***Original Research Article***

# Ovicidal and Larvicidal activities of n-hexane extract of Murraya paniculata(L.) leaves against *Aedes aegypti*

.


## ABSTRACT

**Aims:** *Aedes aegypti* is a widely distributed mosquito and the primary vector of several arboviral diseases attracting major global health concerns. This laboratory study accessed the ovicidal and larvicidal activities of n-hexane extract of *Murraya paniculata* (L.) Jackleaves on eggs and first instar larvae of *Aedes aegypti*.
**Study design:** Conventional bioassay,
**Place and Duration of Study:** Department of Zoology, ETF Laboratory and Central research laboratory, College of Medicine, University of Lagos, Idiaraba, between February and July 2021.
**Methodology:** Eggs and larvae were exposed to various quantities of test plant extract ranging from 20ppm to 80ppm for 72 hours at ambient laboratory temperature(30±0.57 ºc). Probit analyses and analysis of variance were used to analyze the data.
**Results:** Results revealed that the plant extract recorded the highest ovicidal activity of 61% at 80 ppm concentration against the eggs of *Aedes aegypti*, after 72 hours. The $LC_{50}$ values of ovicidal and larvicidal activities at 72hrs were 85.162ppm and 80.270ppm respectively, there was a significant difference between the ovicidal and larvicidal activity of each concentration while mortality increased with increasing concentration.
**Conclusion:** The results suggest that n-hexane extract of *M. paniculata* can be used as an environmentally friendly methodfor the control of *Aedes aegypti*.



*Keywords: Murayya paniculata, Aedes aegypti, Ovicidal, Larvicidal*


## 1. INTRODUCTION

Mosquitoes' importance as medically important insects has been studied throughly recognized. *Aedes aegypti,* for example, is the vector of various arboviral infections that affect humans, including Dengue fever, Chikungunya fever, Yellow fever, and Zika virus, all of which have serious consequences ranging from morbidity to mortality and economic losses [1]. During the 2019 outbreak of dengue fever in Brazil, 1,544,987 cases was recorded with 782 deaths. Additionally, about 132,205 cases of chikungunya resulting in 92 deaths as well as 10,768 cases of Zika virus with three deaths was recorded within the same period [2]. In Nigeria, 163 cases of yellow fever were confirmed in 17 States within 2017 to 2020 [3] and in 2019, these cases was reported to have escalated to an unidentified magnitude to a case fatality

rate of less than one percent in Adamawa State [4,5]. Dengue fever has also been evinced as a growing global health problem affecting more than 2.5 billion people in 100 countries [6].

Several mosquito control strategies have been adopted to minimize the occurrence of the diseases, which focus primarily on eliminating mosquitoes at the different stages their life cycle [7,8]. Transovarian transmission of viruses has been reported to occur in *A. aegypti* [9], hence it will make more sense to kill the immature stages of the mosquito alongside the adults. In areas where endemic mosquito-borne diseases exist, reduction or removal of mosquito breeding sites is frequently used alongside chemical or microbiological compounds that act as ovicides, larvicides, and pupicides [10-13]. As reports of *A.aegypti* resistance to insecticide has become widespread it continues to pose a major threat to public health worldwide [14-17]. Demands for locally available, novel, cheap and reliable mosquito control strategies such as those obtainable in plant materials are high [18], these compounds are biodegradable, less hazardous and rich store house of chemicals of diverse biological activity [12,19]. Insecticidal efficacy of locally available plant species were also determined using powders, solvent extracts (including ethanol, methanol, and Petroleum ether), fixed oils, and essential oils of the candidate plant species' leaves, stem bark, roots, fruits, and seeds [20-22]. Plant essential oils are important natural resources of insecticides, they are selective, biodegradable, environmentally friendly and specificity to target organisms [23]. Many plant extracts such as *Eucalyptus citriodara*, *Syzgium aromaticum*, *Zingiber officinale*, *Azadirachta indica*, *Ocimum basilicum,* have been proven to be highly effective as mosquito repellents against *Aedes spp*, *Culex spp* and *Anopheles spp* [24].

The present study investigates *Murraya paniculata* (L.) Jack, a species of small tree, grown in hot climates in the tropics and subtropics, commonly known as orange jasmine or mock orange [25,26]. In the past, *M. paniculata* has been utilised as decorative and therapeutic plant [27], it has a wide range of soil tolerance and a strong fragrance, it is therefore used as a hedge plant and as a food additive in many Indian and Malay dishes [28]. In traditional medicine, *Murraya paniculata* is used to treat diarrhea, abdominal pain, stomach ache, dysentery, headache, edema, thrombosis, *and* blood stasis. As a result, several phytochemical studies have been conducted on *M. paniculata*. Coumarins and flavonoids, which are active constituents in most natural repellents, were found in the leaf extract [29].

In this present study, the insecticidal potential of n-hexane extract of *Murrayapaniculata* leaf was evaluated on eggs and first instar larvae of *Aedes aegypti.*

## 2. METHODOLOGY

### 2.1 Preparation of plant materials

*Murraya paniculata* leaves were collected from the shrub grown as ornamental plants within the premises of Lagos State University, Ojo. The leaves were confirmed in identification as *M. paniculata* in the Department of Botany, LASU herbarium. The fresh leaves were washed in tap water and then air dried on open benches in the laboratory for over 18 days at temperature of 30±0.57 ºc and 70±0.57% relative humidity. The dried leaves were powdered mechanically using Panasonic® MX 1521 model electrical stainless steel blender. The powder was kept in an airtight container at room temperature until it was used for laboratory extraction.

### 2.2 Preparation of Extracts

Using a Pyrex ® 3740 series Soxhlet extractor, dried powdered leaves of M. paniculata were extracted with n-hexane. The extract was concentrated in a rotavapour at 45$^{o}$C under minimized pressure of 22-26mmhg, and the residue was kept refrigerated at 4$^{o}$C in an amber airtight glass bottle to prevent evaporation and maintain photo stability.

### 2.3 Rearing of test insects *Aedes aegypti*

Larvae of *Aedes aegypti* were collected from water holding containers mainly dis-used tyres within the Ojo campus of Lagos State University using plastic scoops. The larvae where then cultured till adult stage in the laboratory.

Adult *Aedes aegypti* were thereafter reared in Aluminium-wire net-cages with length, breadth and height dimension of 0.5 m × 0.5 m × 0.5 m respectively. Male and female *Aedes aegypti* mosquitoes were maintained on 10% sugar solution soaked in cotton ball and held in petri dish. Female mosquitoes were supplied with human volunteers to feed on their blood which provided them with protein for hatching.

### 2.4 *Aedes aegypti* culture

Adult Female mosquitoes were allowed to lay eggs in 50ml plastic cups lined which contained 30ml water and was lined with filter paper which served as a substrate for mosquito eggs. The filter paper stripe of eggs was dried at temperature of 30±0.57 ºc and 70±0.57% relative humidity and stored in air tight container until its required for laboratory experiment. The eggs of *Aedes aegypti* (500) were collected and allowed to hatch at the same time in plastic container filled with water. The larvae were fed with ground-fish meal until they matured into pupae. The pupae were transferred to the net cage till adult emerged.

### 2.5 Egg Hatchability (Ovicidal ) and Larvicidal bioassay

In 1000mls of water, 1 ml of *M. paniculata*extract was mixed with 2ml of Di-Methyl Sulfur Oxide (DMSO) which served as diluent for faster miscibility in 1000mls of water. For the ovicidal bioassay, serial dilutions were made to achieve 20,40,60,80 ppm for ovicidal bioassay. Twenty eggs were placed into each disposable transparent cup containing 20, 40, 60 and 80 ppm of *M. paniculata* leaf extracts using a magnifying table lens. The bioassay set up consisted ofthree control; negative control (1) with only Dimethyl sulfoxide, negative control (2) with only distilled water and Dimethyl sulfoxide in proportion of 1000ml to 2ml and positive control with only distilled water. Each experiment and control was replicated three times. Eggs were considered non hatching when first instar larvae did not emerge after 72 hrs.

The same procedure was adopted for the larvicidal test, Mortality was recorded daily. Larvae was considered dead, when they floated or failed to move any part of their body with gentle swirling of the exposure cup.

### 2.6 Data analysis

Quantal data from the number of dead eggs or larvae were analyzed using ProbitAnalysis computer software (version 1.5, 2012).Lethal Concentrations (LC) values and associated Confidence Limits (CL) were thereby computed. Lethal concentration values with overlapping confidence limits are considered not to be significantly different.

## 3. RESULTS

### 3.1 Activity of n-hexane extract of *M. paniculata* against *Aedes aegypti* eggs

*Murraya paniculata* extract of showed ovicidal activity on *Ae. aegypti* eggs, at all concentrations, percentage of non-hatching eggs increased with increasingconcentration as shown in Figure 1. There was a significant difference between the ovicidal activity of the extracts at all concentration levels. However, there was no significant difference between the ovicidal activity of the extract at 24 hours, 48hours and 72 hours ($P<0.01$). The negative control test (1) with only Dimethyl sulfoxide, revealed a significant difference in comparison with all concentrations except the 80 ppm concentration ($P<0.01$). Negative control test (2) with distilled water and Dimethyl sulfoxide delayed hatching of *Aedes aegypti* egg for 48 hours, the ovicidal activity of Negative control test (2) was not significantly different from 40, 60 and 80 ppm concentrations. The lethal concentration (LC) values for the ovicidal activity of n-hexane extract of *M. paniculata* leaves against *Aedes aegypti* egg are presented in Table 1.

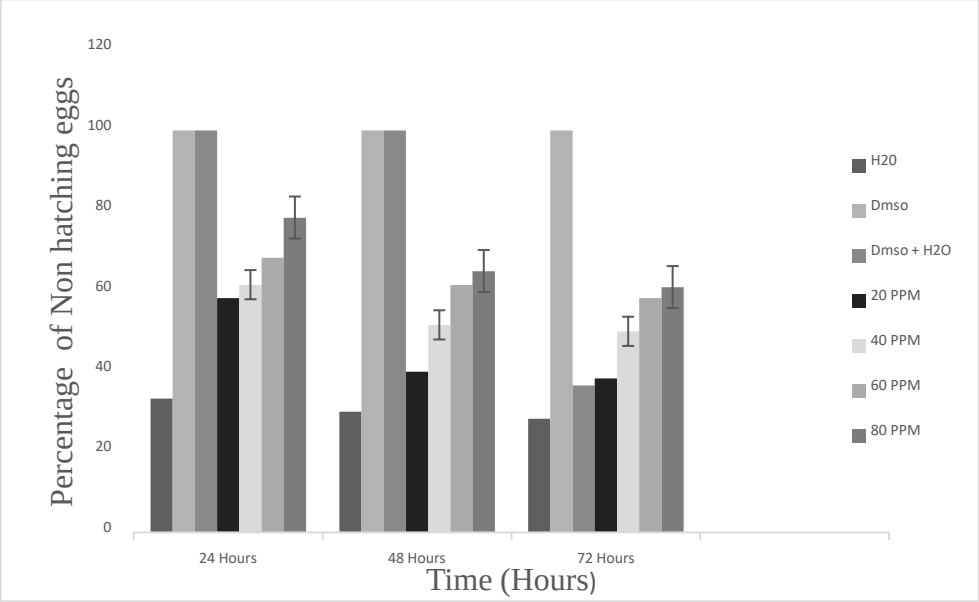


**Fig. 1. Percentage of Non hatching *Aedes aegypti* eggs treated with different concentration of extracts of *Murraya paniculata* at 24, 48 and 72 hours.**

**Table 1. ovicidal activity of n-hexane extract of *M. paniculata* against *Aedes aegypti* eggs**

| *Time of exposure (hours)* | *$LC_{50}$ (PPM)* | *95% confidence limit* | | *$LC_{99}$ (PPM)* | *95% confidence limit* | | *Chi-square* |
|---|---|---|---|---|---|---|---|
| | | **Lower** | **Upper** | | **Lower** | **Upper** | |
| 24 | 44.859 | 5.721 | 104.585 | 3356.088 | 455.096 | $440.4\times10^{15}$ | 1.070 |
| 48 | 73.519 | 40.193 | 269.793 | 1809.399 | 379.063 | $112.5\times10^{8}$ | 0.281 |
| 72 | 85.162 | 48.486 | 1015.065 | 2276.884 | 410.978 | $173.1\times10^{12}$ | 0.097 |

### 3.2 Toxicity of n-hexane extract of *M. paniculata*against first instar larvae of *Aedes aegypti*

The n-hexane extract of *M. paniculata* was toxic to the first instar larvae at all concentrations, toxicity increased with increasing concentration, no mortality was observed in all control set ups (Figure 2). At all concentration levels, there was a substantial difference in the extracts' larvicidal activity. There was no significant variation in the extract's larvicidal activity at 24 hours, 48 hours, or 72 hours (P0.01). The larvicidal effect of the lowest concentration (20 ppm) was not significantly different from the control set ups (P<0.01). Table 2 shows the computed lethal concentration (LC) values for the toxicity of *M. paniculata* n-hexane extract against *Aedes aegypti*egg.

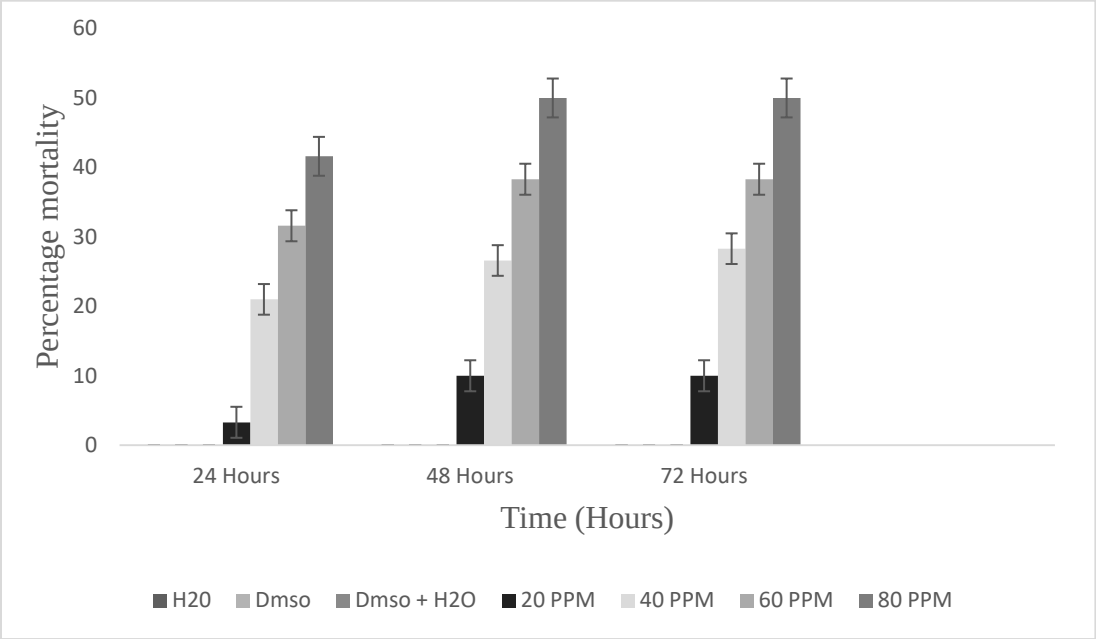


**Fig. 2. Percentage mortality of first instar larvae of *Aedes aegypti* at 24, 48 and 72 hours**

**Table 2. Toxicity of n-hexane extract of *M. paniculata* leaves against first instar larvae of *Aedes aegypti***

| *Time of exposure (hours)* | *$LC_{50}$ (PPM)* | *95% confidence limit* | | *$LC_{99}$ (PPM)* | *95% confidence limit* | | *Chi-square* |
|---|---|---|---|---|---|---|---|
| | | **Lower** | **Upper** | | **Lower** | **Upper** | |
| 24 | 92.848 | 74.738 | 141.149 | 792.310 | 365.964 | 4506.079 | 0.901 |
| 48 | 80.892 | 64.988 | 121.681 | 1035.813 | 427.005 | 8086.789 | 0.039 |
| 72 | 80.270 | 64.421 | 121.029 | 1064.224 | 433.121 | 8669.380 | 0.164 |

## 4. DISCUSSION

Following scientific verification of the harm caused by synthetic pesticides, knowledge and use of botanical insecticides is increasing in underdeveloped nations. It's also becoming more important in poorer countries, where insecticide poisoning is on the rise, creating environmental and health problems [30].Botanical insecticides are considered as a viable alternative to synthetic insecticides due to their lack of cytotoxicity, ease of biodegradability, and mimicking of host metabolism [31]. In comparison to synthetic pesticides, the chemicals in botanical insecticides degrade quickly, making them more environmentally benign [32].In this present study, the n-hexane extract of *M. paniculata* leaves recorded the highest ovicidal activity of 61% at 80 ppm concentration against the eggs of *Aedes aegypti*, at 72 hours, which showed similar results obtained by Govindarajan and Karuppannan [33] using n-hexane leaf extracts of *Eclipta alba L*. against *Aedes aegypti* eggs, n-hexane extracts of *Eclipta alba L* showed ovicidal activity of 78.6% at 350ppm against *Aedes aegypti.* Comparably,Reegan *et al*. [34] used hexane extracts of *Limonia acidissima* L. against *Aedes aegypti* egg and found the *L. acidissima* hexane extract caused 60.0% ovicidal activity at 500 ppm. Also, Wariko and Kumar [35] reported

that the hexane root extract of *Argemone mexicana* caused 91.1% ovicidal activity respectively at highest concentration against *Aedes aegypti*e ggs. Negative control (1) with only Dimethyl sulfoxide debarred *Aedes aegypti* eggs from hatching for 72 hours while only Dimethyl sulfoxide and distilled water delayed hatching for 48 hours, this may be because Dimethyl sulfoxide slowed down embryogenesis in *Aedes aegypti* egg, a study reported by Yamamoto *et al*. [36] showed that Dimethyl sulfoxide could prevent and cause diapause in eggs of *Bombyx mori*, in a similar study by Cvetković *et al*. [37]. Dimethyl sulfoxide showed toxicity against *Drosophilia melanogaster* larvae with an $LC_{50}$ of 0.42% v/v , hence Dimethyl sulfoxide may be more toxic than it was initially considered.

The hatching process in *Aedes aegypti* is a two-stage mechanism with a specific pattern of hatching decrease after the second day [38, 39]. In this aforementioned studies, the percentage of hatching in *Aedes aegypti* eggs was highest on the second day after it was introduced into water similarly in the current study, there was a steady rise in larval emergence in the control, which recorded the highest hatching after 48 hours, whereas hatching was completely delayed in the treated cups. When compared to the eggs in the positive control, egg hatching was delayed in the n-hexane treated cups.

In the present study n-hexane extract of *M. paniculata* showed toxicity to the first instar larvae of *Aedes aegypti* at all concentrations, there was a significant difference between the larvicidal activity of each concentration tested and toxicity increased with increasing concentration, no mortality was observed in all control set ups (Positive control (1) with only distilled water, Negative control (1) with only Dimethyl sulfoxide and Negative control (2) with only Dimethyl sulfoxide and distilled water), There was no significant difference between the larvicidal effect of the lowest concentration (20 ppm) and the control set ups ($P<0.01$).

For larvicidal activity, n-hexane extract of *M.paniculata* showed similar results obtained by Rawani*et al*. [40] who worked on the larvicidal effect of Crude phyto extract of *Murraya paniculata* on first, second, third and fourth instar larvae of *Culex quinquefasciatus, with an* $LC_{50}$ and $LC_{99}$ value of 0.10 and 0.64 at 24 hours respectively, 0.13 and 0.47 at 48 hours and 0.08 and 0.23 at 72 hours. Similarly, Ramar and Jeyasankar (41) tested the larvicidal activity of chloroform extract, ethyl acetate and petroleum ether extractsof *M.paniculata*against first instar larvae of *Aedes aegypti,* Chloroform extract showed highest larvicidal activity against *Aedes aegypti* with the $LC_{50}$ and $LC_{90}$ values of 57.58 and 246.20ppm respectively followed by petroleum ether of larvicidal activity against *Aedes aegypti* with the $LC_{50}$ and $LC_{90}$ values of 83.58 and 289.26ppm respectively, Ethyl acetate extract of *M. paniculata* exhibited the least larvicidal activity among the extracts used. Also, Reegan *et al*. [42] tested the larvicidal activity of purified fractions of a *n*-hexane extract of *Limoniaacidissima* L. (Rutaceae) leaves against *Aedes aegypti,* the $LC_{50}$ for L3 larvae ranged from 4.11 to 23.53 ppm.

Singh *et al*. [43] also reported the larvicidal activity of the leaf extract of *Ocimum canum* against *Aedes aegypti*. The $LC_{50}$ values for 2nd, 3rd and 4th instar larvae were 177.82, 229.08 and 331.13 ppm, respectively.

The larvicidal activity observed can be attributed to the high flavonoid content whiich is one of the major secondary metabolites obtained after phytoconstituent analysis of *M. paniculata* leaves as reported by Sonter *et al*., [44], and hexane extract was also observed to contain a high amount of Cyclohexane and fractionization of the extracts produce six fractions some of which exhibited high antibacterial activities

## 5. CONCLUSION

In conclusion, this study documents the potential of *M. paniculata* as a mosquito control agent, hence it can be used in mosquito vector control. However, there is need for further studies to determine and isolate the active constituents of *M. paniculata* responsible for its insecticidal properties.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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