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Effect of Leaf Extract of *Azadirachta indica* (Neem Tree) on Mosquito Larvae

Bode A. S.[1], Adamu M. Y.[2], Hamawa S. S.[3], Gubya E. E.[4]
[1,3,4]Adamawa State University, Mubi , Adamawa State, Nigeria
[2]Modibbo Adama University, Yola, Adamawa State, Nigeria
saiduahmedbode@gmail.com

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Abstract

The larvicidal effect of neem leaf extract on early fourth-instar larvae of *Anopheles gambiae* was investigated under laboratory conditions of 23–30°C and 70%–75% relative humidity. Neem leaves were extracted using 70% ethanol, and the extract was evaluated at concentrations of 300, 350, 400, 450, 500, and 550 mg/L. Distilled water was used as the control. Each treatment was replicated twice, with 20 fourth-instar larvae of *Anopheles gambiae* exposed to each concentration and an equal number exposed to the control. The larvae were fed with Regal dried yeast during the experiment to minimize background mortality. Larval mortality was assessed after 24 hours of exposure, and the median lethal concentration was determined. The results showed that neem leaf extract exhibited larvicidal activity against *Anopheles gambiae*, with an LC50 value of 349.95 mg/L after 24 hours of exposure, while no mortality was recorded in the control group. The study concludes that *Azadirachta indica* leaf extract has potential as a plant-based biopesticide for mosquito larval control. These findings support further investigation into neem leaves and other plant parts for pesticide development and highlight

the need for public education and institutional support for bio-pesticides as accessible, plant-derived alternatives for vector control.

Keywords: *Anopheles gambiae*; *Azadirachta indica*; Larvicidal Activity; Neem Leaf Extract; Biopesticide

INTRODUCTION

The study of mosquitoes is of immense importance to man, this is because they are able to host and transmit various disease pathogens. Mosquitoes are vectors of disease causing agents fund within almost all tropical and subtropical countries. They are responsible for the transmission of pathogens causing some of the life threatening and debilitating diseases of man, such as malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis (Collins and Paskewitz, 2018). Becker and Asche, (2017) reported that fifty-five per cent (55%) of the world's population are at risk of mosquitoe borne disease in 124 countries. According to the WHO (2022) report malaria is endemic in 117 countries, with some 3.2 billion people living in risk areas all over the world. It further states that each year, there are about 500-800 million clinical cases of malaria worldwide, with over 2 million deaths. About 59% of all clinical cases occur in Africa, 38% in Asia, and 3% in the Americas (Achs and Malaney, 2022).

Malaria mortality is also highest in Africa, accounting for 89% of all deaths, whereas 10% occurs in Asia and less than 1% in the Americas, of all malaria cases caused by *Plasmodium falciparum*, the most deadly human malaria species, 74% are in Africa, 25% in Asia, and 1% in the America. *Anopheles* mosquitoes are the vectors responsible for transmitting of deadly malaria etiological agent plasmodium (Walker, 2020). Despite several efforts in the field of vector control, the medical and economic burden cause by vector borne diseases continues to grow as current control measures fail to cope (Romoser and Stofollano, 2017). There is therefore an urgent need to identify new control strategies that will remain effective, even in the face of growing insecticides and drugs (Foster and Walker, 2020). .

Current vector control strategies include chemical-based control measures, non-chemical based control measures, and biological control agents (Bdione, *et al.*, 2019). Chemical based control measures have dominated over other strategies over the years.

Clements, (2014) reported that repetative use of man-made insecticides for mosquito control disrupts natural ecosystem and the biological control system, and lead to re-emergence of, and increase in mosquito populations. In his studies Gillet, (2017) pointed out that continues use of chemical base insecticide has resulted in the development of resistance, detrimental effect on non-target organism and human health problems. Consequently, they suggested the need for alternative control measures. This leaves biological control as a viable alternative. The use of biological control agents such as predatory fish (Jacobson, 1995) bacteria (Kumar, 1998), protozoa (Chapman, 1974), fungus (Mulla, 2009), nematodes (Harrel, 1993), and plant products (Potter, and Knapp, 2003), had shown promising results in the control of mosquito populations.

Man since his arrival on earth has been face with the problem posed by insects. Insect constitute pest of field crops, stored products and some are parasites or vectors of many parasites that cause human and animal diseases (Jackson *et al.*, 2022). Among these insects, mosquito seems to be the most important. They are man's enemy because their females suck blood and in the process transmit the deadly malaria parasites plasmodium sp. Malaria is wide spread in the tropical and subtropical Africa, part of Asia and Americas. About three million people are at risk of infection in malaria endemic countries, it is estimated that 350 to 500 million cases are reported annually, and between one and three million of such cases end up in death (WHO, 2019). Majority of the victims of malaria attacks in sub-Saharan Africa are pregnant women and young children below the age of five years (snow *et al.*, 2018). Africa alone has about 90 per cent of such malaria related deaths especially among under-fives . Nigeria account for a quarter of all malaria case within the African region (WHO 2019). In Nigeria the main vector of malaria parasite includes the following Anopheles species: An. arabiensis, An. bronchieri An. constani, An. Flavicosta, An. funestus, An. gambiae, An. hancocki, An. hargreavis, An melas and An. moucheti, (WHO, 2020). These vectors breed in wells, overhead tanks, or ground level tanks, used tires, cisterns, roof gutters, coconut shells, open used beverage tins and small pools of water on road surfaces etc. (Harrell, 2021).

Among plants that have evoked interest as a source of bio-insecticide is Azadirachta indica commonly known as Neem has been showed to be a promising source of plant derived insecticide. Content of its leaves, seeds, and bark showed marked insecticidal properties on a wide range of insect species (Isman, 2020). The trees are widely spread in many parts of Nigeria almost in every village. Though there have been works on

the use of Neem plants and other plant extracts for the control of certain insects, (Schmutterer *et al.*, 2019). Mosquitoes have been reported to transmit diseases in human and animal, disease such as malaria, yellow fever, and dengue fever. More so, their noise causes discomfort to man when asleep. The insecticides for controlling mosquitoes are effective but have negative impact on human environment and health. It is in view of this, the research seeks to strategize an alternative means of controlling mosquitoes through the use Neem leaf extracts that has been reported to be cheap and hazard free. To ensure effective mosquito control there is the need to develop and incoperate new alternative tools for integrated vector management where method aimed at reducing adult mosquito bites and control of their aquatic larval stages are combined.The research is restricted to Mubi North L.G.A. of Adamawa.The persistence of mosquitoes on human and animals become a tropical issue of concern in many part of the world and has attracted the attention of many researchers. Information obtained from this study will serve as alternative means of controlling mosquito in the study area and will also serve as an alternative means of controlling mosquitoes in the study area and will also serve as a base line data for those who want to conduct further research in the field.

MATERIALS AND METHODS

Study Area

Mubi North is one of the 21 Local Government Areas in Adamawa State. Mubi north lies between latitude 9°50' N and 10°50' N and longitude 10°10° E (Adebayo and Tukur 2018). The temperature regime of the area is warm to hot throughout the year, because of high radiation income which is relatively evenly distributed throughout the year. However, there is usually a slightly cold period between November and February. There is a gradual increase in temperature from January to April with seasonal maximum occurring in April. Temperature drops slightly at the onset of rains due to the effects of cloudiness. It increases again a bit after the cessation of rains (October-November). This is followed by the hamattan period when temperature drops to the possible minimum. Generally, the mean annual temperature is about 27°c, the hottest month is May with about 35°c and the coldest month is January with less than 13°c (Adebayo and Uyi, 2016).

April is a month of transition between the wet and dry seasons in the area. During this period, there is scanty rainfall in most part of the area. The months of May to

September constitute the wet season. Monthly rainfall increases steadily from May to August which has the highest amounts. Rainfall decreases from September to October at a very sharp rate. The mean annual rainfall ranges from 900mm to 1050mm. Generally, planting of crops begins earlier in mountainous areas than the low land areas due to the orographic factor earlier mentioned. Relative humidity between January and March is low. It starts rising as from April and reaches the maximum in August, the peak of the raining season. Relative humidity starts decreasing as from October following the cessation of rainy season (Adebayo *et al*., 2017).

Plant Collection

Plant sample (Leaves) of *Azadirachta indica* (Neem) were collected from Lokuwa 2 Primary School in Mubi North LGA of Adamawa state, Nigeria. and was authenticated by Mr. Nyam of the Plant Science and Technology Department, University of Jos.

Sample Preparation and Extraction

The leaves of the plant were rinsed with water to remove dirt. They were then spread out on a clean surface and allowed enough time to air dry under shade at room temperature (28 ± 2°C). Dried leaves were then pounded into fine power using mortar and pestle. Ethanol extraction was carried out on the leaf power; ethanol was used because it was found that the active ingredients of Neem leaf are insoluble in water. (Jung *et al*., 2012). A Soxhlet extractor connected to a reflux condenser fitted into a 500ml round bottom flask was used. The powdered leaf was put in a thimble, which is permeable tube covered with cotton wool. 350ml of 70% ethanol was used as extraction solvent and was put into round bottom flask and was placed on electro heating mantle and heat adjusted to 40°C. The solvents were allowed to reflux repeatedly, until refluxing solvent was clear and free from extracts. Ethanol and distilled water were used respectively. The extracted content was then subjected to a rotary evaporator until solvent were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored in sterilized amber colored bottles and maintained at 4°C in refrigerator, using the method described by Bibby and England, (2016).

The percentage yield of the extract was determined as:

$$\% \text{ Yield} = \frac{(\text{Weight of dry extract / Weight of sample extracted}) \times 100}{\text{weight of sample extract}}$$

Culture of Mosquitoes

The Eggs of the *Anopheles gambiae* was collected from a container which was prepared by stocking hay into the water, this fermented and attracts female mosquitoes to lay eggs. This behaviour is instinctive and probably because the larvae will find food after hatching in such water than the clean water Vulule, (2016). The batches of eggs laid on the surface of water were collect and transferred into plastic bowls containing the same from the container. The bowls were covered to prevent the female mosquitoes from laying more egg, which will interface with the batch range.

The Eggs were monitored under laboratory conditions at 23-30°C and 70-75% relative humidity. These hatched after four days, regal dried yeast (75-25 by weight) was then sprayed on the surface of the water in the bowls. The yeast granules disintegrate and serve as food to growing larvae. This helps reduce the mortality to minimal level. To avoid overcrowding the larvae were raised in three separate bowls. At the fourth day of rearing, the larval features such as brushes and fan were very clearly seen because of the size. This is the late third instars larvae which were likely to pupate within the next 48 hours. (Mar, 2017).

Formulation of TEST Solution

For both preliminary and the actual tests carried out, the test solutions were formulated by weighing various milligram (mg) of the Neem leaf extract using mettle balance. These weighed quantities of the extract were then dissolved in 10ml of ethanol and then poured into 1 litre of water each. This give the concentration of the neem leaf extract in mg/l or ppm (milligrams per litter or part per million). Five (5) concentrations were chosen after the preliminary test these are 300, 350, 400, 450, and 500 mg/L (ppm). The actual bioassay was then conducted.

Experimental Design

For each of the five concentrations and the control, two replicates were made. A check made up of 10ml ethanol in 1 litter of water was also included, making seven (7) sets of replication. Twenty (20) early fourth instars larvae of the mosquito larvae were introduced into the seven pairs of the 300ml containers, containing 200ml of the various concentration of the Neem leaf extract, the ethanol (check), and water (control). These were then observed for 24 hours after which mortality was count were taken and average (mean) mortalities of each pair was evaluated.

The experiment was conducted under ordinary laboratory condition and the larvae were feed with regal dried yeast (75-25 by weight) during the test period to avoid death due to starvation. The check and control recorded no mortalities.

RESULTS

Effect of Ethanol Extract of Neem Leaf on Mosquito Larvae

When the leaf extract of *Azadirachta indica* was administrated at the concentration of 300mg/L. 10% mortality was recorded after 24 hours of exposure. At 350mg/L, concentration 50.0% mortality was recorded after 24 hours exposure. At 400mg/L concentration, it caused 67.5 % mortality on the larve. At 450mg/L concentration 82.5% mortality was recorded after 24 hours exposure whereas 500mg/L brought about 92.5% mortality of the mosquito larvae after the same 24 hours of exposure to the Neem leaf extract no mortality was recorded in the check and control experiment.

Analysis of Experimental Data

Table 1: observed mortality rate of mosquito larvae exposed to different concentrations of ethanol leaf extract of Neem (Azadirachta indica).

Quantity of leave Extract (mg)	Volume of Water (L)	Concentration of test solution (mg/L or ppm)	Volume of test solution Administered (ml)	Log Concentration	Mean Mortality after 24hrs	Percentage Mortality after 24hrs (%)
Percent Mortality (24hrs)	1	300	200	2.4771	2.0	10.0
350	1	350	200	2.5441	10.0	50.0
400	1	400	200	2.6021	13.5	67.5
450	1	450	200	2.6532	16.5	82.5
500	1	500	200	2.6990	18.5	92.5

Table 2: probit kill of mosquito larve using ethanol leaf extract of (Azadirachta indica).

s/no	Concentration (mg/L or ppm)	Log concentration	Percentage mortality (%)	Probit kill value
1	300	2.4771	10.0	3.2
2	350	2.5441	50.0	5.0
3	400	2.6021	67.7	5.5
4	450	2.6532	82.5	5.9
5	500	2.6990	92.5	6.4

DISCUSSION

The result of this research reveals that the leaf extract of Neem (Azadirechta indica) has some good degree of toxic effect on mosquito larvae. Neem (*Azadirachta indica)* was tested in the study, and is reported to be eco-friendly and not toxic to vertebrate (Harward *et al*., 2016). It is clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for the control of mosquitos rather than the purified compounds or extracts (Jung *et al*., 2012). 70% ethanol extract of the neem leaf was used. When administered in different concentrations of solution for 24 hours, it was observed during the period that developmental and growth processes such as moulting and pupation were inhibited. Secondly and primarily, it was observed that mortality rate increases with the increases in concentration of the extract.

The highest percentage mortality (92.5%) was recorded when 500mg of extract was administrated for 24 hours. This corresponded with the work of Mlla and Su, (2015) where 500ppm killed 92% of the larvae. The lowest percentage mortality (10.0%) was recorded when 300mg of the extract was administrated for the same period. The calculated f-ratio by analysis of variance was 281.17 which is far greater than the tabulated f-value 4.20. This implies that the Neem leaf extract is very effective against mosquito larvae. Also the 95% confidence limits suggest that neem leaf extract can be used against mosquito larvae. Compared to most other plant extract such as moringa sp and vernonia sp, the neem leaf extract is very effective against mosquito larvae. For instance as seen above 50% mortality was record when 350mg per litre of neem leaf extract was administered for 24hours. While according to Lozano, *et al*., (2016), it took up to 1.0g per 200ml of the leaf extract of the moringa oleifera for 48 hours to record 52% mortality. Hence neem leaf extract is more effective.

CONCLUSION

Result obtained from this study showed that neem leaf extract may serve as an alternative insecticides in the control of mosquito larvae and readily available, inexpensive and hence could serve as more favourable option in the eradication of mosquitoes and other insect pest from our environment.

Recommendation

1. Further research can be conducted on the neem leaf and other parts of tree in relationship pesticide development; this is because it has insecticide properties.
2. Public enlightenment should be carried out to educate the local and rural populace on the use bio-pesticides of plant origin such as neem tree.
3. Government should support research work on bio-pesticides as they are eco-friendly and cheaper. They are also easily formulated and mostly nontoxic to vertebrate.

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APPENDIX I

Number of mosquito larvae introduce into test containers (beakers)

Concentration Replicate	500mg/L	450mg/L	400mg/L	350mg/L	300gm/L	check	Control (water)
A	20	20	20	20	20	20	20
B	20	20	20	20	20	20	20

APPENDIX II

Mortalities of the mosquito larvae after 24 hours

Concentration Replicate	500mg/L	450mg/L	400mg/L	350mg/L	300mg/L	check	Control (water)
A	18	16	14	10	2	0	0
B	19	17	13	10	2	0	0

APPENDIX III

Analysis of variance (ANOVA) for mortalities of mosquito larvae

Replications concentration	A	B	SUM
300	2	2	4
350	10	10	20
400	14	13	27
450	16	17	33
500	18	19	37
TOTAL			121

Treatment variation	Degree of freedom	Sum of square	Mean square	Variance ratio
Treatment	4	337.8	84.35	281.17
Error	5	53.5	0.3	
Total	9	338.9		