Larvicidal Activity of *Thevetia peruviana* Extracts Against *Anopheles* Mosquito Larvae in Mubi, Adamawa Nigeria

Jasini Alexander Wahedi[*], Gambu James Wurma[**], Patrick Deborah[*]

[*] Department of Zoology, Adamawa State University, Mubi, Nigeria.
[**] Department of Biology, Adamawa State College of Education, Hong.

DOI: 10.29322/IJSRP.10.06.2020.p102106
http://dx.doi.org/10.29322/IJSRP.10.06.2020.p102106

Abstract- Mosquitoes constitute a serious public health menace, resulting in millions of deaths worldwide each year. Emergence of insecticide resistance strains of the mosquitoes poses a serious threat and hence calls for alternative control measures. This study assessed the larvicidal efficacy of the ethanol and aqueous extracts of leaves of *Thevetia peruviana* against the larvae of malaria vector *Anopheles* mosquitoes. Bioassay was carried out on 2nd and 3rd instar larval stages of *Anopheles* mosquitoes. The toxicity effect of the treatment was measure in terms of mortality, which was observed for 72 hours, at 24 hours interval. Data collected was subjected to analysis of variance (ANOVA) to determine the larvicidal efficacy of *T. peruviana*. The means were separated using the Least Significant Difference, while the log/probit-regression analysis was used to determine the lethal concentrations of the treatments at 50% and 95% i.e. LC_{50} and LC_{95} respectively. The treatment extracts at 200 and 400 mg/ml significantly recorded higher mortality of the larvae of *Anopheles* mosquitoes when compared with the control (acetone) experiment. The regression co-efficient (R^2) further revealed the superiority of ethanol extract (0.882 ppm) against aqueous extract (0.055 ppm). This was further evident in the LC_{50} (9.193 ppm, 2.42E+13 ppm) and LC_{95} (17.545 ppm, 1037.079 ppm), for ethanol and aqueous extracts, respectively. Therefore, this study further confirms the efficacy of the use of the use of biopesticides as larvicidal in controlling insect pests especially *Anopheles* larvae.

Index Terms- *Anopheles*, Larvicidal, Mosquito, *Thevetia peruviana*.

I. INTRODUCTION

Mosquitoes are the major cause of diseases like malaria, filariasis, dengue fever, encephalitis, yellow fever etc. [1]. Compared to all the countries of the world, Nigeria has the highest cases of malaria, with a record of about 300,000 people dying each year [2]. Malaria equally accounts for about 63% of the disease reported across the country and a burden of over one billion dollars annually [3]. Malaria situation in Nigeria is very burdensome and it affects humanitarian development and can cause serious consequence of underdevelopment [4]. In Adamawa State in the northeast Nigeria, malaria is highly endemic, and childhood morbidity is generally high [5].

As part of the global strategy geared towards the prevention of malaria, the World Health Organization identified vector control as an important component [6], which fortunately is practiceable [7]. One of such methods is by employing the use of larvicides. Generally, insecticides are a quick and efficient way of controlling mosquitoes around the surroundings but, unfortunately, the effect usually does not last long. The effect lasts only when the insecticide is present [8]. Therefore, there is a need to develop new alternative control protocol for efficient and reliable vector control management. The control of aquatic stages, especially the larval stages of these vectors will go a long way in the effort geared towards eliminating these vectors, which is the sole target in this study. Control of vectors at the larval stage is one of the most promising strategies in eradicating malaria today [1, 9, 10].

The uncontrollable and haphazard use of synthetic insecticides for the control of mosquito vectors has led to the development of resistance and some negative impacts on non-target organisms and the environment. And so, there is a need for development of biological effective mosquito control tools other than the use of the conventional insecticides against the vectors in our environment [11]. The development of insecticide resistance apart from its toxic effects on humans has necessitated the deployment of the indigenous method of vector control. However, insecticide resistance, coupled with the environmental and economic burden arising from continuous use of insecticides and the health problems associated with the vectors calls for urgent alternative control measures. Plant products are some of the alternative substitute to insecticides, and have proven to be effective in controlling mosquito larvae in different places [1, 9, 10, 12, 13, 14]. Therefore, this study was designed to search for the larvicidal activity of *Thevetia peruviana* on *Anopheles* mosquito larvae as an alternative control measure for malaria menace.

II. MATERIALS AND METHODS

A. Study Area

The study was carried out in Mubi, Adamawa State, Nigeria. Mubi is located between latitude $10^0$12N and longitude $13^0$10E. It is characterized by two seasons, the dry and the wet season. The climate is tropical with temperature between 15-42^0 in dry

season and relative humidity between 10-45%, and annual rainfall of about 1056mm [15].

B. Collection and Processing of Plant Treatment (Thevetia peruviana)

Leaves of *T. peruviana* was collected from a plantation in Bazza area of Michika Local Government Area, Adamawa State, and was transported to Zoology Laboratory of Adamawa State University Mubi for processing. The leaves were rinsed in water, spread on a clean pavement for proper air-drying at room temperature. Thereafter, the leaves were ground into fine powder using electric blender. 100% ethanol and methanol extracts were obtained from the powdered treatment using soxhlet apparatus of boiling point ranging 60-80℃ for 6 hours [9]. The extracts were filtered through a Buchner funnel with Whatman number filter paper, while 100% aqueous extract was obtained by soaking 100g of powdered treatment in 100ml of distilled water. The extracts were concentrated under reduced pressure between 22 and 25mm Hg at 45℃. Thereafter, were stored at 4℃.

C. Collection and Maintenance of Anopheles Mosquito Larvae

Anopheles larvae were collected from their natural breeding habitats such as abandoned tires, ponds, rice fields, etc. This was done after identifying them from other mosquito larvae by their horizontal resting position on the surface of water [6]. They were reared in enamel trays on powdered yeast and dog biscuits in the ratio of 40:60 respectively [16].

D. Larvicidal Bioassay

The Larvicidal Bioassay was carried out using WHO Standard test procedure [17] on second and third instar larvae i.e. L2 and L3, respectively. *Anopheles* larvae were taken in batches of 25 per experimental beaker in 250ml using a rubber pipette. The control was set up where 25 instar larvae were introduced into 250 ml beaker, with only acetone as treatment. Both the treatment and the control experiments were replicated four (4) times. Treatment beakers were covered with muslin cloth to avoid entry of any foreign material and for proper aeration. The larval mortality was observed for 72 hours at 24 hours interval. During the bioassay, the larvae were not fed [18]. Mortality was regarded when there is no sign of any movement or even after mild touch with glass rod [19], and dead larvae were counted. If 30% mortality was recorded in the control, the experiment was discarded or the mortality was corrected using Abbott's formula [20], as follows:

$$\text{Corrected Mortality} = \frac{\text{Mortality in Test Bottle (\%)} - \text{Mortality in Control Bottle (\%)}}{100\% - \text{Mortality in Control Bottle(\%)}} \times 100$$

E. Data Collection

Mortality counts were carried out at 24 hours interval and lasted for 72 hours. This was performed by counting and retrieving the dead larvae. And incase in a situation where the mortality is >5%, corrected percentage mortality was determined using Abbot's formula [20]. The Lethal Concentrations at 50 and 95% i.e. LC_{50} and LC_{95} were calculated for each treatment using a log/probit-regression method as described by Finney [21].

F. Data Analysis

Data obtained from mortality was subjected to analysis of variance (ANOVA) to determine the larvicidal activity of *T. peruviana.* The means were separated using the Least Significant Difference (LSD). The log/probit-regression analysis was used to determine the lethal concentrations of the treatments at 50% and 95% i.e. LC_{50} and LC_{95} respectively.

III. RESULTS AND DISCUSSION

Table 1 shows the mortality counts of *Anopheles* mosquitoes larvae exposed to ethanol, methanol and aqueous extracts of *Thevetia peruviana* for 72 hours. The result revealed that there was a significant difference (P>0.05) in the number of mortalities recorded throughout the period of exposure with the control (untreated experiment). The treatment extracts especially the ethanol extracts were quick in action as significant number of larvae died after 24 hours. However, the control experiment did not record any mortality throughout the period of experiment as shown in Table 1.

The result indicate significant larvicidal activity (P<0.05) with ethanol extracts. This could be as a result of the various compounds contained in *T. peruviana* like terpenoids, phenolics, flavonoids and alkaloids, which constitute toxic activity against *Anopheles* mosquitoes. Wink [22] reported that the secondary metabolites produced by some plants have been explored for their utility in mosquito control as it has been already proven that some of the compounds are toxic to the target organism but harmless to humans.

This finding is similar to Kamaraj *et al*. [9] who reported a significant (P<0.05) larvicidal activity of the bark of *Annona squamosa* and leaves of *Chrysanthemum indicum* and *Tridax procumbens*, as moderate toxic effect on *Anopheles Subpictus* and *Culex tritaeniorhynchus*, with the highest mortality recorded in methanol extract was observed. Similarly, Thirumalapura *et al*. [23] reported a significant (P<0.05) higher larvicidal activity of methanol plant extracts against larvae of *Aedes aegypti* and *Anopheles stephensi.* Patil *et al*. [10] reported that crude extracts of *Balanites aegyptiaca*, *Nyctanthes arbor tristis*, and *Plumbago zeylanica* significantly controlled the larvae of *Aedes aegypti* and *Anopheles stephensi*.

The result obtained from the regression equation which was obtained from the probit analysis for LC_{50} and LC_{95} of the ethanol and aqueous extracts of *T. peruviana* is shown in Table 2. The result showed that aqueous extract had the least larvicidal effect on larval *Anopheles* mosquitoes compared with the ethanol extracts. The aqueous extract recorded the highest value of

1037.079 ppm that will be required to kill 95% of the *Anopheles* larval population. Meanwhile, ethanol extract recorded a significant lower LC_{50} (9.193 ppm). However, the regression coefficient revealed that ethanol extract of *T. peruviana* had the highest (0.882 ppm) correlation between the treatment concentration and the mortality, while the least (0.055 ppm) was recorded in the aqueous extract. This confirms the effectiveness of *T. peruviana* and ethanol extracts as the most superior treatment on *Anopheles* mosquito larvae. It is a common maxim that a safety of insecticide in an environment is of great importance when used in pest and vector control. However, it does not need to elicit high mortality on the target organism in order to be accepted as insecticidal, and so, *T pruviana* can be adopted and use sustainably as a better alternative to synthetic chemical insecticides.

Table 1: Mortality counts for *Anopheles* mosquito larvae exposed to different extracts of *Thevetia peruviana*

Treatment	Concentration	Exposure Time (Hours)		
		24	48	72
Control	0.0mg/ml	0.00	0.00	0.00
Ethanol Extract	200mg/ml	22.38	23.87	23.87
	400mg/ml	23.13	24.38	24.38
Aqueous Extract	200mg/ml	3.75	19.50	21.66
	400mg/ml	5.61	18.75	21.88
LSD		4.5957	3.2162	1.7812

Values are means of four (4) replicates.

Table 2: Regression probit for LC_{50} and LC_{95} of the alcoholic and aqueous extracts of *T. peruviana* exposed on *Anopheles* mosquito larvae.

	Ethanol (mg/ml)	Aqueous (mg/ml)
Regression equation	Y=5.388x0.467	Y=5E+16-1E+16
R^2	0.882	0.055
LC_{50}	9.193	2.42E+13
LC_{95}	17.545	1037.079

Key note: R^2 = Regression co-efficient, E = Exponential

I. CONCLUSION

The results obtained from this study revealed that *Thevetia peruviana* hold the potentials that could serve as an alternative for effective and efficient mosquito larvae control measure. The ability of the stock solutions of aqueous and ethanol extracts of *T. peruviana* indicates their possibility of their effectiveness as an alternative to the dangerous synthetic insecticides for malaria vector control. For effective and more efficient vector management, complimentary techniques such as the use of plant products against *Anopheles* mosquitoes are advisable in other to curtail their prevalence.

REFERENCES

[1] A. Redwane, H. B. Lazrek, S. Bouallam, M. Markouk, H. Amarouch, M. Jana. Larvicidal activity of extracts from Querus Lusitania var infectoria galls (Oliv). *Journal of Ethnopharmacology*, 2002, 79:1149-1166.

[2] World Health Organization. World Malaria Report. Available at http://www.who.int/malaria/world_malaria_report_2010/en/index.html. 2010.

[3] Federal Ministry of Health (FMoH) (2010). National Malaria and Vector Control rogramme. Guidelines for the implementation of indoor residual spraying for malaria vector control in Nigeria. 2010, pp30.

[4] A. Onwuemele. An assessment of the spatial pattern of malaria infection in Nigeria. *International Journal of Medicine and Medical Sciences*, 2014, 6 (2):80-86.

[5] National Bureau of Statistics (NBS). *Annual Abstract of Statistics*, Federal Republic of Nigeria. 2012, pp3.

[6] World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. ISBN 978 924 150515 4. 2013, pp 18-20.

[7] J. C. Beier, J. Keating, J. I. Githure, M. B. Macdonald, D. E. Impoinvil, R. J. Novak. Integrated vector management for malaria control. *Malaria Journal*, 2008, **7**: Suppl 1. doi:articleS4 10.1186/1475-2875-7-S1-S4.

[8] M. Zaveri. *"Study Links Pesticides to River Contamination". The Daily Californian. The Daily Californian*. 2010, *Retrieved 9 June 2012*.

[9] C. Kamaraj, A. Bagavan, G. Elango, A. Abduz-Zahir, G. Rajakumar, S. Marimuthu, T. Santhoshkumar, A. Abdul-Rahuman. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Indian J. Med. Res*. 2011, 134: 101-106.

[10] S. V. Patil, C. D. Patil, R. B. Salunkhe, B. K. Salunke. Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*. *Tropical Biomedicine*, 2010, 27 (3): 360-365.

[11] M. Govindarajan, M. Rajeswary, S. Arivoli, S. Tennyson, G. Benelli. Larvicidal and repellent potential of Zingiber nimmonii (J. Graham) Dalzell (Zingiberaceae) essential oil: an eco-friendly tool against malaria, dengue, and lymphatic filariasis mosquito vectors? *Parasitol. Res.* 2016, 115, 1807–1816.

[12] A. Jaswanth, P. Ramanathan, K. Ruckmani. Evaluation of mosquitocidal activity of *Annona squamosa* leaves against filarial vector mosquito, *Culex quinquefasciatus* Say. *Indian Journal of Experimental Biology*, 2002, 403: 363-365.

[13] N. G. Das, D. Goswami, B. Rabha. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J. Vector Borne Dis*., 2007, 44:145-148.

[14] N. Senthilkumar, P. Varma, G. Gurusubramanian. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* Liston. *Parasitol. Res*., 2009, 1042:237-244.

[15] A. A. Adebayo, A. L. Tukur. "*Adamawa State in Maps"* Paraclete Publisher Yola, 1999, pp. 27-31.

[16] World Health Organization (WHO) (1996). Report of the WHO informal consultation on the evaluation on the testing of insecticides, *CTD/WHOPES/IC/96.1*. Geneva. 1996, pp69.

[17] World Health Organization. Pesticides and their application for the control of vectors and pests of public health importance. Sixth edition. *WHO/CDS/NTD/WHOPES/GCDPP/2006.1*. Department of Control of Neglected Tropical Diseases, WHO Pesticide Evolution Scheme (WHOPES). Geneva: World Health Organization. 2006.

[18] D. Kumar, R. Chawla, P. Dhamodaram, N. Balakrishnan. Larvicidal Activity of *Cassia occidentalis* (Linn.) against the Larvae of Bancroftian Filariasis Vector Mosquito *Culex quinquefasciatus. Journal of Parasitology Research*, 2014, Volume 2014, Article ID 236838, 5 pages. http://dx.doi.org/10.1155/2014/236838.

[19] M. Y. S. Ali, S. Ravikumar, J. M. Beula. "Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*," *Asian Pacific Journal of Tropical Medicine*, 2013, 3(3): 196-201.

[20] W. S. Abbott. A method for computing the effectiveness of an insecticide. *J Econ Entomol*, 1925, 18(2):265-267.

[21] D. J. Finney. *Probit Analysis*. 3rd edition. Cambridge: Cambridge University Press; 1971.

[22] M. Wink. Modes of action of herbal medicines and plant secondary metabolites. *Medicine*, 2015, 2: 251-286. doi: 10.3390/medicine2030251.

[23] K. M. Thirumalapura, S. S. Kumuda, V. A. Vijayan. Screening of methanolic plant extracts against larvae of *Aedes aegypti* and *Anopheles stephensi* in Mysore. *Journal of Arthropod-Borne Diseases*, 2016, 10(3):305-316.