

Original Article

Biopesticides Activity of Three Medicinal Plants Extracts on the Developmental Stages of Malaria Vector, *Anopheles Gambiae* Giles (Diptera: Culicidae)Kayode David Ileke¹ **Jacobs Mobolade Adesina**^{2*} Adebimpe Olabisi Adeoye³ Isaac Omotayo Olabimi⁴

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Abstract

Background and Purpose: The insecticidal potential of *Carica papaya* (male and female species), and *Spondias mombin* were tested against the developmental stages of the malaria vector; *Anopheles gambiae* in the laboratory at an ambient condition.

Materials and Methods: Methanolic leaves extracts of *S. mombin* and *C. papaya* (male and female species) were carried out using cold extraction method. The extracts were evaporated and concentrated using a rotary evaporator under low pressure to make solvent free. The *An. gambiae* larvae and pupae were exposed to 0.1, 0.2, 0.3, 0.4 and 0.5% concentrations of the plant methanolic extracts for 24 hours and their mortality was recorded.

Results: Results indicated that *C. papaya* (male species) caused significantly higher mortality of the *An. gambiae* larvae and pupae than the other plant extracts tested ($p < 0.05$). It caused 100% larval mortality and 95% pupal mortality at 0.5% concentration. However, it was observed that the larvae were more susceptible to the extracts of all the plants tested. Data were analyzed using Analysis of Variance and log probit analysis.

Conclusion: All the three medicinal plants screened in this work showed high potency to induce mortality of both the larval and pupal stages of the malaria vector and could consequently be used to reduce malaria prevalence in the endemic areas of Nigeria.

Key Words: *Carica papaya*; *Spondias mombin*; *Anopheles gambiae*; Mortality; Malaria

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1. Introduction

Due to the nuisance associated with increasing insect populations, man suffers broadly and significantly both in agriculture and health. In agriculture, the growing parts of crops are severely damaged by insects, leading to loss in revenue. In health, on the other hand, diseases of clinical importance, like malaria, filarial fever, dengue fever, and etc. are transmitted by insect vectors especially mosquitoes (1). Malaria, an important disease transmitted by Mosquito of the Genus *Anopheles* had estimated cases of 228 million and accounted for about 405,000 deaths in 2018 (2). Some 11 countries, mainly in sub-Saharan Africa accounted for 70% of malaria cases and 71% deaths globally (2). These estimates prove the necessity of malaria control both globally and most importantly in sub-Saharan Africa.

An essential requirement in the control of epidemic diseases, such as malaria, Japanese encephalitis, dengue and filarial fever, and etc. that are transmitted by mosquitoes is vector control. Excessive and uncontrolled use of chemical pesticides results in the emergence of pesticide resistance and causes adverse effects on non-target organisms and the environment. This has led to a serious search for the development of new, improved, economical, effective, and safe mosquito control methods. A priority in this search is herbal insecticides of plant origin (3).

Many medicinally important plant extracts have been studied for their biopesticidal efficacy against the different stages (most especially the larval and pupal stages) of the mosquito vector (4). Phytochemicals with biopesticidal potential are now recognized as efficacious alternative insecticides to reinstate chemical

insecticides under the integrated mosquito management programmes (5). Currently, the use of conventional synthetic insecticides has been discouraged due to their high cost, concern for environmental pollution, detrimental effects on human health, and other non-target populations, and increasing insecticide resistance on a global scale. There is, therefore, an increased need for the development of alternative mosquito control agents with different modes of action (6).

Carica papaya (Papaya) is a juicy and tasty fruit belonging to the family, *Caricaceae*. The plant is grown in various parts of the world, including tropical America, India, Europe, and Africa (7). Papaya is generally short lived and also happened to be the first genetically modified fruit consumed by humans because of its nutritional and medicinal properties. The latex, leaves and fruits of Papaya are used traditionally in various locations in the treatment of fever, boils, hypertension, asthma, and rheumatism (8). According to Ukaegbu-Obi et al. (9), it was reported that the fruit and seed extracts of *C. papaya* show significant bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Spondias mombin Linn belongs to the family *Anacardiaceae*, and it is a fructiferous tree found in Nigeria, Brazil, and several other tropical forests of the world (10). The plant is readily common in the South Western States of Nigeria and is traditionally used in folk medicine (10). The fruit of the plant has been used to make jelly, juice, jams, and ice cream mainly in Northeastern Brazil, and their leaves are readily used in traditional medicine for the treatment of several systemic diseases (11). According to several literatures, the leaves

of *S. mombin* have been shown to exhibit antimicrobial, antiedematogenic, hypoglycemic, antioxidant, leishmanicide, and antiviral properties (12-15).

These plants (*C. papaya* and *S. mombin*) have been shown to be effective against larva of other mosquito species (16-17). Therefore, in the present study, the insecticidal potential of the extracts of *Carica papaya* (male and female species) and *Spondias mombin* against the developmental stages of *Anopheles gambiae* was studied.

2. Materials and Method

Mosquitoes' baits consisting of shallow containers with a large surface area and opaque in colour were established in the Hatchery Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba-Akoko, Ondo State, Nigeria. The opaque coloured container was filled with rain water in order to mimic mosquito's natural breeding environment and also to attract adult mosquitoes for oviposition. Small quantity of industrial yeast was sprinkled on the surface of the water and was allowed to decompose slowly as this could nourish the developing larva. Wild mosquitoes were also allowed to freely visit the bait and to lay eggs. Afterward, the containers bearing larvae and pupae of mosquitoes were transferred to the laboratory and identified using morphological keys described by Gillies and De Meillon (18). The larvae and pupae were maintained at the temperature $28 \pm 2^{\circ}\text{C}$ with $75 \pm 5\%$ relative humidity (19). The plants evaluated in this work were leaves of *Spondias mombin* and *Carica papaya* (male and female species). They were obtained in fresh form, free of insecticides from Ibaka, Akungba-Akoko, Ondo State, Nigeria, and

were authenticated at the Plant Science and Biotechnology Department of Adekunle Ajasin University, Akungba-Akoko, Ondo State. Then, these plant leaves were rinsed in clean water to remove dirt and other impurities, cut into smaller pieces before they were air dried in a well-ventilated laboratory, and ground into very fine powder using an electric blender. The powders were further sieved to pass through 1mm^2 perforations. The powders were packed in plastic containers with tight lids and stored in a refrigerator at 4°C prior to use.

Methanolic extracts of *S. mombin* and *C. papaya* (male and female species) were carried out using cold extraction method. About 500g of plant air-dried leaves powders were soaked separately in an extraction bottle containing absolute methanol. The mixture was stirred occasionally with a glass rod and extraction was terminated after 72 hours. 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°C with rotary speed of 3 to 6 rpm for 8 hr. (20). The resulting materials were air dried in order to remove traces of solvents. The crude extracts were kept in a dark bottle; labeled and preserved in the refrigerator till further use. From this stock solution, 1% concentration was prepared by diluting 0.1ml of extract in 9.9ml of solvent (21-23). Larvicidal and pupicidal activity of the plant extracts was carried out at different concentrations by preparing the required stock solutions following the methods described by World Health Organisation (WHO) (24) and Akinkurolere et al. (25). The desired concentrations were achieved by adding $1.0\mu\text{g}$ of the crude extract to 100

mL of solvent. From this, five concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5% of the plant extracts and its derivatives were prepared. The treatments were separately added to 2.5 L of water inside a plastic bowl, and yeast powder was added in order to provide source of food for the introduced larvae. Twenty larvae and pupae of *An. gambiae* were separately introduced into the treated water, and untreated water was set as control. Each treatment was replicated five times. Mortality was observed over a 24-hour period after the introduction of larvae and pupae to notice recovery; 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 (25).

The Statistical Package for Social Science (SPSS) Software, version 16, was used in the analysis of the quantitative data. Data were subjected to analysis of variance (ANOVA), and means were separated using the new Duncan's multiple Range test. The log-Probit model analysis was used regarding the data recorded in the larvicidal and pupicidal bioassay to assess the 50% lethal concentration (LC50), the 90% lethal concentration (LC90), and their 95% confidence limits (26).

3. Results

Larvicidal activity of the selected plant extracts at different concentration on *An. gambiae* are shown in Table 1. No mortality was recorded for control, and at concentration 0.1%, 0.2%, 0.3% and 0.4%, *S. mombin* was 40.00%, 50.00%, 60.00%, and 75.00%, respectively, which was significantly different ($p < 0.05$) from male *C. papaya* with 55.25%, 70.00%, 82.25%,

and 95.00% mortality, respectively for the same concentration levels. Meanwhile, the larvicidal activity of female *C. papaya* with 50.00%, 60.00%, 72.00%, and 82.25%, was not significantly different ($p > 0.05$) from the two plant extracts. At 0.5% concentration, there was documented no significant difference ($p > 0.05$) in the larvicidal activity of the three plant extracts used on the mortality of *Anopheles gambiae*. The probit Chi square test revealed that there was significant difference ($p < 0.05$) within the concentrations of the three plant extracts (Table 2). No survivor (100% mortality) was recorded using male *C. papaya* at 0.5% concentration. For the probit analysis, there was also no significant difference ($p > 0.05$) between the LC50 of male *C. papaya* (0.102%) and female *C. papaya* (0.121%), but male *C. papaya* was more effective to have 50% mortality rate at the lowest concentration of 1.02%. Thus, there was a significant difference ($p < 0.05$) in LC90 of all the plant extracts used, while the effective LC90 was male *C. papaya* (0.331%).

At the pupae stage of *An. gambiae* (Table 3), the survivor was absolute and for 0.1, 0.2, 0.4, and 0.5% concentration of the plant extract, *S. mombin* (25.25%, 32.25%, 55.00%, and 80.00%, respectively) was significantly different ($p < 0.05$) from male *C. papaya* (40.00%, 52.52%, 80.00%, and 95.00%, respectively). Meanwhile, the two plant extracts were not significantly different ($p > 0.05$) with female type of *C. papaya* (30.00%, 40.00%, 70.00%, and 85.00%, respectively). Pupicidal activity of male *C. papaya* (75.00%) and *C. papaya* (f) (65.00%) had no significant differences ($p > 0.05$) on *An. gambiae* at 0.3% concentration. The Lethal Concentration for 50% mortality was significantly different among the three plant extracts

(Table 4). The efficacy of male *C. papaya* against *An. gambiae* was higher for LC50 (0.150%) and LC90 (0.542%). The LC90 of *S. mombin* (1.405%) was also significantly

different ($p < 0.05$) from male *C. papaya* (0.542%), and the two plant extracts were not significantly different ($p > 0.05$) from female *C. papaya* (0.815).

Table 1. The Larvicidal effects of plant extracts on *Anopheles gambiae* Giles

	% mortality				
Treatments/conc.(%)	0.1	0.2	0.3	0.4	0.5
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
<i>S. mombin</i>	40.00 ± 0.33 ^b	50.00 ± 3.25 ^b	60.00 ± 3.25 ^b	75.00 ± 3.75 ^b	90.00 ± 3.25 ^b
<i>C. papaya</i> (m)	55.25 ± 3.75 ^c	70.00 ± 3.25 ^c	82.25 ± 3.75 ^c	95.00 ± 3.75 ^c	100.00 ± 0.00 ^b
<i>C. papaya</i> (f)	50.00 ± 3.75 ^{bc}	60.00 ± 3.25 ^{bc}	72.00 ± 3.25 ^{bc}	82.25 ± 3.20 ^{bc}	97.25 ± 3.75 ^b

Each value is a mean ± standard error of five replicates. Means followed by the same letter along the column are not

significantly different ($p > 0.05$) using New Duncan's Multiple Range Test.

Table 2. LC50 and LC90 of plant extracts on Larvae of *Anopheles gambiae* Giles

	Concentration (%)		
Treatment	LC ₅₀ (LCL – UCL)	LC ₉₀ (LCL – UCL)	X ² (Sig.)
<i>S. mombin</i>	0.167 ^b (0.026 – 0.263)	0.806 ^c (0.428 – 1.184)	11.26 (0.01)
<i>C. papaya</i> (m)	0.102 ^a (0.012 – 0.160)	0.331 ^a (0.218 – 0.444)	12.14 (0.007)
<i>C. papaya</i> (f)	0.121 ^a (0.001 – 0.202)	0.538 ^b (0.308 – 0.768)	13.64 (0.003)

Mean of five replicates. Means followed by the same letter along the column are not significantly different ($p > 0.05$) using New

Duncan's multiple Range test. LCL- Lower Confidence Limit, UCL-Upper Confidence Limit, X² - Chi square, Sig.- Significance.

Table 3. The pesticide effects of plant extracts on *Anopheles gambiae* Giles's pupae

	% mortality				
Treatments/conc.(%)	0.1	0.2	0.3	0.4	0.5
Control	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
<i>S. mombin</i>	25.25 ± 1.75 ^b	32.25 ± 2.75 ^b	40.00 ± 2.25 ^b	55.00 ± 3.75 ^b	80.00 ± 3.25 ^b
<i>C. papaya</i> (m)	40.00 ± 2.25 ^c	52.52 ± 2.75 ^c	75.00 ± 3.75 ^c	80.00 ± 3.25 ^c	95.00 ± 3.75 ^c
<i>C. papaya</i> (f)	30.00 ± 2.25 ^{bc}	40.00 ± 2.25 ^{bc}	65.00 ± 3.75 ^c	70.00 ± 3.25 ^{bc}	85.00 ± 3.75 ^{bc}

Each value is a mean ± standard error of five replicates. Means followed by the same letter along the column were not

significantly different ($p > 0.05$) using New Duncan's Multiple Range Test.

Table 4. LC50 and LC90 of plant extract on Pupae of *Anopheles gambiae* Giles

Treatment	Concentration (%)		
	LC ₅₀ (LCL – UCL)	LC ₉₀ (LCL – UCL)	X ² (Sig.)
S. mombin	0.296 ^c (0.067 – 0.523)	1.405 ^b (0.563 – 2.247)	17.220 (0.001)
C. papaya (m)	0.150 ^a (0.056 – 0.126)	0.542 ^a (0.349 – 0.735)	9.796 (0.020)
C. papaya (f)	0.206 ^b (0.124 – 0.281)	0.815 ^{ab} (0.501 – 0.129)	6.888 (0.076)

Mean of five replicates. Means followed by the same letter along the column are not significantly different ($p > 0.05$) using New Duncan's Multiple Range Test. LCL-Lower Confidence Limit, UCL-Upper Confidence Limit, X²-Chi square, Sig.-Significance.

4. Discussion

A synthetic pesticide is well-known for its adverse effects on the environment, and especially on human and animal health. Biopesticide has been a suitable alternative to synthetic pesticide in terms of its eco-friendly activities. Research has revealed that some botanicals have the potency of eliminating or repelling disease transmitting vectors that are of health importance. Several authors have reported that plant extracts are known to be toxic to different species of mosquitoes and could be used to control the diseases they transmit (27-29).

Male and female *C. papaya* and *S. mombin* used in this study showed positive insecticide potential against the developmental stages of the malaria vector. This aligned with the findings of (16, 17, 30,31) reported the insecticidal activity of *C. papaya* and *S. mombin* against *Aedes aegypti* and *Podagrica uniforma*. As the concentration of these plants extract increased, so did the mortality of the *An. gambiae*. Nevertheless, the bioactivity of these plant extracts varied significantly, and it was observed in this study that larvicidal ability of the three plants was not significantly different at the highest

concentration. *Spondias mombin* had the lowest potency for both larvicidal and pupacidal activity in this study, which may be due to a report given by (30) that the larvicidal activity of *S. mombin* depended on mosquito species and extract or fraction dependents. LC₅₀ and LC₉₀ results revealed that male *C. papaya* was the most potent of the three extracts. Female *C. papaya* also exerted high toxicity against *An. gambiae*, though not as high as observed with *C. papaya* (male). The markedly high toxicity of male *C. papaya* against the larvae and pupae of *An. gambiae* could be due to its strong pungent odour. A number of plants with high pungency have been reported for their bioactivity against insect pest (32-34). According to Kovendan et al. (35), the biological activity of *C. papaya* extract might be due to the various compounds contained, such as chymopapain, papain, glycosides, tannin, saponin, and these compounds may have also jointly or independently contributed to produce biopesticide activity against *An. gambiae*. Oil constituent of the plant extracts could also block oxygen supply to the developmental stages in the water or blockage of the spiracle which will later lead to suffocation and death (36-37).

In this study, when comparing the Lethal Concentration (LC) of *An. gambiae* larvae with its pupae, it was observed that the larvae stage was much susceptible to bioactivity of the three plant extracts used. This was due to the fact that the mosquito larvae feed actively, and by so doing, doses

of plant active components could be ingested, thereby leading to stomach poisoning and death. Saranya et al. (27), Panneerselvam et al. (28), Valentina et al. (38), Ileke and Olabimi (39), Ileke and Adesina (40) also reported the similar observation in their study.

5. Conclusion

The three plant extracts used in this investigation, especially male *C. papaya*, was highly effective against *An. gambiae*. The plants have proved to provide an alternative approach to the control of mosquito. They are natural, safe, and a stable biopesticide to synthetic pesticide. An in depth study on the isolation and purification of active ingredient, mode of action, as well as the field application of this base plant pesticide is needed.

Conflicts of Interest

The authors declare no competing interests.

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