



## A Comparative Study on the Larvicidal Effect of Ethanol Leaf Extracts of *Cymbopogon Citratus* (Lemongrass) and *Ximenia Americana* (Sea Lemon) on *Anopheles* and *Culex* Larva

By Abdullahi Hasan Amoto, Muhammad Yusha'u, Umar Sani Inuwa,  
Salawudeen Shuaibu Omeiza & Abdulrahman Itopa Suleiman

Bayero University

**Abstract-** *Aim:* Synthetic insecticides are widely being used for the control of mosquitoes. However this is faced with many challenges among which are development of insecticide resistance by the mosquitoes, damage to the environment, effect on human health and non-target organisms. This study thus aimed at comparing the larvicidal effect of leaves extracts of *Cymbopogon citratus* (Lemon grass) and *Xymenia-americana* (Sea lemon) against *Anopheles* and *Culex* larvae.

**Method:** The leaves components were extracted and phytochemical analyses of the extracts were also done. *Anopheles* and *Culex* larva were collected and identified using morphological features. Larvicidal susceptibility test were carried out using WHO standard method and the mortalities were observed after 24 hours and 48 hours of exposure.

**Keywords:** *cymbopogon citratus*, *xymenia americana*, *anopheles*, *culex*, *laevae*.

**GJSFR-C Classification:** DDC Code: 398.2 LCC Code: PZ8.1.A213



A COMPARATIVE STUDY ON THE LARVICIDAL EFFECT OF ETHANOL LEAF EXTRACTS OF CYPBOPOGON CITRATUS LEMONGRASS AND XIMENIA AMERICANA SEA LEMON ON ANOPHELES AND CULEX LARVA

Strictly as per the compliance and regulations of:



RESEARCH | DIVERSITY | ETHICS



# A Comparative Study on the Larvicidal Effect of Ethanol Leaf Extracts of *Cymbopogon Citratus* (Lemongrass) and *Ximenia Americana* (Sea Lemon) on *Anopheles* and *Culex* Larva

Abdullahi Hasan Amoto <sup>a</sup>, Muhammad Yusha'u <sup>a</sup>, Umar Sani Inuwa <sup>b</sup>, Salawudeen Shuaibu Omeiza <sup>c</sup>  
 & Abdulrahman Itopa Suleiman <sup>¶</sup>

**Abstract- Aim:** Synthetic insecticides are widely being used for the control of mosquitoes. However this is faced with many challenges among which are development of insecticide resistance by the mosquitoes, damage to the environment, effect on human health and non-target organisms. This study thus aimed at comparing the larvicidal effect of leaves extracts of *Cymbopogoncitratus* (Lemon grass) and *Xymenia-americana*(Sea lemon) against anopheles and culex larvae.

**Method:** The leaves components were extracted and phytochemical analyses of the extracts were also done. Anopheles and culex larva were collected and identified using morphological features. Larvicidal susceptibility test were carried out using WHO standard method and the mortalities were observed after 24 hours and 48 hours of exposure.

**Results:** Higher mortality was observed in *anopheles* larvae exposed to ethanol leaf extracts of *C. citratus* after 24 hours of exposure (LC50 5.905mg/ml and LC90 16.241mg/ml) compared to the ethanol leaf extract of *X.americana* with the lower mortality after 24 hours of exposure (LC50 7.617mg/ml and LC90 43.471mg/ml). Against *culex* larvae, higher mortality was also observed in ethanol leaf extract of *C. citratus* after 24 hours of exposure (LC50 6.851mg/ml and LC90 25.678mg/ml) compared to ethanol leaf extract *X. americana* with the lower mortality after 24 hours of exposure(LC50 10.626mg/ml and LC90 54.434mg/ml).

**Conclusion:** The result suggest that the ethanol leaf extract of the plants used possessed significant larvicidal activities against the *anopheles* and *culex* laeva. However, the *C. citratus* extract is more potent than that of the *X. Americana* extract. These show that they may be considered as natural sources for the production of natural larvicides.

**Keywords:** *cymbopogon citratus*, *xymenia americana*, *anopheles*, *culex*, *laevae*.

## I. INTRODUCTION

Mosquitoes are the most important single group of insects in terms of public health, which transmit a number of diseases such as malaria, filariasis, dengue and zika virus, causing millions of deaths every year [28, 4]. Malaria is transmitted by female anopheles mosquito from person to person. Various species have been found to be the vectors in different parts of the world. *Anopheles gambiae* complex is the principal vector in Africa [7]. Vector control strategies have traditionally focused on killing mosquitoes using a variety of insecticides. Environmental management (through reduction or removal of mosquito breeding sites) has often been used alongside chemicals or microbiological ovicides, larvicides and pupicides [31] in areas where endemics mosquito-borne diseases occur. The use of synthetic insecticides has to be regulated given that the development of insecticide resistance is widespread and that there is concern regarding the damage of the environment, effect on human health and non-target organisms [21]. Hence there is a need to develop and incorporate new alternative insecticidal agent.

In recent years, the emphasis on control of the mosquito population has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, which are generally of botanical origin. Plant products have been used traditionally by human communities in many part of the world against the vector and pest species of insects [25]. The plant derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides [27]. The demand for plant-based insecticides is that they are non-toxic, easily available at affordable prices, biodegradable and show broad spectrum, target specific activities against various species of mosquitoes. A lot of phytochemicals extracted from various plant species have been tested for their larvicidal actions against mosquitoes [25]. The use of active toxic agent from plant extract as an alternative mosquito control strategy was dated back to

**Author a & p:** Department of Microbiology, faculty of natural and pharmaceutical sciences, Bayero University, Kano PMB 3011, Kano Nigeria. e-mail: dramoto005@gmail.com

**Author Q:** Department of Biochemistry, faculty of Basic Medical Sciences, Bayero university, Kano PMB 3011, Kano Nigeria.

**Author ¶:** Department of Biochemistry, Kogi State University, Ayingba, PMB 1005, Kogi Nigeria.

ancient time as such many studies on plant extract and their active constituent compounds against mosquito larvae have been carried out in different parts of the world [16, 18]. Although, there are several reports on the antibacterial effect of *Ximenia Americana*, there is dearth of information on the larvicidal effect of the plant, thus this work is aimed at comparing the larvicidal effect of ethanol leaf extracts of *Cymbopogon citratus* and *Ximenia americana*.

## II. MATERIALS AND METHODS

### a) Plant identification and harvesting

The plant samples of *C. citratus* and *X. americana* leave were collected from the botanical garden behind Aminu Kano Teaching Hospital (AKTH) and Jibga Town in Bebeji Local Government Area (LGA) Kano respectively. Each plant specimen was submitted to the Herbarium division of Plant Science Department, Bayero University Kano (BUK) and then identification was confirmed in the laboratory according to Delziel [9].

### b) Processing of samples to remove pesticides residues

Harvested plant samples were washed with tap water, a commercial detergent solution and rinsed again with distilled water to remove all traces of pesticides [33]. The leaves were then shade dried and electric blender was used to grind the dried leaves of the plant into powder at pharmacology laboratory, department of Pharmacology, Bayero University Kano.

### c) Extraction of leaves component

The plant powder was extracted according to the method of Anees [2], using soxhlet extraction device. Fifty grams each of the plant fine powder of *Cymbopogon citratus* and *Ximenia americana* were extracted using the soxhlet extraction with ethanol as a solvent. The solvent ethanol was then evaporated to obtain ethanolic extract of the leaves using rotary evaporator.

### d) Phytochemical Screening

The plant extracts were analyzed for the presence of Alkaloids, flavonoids, saponins, phytosteroids and tannins as described by Ngbede et al. [22] and Amzad et al. [1].

#### i. Test for alkaloids

About 1g powder sample was mixed with 3ml of ammonia solution in a conical flask. It was then allowed to stand for 3 minutes to evaluate for free alkaloids. Chloroform (10ml) was added to the conical flask, shaken and then filtered. The chloroform was evaporated from the crude extract by water bath and Mayer's reagent (3ml) was added. Observation for cream precipitate was done and result was recorded.

#### ii. Test for flavonoids

About 1ml of stock solution was taken in a test tube, four drop of dilute NaOH solution was added. An

intense yellow colour appeared in the test tube. It became colorless upon addition of few drop of dilute acid which indicate the presence of flavonoids.

#### iii. Test for saponins

About 1ml of stock solution was taken in a test tube and diluted with 20ml of distilled water. It was shaken by hand for 15minutes. Observation for foam layer was done and the result recorded.

#### iv. Test for phytosteroids

About 1ml of the crude plant extract was taken and mixed with chloroform (10ml) and then equal volume of concentrated sulphuric acid was added to the mixture. Observation for colour change was done and result recorded.

#### v. Test for tannins

About 3ml of the crude extract mixed with chloroform and 1ml of acetic anhydride was added. Finally, 1ml of sulphuric acid was added carefully by the side of test tube to the solution. A green color shows the presence of tannins.

#### vi. Test for resins

About 5ml of petroleum ether was added to 1g of the powdered extract. Equal volume of copper acetate solution was added and shaken vigorously, then allow to separate. A green color indicates the presence of resins.

#### e) Mosquito larval collection and identification

*Anopheles* larvae were collected from rice field, and small temporary rain pools in Doko town Garki L.G.A, Jigawa State, Northern Nigeria while *Culex* larvae were collected from drainage behind provost office of college of health sciences, Aminu Kano Teaching Hospital, Kano. Larvae were collected by dipping method using entomological larval spoons, plastic cups and suitable containers. The larvae were processed at site of collection, worms and other insects were removed according to the method described by Cheah et al., [6].

Morphological features such as presence or absence of siphons, resting position to water surface contained in taxonomic keys were used to identify the *Anopheles* and *Culex* larvae as described by Gillies and Coetzee [13].

#### f) Transportation of mosquito larvae

All specimen collected from a particular breeding place was kept in a plastic bucket and labeled. (Date and site of collection). The buckets were not filled to the brim in other to allow air space for the larvae to breath. The buckets were well covered before they were transported to the laboratory [29].

#### g) Rearing of mosquito larvae

The collected larvae were kept inside in a rearing bowl, at 27°C. The larvae were fed daily with yeast according to WHO [34].

*h) Larvicidal effect of the plant extracts*

The efficacy of the plants extracts as larvicides against the *Anopheles* and *culex* mosquito larvae were evaluated in accordance with guidelines of World Health Organization (WHO) standard method [34]. Ten milliliter (10 ml) of the solution for each concentration 30mg/ml, 20mg/ml, 10mg/ml and 5mg/ml was placed in a small plastic container, mixed thoroughly with 90 ml of distilled water. Batches of 25 matured larvae in four replicates were exposed to each of test concentrations. During the period of exposure larvae were fed with yeast [34]. The control contains 100 ml of distilled water with 0.2 ml of ethanol.

*i) Determination of mortality*

The effect of each plant extract was carefully monitored. Larvae were considered dead if they were unable to move after touching with a needle. Moribund larvae were unable to rise to the surface when the water was disturbed. The mortality was observed counted and recorded after 24 and 48 hours of exposure and the percentage mortality was computed using the expression below according to WHO [34].

$$\text{Percentage Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

*j) Statistical analysis*

The statistical tools used in this study include; Arithmetic mean to get the average number of dead larvae and percentage mortality as well Probit Analysis

*Table 3.1: Phytochemical constituents of the plants extracts*

Plants	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Resins
<i>C. citratus</i>	+	+	+	+	+	+
<i>X. americana</i>	-	+	+	-	+	+

+: Phytochemical presence,

-: Phytochemical absent.

*b) Larvicidal activities of plants extracts against *Anopheles* larvae*

The result shows the larvicidal effects of *C. citratus* and *X. americana* against *Anopheles* larvae. At concentration of 5mg/ml *C. citratus* shows 45% mortality after 24 hours and 65% mortality after 48 hours of exposure. At concentration 10mg/ml it shows 73% mortality after 24 hours and 87% after 48 hours. At concentration of 20mg/ml it shows 90% mortality after 24 hours and 100% after 48 hours. At concentration of 30mg/ml it shows 99% mortality after 24 hours and 100% after 48 hours while *X. americana* on the other hand shows 38% mortality after 24 hours and 53% after 48 hours at concentration of 5mg/ml. At concentration of 10mg/ml it shows 54% mortality after 24 hours and 69% after 48 hours. At concentration of 20mg/ml it shows 72% mortality after 24 hours and 92% after 48 hours. At concentration of 30mg/ml it shows 85% mortality after 24 hours and 100% after 48 hours. There is significant association between *Anopheles* larvae

using SPSS 20 software to calculate LC<sub>50</sub> and LC<sub>90</sub> values to determine lethal concentrations of the plant extracts on mosquito larvae at 24 and 48 hours of treatment. Analysis of variance (ANOVA) and a two tailed type one t- test was used to determine whether there exists a significant difference in the mean mortality over the period of observation with the 5% confidence interval.

### III. RESULTS

*a) Phytochemicals constituents of the plants extract*

The result shows the phytochemical constituents of the *C. citratus* and *X. americana* leave extracts. As shown on table 3.1, *C. citratus* contains alkaloids, saponins, flavonoids, phytosteroids, tannins and resins while *X. americana* contains saponins, flavonoids, tannins and resins only, it does not contain alkaloids and phytosteroids.

**Table 3.2:** Larvicidal effects of plants extracts of against *Anopheles* larvae

Plant type	Concentration (mg/ml)	n	Percentage Mean 24 hours	Mortality 48 hours
Control		25	0	0
<i>C. citratus</i>	5	25	45.00±2.00	65.00±3.83
	10	25	73.00±2.00	87.00±2.00
	20	25	90.00±2.31	100.00±0.00
	30	25	99.00±2.00	100.00±0.00
P- value			0.0000	0.0000
<i>X. americana</i>	5	25	38.00±2.31	53.00±2.00
	10	25	54.00±2.31	69.00±3.83
	20	25	72.00±3.27	92.00±0.00
	30	25	85.00±2.00	100.00±0.00
P- value			0.0000	0.0000

c) *Larvicidal activities of plants extracts against Culex larvae*

The result shows the larvicidal effects of *C. citratus* and *X. americana* against *Culex* larvae. At concentration of 5mg/ml *C. citratus* shows 37% mortality after 24 hours and 67% mortality after 48 hours of exposure. At concentration 10mg/ml it shows 68% mortality after 24 hours and 90% after 48 hours. At concentration of 20mg/ml it shows 83% mortality after 24 hours and 100% after 48 hours. At concentration of 30mg/ml it shows 92% mortality after 24 hours and

100% after 48 hours while *X. americana* on the other hand shows 28% mortality after 24 hours and 40% after 48 hours at concentration of 5mg/ml. At concentration of 10mg/ml it shows 47% mortality after 24 hours and 67% after 48 hours. At concentration of 20mg/ml it shows 67% mortality after 24 hours and 84% after 48 hours. At concentration of 30mg/ml it shows 81% mortality after 24 hours and 100% after 48 hours. There is significant association between *Anopheles* larvae mortality and the concentration of the extracts at  $P<0.05$ .

**Table 3.3:** Larvicidal effects of plants extracts of against *Culex* larvae

Plant type	Concentration (mg/ml)	n	Percentage Mean 24 hours	Mortality 48 hours
Control		25	0	0
<i>C. citratus</i>	5	25	37.00±2.00	67.00±2.00
	10	25	68.00±4.62	90.00±2.31
	20	25	83.00±2.00	100.00±0.00
	30	25	92.00±0.00	100.00±0.00
P- value			0.0000	0.0000
<i>X. americana</i>	5	25	28.00±0.00	40.00±0.00
	10	25	47.00±2.00	67.00±2.00
	20	25	67.00±2.00	84.00±0.00
	30	25	81.00±2.00	100.00±0.00
P-value			0.0000	0.0000

d) *Comparative larvicidal effects of plants extracts against Anopheles larvae*

At concentration of 5mg/ml *C. citratus* shows 45% mortality while *X. americana* shows 38% with a p-value of 0.8184 after 24 hours of exposure and after 48 hours *C. citratus* shows 65% while *X. americana* shows 53% with a p-value of 0.0000. At concentration of 10mg/ml *C. citratus* shows 73% mortality while *X. americana* shows 54% with a p-value of 0.8184 after 24 hours of exposure and after 48 hours *C. citratus* shows 87% while *X. americana* shows 69% with a p-value of

0.8184. At concentration of 20mg/ml *C. citratus* shows 90% mortality while *X. americana* shows 72% with a p-value of 0.5825 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 92% with an undefined p-value. At concentration of 30mg/ml *C. citratus* shows 99% mortality while *X. americana* shows 85% with a p-value of 1.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 100% with an undefined p-value.

**Table 3.4:** Comparative larvicidal effects of plants extracts against *Anopheles* larvae  
Concentration of plant extract (mg/ml)

Plant type	Time (hrs)	Concentration of plant extract (mg/ml)			
		5	10	20	30
<i>C. citratus</i>	24	45.00±2.00	73.00±2.00	90.00±2.31	99.00±2.00
<i>X. americana</i>		38.00±2.31	54.00±2.31	72.00±3.27	85.00±2.00
P-value		0.8184	0.8184	0.5825	1.0000
<i>C. citratus</i>	48	65.00±3.83	87.00±2.00	100.00±0.00	100.00±0.00
<i>X. americana</i>		53.00±2.00	69.00±3.83	92.00±0.00	100.00±0.00
P- value		0.0000	0.8184	-	-

e) Comparative larvicidal effects of plants extracts on *Culex* larvae

The result shows the comparative larvicidal effects of *C. citratus* and *X. americana* against *Culex* larvae. At concentration of 5mg/ml *C. citratus* shows 37% mortality while *X. americana* shows 28% with a p-value of 0.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 67% while *X. americana* shows 40% with a p-value of 0.0000. At concentration of 10mg/ml *C. citratus* shows 68% mortality while *X. americana* shows 47% with a p-value of 0.2025 after 24 hours of exposure and after 48 hours *C. citratus* shows

90% while *X. americana* shows 67% with a p-value of 0.8184. At concentration of 20mg/ml *C. citratus* shows 83% mortality while *X. americana* shows 67% with a p-value of 1.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 84% with an undefined p-value. At concentration of 30mg/ml *C. citratus* shows 92% mortality while *X. americana* shows 81% with a p-value of 0.0000 after 24 hours of exposure and after 48 hours *C. citratus* shows 100% while *X. americana* shows 100% with an undefined p-value.

**Table 3.5:** Comparative larvicidal effects of plant extract against *Culex* larvae

Plant type	Time (hrs)	Concentration of plants extract (mg/ml)			
		5	10	20	30
<i>C. citratus</i>	24	37.00±2.00	68.00±4.62	83.00±2.00	92.00±0.00
28.00±0.00		47.00±2.00	67.00±2.00	81.00±2.00	<i>X. americana</i>
P-value		0.0000	0.2025	1.0000	0.0000
<i>C. citratus</i>		4867.00±2.00	90.00±2.31	100.00±0.00	100.00±0.00
<i>X. americana</i>		40.00±0.00	67.00±2.00	84.00±0.00	100.00±0.00
P- value		0.0000	0.8184	-	-

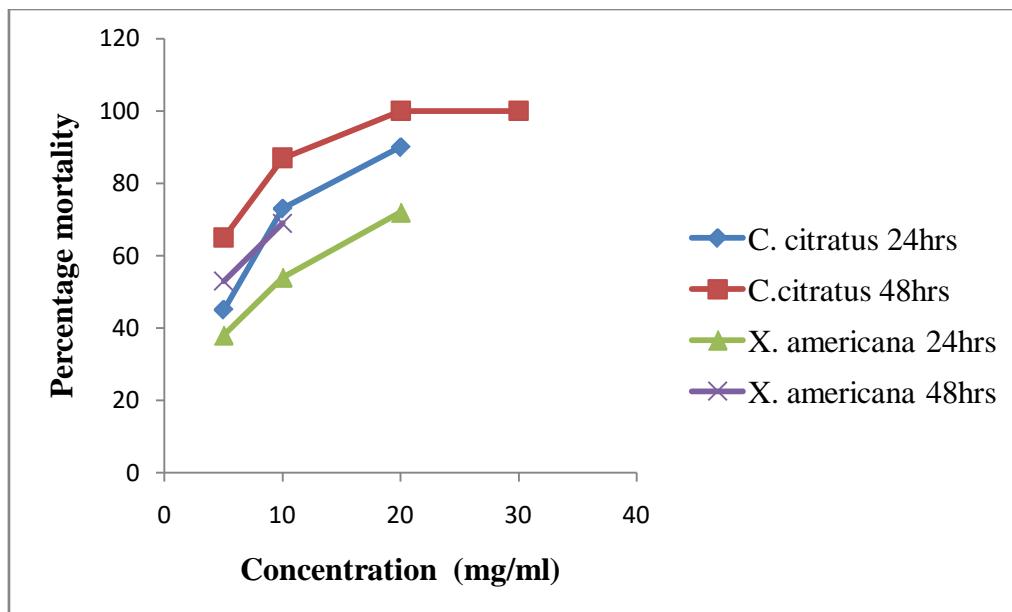


Figure 3.1: Percentage mortality of anopholes in plants extracts

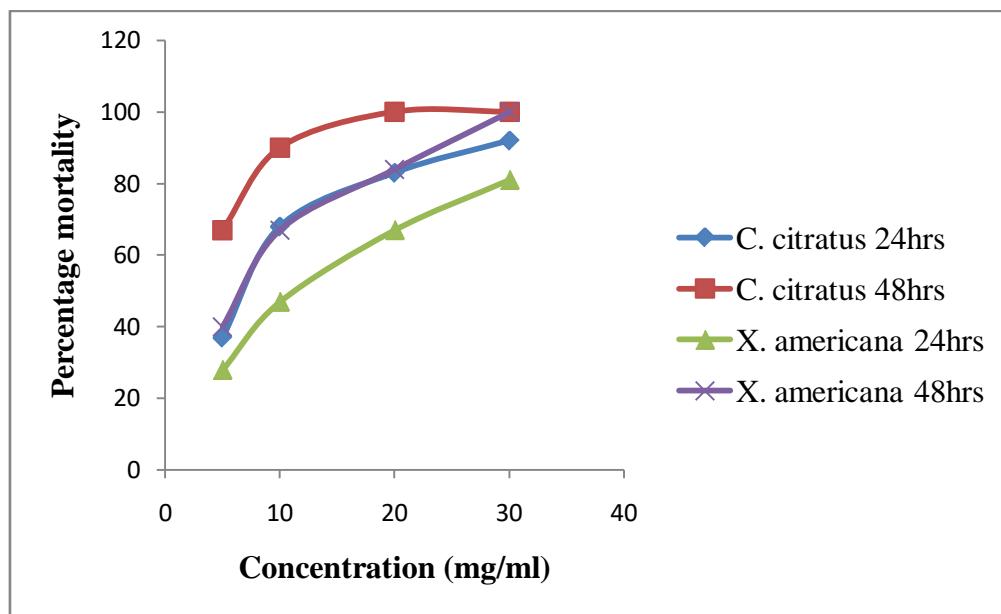


Figure 3.2: Percentage mortality of culex in plants extracts

f)  $LC_{50}$  and  $LC_{90}$  of plants extracts on *Anopheles* larvae

The result of probit analysis on *Anopheles* larvae to different concentration of *C. citratus* after the period of exposure showed that the lethal concentration capable of killing 50% of the larvae  $LC_{50}$  after 24 and 48 hours were 5.095 and 3.731mg/ml and  $LC_{90}$  were 16.241 and 9.391mg/ml respectively. While for *X. americana*  $LC_{50}$  were 7.617 and 5.377mg/ml and  $LC_{90}$  were 43.471 and 16.911mg/ml after 24 and 48 hours respectively.

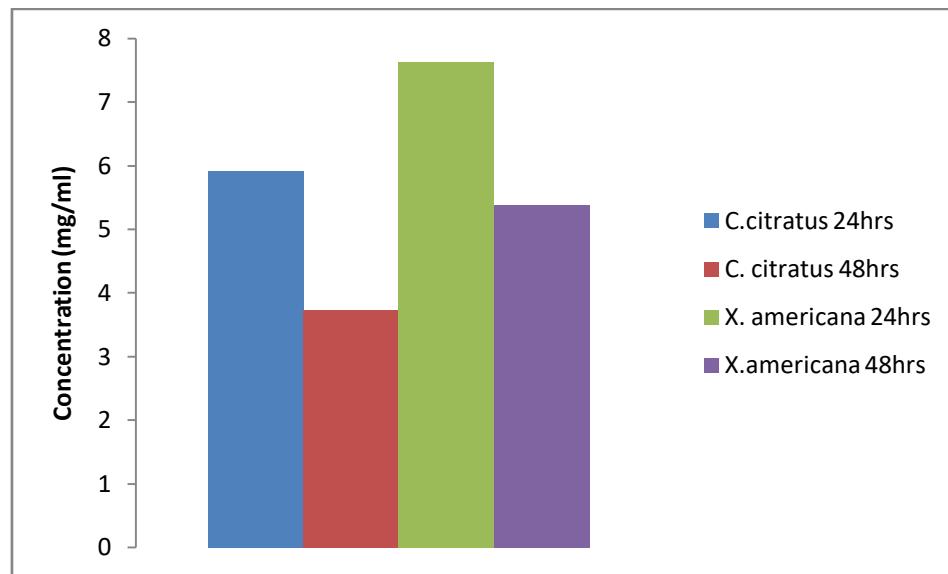


Figure 3.3: LC<sub>50</sub> of extract of the plants against anopheles larvae

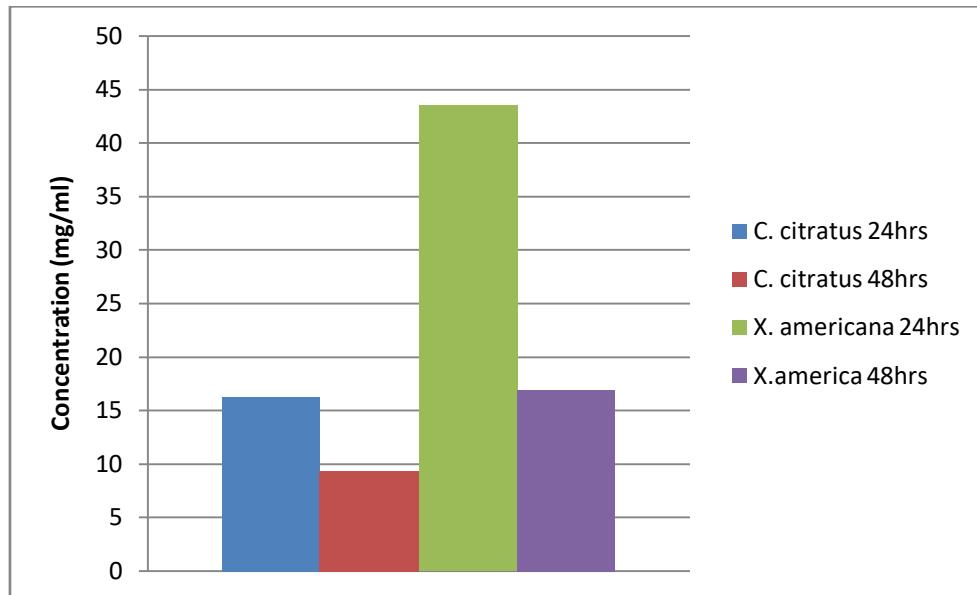


Figure 3.4: LC<sub>90</sub> of extract of the plants against anopheles larvae

g) LC<sub>50</sub> and LC<sub>90</sub> of plants extracts on Culex larvae

The result of probit analysis on *Anopheles* larvae to different concentration of *C. citratus* after the period of exposure showed that the lethal concentration capable of killing 50% of the larvae LC<sub>50</sub> after 24 and 48 hours were 6.851 and 3.7741mg/ml and LC<sub>90</sub> were 25.678 and 8.584mg/ml respectively. While for *X. americana* LC<sub>50</sub> were 10.626 and 6.534mg/ml and LC<sub>90</sub> were 54.434 and 20.130mg/ml after 24 and 48 hours respectively.

Table 3.8: LC<sub>50</sub> and LC<sub>90</sub> of plants extracts on *Culex* larvae

Plants	Times (hrs)	LC <sub>50</sub> (mg/ml)	LC <sub>90</sub> (mg/ml)
<i>C. citratus</i>	24	6.851	25.678
	48	3.774	8.584
<i>X. Americana</i>	24	10.626	54.434
	48	6.534	20.130

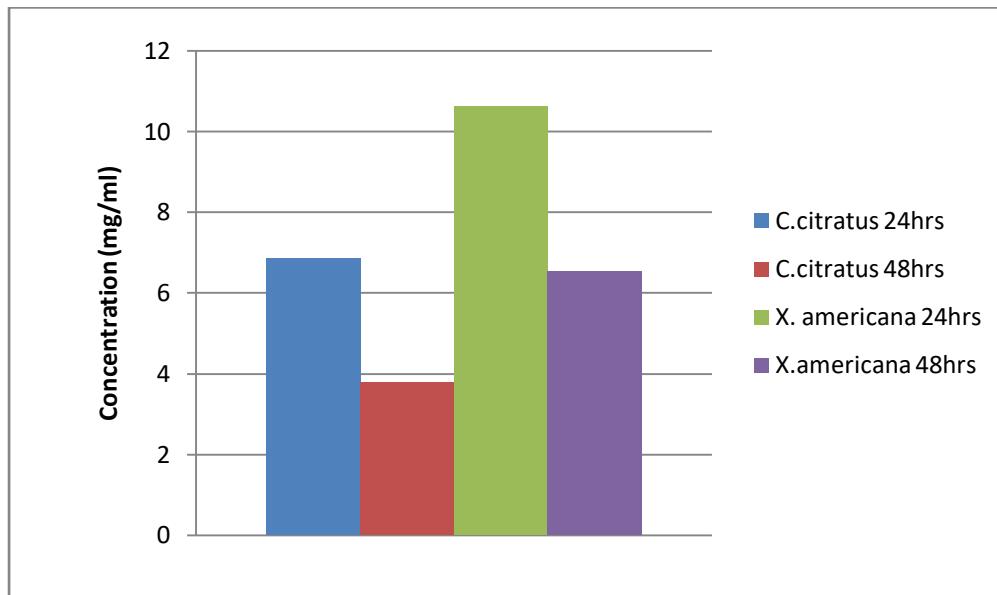


Figure 3.5: LC<sub>50</sub> of extract of the plants against culex larvae

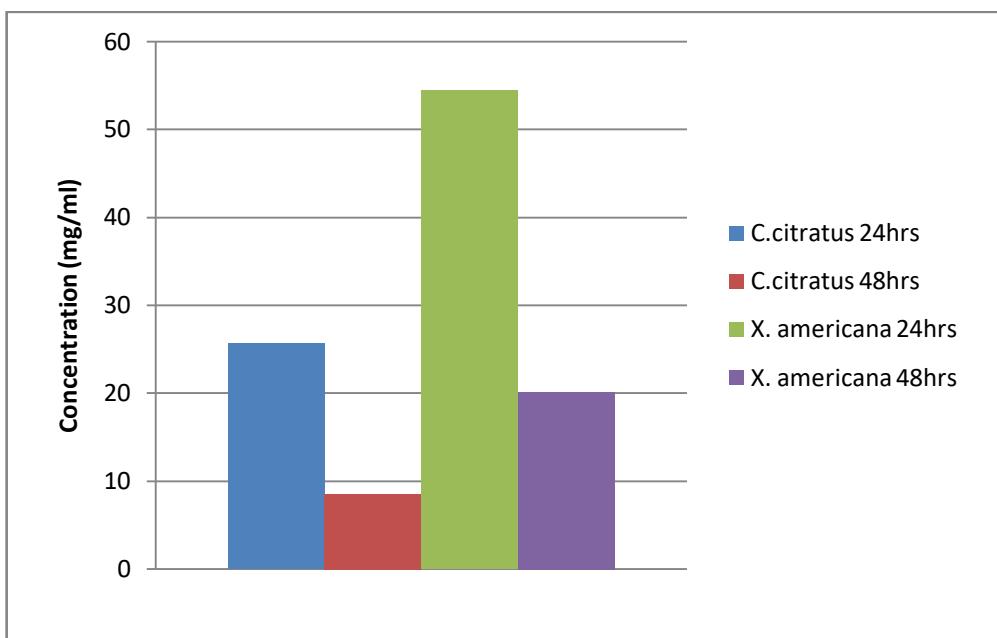


Figure 3.6: LC<sub>90</sub> of extract of the plants against culex larvae

#### IV. DISCUSSION

Result of the phytochemical constituents *C. citratus* show that it contains alkaloids, saponins, flavanoids, phytosteroids, tannins and resins.

Researchers such as Hasim *et al.* [14], identified the same phytochemicals in *C. citratus*. Egunyomi *et al.* [11] worked on the phytochemical component of Nigerian medicinal plants and found the above mentioned active components in *Citrus sinensis* and *C. citratus*. Otabor *et*

al.[24]also reported the presence of the above components in the methanolic extract of *C. citratus*.

However, *X. americana* contains saponins, flavanoids, tannins and resins only, it does not contain alkaloids and phytosteroids which is in line with the findings of Shagal *et al.* [32], Ogunleye and Ibiotoye [23] also reported the absence of alkaloid in *X. americana*. This variation may be due to geographical location and soil type. More so, Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, they protect plants from diseases, damage and also contribute to the plants colour, aroma and flavor [30]. Insecticidal effects of plants extracts vary not only according to plant species, mosquito species, geographical variation and plant part used, but also due to method of extraction followed and the polarity of the solvent used during extraction, Phytochemicals are extracted either from the whole body of little herbs or from various parts like fruits, leaves, stem bark and roots etc of larger plants or trees. In all cases where the most toxic substances are concentrated are usually extracted for mosquito control [3].

From the mortality bioassay of the ethanolic leaves extract of *C. citratus* and *X. americana* shows a proven larvicidal effect against both the mosquito larvae tested (*Anopheles* and *Culex*) at different concentrations. The highest concentration of 30mg/ml from *C. citratus* shows 99% mortality within 24 hours of exposure against *Anopheles* larvae while against *Culex* larvae the same extract at same concentration shows 92% mortality. Similarly, other concentrations, 20mg/ml and 10mg/ml shows almost 100% mortality on both *Anopheles* and *Culex* mosquito larvae after 48 hours of exposure with the same plant extract. The 5mg/ml concentration shows 65% mortality against *Anopheles* larvae and 67% mortality against *Culex* larvae after 48 hours of exposure with the same plant extract.

In contrast, the highest concentration (30mg/ml) of *X. americana* leave extract shows 85% mortality within 24 hours of exposure against *Anopheles* larvae and 81% against *Culex* larvae. Other concentrations, 20mg/ml and 10mg/ml shows more than 50% mortality against both *Anopheles* and *Culex* mosquito larvae after 48 hours of exposure with the same plant extract.

The leaves extract of *C. citratus* and *X. americana* were found to be effective in killing the mosquito larvae, but *C. citratus* extract have more effect on the larvae compared to *X. americana* extract. The results of larvicidal activity showed that the percentage mortality of larvae increases with the increase in concentration of the extract with prolong exposure to treatment. However, in all the experiments no mortality was recorded in the control group which shows that the mortality observed in treating the larvae with extract is due to the presence of some active ingredients that are proven to have some larvicidal properties against mosquito larvae as described by Anupam *et al.*, [3].

Higher percentage mortality was observed on *anopheles* larvae than on *culex* larvae which could be as a result of their breeding habitat, *Anopheles* larvae breed in a fresh water that are free of pollutant, therefore find the plant extracts more toxic than *Culex* larvae which breed in a stagnant water containing pollutants and other toxic substances, therefore they are more resistant to the plant extracts. The comparison of percentage mean mortality of larvae in extract of *C. citratus* and that of *X. americana* showed significant difference at various concentrations and hours of exposure while some showed no significant difference at all concentration and hours of exposure. This could be due to the possession of various phytochemicals constituents that are responsible for the larval mortality, some of these constituents are common within the plants while some are not, therefore the variation in the percentage mortality of the larvae could be as a result of the phytochemical constituents present in the plants. The result of this research shows that leave extracts of *X. americana* has larvicidal effect on mosquito larvae and it could be due to the presence of secondary metabolites present in the plant such as flavonoids, saponins, tannins, and resins. Flavonoids have been reported to has insecticidal properties acting as a mitochondrial poison, which blocks the electron transport chain and prevent energy production (Musau *et al.*, 2016). Flavonoids have also been reported to inhibit Acetylcholinesterase, act as an insect growth regulator and antifeedant (Jagruti *et al.*, 2014). Saponins on the other hand are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking the cuticle membrane of the larvae, eventually disturbing the membrane which is the main cause for larval death [15].

However, in this study probit analysis shows that *C. citratus* has the highest effects against *Anopheles* and *Culex* larvae with  $LC_{50}$  5.905mg/ml and  $LC_{90}$  16.241mg/ml while against *Culex* larvae with  $LC_{50}$  6.851mg/ml and  $LC_{90}$  25.678mg/ml. The result shows that *C. citratus* has the least concentration required to kill 50% and 90% of the larvae within 24 hours of exposure, compared to *X. americana* which has the least effect by having the highest concentration required to kill 50% and 90% of the larvae within the time of exposure with  $LC_{50}$  7.617mg/ml and  $LC_{90}$  43.471mg/ml against *Anopheles* larvae while  $LC_{50}$  10.626mg/ml and  $LC_{90}$  16.241mg/ml against *Culex* larvae. Ebe *et al.* [10] in their findings also revealed that *C. citratus* have some larvicidal effects when tested against *Anopheles gambiae*, *Aedes egypti* and *Culex quinquefasciatus*. Musa *et al.*[19] also revealed that *C. citratus* have some larvicidal and insecticidal effect when tested against *Anopheles* mosquitoes. Several researchers studied the components of essentials part of plants such as bark [15] oil from the leaves [19] etc against different species



of insects which were proven to have some insecticidal and larvicidal properties.

However, the nature of the bioactive components of plants activities depend on the nature of the solvent used during the extraction. In this study ethanol was used which is among the good solvent for extraction of polar organic compounds. Different compounds have been found to be present in the plants used such as flavonoid, alkaloid, saponin, tannin, resin and phytosteroid which have some insecticidal properties. Furthermore, Eliman *et al.*, [12] suggest that the use of plants available stand a better option when compared to chemicals for the control of mosquito larvae as the affect non-target organisms and environmental hazards.

## V. CONCLUSION

Plants derived natural products possess a number of phytochemicals that have been proven for larvicidal effect against mosquitoes and that they are non-toxic, easily available at affordable prices, biodegradable and show broad spectrum, targets specific activities. The result for phytochemical screening indicated that alkaloid, flavonoid, saponin, tannin, steroid and resin were present in *C. citratus* while alkanoid and steroid were absent in *X. americana*. The leaves extract of *C. citratus* kills more mosquito larvae than that of *X. americana*. Therefore, in this study it was concluded that the leaves extract of *C. citratus* was found to be more effective against both species of mosquitoes exposed (*Anopheles* and *Culex*) compared to the leaves extract of *X. americana*. The higher mortality of the mosquito larvae recorded with *C. citratus* maybe due to the presence of more bioactive compounds found in it compared to *X. americana*.

This study shows that treating the larvae with plant extracts prevent them from molting to pupae while in the control group the larvae successfully molted and emerged into larvae.

### Competing Interest

The authors of this article declare no conflict of interest through the processes of this work.

## ACKNOWLEDGEMENT

Our profound gratitude goes to the entire members of staff of the department of Microbiology, Bayero University Kano Nigeria for their academic and technical support for the success of this work. A special thanks also goes to the staff members of pharmaceutical laboratory section of Aminu Kano Teaching Hospital, Kano for their unflinching support during the data collections phase of this work.

## REFERENCES RÉFÉRENCES REFERENCIAS

1. Amzad MK, Hossain KA, Salim ZH, Al-mijizy AM, Weli QA. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pacific Journal of Tropical Biomedicine*. 2013;3(9):705-710.
2. Anees A, Abbas F, Sufia H, Khoo WD. Optimization of soxhlet extraction of *herbaleonuri* using factorial design of experiment. *International Journal of chemistry* 2010; 2(1):198.
3. Anupam G, Nandita C, Goutam C. Plant extract as potential mosquito larvicide. *Indian Journal of Medical Research*. 2012;135(5): 581-598.
4. Borah R, Kalita MC, Kar A, Talukdar AK. Larvicidal efficacy of *Toddaliaasiatica*(Linn.) Lam against two mosquito vectors *Aedesaegypti* and *Culexquinquefasciatus*. *African Journal of Biotechnology*. 2010; 9(16):2527-2530.
5. Cheah S. Toxicity and sublethal effects of *Artemisia annua* Linnaeus on *Aedes aegypti*, *Aedes albopictus*, *Anopheles sinensis* and *Culex quinquefasciatus*. *New England journal of medicine*. 2014;371(5):411-423
6. Cheah S, Tay J, Chan L. Larvicidal , oviposition and ovicidal effects of *Artemisia annua* (Asterales: Asteraceae) against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*. 2013; 112: 3275-3282.
7. Coetzee M. Distribution of the African malaria vectors of the *Anopheles gambiae* complex. *American Journal of Tropical Medicine*. 2010; 70: 103-104.
8. Connelly CR, Bolles E, Culber D, De Valerio J, Donahoe M, Gabel K, Jordi R, McLaughlin J, Neal A, Scalera S, Toro E, Walter J. Florida Resident's Guide to Mosquito Control, Integrated Pest management for Mosquito Reduction around Homes and Neighborhoods. *University of Florida*, USDA-NIFA.2014; 22-37.
9. Dalziel JM. The useful plants of tropical west Africa. *Crown agents for overseas and Government and adains* 5<sup>th</sup> ed. London press;1937
10. Ebe E, Ifeyinwa M, Roselyn F, Njoku T, Chinedu I, Emanuel E. Larvicidal effect of *cymbopogon citratus* root and leaf on first instar larval stage of *Anophelesgambiae*, *culexquinquefasciatus* and *Aedesaegypti*. *Journal of Environmental Toxicology and Public Health*. 2015; 1:41-13.
11. Egunyomi A, Gbadamosi IT, Osinama KO. Comparative effectiveness of ethanol botanical mosquito repellents. *Journal of Biological Science*. 2010; 36: 2382-23008.
12. Eliman AM, Elimalik KH, Ali FS. Efficiency of leaves extract of *clattropisprocera* art in controlling *Anopheles gambiai* and *culex quinquefasciatus* mosquitoes. *Saudi journal of Biological Science*. 2009; 23:15-19

13. Gillies MT, Coetzee M. A supplement to the anophelinae of Africa south of the sahara (Afrotropical Region). South African Institute for Medical Research, Johannesburg. *Publication of the South African Institute for Medical Research*. 1987;55:1-143

14. Hasim S, Falah RD, and Ayunda DN. Potential of lemongrass leaves as prevention for oil oxidation. *Journal of Chemical and Pharmaceutical Research*. 2015; 7(10):55-60.

15. Jagruti M, Kevalia J, Patel P. Mode of action of phytochemicals in target insect body. *International Journal of Pharmaceutical Science*. 2014; 5(521): 365 – 373.

16. Kamaraj C, Abdul Rahman A, Bagavan A, AbdulZahir A, Elango G, Kandan P. Larvicidal Efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culexquinquefasciatus* (Diptera: Culicidae). *Tropical Biomedicine*. 2010; 27: 211–219.

17. Maheswaran R, Ignacimuthu S. A novel herbal formulation against dengue vector mosquitoes. *Aedesaegypti* and *Aedesalbopictus*. *Journal of Parasitology Research*. 2012;110

18. Maheswaran R, Ignacimuthu S, Sathish S. Larvicidal activity of *Leucasaspera* against the larvae of *Culexquinquefasciatus* and *Aedesaegypti*. *International Journal of Integrative Biology*. 2008; 2(3):214

19. Musa AR, Aleiro BL, Aleiro AA, Tafinta IY. Larvicidal and insecticidal effect of *Cymbopogoncitratus* (Lemongrass) on *Anopheles* mosquitoes in Sokoto State, Nigeria; *Annals of Biological Sciences*.2015; 2(1):19-22.

20. Musau JK, Mbaria JM, Nguta JM, Mathiu M. Phytochemical composition and larvicidal properties of plants used for mosquito control in Kwale County, Kenya. *International Journal of Mosquito Research*. 2016;3(3): 12–17.

21. Naqqash M, Gokce A, Bakhsh A, Salim M. Insecticide Resistance and its molecular basis in urban insect pest. *Parasitology Research*. 2016; 115: 1363-1373.

22. Ngbede J, Yakubu, RA, Nyam DA. Phytochemical screening for active compound in *Canarium schweinfurthii* (Atile) leaves from Jos North, Plateau State, Nigeria. *Research Journal of Biological Sciences*. 2008; 3(9):1076-1078.

23. Ogunleye DS, Ibiotoye SF. Studies of Antimicrobial activity and chemical constituent of *Ximenia americana*. *Tropical Journal of Pharmaceutical Research*. 2003; 2(2):239-241.

24. Otabor JI, Rotimi J, Opoggen L, Egbon IN, Uyi OO. Phytochemical constituents and larvicidal efficacy of methanolic extracts of *Cymbopogon citratus*, *ocimumgratissimum* and *Vernoniaamygdalina* against *culex quinquefasciatus* larvae. *Journal of applied science and environmental management*. 2019; 23(4):701-709.

25. Pavela R. Possibilities of botanical insecticides exploitation in plant protection. *Pesticide Technolgy*. 2007; 1:47-52.

26. Pavela R. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* larvae (Diptera: Culicidae). *Parasitology Research*. 2008; 102:555-559.

27. Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi R, Tippawangkosol P, Riyong D, Tuetun B, Chaiyasisit D. Aromatic plant-derived essential oil: An alternative larvicide for mosquito control; *Fitoterapia*. 2007;8(3):205-10.

28. Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir,AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera:Culicidae). *Parasitology Research*. 2009; 104: 1365-1372.

29. Rufalco-moutinho P, Schweigmann N, Pimentel D, Anice M, Sallum M. Acta Tropical larval habitats of *Anopheles* species in a rural settlement on the malaria frontier of southwest Amazon. Brazil. *Acta Tropica*. 2016;164:243-258

30. Saxena M, Saxena J, Rajeev N, Signh D, Abhishek G. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*. 2013; 1(6):819

31. Semmler M, Abdel-Ghaffar F, Al-Rasheid KAS, Mehlhorn H. Nature helps from research to products against blood sucking arthropods. *Parasitology Research*. 2009; 105(6):1483-7.

32. Shagal MH, Kubmarawa D, Bamina S. Evaluation of Antimicrobial property of *Ximenia americana*. *Journal of Biotechnology and Pharmaceutical Research*. 2013; 4(6):99-102.

33. Sheikh SA, Nizamani SM, Jamali AA, Panhwu AA. Removal of pesticides residues from okra vegetables through traditional processing. *Journal of basic applied science*.2012;8:79-84.

34. World Health Organization (2005). Guide lines for laboratory and field testing of mosquito larvicides. World Health Organization Communicable disease control, prevention and eradication. WHO Pesticides Evolution Scheme. WHO/CDS/WHOPE S|CGCD, 13.

