correlations of mean mortality values and mean values of physicochemical parameters of ethanolic leaf extract of *Ricinus communis*

| Pearson Correlation | Mortality | Temp | PH | DO | TDS | COND |
|---|---|---|---|---|---|---|
| Mortality | 1 | .737** | .624** | .154 | −.372 | −.449* |
| Temp | .737** | 1 | .779** | .531** | −.318 | −.560** |
| PH | .624** | .779** | 1 | .567** | −.435* | −.705** |
| DO | .154 | .531** | .567** | 1 | −.129 | −.552** |
| TDS | −.372 | −.318 | −.435* | −.129 | 1 | .464* |
| COND | −.449* | −.560** | −.705** | −.552** | .464* | 1 |

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

**Table 4** Correlations of mean mortality values and mean values of physicochemical parameters of ethanolic leaf extract of *Allium sativum*

| Pearson Correlation | Mortality | Temp | PH | DO | TDS | COND |
|---|---|---|---|---|---|---|
| Mortality | 1 | .713** | .611** | .396 | −.110 | −.448* |
| Temp | .713** | 1 | .813** | .643** | −.140 | −.717** |
| PH | .611** | .813** | 1 | .725** | −.289 | −.877** |
| DO | .396 | .643** | .725** | 1 | −.460* | −.890** |
| TDS | −.110 | −.140 | −.289 | −.460* | 1 | .543** |
| COND | −.448* | −.717** | −.877** | −.890** | .543** | 1 |

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

**Table 5** Correlations of mean mortality values and mean values of physicochemical parameters of ethanolic leaf extract of *Cymbopogon citratus*

| Pearson Correlation | Mortality | Temp | PH | DO | TDS | COND |
|---|---|---|---|---|---|---|
| Mortality | 1.000 | .704** | .510* | .011 | −.148 | −.542** |
| Temp | .704** | 1.000 | .652** | .364 | −.321 | −.671** |
| pH | .510* | .652** | 1 | .535** | −.564** | −.812** |
| DO | .011 | .364 | .535** | 1 | −.839** | −.534** |
| TDS | −.148 | −.321 | −.564** | −.839** | 1 | .596** |
| COND | −.542** | −.671** | −.812** | −.534** | .596** | 1 |

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

supported by Devi et al. (2014) who reported that activity of immature mosquitoes depends to a large extent on the temperature of the water they inhabit. This, however, disagrees with Minakawa et al. (1999) who reported that moderately high temperatures are necessary for the optimum growth of *Anopheles* larvae. Electrical conductivity had a significant correlation with mortality. By implication, its increase led to an increase in mortality. This corroborates with Edillo et al. (2006) and Yee & Juliano (2007).

Contrarywise, dissolved oxygen did not have a significant correlation with mortality. The survival of larvae depends on their ability to take up oxygen via their cuticle. At low dissolved oxygen, larvae survive longer at lower temperatures, presumably because of the reduced respiration rate. This finding is in agreement with Muturi et al. (2008). It will be important to also point out that availability of oxygen in water is an affirmative indication for growth, while its deficiency is a pointer of pollution. Total dissolved solids of the leaf extracts did not have a significant correlation with mortality of *Anopheles* larvae.

Basically, total dissolved solids cause toxicity through increase in salinity, change in the ionic composition of the water and toxicity of individual ions.

## Conclusions

Ethanolic leaf extracts of *C. citratus, A. sativum* and *R. communis* have promising larvicidal properties. However, C. *citratus* recorded the highest mortality rate on *Anopheles* larvae, followed by *A. sativum* while *R. communis* had the least mortality rate. These plants can be used as alternatives to synthetic insecticides considering the fact that they are bio-degradable, ecologically friendly, safe for non-target organisms and do not lead to an aftermath problem and still have properties that can reduce pest populations.

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**Author contributions**

All authors provided all materials used for this study, designed the research and participated in the laboratory activities. EA coordinated laboratory stage while OCA supervised. All authors handled, statistical analysis, manuscript preparation while EIN edited it. The final version was read and approved by all authors.

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**Availability of data and materials**

We declare that data generated from this study are readily available as well as information about material used.

## Declarations

**Ethical approval and consent to participate**

Ethical approval Handling of experimental animals used in this research was in accordance with recommendation of the Committee on Ethical Issues Concerning Animal Research Outside the Laboratory (Nisbet & Paul, 2004). Consent to participate was not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

Authors declare that there is no conflict of interest among authors.

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## Publisher's Note