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Research article

Larvicidal effect of *Azadirachta indica, Moringa oleifera,* and *Carica papaya* on the development of *Anopheles gambiae* (Diptera: Culicidae) larvae

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Abstract:
A wide range of pyrethroid and organochlorine resistance has been observed across most African countries. Therefore, using eco-friendly plant botanicals to control Anopheles larvae offers a cost-effective alternative method for larval management. This study assessed the larvicidal effect of *Azadirachta indica*, *Moringa oleifera*, and *Carica papaya* on the growth of *Anopheles gambiae* larvae (Diptera: Culicidae). *Azadirachta indica, M. oleifera*, and *C. papaya* leaves were obtained, processed and crushed into powder using a Qasa electric grinder. *Anopheles* larvae were collected from natural breeding sites using dippers and nets. Twenty live larvae were subjected to test cups filled with 100 ml of distilled water containing *A. indica*, *M. oleifera*, and *C. papaya* leaf powder at concentrations of 0.001, 0.005, and 0.01 g/ml. The larvae were exposed to these treatments for 24 and 48 hours, respectively, with four replicates for each concentration. The *Anopheles* larvae showed 100% mortality within 24 hours when exposed to 0.01 g/ml of *C. papaya*. Similarly, 0.01 g/ml of *M. oleifera* resulted in mortality rates of 45% at 24 hours and 100% at 48 hours. In contrast, *A. indica* leaf powder showed the least effectiveness, with mortality rates of 2.5% and 30% observed after 24 and 48 hours of exposure. A strong positive linear relationship was observed between the concentration of *A. indica* ($r^2 = 0.83$), *M. oleifera* ($r^2 = 0.66$), and *Anopheles* larvae mortality. *C. papaya* exhibited superior larvicidal efficacy with higher mosquito mortality and a lower LC_{50} value. The mortality of *Anopheles* larvae exposed to *A. indica* and *M. oleifera* depended on concentration and duration. *A. indica*, *M. oleifera*, and *C. papaya* leaf powder can minimize and compliment measures towards control and inhibit development of *Anopheles* larvae.

Keywords: *Anopheles* larvae, *Azadirachta indica*, Biological control, *Carica papaya*, larvicidal effect, *Moringa oleifera*

Introduction

Anopheles mosquitoes (Diptera: Culicidae) are small insects with piercing and sucking mouthparts (Okwa, 2019). Females are hematophagous feeders, feeding on humans and animals, while males feed on nectar. There are about 400 identified species globally (WHO, 2019), 140 species in sub-Saharan Africa (WHO, 2014), and about 27 species in Nigeria (Okwa, 2019). *Anopheles gambiae, An. coluzzii, An. arabiensis* and *An. funestus* has been recorded as the major vector of malarial protozoan parasites, *Plasmodium falciparum* in Africa (Sinka *et al*., 2012), *An. rivolorum, An. pharoensis, An. ziemanni, An. implexus, An. squamosus* can only be observed as vectors after eradication of major vectors, thus, becoming secondary vectors (Afrane *et al*., 2016). The frequent application of synthetic insecticides in mosquito control has disrupted natural biological control systems, causing rebounds in mosquito populations. This has consequently induced resistance, and adverse effects on non-target organisms, and raised environmental and human health concerns, prompting the exploration of alternative control measures (Prabhu *et al*., 2011). A wide range of pyrethroid and organochlorine resistance has been observed across most African countries (Elissa *et al*., 1993; Keïta *et al*., 2021; Boussougou-Sambe *et*

al., 2023; Teshome *et al*., 2023). Therefore, using eco-friendly plant botanicals to control *Anopheles* larvae offers a cost-effective alternative method for larval management.

Azadirachta indica A. Juss (Sapindales: Meliaceae) has its origin in India and has spread over the years to other tropical areas of the world (Popoola *et al*., 2017a). It has been identified with medicinal, repellent, and pesticidal properties (Popoola *et al*., 2017a). Among the complex secondary metabolites, azadirachtin-A predominates in quantity, with azadirachtin-E emerging as a potent regulator of insects (Achio *et al*., 2012; Nicoletti and Murugan, 2013). Success in using neem has been reported in veterinary pests and pests of public health importance (Mulla and Su, 1999). Moreover, *Moringa oleifera* Lam. (Brassicales: Moringaceae family), commonly referred to as the miracle tree, is a tree found in the sub-Himalayan tracts of Northern India (Ramachandran *et al*., 1980). It contains phytochemicals that serve a crucial function as bioinsecticides, inducing morphological changes in the digestive tract, disrupting digestive enzymes, exhibiting trypsin inhibitor activities (Pontual *et al*., 2018), and influencing egg hatchability (Santos *et al*., 2012). Some larvicidal results of *M. oleifera* have been reported on larvae of *Aedes aegypti* and *An. stephensi* (Coelho *et al*., 2009; Silva *et al*., 2019).

Furthermore, the seed and leaves of *Carica papaya* Linn. (Brassicales: Caricaceae) are known for their rich phytochemical compounds such as alkaloids carpaines, flavonoids, saponins and tannins (Yogiraj *et al*., 2014; Hayatie *et al*., 2015). The acute action of alkaloid carpaines on the nervous system of insect larvae has been validated (Chandrasekaran *et al*., 2018; Ilham *et al*., 2019). Mortality of 100% susceptibility on *Aedes aegypti* and *Culex quinquefasciatus* larvae has also been reported by Chandrasekaran *et al*. (2018). In the present study, an attempt was made to determine the larvicidal effect of *Azadirachta indica, Moringa oleifera,* and *Carica papaya* on the development of *Anopheles gambiae* sl.

Study area

The study was conducted in Calabar Municipality, Cross River State, Nigeria. Calabar Municipality serves as the capital of Cross River State, with an area of 331.551 km^2. The area features a tropical climate with high humidity and substantial rainfall, fostering an ideal environment for mosquito breeding. The municipality includes both urban and peri-urban zones, with numerous water bodies such as swamps and stagnant pools that serve as potential mosquito breeding sites. Poor drainage systems in residential areas often lead to water accumulation, creating additional breeding habitats. Additionally, the municipality's dense vegetation and overgrown areas offer ample resting and breeding sites for mosquitoes. The study points where larvae were collected is an area dominated by the Fulani and Hausa tribes who are predominantly truck drivers. The area serves as a permanent breeding site for mosquito larvae.

Collection of larvae

The larvae of *Anopheles* species used in this study were obtained from stagnant water at Lemna Truck Park, Calabar in August 2022. Collected larvae were transported to the insectary facility at the Calabar Institute of Tropical Disease Research and Prevention, University of Calabar (04˚57.935'N, 008˚ 19.186'E). Larvae were fed with grounded yeast and cabin biscuit (10 tablets to 1 piece of biscuit) and were maintained in a controlled environment of 27±2˚C and 75±20% temperature and relative humidity respectively.

Preparation of plant powder

Matured leaves of *A. indica, M. oleifera* and *C. papaya* were obtained within the University of Calabar campus with permission from the Herbarium unit of the Department of Plant and Ecological Studies as required by the institution. The leaves were air dried, pulverized into powder using a Qasa electric grinder (QBL-18L40) and then sifted through a mesh (180 µm) to acquire a fine powder. The resulting powder was subsequently packed into polyethene bags and stored in a refrigerator for use within a twelve-hour timeframe (Popoola *et al*., 2017b).

Larvicidal bioassay

The larvicidal activity of the leaf powders of *A. indica, M. oleifera* and *C. papaya* were evaluated according to WHO (2005). Fourth instar larvae of *Anopheles* species were used for the study. Leaf powders were measured in amounts of 0.1 g, 0.5 g, and 1.0 g, and then placed into test cups with 100 ml of distilled

water, resulting in concentrations of 0.001 g/ml, 0.005 g/ml, and 0.01 g/ml. Twenty live larvae were placed in each of the experimental cups, and muslin cloth was used to cover them, secured with a rubber band to allow for ventilation. Control was set up without the leaf powders. Each experiment was replicated four times. Mortality was observed within 24 and 48 hours in the test cups subjected to treatment, in contrast to the control group (Kudom *et al*., 2011).

Statistical analysis

The mortality observed was recorded and the data obtained was used to calculate the lethal concentration (LC_{50}) of *A. indica* and *M. oleifera* on *Anopheles* larvae. The lethal concentration, 96 hours LC_{50} was computed using probit analysis (Finney, 1979). The control mortalities were corrected using the formula described by Chil *et al*. (2018):

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control Mortality}} \times 100$$

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

Results

Percentage mortality of *Anopheles* mosquitoes when exposed to *A. indica*, *M. oleifera*, and *C. papaya* indicates that *Anopheles* larvae exhibited susceptibility to *C. papaya* leaf powder at a concentration of 0.01 g/ml after 24 hours of exposure. After 48 hours, susceptibility was observed at concentrations of 0.005 and 0.01 g/ml, while a concentration of 0.001 g/ml exhibited suspected resistance with 95% mortality. This was followed by *M. oleifera* leaf powder, which exhibited 100% mortality only at a concentration of 0.01 g/ml after 48 hours of exposure. *Azadirachta indica* leaf powder displayed the lowest mortality rate, indicating greater resistance across all tested concentrations at 24 and 48 hours of exposure compared to other treatments (Figure 1).

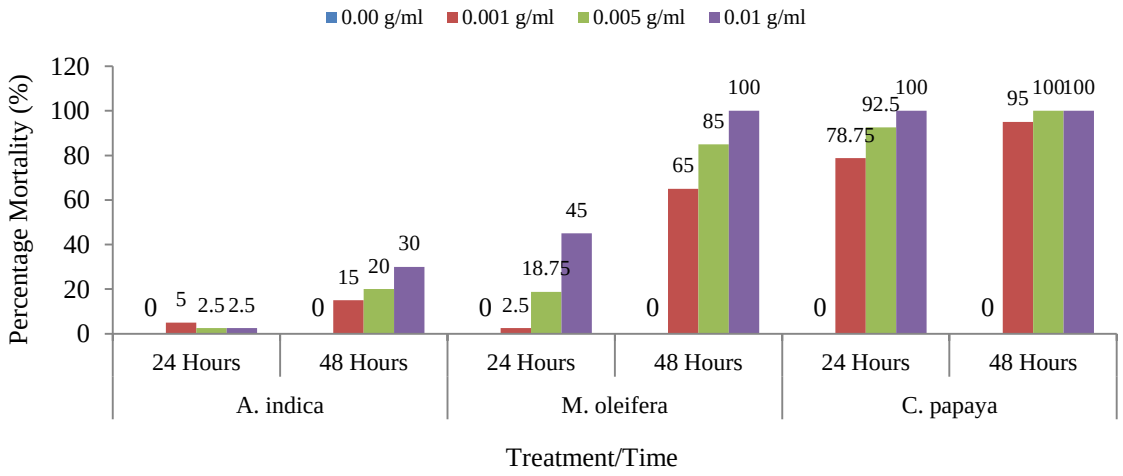

Figure 1**:** Susceptibility of *Anopheles* larvae exposed to *A. indica*, *M. oleifera* and *C. papaya* after 24 and 48 hours

The regression analysis conducted indicated a significant dependency of mortality on concentration for *A. indica* and *M. oleifera*. A correlation coefficient and coefficient of determination (r^2) of 0.83 (*A. indica*) and 0.66 (*M. oleifera*) were derived for *Anopheles* mosquitoes. These values indicate a strong, positive linear relationship between the concentration of *A. indica*, *M. oleifera* and *Anopheles* mortality (Figures 2 and 3). However, in *Carica papaya*, the regression analysis demonstrated a weak linear correlation between concentration and *Anopheles* mortality, yielding an r^2 value of 0.38 (Figure 4). The 96-hour LC_{50}, along with a 95% confidence limit, revealed that *C. papaya* exhibited the highest

potency against *Anopheles* larvae, with an LC_{50} of 0.00 g/ml. *M. oleifera* followed closely with an LC_{50} of 0.001 g/ml, while *A. indica* demonstrated the least effect, with an LC_{50} value of 0.401 g/ml (Table 1). There was a significant difference in the impact of *M. oleifera* ($P < 0.001$) and *A. indica* ($P = 0.007$) leaf powder concentrations on the mortality of *Anopheles* larvae. However, *C. papaya* ($P = 0.224$) showed no statistical significance.

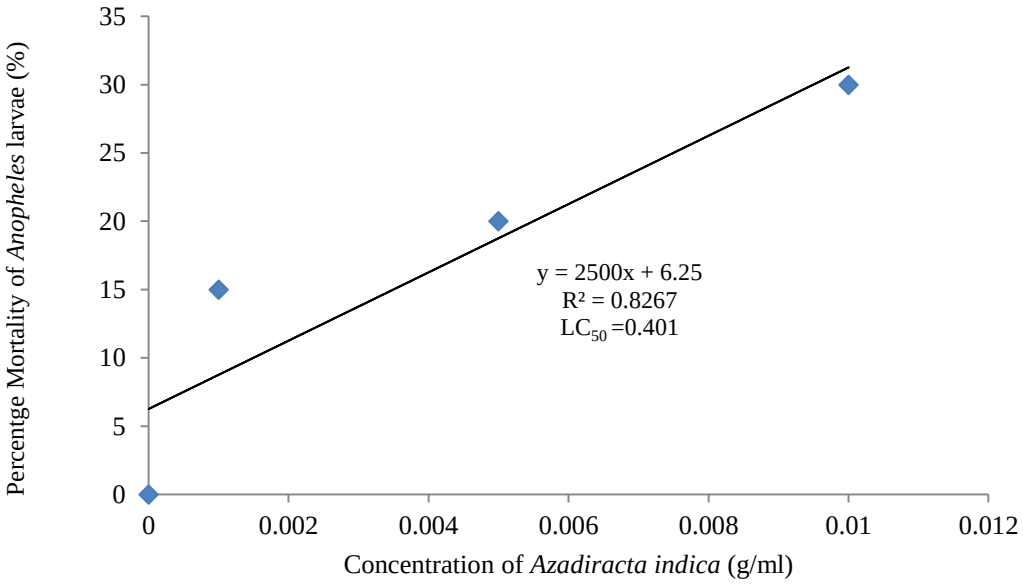

Figure 2**:** LC_{50} probit transformation graph of *Anopheles* larvae treated with *A. indica*

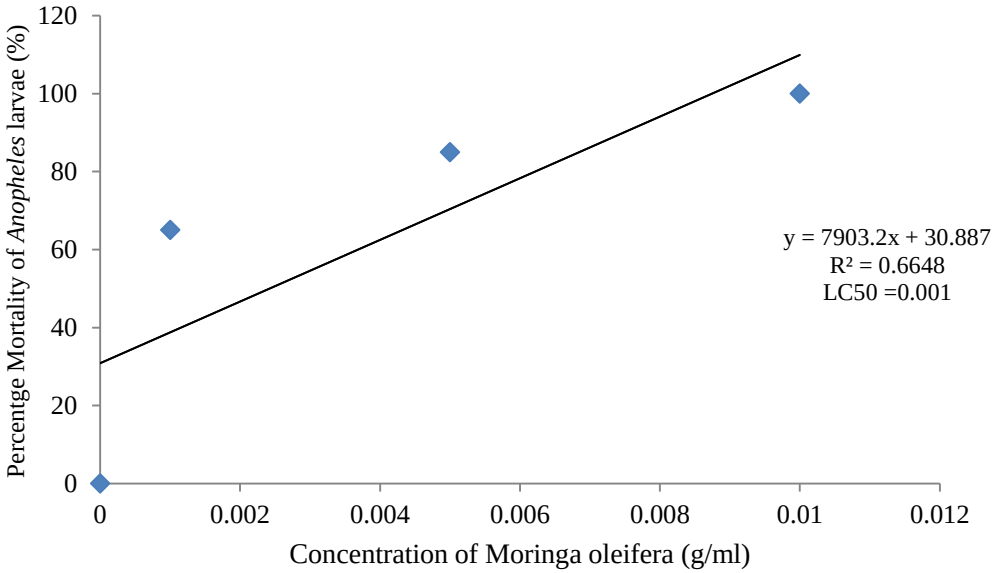

Figure 3: LC_{50} probit transformation graph of *Anopheles* larvae treated with *M. oleifera*

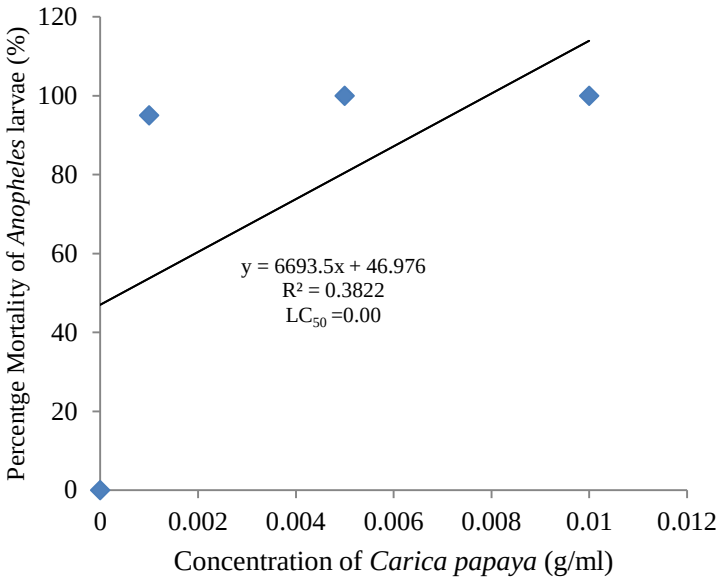

Figure 4**:** LC_{50} probit transformation graph of *Anopheles* larvae treated with *C. papaya*

Table 1: Lethal concentration for *A. indica, M. oleifera,* and *C. papaya* leaf powders against *Anopheles* larvae after 48 hours of exposure

Leaf powders	LC_{50} g/ml	LC_{90} g/ml	LC_{99} g/ml	Confidence Limit	
				Upper	Lower
A. indica	0.401	529.0	183717.8	0.856	0.33
M. oleifera	0.001	0.006	0.039	1.765	0.824
C. papaya	0.00	0.001	0.002	7.257	-1.701

DISCUSSION

The result showed that *A. indica* leaf powder possesses insecticidal properties on *Anopheles* larvae after treatment compared to the control. However, the low percentage mortality of 2.5% and 30% after 24 and 48 hours of exposure did not confer confidence for the leaf powder, though this may have increased over longer hours of exposure. In contrasts a higher percentage of mortality within the same duration of exposure was reported (Kudom *et al*., 2011; Wahedi *et al*., 2020). The variation in the mortality percentage may be attributed to the different forms in which the *A. indica* leaf was utilized. Wahedi *et al*. (2020) achieved 100% mortality after 72 hours using aqueous extract and within 24 hours using ethanolic extract against Anopheles larvae in Mubi. Furthermore, Kudom *et al*. (2011) employed an identical concentration of *A. indica* leaf powder which resulted in a complete mortality rate against *Culex quinquefasciatus* within a 24-hour timeframe, whereas the crude aqueous extract from the leaves exhibited a delayed impact on *Cx. quinquefasciatus* larvae by achieving a mortality rate exceeding 95% over an extended period of up to 120 hours at a concentration of 0.01 g/mL. *Aedes aegypti* larvae exhibited susceptibility to *A. indica* leaf acetone and root chloroform extracts, resulting in 100% mortality at a concentration of 1000 ppm within a 24-hour timeframe (Nour *et al*., 2012).

Numerous studies reported 100% mosquito larvae mortality within 24 hours using various solvents for *M. oleifera* extracts, the mortality rates for *An. gambiae* larvae in this study were only 45% and 100% after 24 and 48 hours, under the treatment concentration of 0.01g/ml. It has been used against *Aedes aegypti* to obtain 99.2% larval mortality within 24 hours (Ferreira *et al*., 2009). Kumar *et al*. (2013) reported 93.3% larval mortality on *An. stephensi* within 48 hours. Prasad and Parven (2016) similarly found the use of *M. oleifera* extracts promising in the control of *An. stephensi* when compared with other botanicals. Furthermore, *M. oleifera* recorded 100% mortality in *Culex quinquefasciatus* larvae within 3 hours of exposure (Afolabi and Olonisakin, 2022).

Carica papaya leaf powder was more effective on the larvae of *the Anopheles* mosquito used in this study. Mortality of 100% was recorded after 24 hours of exposure. The potency of the leaf

powder could be associated with the active phytochemicals (Julaily and Setyawati, 2013; Wijanarko, 2017). These include cysteine protease enzymes such as papain and kimopapain. Ilham *et al.* (2019) reported the fatal effect of *C. papaya* leaf extract on *Aedes* spp. larvae. Ahmad and Adriyanto (2019) observed the effectiveness of *C. papaya* leaf extract when used against *Culex quinquefasciatus*.

The leaf powders from *A. indica*, *M. oleifera*, and *C. papaya* used in this study exhibited larvicidal properties. Despite their comparable content of active compounds, including alkaloids, terpenoids, flavonoids, saponins, and non-protein amino acids, these compounds can potentially act as stomach poisons, growth inhibitors, respiratory toxins, and anti-feedants (Singh *et al*., 2020; Xie *et al*., 2020; Souto *et al*., 2021).

Conclusion and Recommendation

The findings of this study demonstrated the larvicidal effectiveness of *Azadirachta indica*, *Moringa oleifera*, and *Carica papaya* leaf powder against *Anopheles gambiae* larvae. Among the three plant extracts, *C. papaya* and *M. oleifera* exhibited the highest larvicidal efficacy resulting in 100% mortality against *Anopheles* larvae within 24 and 48 hours at 0.01 g/ml while *A. indica* showed comparatively lower effectiveness, its larvicidal activity increased with concentration and duration of exposure. The study also revealed a strong positive linear relationship between the concentration of the plant extracts and *Anopheles* larvae mortality. These findings highlight the potential of *A. indica*, *M. oleifera*, and *C. papaya* leaf powder as alternative and effective control measures for inhibiting the development of Anopheles larvae, thus contributing to the management of mosquito-borne diseases.

Conflicts of Interest

The authors declare no conflicts of interest.

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