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**Larvicidal Effects of Eucalyptus and Tamarind Methanolic Leaves**  
**Extracts Against *Anopheles gambiae* Complex**

Nazifi. U. U<sup>1</sup>., Yayo.A.M<sup>1</sup>., Sadiq.A<sup>2</sup> and Sani Z. R<sup>3</sup>

<sup>1</sup>Department of Medical Microbiology and Parasitology College of Health Science, Bayero University Kano, Nigeria

<sup>2</sup>Department of pure and industrial chemistry, Bayero University Kano, Nigeria.

<sup>3</sup>Department of Biological Sciences, Federal University Gusau, Zamfara State, Nigeria

Corresponding Author's Email & Phone No.: [nazifusmanuba@gmail.com](mailto:nazifusmanuba@gmail.com), 08069080719

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**ABSTRACT**

Synthesis of Natural insecticides from plant origin against mosquito vectors have been the main concern for research due to their high level of eco-safety. Controls of mosquitoes in their larval stages are an ideal method. The present study was specifically conducted to compare the larvicidal efficacy of methanolic leaves extract of tamarind and eucalyptus against *Anopheles gambiae* complex. Probit analysis reveals the differential pattern of mortality among the studied plants with tamarind having the highest number of mortality recorded (LC<sub>50</sub> 246.275 µg/ml and LC<sub>90</sub> 1937.015 µg/ml). This result showed that it has the least concentration to kill 50% and 90% of the larvae while eucalyptus has the highest concentration (LC<sub>50</sub> 430.828 µg/ml and LC<sub>90</sub> 2284.741 µg/ml) to kill the 50% and 90% of the larvae within 24 hours of exposure. However, the result shows that mortality increases with prolong exposure time. Therefore, results suggested that extract of the two plants possess significant larvicidal activity and might be considered natural source for production larvicides.

**Keywords:** Larvicidal, *Anopheles gambiae* complex, Medicinal plants, leaves extract, Africa, Malaria, Vector

**1.0 INTRODUCTION**

Mosquitoes act as vectors for most of the life threatening diseases. Malaria is transmitted from man to man by the female *Anopheles* mosquito, one of the most capable vectors of human disease. Various species have been found to be the vectors in different parts of the world. *A. gambiae* complex is the principal vector in Africa (Coetzee, 2004). The use of synthetic insecticides such as organochlorides (DDT) in mosquito control resulted in environmental hazards due to persistence and accumulation of the non-biodegradable chemicals in ecosystem as well as biological magnification through the food chains. This led to development of insecticide resistance among vector species and toxic effect in human health and non-target organisms (Anupamet *al.*, 2012). The situation demands renewed alternative insecticidal agents with high bio control potentiality but which cause little or no harmful effect to environment and human health (Hussainet *al.*, 2017).

One possible strategy is the rational localization of bioactive products from phytochemicals by systematically exploring the global floral biodiversity. There is currently an ever-increasing demand for plant-based insecticides as they are nontoxic, easily available at affordable prices, biodegradable and show broad spectrum target specific activities against different species of vector mosquitoes. Furthermore, unlike the conventional commercial insecticides that are based on single active ingredient, plant derived insecticides comprise botanical blends of secondary metabolites act concertedly on both behavioral and physiological processes (Anupamet *al.*, 2012).

Application of active toxic agents from plant extracts as an alternative mosquito control strategy was dated back to ancient times; hence many studies on plant extracts and their active constituent compounds against mosquito larvae have been conducted around the world (Kamarajet *al.*, 2010, Maheswaranet *al.*, 2012).

## 2.0 MATERIALS AND METHODS

### 2.1 Collection of the Plant Samples

The leaves of Eucalyptus and Tamarind were collected from the trees growing in the botanical garden of Bayero University Kano. The taxonomic identification of the plants was established in the herbarium, Plant Biology Department, Bayero University Kano. The leaves were rinsed with distilled water air dried for several days at room temperature. Dried leaves of the plant samples were pulverized into a fine powder using mortar and pestle to enhance the penetration of the extracting solvents into the plant cells. The powders were sieved using a sieve and kept in a dried polythene bag (Aleiro, 2013).

### 2.2 Extraction of Plants

The cold extraction method was used. 125g each of pulverized plant samples was percolated in a glass bottle. This involves leaving the pulverized plant leaves to soak in a methanol in the 1:5 (w/v) ratio (Ihsan, 2015) cold maceration was performed at room temperature and the mixture was kept for fourteen days with occasional shaking and then filtered through Buckner funnel (whatman filter paper). The resulting filtrates were then concentrated in a rotary evaporator allowed to dry and weighed (Ashwaniet *al.*, 2014).

### 2.3 Phytochemical Screening

The extracts were analyzed for the presence of Alkaloids, Saponins, Tannins, Steroid, resins and flavonoids, according to standard methods described by Ngbedeet. *al.*, 2008 and Amzadet *al.*, 2013.

### 2.4 Preparation of the Test Dose

500mg of the extract was dissolved in 50ml distilled water to obtain 10,000µg/ml stock solution. From the stock solution, different test concentrations were prepared with distilled water as follows; 1000µg/ml, 100µg/ml, 10µg/ml.

### 2.5 Mosquito Larval Collection

Larvae of *Anopheles* mosquitoes were collected from stagnant water, rice field, and small temporary rain pools in Doko town Garki L.G.A of Jigawa state Northern Nigeria. Most species prefer clean, unpolluted water (Gimniget A, 2001). The dipping technique was employed the dipper was lower gently in an angle of 45° just below the surface so that water

flows in together with any larvae that might be present. (UMCP, 2005).

### 2.6 Morphological Identification of the Mosquito Laevae

This was achieved using some identification keys (Coetzee *et al.*, 2000, Harrison, 2005). The mosquito larva has a well-developed head with mouth brushes used for feeding, a large thorax and a nine-segment abdomen. It has no legs, in contrast to other mosquitoes; the *Anopheles* larva lacks a respiratory siphon, so it positions itself so that its body is parallel to the surface of the water. In contrast, feeding larva of a non Anopheline mosquito species attaches itself to the water surface with its posterior siphon, with their body pointing downwards (Coetzee *et al.*, 2000).

### 2.7 Mosquito Larvicidal Bioassay

The efficacy of the plant extracts as larvicides against the *Anopheles gambiae* complex mosquito was evaluated in accordance with guidelines of World Health Organization (WHO). 10 ml of the solution for each concentration (1000µg/ml, 100µg/ml), and 10µg/ml were placed in a small plastic container, mixed thoroughly with 90 ml of distilled water. A batch of 20 mature larvae in four replicates was exposed to each of test concentrations. During period of exposure larvae were fed with yeast (WHO, 2005). The control contains 100 ml of distilled water with 0.2 ml of methanol. A mortality count was taken 24 hrs, 48hrs and 72 hrs after treatment (Love *et al.*, 2013). 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 (Bekeleet *al.*, 2014).

### 2.8 Determination of Mortality

The effect of each plant extract was monitored through careful counting the number of dead larvae after 24, 48 and 72 hours of treatment, and the percentage mortality was computed using the following expression (Kovendan *et al.*, 2014).

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

## 2.9 Data Analysis

Data generation was obtained using Arithmetic Mean to get the average number of dead mosquito larvae and percentage as well as Probit Analysis using SPSS 16.0 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 24,48 and 72 hours of treatment (Finney,1971) also a two tailed type one T-TEST was carried out to determine whether there exist a significant deference in the mean percentage

mortality over the period of observation with the 5% confidence interval.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Phytochemical composition of the extract

The study carried out to determine the phytochemical constituents of the plants extract revealed the presence of secondary metabolites of pharmacological significance as shown in the table below:

Table 1. Phytochemical Component of the Plants Extract

PLANTS	PHYTOCHEMICAL COMPONENTS					
	ALKALOIDS	SAPONINS	FLAVONOIDS	STEROIDS	TANNINS	RESINS
Tamarind	++	+++	-	-	+++	+
Eucalyptus	+	++	-	++	+++	-

Legend: + = Phytochemical presence, - =Phytochemical absent. AL= Alkaloid, SAP= Saponin..... etc

Table 2.Mortality Bioassay of the Plants Extract against *Anopheles Gambiae* Complex

PLANT	CONCENTRATION (µg/ml)	PERCENTAGE MEAN MORTALITY			CI	P-Value
		24 HOURS	48 HOURS	72 HOURS		
EUCALYPTUS	10	0	8.75	21.25		0.035
	100	13.75	31.25	40	4.691	0.001
	1000	46.25	75	88.75	4.691	-
TAMARIND	10	0	3.75	11.25	-	0.058
	100	13.75	23.75	70	4.691	0.066
	1000	73.75	96.25	100	4.691	0.014

Table 3. Lc<sub>50</sub>and Lc<sub>90</sub> of the Plants Extract Against *Anopheles Gambiae* Complex

PLANTS	TIME (HOURS)	LC <sub>50</sub> (µg/ml)	LC <sub>90</sub> (µg/ml)
TAMARIND	24	246.275	1937.015
	48	144.275	4285.553
	72	96.825	7056.411
EUCALYPTUS	24	430.828	2284.741
	48	243.979	1833.946
	72	47.997	234.183

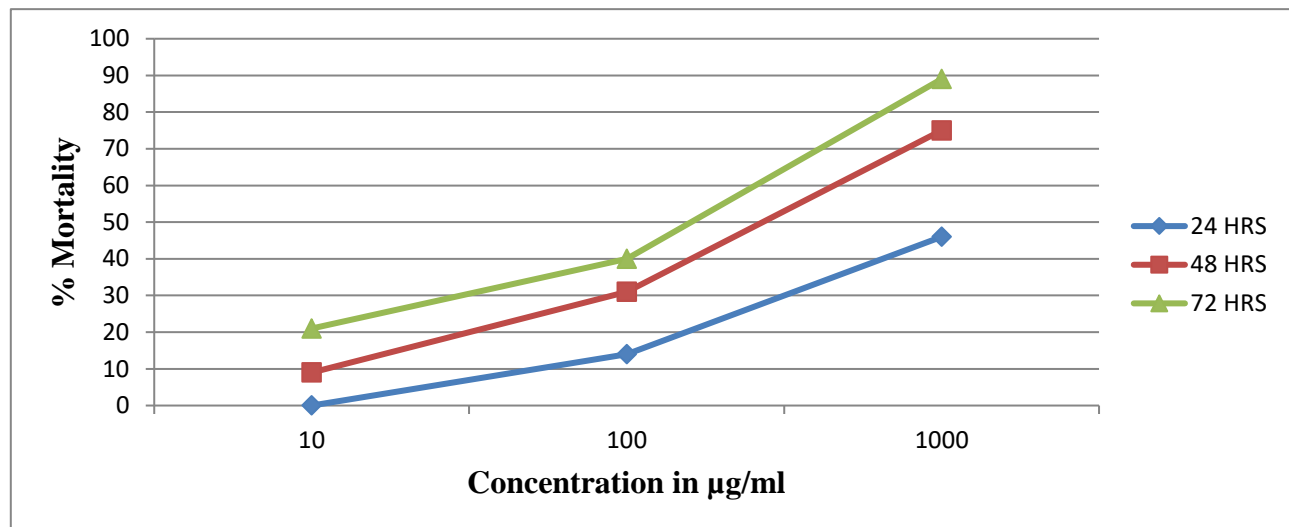


Fig 1. Effect of Eucalyptus Leaves Extracts Against *A. Gambiae* Complex 3<sup>rd</sup>/4<sup>th</sup> Instars Larvae

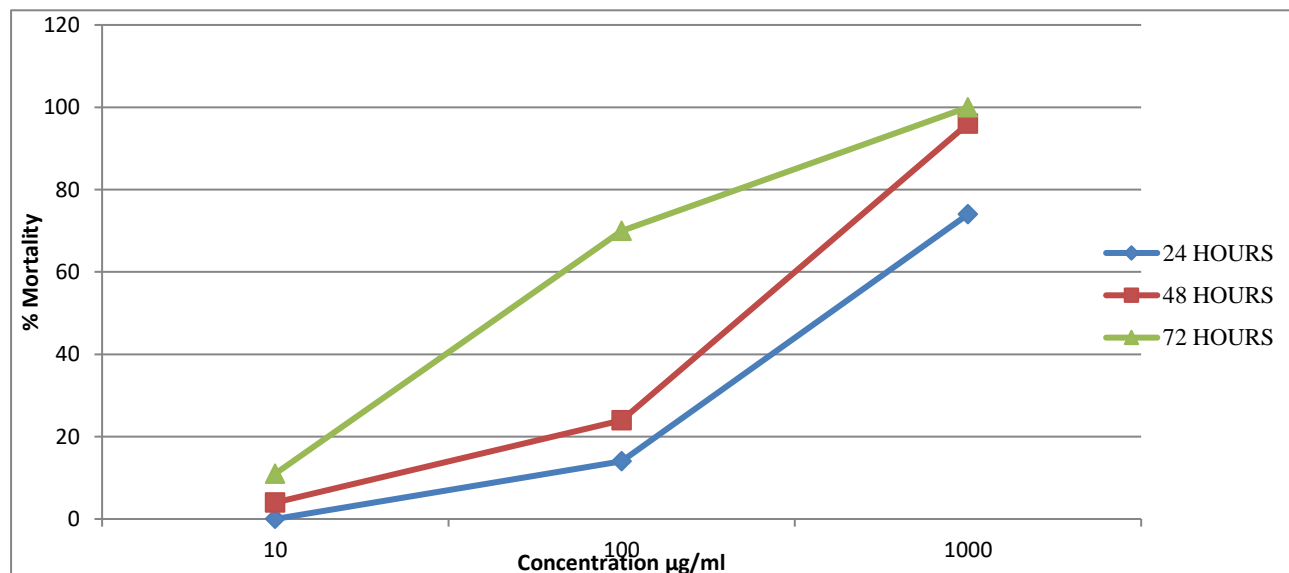


Fig 2. Effect of Tamarin Leaves Extracts Against *A. Gambiae* Complex 3<sup>rd</sup>/4<sup>th</sup> Instars Larvae

### 3.2 DISCUSSION

All the two methanol extracts in this study were rich in alkaloids, saponins and tannins, also with varying amount of flavonoids, steroids and resins. Researchers such as Vinayagam *et al.* (2008), identified the above mentioned components in banana, eucalyptus and tamarind using the same solvent when tested against *Anophlesstephensi*. Also Jagrutiet *al.* (2014) identified such components in *Chromolaenaodorata* when tested against *Culextritaeniorhynchus*.

Phytochemicals are basically secondary metabolites that serve as a means of defense mechanism of the

plants to withstand the continuous selection pressure from herbivore predators and other environmental factors. Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction (Jagrutiet *al.* 2014).

A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots of larger plants

or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control. (Anupametal 2012). These compounds detected can act as; an active ingredients with insecticidal properties. The activities of the crude plant are often attributed in the complex compounds which have been tested earlier by several investigators; Das and Ansari (2003).

From the mortality bioassay of the methanolicleaves extract of the plants (Eucalyptus and Tamarind) shows a proven larvicidal effect against *Anopheles gambiae* complex at different concentrations. The highest concentration of 1000828  $\mu\text{g/ml}$  shows 100% mortality in tamarind within 72hours of exposure. Also at 100 $\mu\text{g/ml}$  and 10  $\mu\text{g}$  shows effects on the mosquito larvae after 48 hours of exposure in the same plants extract. However, in tamarind the highest concentration shows more than 50% mortality within 24 hours of exposure with little effects in other concentrations within stipulated period of time while with prolong exposure(after 72 hours) the extract proven some larvicidal effects in eucalyptus(table 3) the highest concentration also shows more than 50% mortality after 24 hrs of treatments. This result showed that mortality increases with increase in concentrations with prolong exposure to treatment in all the studied plants conducted.

In all the categories' no mortality was recorded in the control groups, which shows that the mortality encounter in the test groups were attributed due to the presence of some active ingredients that proven to have some larvicidal properties against mosquito larvae as described by Anupamet *al.*(2012).

In the present study, probit analysis showed that tamarind has the highest effect on the *Anopheles* mosquito larvae among the studied plants with  $\text{LC}_{50}$  430.828  $\mu\text{g/ml}$  and  $\text{LC}_{90}$  2284.741  $\mu\text{g/ml}$ . This result shows tamarind has the least concentration to kill 50% and 90% of the organisms within 24hours of exposure. Kamaraj *et al.*(2011),their findings also revealed tamarind proven to have some larvicidal effects when tested against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Many researchers had studied the components of the essential parts of the plants such as oil from the leaves (Musa *et al*, 2015), bark of the plants (Jagrutiet *al*, 2014) etc, against

different species of plants which were proven to have some medicinal properties. However, the natures of the phytochemicals components activities depend on the nature of the solvent used during the extraction. In these studies methanol was used being a good solvent for extraction of polar organic components while non polar compound can be used to extract on polar organic components.

According to Webbe and Lambart (1983) as well as Azamatullahet *al.* (2011) that alkaloids, saponins and tannins were proven to have some insecticidal properties which were detected in all the plant extracts that have been studied in this research. Alkaloids have been identified as one of the largest groups of chemical arsenals produced by plants. Many of these secondary metabolites are derived from amino acids and include an enormous number of bitter, nitrogenous compounds.

#### 4.0 CONCLUSION

In the present study, the treatment with medicinal plants rendered the *Anopheles gambiae* complex larvae inactive, motionless and death. Mortality of the larvae within 24 hours of exposure was observed in the treatments. The present findings have important implications in the practical control of mosquito larvae in the polluted aquatic ecosystem. The plants studied are available in large quantities and the extracts are easy to handle, inexpensive and safe natural products for mosquito control. In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for non-target animals and do not pose any residue problem but are still able to suppress pest populations. It may be concluded that the nature possesses numerous medicinal plants, which may be useful for control of vector borne diseases. Out of the two plants tested eucalyptus was found to be less toxic when compared to the tamarind. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant. Treatment with these plants leaves extracts has further shown that many of the larvae treated with higher concentration did not successfully moult to pupae.

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