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BIOEFFICACY OF LEAVES EXTRACTS OF FIVE SELECTED PLANTS AGAINST LARVAE OF MALARIA MOSQUITO SPECIES *Anopheles gambae* AND *Culex quenquifasciatus* AND FORMULATION OF LARVICIDE

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Abstract

Plants are rich sources of alternative agents for the control mosquito because they possess bioactive chemicals which act against mosquito species including specific target insects and are eco-friendly. The need for a more effective larvicide with broad host specificity leads to this study which evaluated the larvicidal efficacy of aqueous and ethanolic leaf extracts from Jatropha curcas, Azadirachta indica, Senna tora, Ficus thonningii and Delonix regia against the larvae of Anopheles gambiae and Culex quenquifasciatus. Larvicidal bioassay showed concentration dependent activity of both ethanolic extracts of Ficus thoningii and Delonix regia were indicated a positive correlation of LC_{50} and LC_{90} at 0.021, 0.004 and 1.312, 0.226 respectively against Anopheles gambiae, while Azadirachta indica, Senna tora and Ficus thoningii indicated a positive correlation of LC_{50} and LC_{90} at 0.032, 0.376, 0.231 and 0.467 5.433, 3.337 respectively against Culex quenquifasciatus. The LC_{50} and LC_{90} values of aqueous extracts were determined based on the transformed PROBIT analysis and were observed to be 0.017, 0.058, 0.035 and 0.206, 0.716, 0.438 for Jatropha curcas, Azadirachta indica and Delonix regia respectively, indicated a positive correlation against Anopheles gambiae, while Jatropha curcas, Azadirachta indica, Senna tora and Ficus thoningii also indicated a positive correlation of LC_{50} and LC_{90} at 0.008, 0.029, 0.100, 0.018 and 0.069, 0.260, 0.883, 0.159 respectively against larvae of Culex quenquifasciatus. The aqueous extracts were found to be more potent than the ethanolic extract. Jatropha curcas, and Azadirachta indica were more effective against the larvae of malaria mosquito Anopheles gambiae and Culex quenquifasciatus.

Keywords: Plant extract, *Anopheles gambiae, Culex quenquifasciatus,* larvae, malaria

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Introduction

Mosquitoes are vectors responsible for the transmission of various diseases, such as malaria, yellow fever, dengue fever and other infections. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito borne disease is by killing mosquito at larval stage. The current mosquito control approach is based on synthetic insecticides (Anupam Ghosh *et al.,* 2012) which created many problems like insecticide resistance, environmental pollution and toxic side effect on human begins. One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of biological origin as a simple and sustainable method of mosquito control. A wide selection of plants from herbs, shrubs and large trees were used for extraction of mosquito toxins (Mikolajczak, *et al.*, 1989). Phytochemicals were extracted either from the whole plant or from various parts of plant, like fruits, leaves, stem, bark, roots etc.

A large number of plant products have been reported to have mosquito larvicidal and repellent activity against adult mosquitoes. The discovery of insecticide activity in *Asteraceae* species has stimulated interest in this plant family as part of the search for new plant derived insecticides (Rawls, 1986; Prashant *et al.,* 2006; Masotti *et al.,* 2012). In recent years, essential oils have received much attention as potent bioactive compounds against various mosquito species (Tripathi *et al.,* 2009). WHO has declared the mosquitoes as "public enemy number one" (WHO, 2021). Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 241,000,000 people every year globally (World malaria report 2021) and 68,000,000 of the Nigerian population (World malaria report 2021 in Nigeria). They are nuisance to human beings and are the major vector for the transmission of life-threatening diseases.

In Nigeria malaria is one of the most important cause of direct and indirect infant, child and adult mortality with approximately two to three million new cases arising every year. Malaria is transmitted through bites of parasite infected Anopheles species (Beadle *et al,*. 1994), Culex species also transmit lymphatic filariasis and possibly Zika virus (Diallo *et al.*, 2014).

Larvicides kill the mosquitoes in the juvenile stages before they emerge in to hematophagous adults. Larvae are bound to their habitats, and hence, the control operations are much easier with larvicides. Larval control mainly relies on application of synthetic chemical control agents. Although effective, repeated use of chemical insecticides has resulted in resistant mosquitoes and environmental pollution (Liu 2015; Lawler *et al.* 2003, 2007) and the limited success of biocontrol programs on *Culicidae* (Benelli 2015a) has spurred the need for search for new insecticides (Liu 2015). Plant products are coming up as a promising alternative for synthetic chemical agents for insect management or control.

Materials and Method

Sample collection

The leaves of five locally available plants *Jatropha curcas, Azadirachta indica, Senna Occidentalis, Thonningii Ficus, and Delonix regia* were collected from different localities in Dawakin Tofa and Kura region of Kano State and kept separately in plastic bags and brought to the laboratory for extraction and small quantity were identified at the Botanical unit of Bayero University Kano. The leaves of the plants were dried for 30 days in the shade at room temperature.

Extraction

The dried leaves were powdered mechanically (50g) using electrical stainless-steel blender and extracted with ethanol (250 ml) for 2hrs in a Soxhlet apparatus separately until exhaustion *(*WHO 2004). The extracts were filtered through a Buchner funnel with Whatman filter paper and the filtrate was collected in a beaker. The filtrates were pooled and concentrated using an oven at 80ºC under low pressure for 24hrs until the solvent completely evaporated. The extract of each plant thus obtained in a 10ml sample container was lyophilized and then kept at room temperature 27±2ºC until testing of mosquito larvicidal activity. Standard stock solutions were prepared at 1% and from the stock solution various concentrations were prepared.

Test mosquitoes

The larvae of *Anopheles Gambiae* and *Culex Quinquefasciatus* were collected from the sewage water of Tukuntawa, Municipal LGA, Kano State and kept in 500ml plastic container containing tap water and all the experiments were carried out at 27±2ºC and 75-85 % relative humidity.

Larvicidal bioassay

The larval bioassay tests for the larvicidal effect of ethanolic extracts derived from five different plants were carried out in accordance with the WHO standard method (WHO, 1981, 2005). For the bioassay test, 5g of each plant extracts were taken and 3 dilutions were made in distilled water (5g/10mL, 0.5g/10mL and 0.05g/10mL). Then 10 larvae were taken in different 500mL plastic container containing 2mL of different concentrations of the plant. The control was set up with ethanol. The number of dead larvae was counted after 24hrs of exposure and the percentage mortality was reported from the average of five replicates. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Dead larvae were removed as soon as they were discovered to prevent decomposition, which may cause rapid death of the remaining larvae.

Dose-response bioassay

From the stock solution, different concentrations ranging from 1/10mL were prepared. Based on the preliminary screening results, crude of different solvents were prepared from the leaves extracts of *Jatropha curcas*, *Azadirachta indica*, *Senna Occidentalis*, *Thonningii Ficus* and *Delonix regia* and subjected to dose-response bioassay for larvicidal activity against the larvae of *Anopheles* species and *Culex* species. The numbers of dead larvae were counted after 24hrs of exposure, and the percentage mortality was reported from the average of five replicates.

Results and Discussion

Mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adult is temporary, unsatisfactory and polluting to the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. The vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides (Chandre *et al.*, 1998). The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose.

The application of botanicals for the control of mosquitoes is recommended (Alkofahi *et al.*, 1989), because they minimize the accumulation of harmful residues in the environment. The crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Mayura *et al.*, 2007). The activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the preliminary screening, potential larvicidal activity of five plants as crude aqueous and ethanolic extracts in different concentrations was noted.

The mean mortality of ethanolic plants extract against *Anopheles gambiae* was illustrated in table I and II The plants extract of *Ficus thoningii, Delonix regia* at conentrations of 5g/10ml and *Delonix regia* at concentration of 0.5g/10ml shows highest mortality of all the 10 tested larvae, and lowest mortality of 0 in the plant extract of *Senna tora* at 0.05g/10ml.

Table I: Mean mortality of Ethanolic extracts of leaves of five plants against *Anopheles* and *Culex* species after 24 hours

S/N	**Plant extracts**	**Tested Larvae**	***Anopheles* species**			***Culex* species**		
			5g /10ml	0.5g /10ml	0.05g /10ml	5g /10ml	0.5g /10ml	0.05g /10ml
1	Control (ethanol)	10	0	0	0	0	0	0
2	*Jatropha curcas*	10	5	5	2	9	8	6
3	*Azadirachta indica*	10	7	5	2	10	8	7
4	*Senna occidentus*	10	6	4	0	10	5	1
5	*Ficus thonnagii*	10	10	7	7	10	8	0
6	*Delonix regia*	10	10	10	7	8	5	2

Also, the ethanolic plants extract against *Culex quenquifasciatus* in table 1 shows that, the plant species *Azadrichta indica, Senna tora, Ficus thoningii* at concentration of 5g/10ml and *Ficus thoningii* at concentration of 0.5g/10ml shows highest mortality of all the 10 tested larvae, and lowest mortality of 0 in the plant extract of *Ficus thoningii* at concentration of 0.05g/10ml.

All plant extracts show moderate toxic effect on *Anopheles* and *Culex* species after 24hrs of exposure at different concentrations. Mosquito larval mortality was examined at different concentration of the extracts. Mortality profile indicates an increase with increasing concentrations and exposure duration and the highest mortality was recorded in the highest concentrations.

Table II: Mean mortality of Aqueous extracts of leaves of five plants against *Anopheles* and *Culex* species after 24 hours

S/N	Plant extracts	Tested Larvae	*Anopheles* species			*Culex* species		
			1g /10ml	0.1g /10ml	0.01g /10ml	1g /10ml	0.1g /10ml	0.01g /10ml
1	Control (water)	10	0	0	0	0	0	0
2	*Jatropha curcas*	10	10	7	5	10	9	6
3	*Azadirachta indica*	10	10	5	2	10	7	3
4	*Senna Occidentus*	10	9	7	2	10	5	0
5	*Ficus Thonnagii*	10	7	4	0	10	8	4
6	*Delonix regia*	10	10	6	3	8	5	1

The mosquito larvae mortality was examined at different concentration of the five selected plant extracts and topical application bioassays were performed. The results revealed that, among the 5 plants extract tested, *Delonix regia*, was the most toxic ethanolic extracts of leaves of five plants against *Anopheles gambiae* with LC_{50} and LC_{90} value at 0.004 and 0.226 respectively, followed by *Ficus thoning* with an LC_{50} and LC_{90} value at 0.021 and 1.312 respectively, *Azadirachta indica* with an LC_{50} and LC_{90} value at 0.692 and 43.399 respectively, while *Ficus thoning* and *Senna tora* does not show any significant effect against *Anopheles gambiae* at LC_{50} and LC_{90} table III. This result is comparable to Singh *et al.* (2006) who observed the larvicidal activity of *Ocimumcanum* oil against *Culex quenquifasciatus* and *Anopheles gambiae*.

Table III: Larvicidal activity of Ethanolic extracts of leaves of five plants against *Anopheles Gambiae*

Plant extract	LC 50 (95% C.I)	LC 90 (95% C.I)	SLOPE (SE)	p_{value}	χ^2, df=11
Control	0	0	-3.673 (3.321)	p=.269	7.258 P=.778
Jatropha curcas	1.289(0. .267– 8.711)	80.860(11.188– 5710.158)	-0.079 (.244)	p=.747	
Azadirachta indica	.692(0.132 – 4.055)	43.399 (6.607 – 2220.608)	0.114 (.246)	p=.644	
Senna tora	2.726(0.533 – 24.515)	170.960(20.126 – 17835.590)	-0.310 (.256)	p=.226	
Ficus thoning	0.021(0.001 – 0.124)	1.312(0.225 – 17.257)	1.198 (.296)	p=.000	
Delonix regia	0.004(0.000 – 0.035)	0.226(0.022 – 2.966)	1.742 (.373)	p=.000	

The mosquito larval mortality was examined at different concentrations of the 5 selected plants extracts, in Table IV. The results revealed that the aqueous extract of the 5 plants were found to be significantly toxic against *Culex quenquifasciatus. Azadirachta indica* was the most toxic ethanolic extracts with LC_{50} and LC_{90} value of 0.032 and 0.467 respectively, followed by *Jatropha curcas* with an LC_{50} and LC_{90} value at 0.068 and 0.985 respectively;

Ficus thoning with an LC_{50} and LC_{90} value at 0.231 and 3.337 respectively. While the *Senna tora* and *Delonix regia* were the least effective extract with LC_{50} and LC_{90} value at 0.376 and 5.433 for *Senna tora* 0.500 and 7.228 for *Delonix regia* respectively.

This result is also comparable to results by Amer and Mehlhorn (2006) that, the most effective oils were those of *Litsea (Litsea cubeba*), *Cajeput (Melaleuca leucadendron*), *Niaouli (Melaleuca quinquenervia), Violet (Viola odorata)* and *Catnip (Nepeta cataria)*, which induced a protection time of 8hrs at the maximum and a 100% repellency against Aedes, *Anopheles* and *Culex* species. This revealed that mortality rate of exposed larvae decreased as the concentration of the plants extract increases.

Table IV: Larvicidal activity of Ethanolic extracts of leaves of five plants against *Culex quenquifasciatus*

Plant extract	**LC 50 (95% C.I)**	**LC 90 (95% C.I)**	**SLOPE (SE)**	**p_{value}**	**χ^2, df=11**
Control	0	0	-3.772 (2.703)	p=0.163	13.596 P=0.256
Jatropha curcas	0.068 (0.017-0.213)	0.985 (0.314 – 4.263)	1.289 (0.302)	p=.000	
Azadirachta indica	0.032 (0.007-0.114)	0.467 (0.133 – 2.101)	1.647 (0.340)	p=.000	
Senna tora	0.376 (0.116-1.198)	5.433 (1.650 – 31.196)	0.469 (0.280)	p=.094	
Ficus thoning	0.231 (0.066-0.748)	3.337 (1.006 – 18.073)	0.703 (0.293)	p=.016	
Delonix regia	0.500 (0.165-1.514)	7.228 (2.279 – 40.638)	0.333 (0.263)	p=.206	

In table V the results revealed that the water extracts of the 5 plants show significant mosquito larval mortality. *Jatropha curcas* being the most toxic against *Culex quenquifasciatus* with an LC_{50} and LC_{90} value at 0.008 and 0.069 respectively, followed by *Ficus thoning* with an LC_{50} and LC_{90} value at 0.018 and 0.159 respectively. *Azadirachta indica* with an LC_{50} and LC_{90} value of 0.029 and 0.260 and *Senna tora* has an LC_{50} and LC_{90} of 0.100 and 0.883; and the least effective water extract was *Delonix regia* with an LC_{50} and LC_{90} of 0.129 and 1.135 respectively.

Table V: Larvicidal activity of Aqueous extracts of leaves of five plants against *Culex quenquifasciatus*

Plant extract	**LC 50 (95% C.I)**	**LC 90 (95% C.I)**	**SLOPE (SE)**	**p_{value}**	**χ^2, df=11**
Control	0	0	-3.125 (3.239)	p=.335	4.048 P=.968
Jatropha curcas	0.008(0.002– 0.023)	0.069 (0.023 –0.253)	2.858 (0.471)	p=.000	
Azadirachta indica	0.029(0.010 – 0.078)	0.260 (0.096– 1.000)	2.075 (0.405)	p=.000	
Senna tora	0.100 (0.036 –0.277)	0.883 (0.315 – 3.784)	1.355 (0.357)	p=.094	
Ficus thoning	0.018 (0.006 – 0.049)	0.159 (0.058– 0.593)	2.364 (0.428)	p=.016	
Delonix regia	0.129 (0.050–0.335)	1.135 (0.425– 4.666)	1.207 (0.331)	p=.206	

Topical application bioassays were performed and the results were summarized in Table VI. The results revealed that, among the 5 plants water extract tested, *Jatropha curcas*, was the most toxic against *Anopheles gambiae* with an LC_{50} and LC_{90} of 0.017 and 0.206 respectively, followed by *Delonix regia* with an LC_{50} and LC_{90} value of 0.035 and 0.438 respectively, then *Senna tora* with an LC_{50} and LC_{90}value at 0.0049 and 0.605 respectively, followed by *Azadirachta indica* with an LC_{50} and LC_{90}value at 0.058 and 0.716 respectively, and the least effective extract with water plant extract was *Ficus thoning* with an LC_{50} and LC_{90}value at 0.281 and 3.472 respectively,

Table VI: Larvicidal activity of Aqueous extracts of leaves of five plants against *Anopheles gambiae*

Plant extract	**LC 50 (95% C.I)**	**LC 90 (95% C.I)**	**SLOPE (SE)**	**p_{value}**	**χ^2, df=11**
Control	0	0	-2.892 (2.351)	p=0.219	5.404 P=0.910
Jatropha curcas	0.017 (0.005 – 0.050)	0.206 (0.068 – 0.862)	2.088 (0.380)	p=.000	
Azadirachta indica	0.058 (0.019 – 0.171)	0.716 (0.236 – 3.431)	1.452 (0.343)	p=.000	
Senna tora	0.049 (0.016 – 0.141)	0.605 (0.205 – 2.713)	1.538 (0.339)	p=.000	
Ficus thoning	0.281 (0.096 – 0.885)	3.472 (1.078 – 19.507)	0.647 (0.302)	p=.032	
Delonix regia	0.035 (0.011 – 0.104)	0.438 (0.146 – 1.978)	1.703 (0.357)	p=.000	

Conclusion

From the results of LD_{50} and LD_{90} above, Jatropha *curcas* is the most effective for both ethanolic and aqueous extract on both *Anopheles* and *Culex* species. It was followed by the *Ficus thoning* which effects both *Anopheles* and *Culex* on the two extracts of ethanolic and aqueous, with relatively weak effect on *Anopheles* aqueous extract. The result suggests that plant crude extracts have the potential to be used as an ideal eco-friendly approach for control

of disease vectors. Further research should be carried out on other locally available plants to further investigate their potency as potential larvicide against the malaria mosquitoes of *Anopheles gambiae* and *Culex quenquifasciatus.*

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References

Alkofahi, A; Rupprecht, J.K; Anderson, J.E; Mc Laughlin, J.L; Mikolajczak, K.L; Bernar, A.S (1989): Search for New Pesticides from Higher Plants. ACS Symposium Series vol. 387 ch. 3 pp 25-43

Amer A, Mehlhorn H., (2006).Repellency effect of forty-one essential oils against Aedes, Anopheles and Culex mosquitoes. Parasitol Res. 99:478-490.

AnupamGhosh, Nandita Chowdhury, Goutam Chandra.2012. Plant extracts as potential mosquito larvicides. Indian J Med Res 135, pp 581-598`.

Chandra, S. A. Singh, P. K. Tomar and A. Kumar., (2011). Evaluation of Physicochemical characteristics of various River water of India. E- Journal of chemistry. 8 (4): 1546-1555.

Chima RI, Goodman CA, Mills A. The economic impact of malaria in Africa: a critical review of the evidence. Health Policy 2008;63(1):17-36.

Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y, Hanley KA, Buenemann M, Weaver SC, Diallo M., (2011). Zika virus emergence in mosquitoes in southeastern Senegal. PLoS One. 2014;9:e109442.

Funmilayo B. E. and IkemC. O., (2020). Ovicidal and Larvicidal Activities of Ethanolic Leaf Extracts of three Botanicals Against the Malaria Vector - Anopheles Gambiae. International Annals of Science. ISSN: 2456-7132 Volume 9, Issue 1, pp. 111-121. DOI: https://doi.org/10.21467/ias.9.1.111-121.

Funmilayo G. F., Francis B. A., Oluyemi J. O., and Oluwatosin R. O.,(2020). Larvicidal Activity of Some Plants Extracts and Their Partitioned Fractions against Culex quinquefasciatus. International Journal of TROPICAL DISEASE & Health vol. 41. pp. 23-34. DOI: https://doi.org/10.9734/ijtdh/2020/v41i1130332.

Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HC, et al. The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution 2013;67(4):1218-30.

Maurya P., Mohan L., Sharma P., Batabyal L., Srivastava C. N. 2007. Larvicidal efficacy of Aloe barbadensis and Cannabis sativa against the malaria vector Anopheles stephensi (Diptera: Culicidae). Entomol Res. 37: 153-156.

Mohan L, Sharma P, Shrivastava CN. Evaluation of Solanumxanthocarpum extract as a synergist for cypermethrin against larvae of filarial vector Culex quinquefasciatus (Say) Entomol Res. 2006; 36:220–5.

Singh, R. K., Dhiman, R. C., and Mittal, P. K., (2006). Mosquito larvicidal properties of Momordica charantia Linn (family: Cucurbitaceae). J. Vect. Borne Dis. 43, 88–91.

Rawls RL, (1986). Experts probe issues, chemistry of light activated pesticides. Chem Eng News, 22:2124.

Report of the WHO informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. Geneva: WHO; 1996. World Health Organization; p. 69.

Singh, R. K., Dhiman, R. C., and Mittal, P. K., (2006). Mosquito larvicidal properties of Momordica charantia Linn (family: Cucurbitaceae). J. Vect. Borne Dis. 43, 88–91.

WHO. Malaria policy advisory group (MPAG) meeting report, (October 2021). Geneva: World Health Organization; 2021.

WHO (2007): World Health Organization guideline on good agricultural and collection practices (GACP) for medicinal plants Geneva.

WHO. World Health Organization. (2005). Guideline for Laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCPP/2005.

WHO. World malaria report (2014). Geneva: World Health Organization; 2014.

Woodbridge A. Foster and Edward D., (2002). Science Direct. Walker. Medical and Veterinary Entomology.

World Health Organization (2023): World Malaria Report Geneva: World Health Organization, 2023.