Original Research Article

Anti-mosquito Larval and Pupal Efficacy of *Tithonia diversifolia* and *Momordica charantia* Leaves Extracts against Malaria Vector, *Anopheles gambiae* Gile (Diptera: Culicidae)

ABSTRACT

Aim: Emergence of resistance by mosquito vectors and harmful effects of chemicals on human and its environment have been major problems encountered in vector control through the use of synthetic insecticides, thus there is a need for development of insecticides of plant derivatives. This research is aimed at using extracts of *Tithonia diversifolia* and *Momordica charantia* against the developmental stages of *Anopheles gambiae*.

Place and Duration of study: The research was conducted at the Federal University of Technology, Akure, Nigeria between the months of March to June, 2017.

Methodology: Larvae and pupae of *Anopheles gambiae* were reared in the laboratory at ambient temperature of 28±2°Cand relative humidity of 75±5%. The leave extracts of *T. diversifolia* and *M. charantia* were extracted with methanol and were prepared at concentrations, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. The larvae and pupae of *A. gambiae* were exposed to these concentrations of the plant extract for 24 hours. Mortality of the larvae and pupae was monitored and recorded. Probit analysis was used to determine the LC_{50}.

Results: Result from this research revealed that at all levels of concentrations, mortality of both the larvae and pupae of this insect increased with increase in the concentrations regardless the type of plant extract used. The leave extract of *T. diversifolia* having a lower value of LC_{50} (larvae: 0.20 %; Pupae: 0.27 %) is more potent than extract from *M. charantia* having a higher value of LC_{50} (larvae: 0.31%; Pupae: 0.44%) after 24 hours Post Treatment of larva and pupae of *A. gambiae*. *T. diversifolia* had significant effect on the larvae of *A. gambiae* with percentage mortality ranges from 23.33-100% within 24hrs of exposure when compared with *M. charantia* that had 16.67-100% of mortality larvae of *A. gambiae.*

Conclusion: The results obtained from this research revealed that extracts from the two plants exhibit great insecticidal properties against larvae and pupae of *A. gambiae*. Therefore, more exploration on the use of these plants for the development of insecticides at commercial should be done.

Keywords: Malaria vector; *Anopheles gambiae*; Larvae; pupae; *T. diversifolia* and *M. charantia*

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1. INTRODUCTION

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Mosquitoes are insects that constitute major public health problem as they have been incriminated to be vectors of different parasitic diseases that affect humans. Among the disease they vector are, malaria, yellow fever, Japanese encephalitis, filariasis, and dengue fever. Annually, report has shown that mosquitoes are capable of transmitting various types of diseases to approximately 700 million people [1].

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Mosquito breed in a variety of habitat where there is stagnant water and the breeding sites vary from large and permanent collections of water such as swamps and pools of water to small collection of temporary

water such as tree-holes, plant axils, tyres, coconut shells, foot-prints of man and animals [2]. Specifically, female *Anopheles* mosquitoes are involved in the transmission of malaria in endemic region.

In tropical and subtropical regions of the world, malaria problem has been on the increase due to increasing abundance of mosquito vectors. Of recent, one of the major means of controlling malaria is through vector control. Means through which mosquito vector problem can be solved is by killing the mosquito at the larva stage before emergence to the adult stage. Use of synthetic insecticide has been employed to achieve this, due to the harmful effects of the chemical on human, mosquitoes developing resistance and mosquito resurgence [3] other alternative source such as the use of botanicals have been used to develop insecticides.

Botanicals are good alternative agents for vector control due to their high bioactive constituents which are potent against specific target insects, environmentally safe and biodegradable. In resents times, more attention is shifting to phytochemical insecticides because they are considered to be more environmentally biodegradable and safer than synthetic [4]. It has been reported that plant extract has high effectiveness against mosquito larvae [5; 6; 7]. For instance, report has shown that plant alkaloids like nicotin, anabasin, and lumpinin extracted from the Russian weed, *Anabasin aphylla* killed the larvae of *Culex sp* [7].

Though use of synthetic insecticides are effective, but has caused so many problem such as posing health issues to human and insecticide resistance [8]. Therefore, there is a need for more research to discover plants with insecticidal properties that are eco-friendly, biodegradable and nontoxic for the development of insecticides which can be use as vector control. Thus, this research is aimed at using extracts of *T. diversifolia* and *M. charantia* against the developmental stages of malaria vector (*Anopheles gambiae*).

2. MATERIALS AND METHODS

2.1. Study Area

The research was carried out during the rainy season, between the months of March to June, 2017 at the federal university of Technology, Akure. Akure is the state capital of Ondo State located in the rain forest zone between latitude 7°15'0"N and longitude 5°11'42"E.

2.2. Collection of mosquito eggs/ Rearing of mosquito Larvae and Pupae

Mosquito baits, consisting of shallow containers with a large surface area were established under a partial shade in an open field area behind the laboratory of Biology Department, School of Science, Federal University of Technology Akure, Ondo State, Nigeria. The container was allowed to be filled with rain water to mimic mosquito natural breeding environment and to attract adult female for oviposition. Two grams (2g) of dried granular yeast was sprinkled on the surface of the water and allowed to decompose slowly to nourish developing larvae after emergence. Wild mosquitoes were allowed to freely visit the bait and lay eggs. After eggs had been laid into the water by adult mosquitoes, the setup was transported to the Entomology laboratory of Biology Department. After few days, the larvae and pupae emerged. The emerged larvae and pupae were identified and separated into different species using morphological keys. The whole setup was maintained at temperature of 28±2°C and 75±5% relative humidity.

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Comment [i7]: The reference of the morphological key must be mentioned.
Have you used mosquito identification software ..!? If so, you can mention it too ..

2.3. Collection of plant materials

Leaves of *T. diversifolia* and *M. charantia* were collected at Aba phase II off Awule in Akure in Ondo State, Nigeria. The collected plant material was authenticated at the Department of Crop Science and Pest Management of the Federal University of Technology Akure, Ondo State, Nigeria. The plants materials were washed thoroughly with tap water and air dried at ambient temperature (28±2) in the laboratory. The dried plant was pulverized into fine powder using an electric blender. The powders were packed in plastic container with tightly lid and stored in a refrigerator at 4°C before used.

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2.3.1. Extraction of Plant Materials

Methanolic extracts of *T. diversifolia* and *M. charantia* were carried out using cold extraction method. Two hundred grams of *T. diversifolia* and *M. charantia* powders were soaked separately in an extraction bottle containing 100 ml of absolute methanol for 72 hours. The mixture was agitated occasionally with a glass rod and extraction was terminated after 72hours. The resulting mixture was filtered using a double layer of whatman No.1 filter paper and the solvent was evaporated using a rotary evaporator at 30 to 40 ^{o}C with rotary speed of 3 to 6 rpm for 8hrs [9]. The resulting materials were air dried in order to remove trace of solvent. The crude extracts were kept in a dark bottle labeled and prepared in the refrigerator until needed.

2.4. Toxicity Test

2.4.1. Effect of *T. diversifolia* and *M. charantia* extracts on the larvae and pupae of *A. gambiae*

Larvicidal and pupacidal activity of the plant extracts was carried out at different concentrations by preparing the required stock solutions following the standard procedure [10]. The desired concentrations

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were achieved by adding 1.0 µg of the crude extract from leaf into 100ml of distilled water. From these, five concentrations of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of the plant extracts were prepared into a petri dish of 9cm diameter and 3cm depth. The treatments were separately added to 2.5 Liters of water inside a bowl and yeast powder was added in order to provide source of food for the introduced larvae. Twenty (20) larvae and pupae of *Anopheles species* were separately introduced into treated and untreated water (control). Each treatment was replicated three times. Mortality was observed and recorded over 24 hours after the introduction of larvae and pupae to notice a recovery time of 5 minutes was allowed.

The larval mortality in treatments was corrected for the controls. Larvae and pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe.

2.5. Statistical Analysis

Data obtained from the research were subjected to analysis of variance (ANOVA). Means were separated using Duncan's Multiple Range test. Probit analysis was carried out to determine the concentration of leaves extracts of *T. diversifolia and M. charantia* lethal to 50% (LC_{50}) of larvae and pupae of *A. gambiae.* All data were analysed using Statistical Package for Social Sciences (SPSS) version 20.

3. RESULTS

3.1 Effect of 0.1 % Concentration of Plant Extracts of *T. diversifolia* and *M. charantia on A. gambiae*

Table 1 shows the mean percentage mortality of larvae and pupae of *Anopheles species* at 24 hours of post treatment exposed concentration level (0.1%) of *T. diversifolia and M. charantia,* which was the lowest concentration. The mortality of *A. gambiae* larvae and pupae increased with increase in plants extracts concentration. Generally, the mortality values of extract treated larvae and pupae was significantly higher ($p>0.05$) than those of the untreated larvae and pupae as regard of the concentration of plants. At 0.1%, there was no significant difference between *T. diversifolia* and *M. charantia* as the mortality of the larvae and pupae was 23.33 % and 11.67 %. At all rate, when treated on the pupae at same concentration, *T. diversifolia* showed no significant different from *M. charantia* while the mortality of larvae and pupae of *M. charantia* were 16.67 % and 7.67 % respectively.

Table 1: Percentage Mortality of *A. gambiae* at 24hrs Post Treatment with 0.1 % of Plant Extracts of *T. diversifolia* and *M. charantia.*

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Plant Extracts	Developmental stages	
	Larvae	Pupae
T. diversifolia	23.33±1.67[b]	11.67±0.67[b]
M. charantia	16.67±1.67[b]	11.67±1.67[b]
Control	0.00±0.00[a]	0.00±0.00[a]

Each value is a mean± standard error of three (3) replicate. Mean having the same superscript along the column are not significantly different (p>0.05) using Turkey's Test.

3.2. Effect of 0.2 % Concentration of Plant Extracts of *T. diversifolia and M. charantia on A. gambiae*

Table 2 presents the mean percentage mortality of larvae and pupae of *Anopheles species* at 24 hours of post treatment exposed concentration (0.2%) level of *T. diversifolia* and *M. charantia*. The mortality of *A. gambiae* larvae and pupae increased with increase in plants extracts concentration. However, it was observed that there was no significantly difference ($p<0.05$) between the larvae treated with *T. diversifolia* and *M. charantia*. Also, larvae treated with *T. diversifolia* caused 38.33% mortality while mortality rate of 25.00% was recorded for the pupae. At all rate when treated on the pupae at same concentration, *T. diversifolia* was significantly higher ($p>0.05$) than the *M. charantia.* As the mortality on the larvae of was *M. charantia* 23.33% while on the pupae was 15.00%.

Table 2: Percentage Mortality of *A. gambiae* at 24 hours Post Treatment with 0.2% of Plant Extracts of *T. diversifolia, M. charantia*

Plant Extracts	Developmental stages	
	Larvae	Pupae
T. diversifolia	38.33±1.67[c]	25.00±2.89[b]
M. charantia	23.33±1.67[b]	15.00±0.00[b]
Control	0.00±0.00[a]	0.00±0.00[a]

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Each value is a mean± standard error of three (3) replicate. Mean having the same superscript along the column are not significantly different (p>0.05) using Turkey's Test.

3.3. Effect of 0.3% Concentration of Plant Extracts of *T. diversifolia and M. charantia on A. gambiae*

Result shows the mean percentage mortality of larvae and pupae of *Anopheles species* at 24 hours of post treatment exposed concentration level (0.3 %) of *T. diversifolia and M. charantia*. At 0.3 % the larvae treated with *T. diversifolia* was significantly higher (p<0.05) than *M.charantia* and achieved 56.67 % mortality on the larvae and 36.67 % on the pupae. However, at same concentration when treated on the pupae, they were significantly different (p<0.05) from each other. *M. charantia* on the larvae and pupae achieved 35.00% and 21.67% mortality respectively (Table 3).

Table 3: Percentage Mortality of *A. gambia* at 24hrs post treatment with 0.3% of Plant Extracts of *T. diversifolia* and *M. charantia*

Plant Extracts	Developmental stages	
	Larvae	Pupae
T. diversifolia	56.67±1.67^{c}	36.67±1.67^{c}
M. charantia	35.00±2.89^{b}	21.67±1.67^{b}
Control	0.00±0.00^{a}	0.00±0.00^{a}

Each value is a mean± standard error of three (3) replicate. Mean having the same superscript along the column are not significantly different (p>0.05) using Turkey's Test.

3.4. Effect of 0.4 % Concentration of Plant Extracts of *T. diversifolia and M. charantia on A. gambiae*

Table 4 presents the mean percentage mortality of larvae and pupae of *Anopheles species* at 24 hours of post treatment exposed concentration level (0.4 %) of *T. diversifolia* and *M. charantia*. The mortality of *A. gambiae* larvae and pupae increased with increase in plants extracts concentration. At 0.4 % concentration, there was no significant difference between *T. diversifolia* and *M. charantia* when used to treat the larvae of *A. gambiae* meanwhile both larvae and pupae treated with *T. diversifolia* gave 100% mortality. However, the pupae treated with *T. diversofolia* were significantly different from *M. charantia.* Mortality of larvae and pupae were recorded as 100.00 % and 70.00 % when treated with *M. charantia.*

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Table 4: Percentage Mortality of *A. gambiae* at 24hrs Post Treatment with 0.5 % of Plant Extracts of *T. diversifolia* and *M. charantia*

Plant Extracts	Developmental stages	
	Larvae	Pupae
T. diversifolia	100.00±0.00[b]	100.00±0.00[c]
M. charantia	90.00±2.89[b]	70.00±2.89[b]
Control	0.00±0.00[a]	0.00±0.00[a]

Each value is a mean± standard error of three (3) replicate. Mean having the same superscript along the column are not significantly different (p>0.05) using Turkey's Test.

3.5. Effect of 0.5% Concentration of Plant Extracts of *T. diversifolia* and *M. charantia on A. gambiae*

Table 5 presents the mean percentage mortality of larvae and pupae of *Anopheles species* at 24 hours of post treatment exposed concentration level (0.5%) of *T. diversifolia* and *M. charantia*. The mortality rate of *A. gambiae* larvae and pupae increased with increase in plants extracts concentration. At 0.5 % there was no significant difference (p>0.05) between the larvae treated with *T. diversifolia* and *M. charantia* and meanwhile mortality rate of the larva and pupae were both 100 %. However, the pupae at same level of concentration showed no significantly difference (p>0.05) between *T. diversifolia* and *M. charantia* meanwhile 90.00 % and 70.00 % mortality rate was observed for both larvae and pupae respectively when treated with *M. charantia*.

Table 5: Percentage Mortality of *A. gambiae* at 24hrs Post Treatment with 0.5% of Plant Extracts of *T. diversifolia* and *M. charantia*

Plant Extracts	Development stages	
	Larvae	Pupae
T. diversifolia	100.00±0.00[d]	100.00±0.00[c]

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M. charantia	100.00±0.00[b]	80.00±2.89[b]
Control	0.00±0.00[a]	0.00±0.00[a]

Each value is a mean± standard error represents three (3) replicate. Mean having the same alphabet down the column are not significantly different (p>0.05) using turkey's HSD (Honestly Significantly Difference).

3.6. Effect of Lethal Concentrations LC_{50} of Extracts of *T. diversifolia and M.charantia* on the Mortality of Developmental Stages of *An. Gambiae*

Table 6 shows the concentration of leaf extracts of *T. diversifolia* and *M. charantia* required to kill 50% of larvae and pupae after 24 hours. It was observed that the LC_{50} values of leaf extract of *M. charantia* (larvae: 0.31%; Pupae: 0.44%) was higher than that of *T. diversifolia* (larvae: 0.20 %; Pupae: 0.27 %) after 24 hours Post Treatment of larva and pupae *A. gambiae*.

Table 6: LC_{50} of *T. diversifolia* and *M. charantia* extracts required to achieve 50 % Mortality of Larva and Pupae of *A. gambiae* after 24 hours Post Treatment.

PLANT EXTRACTS	Developmental stages	
	LARVAE (LC_{50})	PUPAE (LC_{50})
T. diverifolia	0.20 (0.16-0.24)	0.27 (0.22-0.32)
M. charantia	0.31 (0.26-0.39)	0.44 (0.36-0.59)
Control	0.00±0.00a	0.00±0.00a

3.7. Phytochemical Screening of *T. diversifolia* and *M. charantia*

Result showed the phytochemical screening of the methanol extracts of *T. diversifolia* and *M. charantia* leaves. Most of the phytochemical present in both plant extracts were identical except flavonoids which is present in *M. charantia* only (Table 7).

Table 7: Phytochemicals present in Methanol and Aqueous Extracts of *T. divrsifolia* and *M. charantia* Leaves

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Phytochemical constituent	Methanol extract of *T. divrsifolia*	Aqueous extract *of T. divrsifolia*	Methanol extracts of M. *charantia*	Aqueous extract *of M. charantia*
Alkaloids	+	+	+	+
Tanins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	-	-	+	+
Cardiac glycosides	+	+	+	+
Steroids	+	+	+	+

4. DISCUSSION

Emergence of resistance by mosquito vectors and harmful effects of chemicals on human and environment have been major problems encountered in vector control through the use of synthetic insecticides. In effort toward to solving these issues, research is tending towards the use of botanicals to development of insecticide due to the fact that compounds of plant origin are safer to use, without phototoxic properties and leave no scum in the environment [11]. Thus, the effects of extracts of *T. diversifolia* and *M. charantia* against the developmental stages of *A. gambiae* were evaluated in this study. In this study, it was observed that leave extracts of *T. diversifolia* and *M. charantia* showed very high mortality effect against larvae and pupae of *A. gambiae*. However, the mortality effect varied with the plant and concentration of the extracts used.

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Results obtained from this present study revealed that leave extract of *T. diversifolia* having a lower value of LC_{50} (larvae: 0.20 %; Pupae: 0.27 %) is more potent than extract from *M. charantia* having a higher value of LC_{50} (larvae: 0.31%; Pupae: 0.44%) after 24 hours Post Treatment of larva and pupae of *A. gambiae*. *T. diversifolia* been more potent against the larvae and pupae of *A. gambiae* could be as a result of the strong pungent odour of the plant. Previous researches have established that plants with strong pungent odour have high biological activity against insect pest [12; 13; 14]. Also, *T. diversifolia* having high potency against the developmental stages of *A. gambiae* could be as a result of the phytochemical constituent of the plant because phytochemical analysis of its leave extracts showed that the plant consist of alkaloids, cardiac glycosides, tannin, saponin and steroids. This result of the high effectiveness of *T.*

diversifolia is in agreement with the findings of [15], who reported in their work that of all the six plants extracts that was tested against *A. gambiae*, *T. diversifolia* and *R. communis* caused the highest mortality in females of *A. gambiae*. Previous studies have also confirmed that *T. diversifolia* consist of alkaloids, a compound known to possess a high level of biological activity against insect vectors [16; 17], therefore, this compound could have given the plant its potent power against the developmental stages of the mosquito. Also, Considerable reduction in the population of *Anopheles gambiae* due to plant extracts could be linked to the ability of the extract to block oxygen supply to the developmental stages in the water or blockage of the spiracle which will later leads to suffocation and death [18]. Although *M. charantia* has lesser potency compared to *T. diversifolia*, it also exerted high toxicity against the two developmental stages of *A. gambiae*.

Result obtained from this study also showed that larvae of *Anopheles gambiae* was more susceptible to the treatment of the plant extracts than the pupae as there was 100% mortality of larvae at 0.3% concentration of *T. diversifolia* after 24 hours Post Treatment. This is similar to the findings of other researchers who worked on the developmental stages of mosquito, observing a higher mortality rate of larvae than pupae in their research [19; 20; 14]. The larvae having higher mortality rate could be as a result of the mosquito larvae actively swimming around in water, by so doing, doses of plant active components could be ingested, thereby leading to stomach poisoning [21]. During this research, it was observed that the extracts from the two plants affected the swimming ability of the larvae and pupae of the insect, this could have also affected the intake of oxygen by the developmental stages as that may not have the ability to move to the surface of the water for oxygen thereby reducing their chances of survival [22].

5. CONCLUSION

This present study revealed that leave extracts of *T. diversifolia* and *M. charantia* contain phytochemicals that showed high toxicity effect on larvae and pupae of *A. gambiae* leading to significant high mortality rate of the two developmental stages. Therefore, more research should be carried out on these plants in order to formulate insecticides at commercial level. Also, use of insecticides derived from plant products should be encouraged more than use of synthetic insecticides as this would be a great means to solving problems such as emerging resistance in insect vectors and harmful effects of chemicals on human and its environment.

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Comment [i26]: The references must be reviewed and corrected according to the notes to the authors IJTDH.

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Comment [i27]: Change..Use a recent reference.

Comment [i28]: The recent reference is : WHO, "Guidelines for laboratory and field testing of mosquito larvicides," Tech. Rep.Who/cds/whopes/gcdpp/2005.13, 2005. The reference (10) must be replaced.

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Comment [i29]: In italic.