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Larvacidal Effects of Methanolic and Ethanolic Leaf Extracts of *Xienemia americana* against Female *Anopheles gambiae* S.L. (*Culicidae*: *Diptera*).

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ABSTRACT
Anopheles gambiae complex is the most important vector of malaria in sub-Saharan Africa. It is thought that Nigeria comprises about 45 species of *Anopheles* mosquitoes of which *Anopheles gambiae* is the most prevalent. Extract of different concentration were evaluated at the level of 2.00 3.00, 4.00, 5.00 and 0.00 (Control) mg/ml. Distilled water only was used as control. Twenty (20) larvae were put into four (4) cups containing 100ml of the test solution of each concentration. Larval to pupa mortality were recorded and calculated after 24, 48 and 72h after treatment. Mortality results were used to determine LC_{50} and LC_{90} using probit analysis. The larvicidal activity of methanol and Ethanolic extracts of the *X. Americana* were evaluated on 4th instar larvae of *Anopheles gambiae*. From the results concentration of *X. Americana* with ethanol has shown high larvicidal effect with 10%, 12%, 3% adult emergence when compare with control. While Methanol concentration reveals low larvicidal effect with 16 % adult emergence. Treatment group *X. Americana* showed highest survival from pupa to adult (28, 18% and 6%) recorded, this means concentration of ethanol has larvicidal effect against *An. gambiae*. However, methanol concentration shows low activity with 45, 21 and 16% followed by *X. Americana* with 18, 15 and 10% recorded the highest larvicidal activity among all the concentration with methanol respectively. Concentration of 5.0 mL, 12.0 mL, 20.0 mL, 25.0, 30.0mL compare to control group. All the extracts proved to be toxic to the test organism, though there was a remarkable difference in the concentrations and timing of their activity.

Keywords: Larvicidal; Ethanol; Methanol; *X. Americana; Anopheles gambiae*

INTRODUCTION
Mosquito control methods mainly rely on the chemical insecticides, but has led to environmental pollution (Barnard, 1999). Mosquito control has become more difficult due to the unsystematic use of synthetic chemical insecticides which have inauspicious effect on the environment (Das *et al*., 1997), they also effect man and animals because they are not properly degradable and spread their toxic effect (Akhtar *et al.*, 2009). The larval stages of mosquitoes can easily eradicate for control operations because they are less movable in larval forms than the adults (Benelli, 2015). To control or eliminate mosquito population highly efficacious pesticides have been employed. These pesticides are threatened due to the developing resistance of mosquitoes against them (Cheng *et al.*, 2004). Therefore, alternative biological mosquitocides are urgently needed. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Arsenaul *et al*., 2008).

Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal and Adulticidal properties (De Souza *et al*., 2011). Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects (El-Badry, 2010).

Plant extracts are best options for eradication of mosquitoes as they are less harmful to environment and non-targeted species (Conti *et al.*, 2010). There have been many attempts to assay the activity of plant extracts against vectors of human disease, in particular through the utilization of plants for which such knowledge exists (Hafeez *et al*., 2011). Plant extracts contain botanical insecticides or phytochemicals that could be used to limit reproduction and survival of various pest species including mosquitoes (Haque *et al.*, 2009). Mosquitoes are of much concern to public health and well-beings of the global human population (James, 2016). Since these mosquitoes transmit a number of dreadful diseases like filarial, malaria, elephantiasis and dengue, control measures using non-conventional insecticides like botanicals and phytochemical derivatives are gaining much attention in recent days due to a number of favorable reasons (Gericke, 2002).

MATERIAL AND METHODS

Study Area

The study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Health Science, Al-istiqama University Sumaila Kano

Collection and Identification of *An. gambiae*

Anopheles gambiae larvae was obtained from the insectary unit, Department of Biochemistry, Bayero University Kano. Insectare authenticated by taxonomist from the Department of Zoology, Ahmadu Bello University, Zaria.

Mosquito Rearing Protocol

Healthy emerged adult females and males *An. gambiae* were reared and remain inside the insectary for at least 5days for mating to take place. Adults was fed with 10% sucrose before then fed with blood meal after 3-5 days (Des *et al.*, 2007). After each blood meal feeding exercises, successfully fed mosquitoes will become engorge with red colorations abdomen (Clements, 1992) and lay eggs immediately overnight. Beaker was placed inside the cage containing water and a piece of filter paper for oviposition.

Eggs were laid on filter paper over night. Filter paper containing eggs will be placed in a plastic tray with 300ml of distilled water and allowed to hatching into larva (Des *et al.*, 2007). Developing larvae was fed with pinch yeast every day and the use of clean water is also important for refreshing the environment after every feed. Separate pupa from larvae was done daily and placed into a plastic bowl for adult to emerge after 2-3days, inside the insectary (Edillot *et al.*, 2007). Colonies were maintained and all experiments were carried out at a room temperature of 25 ± 2ºC and 80 ± 10% relative humidity (Clements, 1992).

Collections and identifications of plant materials

Plants materials were obtained from Goron-maje ward, Dambatta LGA, Kano State. The plants were authenticated by an expert in plant taxonomy at the Department of Plant Biology, Bayero University Kano.

Processing of plant leaves

Healthy leaves of *X. amaericana* were washed with tap water, cut into small pieces and air dried. After the leaves were completely dry, they were grounded into powder (Jeyabalan *et al*., 2003).

The powdered leaf was extracted with different solvents using Soxhlet extraction method.

Bioassy

The method describes by Sharma *et al*., 2020 was also adopted. Extract of different leaf were evaluated at the level of 2.00 3.00, 4.00, 5.00 and 0.00 (Control) mg/ml. Distilled water only would be used as control (WHO, 1996). Twenty (20) larvae were put into four (4) cups containing 100ml of the test solution of each concentration. Larval to pupa mortality would be recorded and calculated after 24, 48 and 72h after treatment. Mortality results were used to determine LC_{50} using probit analysis (Sharma *et al*., 2020).

Statistical Analysis

All data analyzed were computed using SPSS (Microsoft Co.) Standard deviation was calculated based on the mean values of the experiments to compare between means treated and untreated blood meal with a control groups. Probitanalysis were also use to determine the LC_{50} andLC_{90} between concentration and percentage mortality at various concentrations.

RESULTS

The larvicidal activity of Methanol and ethanoic extracts of the *X. Americana* were evaluated on 4th instar larvae of *Anopheles gambiae*. All the extracts tested exhibited larvicidal potential against the test organism. All the extracts proved to be toxic to the test organism, though there was a remarkable difference in the concentrations and timing of their activity. The lethality pattern of the various extracts on the *Anopheles* larvae is shown in Table 1 revealed that of the three extracts used in this research, only four (Methanolic and Ethyl acetate) exhibited 50% mortality at 24 h, whereas the ethanoic extracts could not achieve a 50% mortality.

The ethanoic leaf extracts of *X. Americana* against 3rd and 4th instar larvae at extract concentration of 5.00 mg/ml and 24 h exposure time, the test organism showed 50% mortality on *X. americana*. This 50% mortality was also obtained after 48 h of test at a concentration of 0.5 mg/ml. However, 100% mortality was achieved by extract concentration of 4.00 mg/ml with at 72 h of test.

Methanolic leaf extract of leaf extracts of *X. americana* against 3rd and 4th instar larvae at concentrations ranging from 0.5 mg/ml to 5.00 mg/ml revealed that the methanol leaf extract of *X. Americana* could not kill 50% of the test organism at test times of 24 and 48 h. However, at 72 h, 95% mortality of *X. americana* was exhibited at concentrations of 3.00 mg/ml to 5.00 mg/ml. LC_{50} and LC_{95} Leaf Extracts of *X. americana* at Varying Time Intervals.

Table 1: Effect of ethanol extract of *X. americana* concentration on larva developmental stages of *An.gambiae*

Concentration (ppm)	Larval Mortality No.	Survival to pupae No.	Adult hatched No.	Inhibition of Adult Emergency (%)
Ethanol				
5.0ml	18	41	38	45
10.0ml	27	33	18	21
15.0ml	21	18	14	16
20.0ml	22	5	4	4
25.0ml	17	3	1	0.0
30.0ml	24	5	3	0.0
Control	5	91	6	-

*Used the equation Abbot (Abbott, 1993) to the percentage inhibition of treatments, according to those in control (Untreated). With six (6) replicates (larvae/replicate)

Table 2: Effect of methanol extract of *X. americana* concentration on larva developmental stages of *An.gambiae*

Concentration (ppm)	Larval Mortality No.	Survival to pupae No.	Adult hatched No.	Inhibition of Adult Emergency (%)
Methanol				
5.0ml	11	31	20	20
10.0ml	24	12	18	7
15.0ml	12	15	6	14
20.0ml	26	22	18	15
25.0ml	20	36	12	10
30.0ml	25	14	8	9
Control	5	59	31	0.0

*Use d the equation Abbot (Abbott, 1993) to the percentage inhibition of treatments, according to those in control (Untreated). With six (6) replicates (larvae/replicate)

Table 3: LethalityLC_{50} and LC_{95} Ethanol and Methanol Leaf Extracts *X. americana* at Varying Time Intervals

		LC_{50} and LC_{95} at 95% C.L					
Solvent 24hrs	Lethality	Conc. (mg/ml)	Lower Bound	Upper Bound	Log Conc.	Lower Bound	Upper Bound
Ethanol	LC_{50}	3.141	2.399	4.286	0.497	0.380	0.632
	LC_{95}	42.154	22.846	110.030	1.625	1.359	2.042
Methanol	LC_{50}	0.352	0.199	0.529	-0.453	-0.702	-0.277
	LC_{95}	4.730	3.246	7.911	0.675	0.511	0.898

LC_{50} = Lethal concentration that kills 50% of exposed larvae; Control= Nil mortality; SE= Standard error; Comparing experimental and control group, with a significant level established at $P<0.05$ and were ANOVA was significant

DISCUSSION

Crude extract of *X. americana* has specifically been reported to inhibit metamorphosis thereby disallowing pupation or adult emergent of the mosquito (Kabaru and Gichia, 2001). The results of this study agree with the finding of Okumu *et al.* (2007) where it was reported that *X. americana* and *C. micratum* is highly toxic to mosquito and delay pupation on *Aedes* and *Anopheles*. Exposure of *A. gambiae* larvae to sub lethal doses of *C. micartum* and catnip leaves extract in the laboratory prolonged larvae development and pupation (Su and Mulla, 1999). The early instars larvae were more susceptible than the later ones and the pupae, which was not much affected by all the solvents. These phyto-chemicals have earlier been reported to have larvicidal and insecticidal abilities (Sofowora, 1993).

The lethality pattern of the various extracts on the *Anopheles* larvae is shown in table 2, it was evident that at extract concentration of 5.00 mg/ml and 24 h exposure time, the test organism showed 50% mortality on *X. americana*. This 50% mortality was also obtained after 48 h of test at a concentration of 0.5 mg/ml on *C. micrantum*. However, 100% mortality was achieved 4.00 mg/ml with *A. citrodora* at 72 h of test (Su and Mulla, 1999).

CONCLUSION

It can be concluded that based on different solvent (ethanol and methanol) *X. americana* extract exhibited larvicidal properties. *X. americana* extract can be employed as effective mosquitocidal agent against larvae of female *Anopheles* mosquitoes having recorded on different in this study. It is recommended that further research studies should be conducted with a view to assessing the ovicidal activity of *X. americana* against the developmental stages of *Aedes* and *Anopheles* mosquitoes.

REFERENCES

Aktar, W., Sengupta, D. and Chowdhury, A. (2009); Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Journal of Toxicology*, (2):1-12.

Arsenal, J. and Malaney, P. (2008).Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors. Asian Pacific *Journal of Tropical Medicine*, 2011, 698-705

Barnard, A.R (2016); In vitro effects of some plant extracts on the development of *Culexquinquefasciatus*in the laboratory, *Journal of Mosquito Research*.

Benelli, W. S. (2005); A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 256 – 257.

Cheng, A.R, and A.M. Adesina (2004); Larvicidal Efficacy of *Cola gigantea*, *Malacanthaalnifolia* and Croton zambesicus Extracts as PhytoinsecticidesAgainst Malaria Vector *Anopheles stephensi* (Diptera: culicidae), *Journal of Mosquito Research*, 5(5), 1-5.

Conti, B., Canale, A., Bertoli, A., Gozzini, F. and Pistelli, L. (2010); Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedesalbopictus (Diptera: Culicidae). Parasitogy Resource*: 107:1455–1462.

Das T.J. Hemen and N. Onyezili (1997); The Effect of Dried Leaves Extract of *Hyptissuaveolens*on Various Stages of Mosquito Development in Benue State, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences.*

De Souza, T. M., Farias, D. F, Soares, B. M, Viana, M. P, Lima, G.P.G, and Machado, L.K. A, et al. (2011) Toxicity of Brazilian plant seed extracts to two strains of *Aedesaegypti* (Diptera: Culicidae) and non-target animals. *Journal of Medical Entomology;* 48:846-851.

Downe, A.E.R. and Archer, J.A. (1975). The effects of different blood-meal sources on digestion and egg production in CulextarsalisCoquillet (Diptera: Culicidae) *Journal Medical Entomology*.; 12:431–437.

El-badry E.R, (2010); Response of *C. maculates* fabricius (Coleoptera: *Chrysomelidae: Bruchinae*) to extract of cashew kernel. *Journal of plant Disease protect*, 118(2): 75-79

El-Badry, A.A. and K.H. Al-Ali, (2010). Prevalence and seasonal distribution of dengue mosquito, Aedesaegypti (Diptera: Culicidae) in Al-Madinah Al- Munawwarah, Saudi Arabia. *Journal of Entomology.*,7: 80-88

Gericke A., Govere J. M. and Durrheim D. N. (2002) Insecticide susceptibility in the South African malaria mosquito Anopheles arabiensis (Diptera: Culicidae). *South Africa Journal of Science* (98):205–208

Hafeez, F., Akram, W., Shaalan, E. A. (2011); Mosquito larvicidal activity of citrus limonoids against *Aedesalbopictus.Parasitology Resource*; 109:221–229

Haque, U., Ahmed, S.M., Hossain, S., Huda, M., Hossain, A., Alam, M.S., Mondal, D., Khan, W.A., Khalequzzaman, M., Haque, R. (2009). Malaria Prevalence in Endemic districts of Bangladesh..;4:1-12

James, B. Kitzmiller and Done, W. (2016). Techniques for rearing Culex mosquitoes. The University of Notre Dame Mick sources: *The American Midland Naturalist*, 52(1): 253-256.

Jayabalan SA., Banjo A.D., Lawal O.A., and Jonathan K., (2003); The efficacy of some plant extracts on *Anopheles gambae* Mosquitoes larvae. *Academic Journal of Entomology*, 2(1): 31-35

Kabaru M.A and Gichia (2001); Molecular Characterization of Mosquitoes of *Anophelesgambiae* Species Complex (Diptera: Culicidae) from Sudan and Republic of Southern Sudan. *Journal of Mosquito Research*, 4(13), 1-10.

Kabaru M.A., Abubakar, M.G., Umar, R.A., Gwarzo, M. S., Muhammad, M. and Ibrahim. M. (2011). Studies on the larvicidal efficacy of aqueous extracts of *Striga hermonthica* (Delile) Benth and *Mitracarpusscaber*(Zucc) on *Culexquinquefasciatus*(culicidae)mos quito larvae.*Journal of Medicinal Plants Research*, 5(21): 5321-5323.

Okumu K. and Morsy TA. (2007); The larvicidal activities of the peel oils of three citrus fruits against *C. pipiens*. *Journal of EgyptianSociety of Parasitology*, 29: 347-352.

Sharma, R. N., Tare, V., Pawar, P. and Vartak, P. H. (1992); Toxic effect of some plant oils and their common, *Journal of Biology Chemical*, (284): 2203-13.

Su O, and Mulla (1999); Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary *Journal of Toxicology*; (2):1-12.