



Ileke Kayode David and Adesina Femi Peter*

Department of Biology, School of Science, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

Received: 19 November, 2018

Accepted: 27 November, 2018

Published: 28 November, 2018

***Corresponding author:** Adesina Femi Peter, Department of Biology Federal University of Technology Akure, Ondo State, Nigeria, Tel: +2347033028164; E-mail: femi.adesina@outlook.com

Keywords: Clerodendrum; Anopheles gambiae; Plant extract; Larvae; Pupae

<https://www.peertechz.com>



Research Article

Bioefficacy of Larvicidal and Pupicidal properties of *Clerodendrum capitatum* and *Bridelia machrantha* leaves extracts against Malaria Vector, *Anopheles gambiae* giles [Diptera: Culicidae]

Abstract

Studies were conducted under ambient condition to assess the bio-efficacy of *Clerodendrum capitatum* and *Bridelia machrantha* leaves against developmental stages (larva and pupa) of malaria vector, *Anopheles gambiae*. One hundred millimeter (100mm) of aqueous solutions of the various plant extracts at various concentrations of 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L were each put in a labelled transparent bowl. Twenty (20) 3rd/4th larval instar were introduced separately into the *C. capitatum* and *B. machrantha* extracts along with a set of control containing rain water without any test solution and all tested concentrations were replicated four times. Similarly, Twenty- two days old pupae of *Anopheles* mosquito were introduced separately into the various plant extracts. The number of dead larvae and pupae were counted and recorded accordingly after 24 hours of treatment. The results showed that Larvae and pupae mortality of *An. gambiae* occurred in a dosage-dependent manner. There was no significant differences ($p > 0.05$) in toxicity level of the two plant extracts on *An. gambiae* larvae at concentrations 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L. *Clerodendrum capitatum* at concentrations 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L caused 30%, 47.5%, 60%, 90% and 100% mortality of *An. gambiae* larvae after 24 hours of exposure, respectively. *Bridelia machrantha* extract caused 22.5%, 35%, 50%, 77.5% and 92.5% mortality of *An. gambiae* larvae after 24 hours of exposure at rates 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L, respectively. Similar trend of results were recorded on the pupae. *C. capitatum* had lower LC₅₀ and LC₉₀ recorded when compared to *B. machrantha* that had the higher value on both larvae and pupae. The use of natural insecticide in the implementation of national policy for the control of malaria vectors should be promoted as they are easily degradable, medicinal and eco-friendly.

Introduction

One of the insects that are of medical importance is mosquito, it is more predominant in tropical countries, thereby putting up to 3.2 billion people at risk of malaria [1]. It abhors parasite (*Plasmodium* spp.) that can cause diseases like malaria, filariasis, yellow fever and denque on human [2] and it has resulted to 44,500 deaths out of 216 million malaria cases reported in the world [1]. This disease is transmitted only by the Anopheline mosquitoes from the order Diptera. Out of 400 species of *Anopheles* described, 40 species are important vectors of human malaria worldwide [3]. Many species has been identified to transmit malaria in Nigeria and *Anopheles gambiae* was reported as a primary vector of malaria among other species in the southern Nigeria [4-10].

Mosquito net, repellent cream and paint, insecticide and mosquito coil among others are well known as mosquito control methods in Nigeria. Over the decades, synthetic insecticide has been the most used because of its effectiveness. Nevertheless, its negative effects on human health, non target organisms (including aquatic animals) and environment are a major concern. It has also make mosquito population develop resistance against its application [5,9-13].

Searching for new compounds that are highly effective against mosquito and at the same time that are eco-friendly, degradable, not toxic to humans and non target organisms, and inexpensive has being a current trend in research. Several researchers have suggested aromatic plants and their essential oils as natural insecticides for pest control because they have few harmful effects on ecosystem structure and function [14].

Eggs, larvae, pupae and adults of different species of mosquito had been subjected to different plant extracts and volatile oils to assess the insecticide ability of botanicals [8,10,15–18]. Therefore, the present study investigates the toxicity of *Clerodendrum capitatum* and *Bridelia machranth* extract on the larvae and pupae of *Anopheles gambiae*.

Materials and Methods

Collection and rearing of larva and pupa mosquito

Mosquito baits, consisting of shallow containers with a large surface area were established under a partial shade in an open field. The clean transparent white bucket was filled with rain water to mimic mosquito natural breeding environment and to attract adult female for oviposition. Ten grams (10g) of yeast (Bakers' yeast) were sprinkled on the surface of the water and allowed to decompose slowly to nourish the developing larvae. Wild mosquitoes were allowed to freely visit the baits and to lay eggs. The water was monitored for 3–5 days for the development of the egg and first instar larva. These larvae were taken into the laboratory. In the laboratory, the larvae were identified into species level using the morphological keys [19]. The *Anopheles* larvae were separated from the mixed culture and transferred into another plastic container containing rain water. Some of the larvae were used for the larvicidal tests. The *Anopheles* larvae were further nurtured to pupae for 3–5 days for pupicidal tests.

Collection of plant materials

The plants evaluated in this work were *Clerodendrum capitatum* and *Bridelia machrantha* leaves. The fully developed leaves of *C. capitatum* and *B. machrantha*, free of insecticides were obtained in fresh form a farm at Ibule Soro, Akure, Ondo State, Nigeria. They were taken to the Crop, Soil and Pest management Department, Federal University of Technology, Akure, Ondo State for authentication by Plant Taxonomist.

Extraction of plant materials

The leaves of *C. capitatum* and *B. machrantha* were washed with distilled water, shade dried, cut into small pieces and air dried for 14 days in the laboratory. The seeds of *M. myristica* were also air dried for 21 days before pulverized into fine powders using an industrial electric pulverizing machine at the Department of Animal Production and Health Laboratory of the Federal University of Technology Akure. The powders were further sieved to pass through 1mm² perforations and kept in air-tight plastic containers for storage before use at ambient temperature (28 ± 2) °C.

About 400g of *C. capitatum* and *B. machrantha* powders were soaked separately in an extraction bottle containing 500ml of absolute n-hexane for 3 days. The mixture was stirred occasionally with a glass rod and extraction was terminated after 3 days. Filtration was carried out using a double layer of Whatman No. 1 filter papers and solvent evaporated using a rotary evaporator at 30 to 40°C with rotary speed of 3 to 6 rpm for 8 hours [20]. The resulting extracts were air dried in order to remove traces of solvent. The extracts were kept in labeled plastic bottles till when needed.

Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 4g of the crude extracts in 1Litre of water. From these stock solutions, different concentrations of 25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L were prepared and these aqueous solutions were used for the various experiments.

Bioassay

Toxicity of Extracts on Larvae of Mosquito: One hundred millimeter (100mm) of aqueous solutions of the various plant extracts at various concentrations of 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L were each put in a labelled transparent bowl. Twenty (20) 3rd/4th larval instar obtained from the culture were introduced separately into the various plant extracts along with a set of control containing rain water without any test solution and all tested concentrations were replicated four times. The number of dead larvae were counted and recorded accordingly after 24 hours of treatment.

Dead larvae were those incapable of rising to the surface or without the characteristic diving reaction when the water was disturbed [21].

$$\% \text{Larval Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times \frac{100}{1}$$

The LC₅₀ and LC₉₀ were carried out by Probit analysis.

Toxicity of extracts on pupae of mosquito

Also, similar experiment as described above was carried out for pupae of *Anopheles gambiae*. One hundred millimeter (100mm) of aqueous solutions of the various plant extracts at various concentrations of 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L were each put in a labelled transparent bowl. Twenty (20) two (2) days old pupae of *Anopheles* mosquito were introduced separately into the various plant extracts. They were replicated four times and rain water was used as control. The number of dead pupae were counted and recorded accordingly after 24 hours of treatment.

$$\% \text{Pupal Mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times \frac{100}{1}$$

Phytochemical Screening of *C. Capitatum* and *B. Machrantha* Leaves

Chemical tests were carried out on the aqueous and methanol extracts of *C. capitatum* and *B. machrantha* leaves for the qualitative determination of phytochemical constituents using standard procedures as described by Harborne [22]. Trease and Evans [23], Sofowora [24].

Statistical analysis of 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 done to 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.

Results

Contact toxicity of *C. capitatum* and *B. machrantha* Extracts on *An. gambiae* Larvae

Effects of *C. capitatum* and *B. machrantha* extracts on percentage mortality of *An. gambiae* larvae is presented in table 1. Larvae mortality of *An. gambiae* occurred in a dosage-dependent manner. There was no significant differences ($p > 0.05$) in toxicity level of the two plant extracts on *An. gambiae* larvae at concentrations 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L. *Clerodendrum capitatum* at concentrations 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L caused 30%, 47.5%, 60%, 90% and 100% mortality of *An. gambiae* larvae after 24 hours of exposure, respectively. *Bridelia machrantha* extract caused 22.5%, 35%, 50%, 77.5% and 92.5% mortality of *An. gambiae* larvae after 24 hours of exposure at rates 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L, respectively. Generally, *An. gambiae* larvae mortality increased with increase in hours of exposure.

Contact toxicity of *C. capitatum* and *B. machrantha* Extracts on *An. gambiae* Pupae

Table 2 shows the effects of contact toxicity of *C. capitatum* and *B. machrantha* extracts on percentage mortality of *An. gambiae* pupae after 24 hours of exposure. Mortality of *An. gambiae* pupae occurred in a dosage-dependent manner. There was no significant differences ($p > 0.05$) in toxicity level of the *C. capitatum* and *B. machrantha* extracts on *An. gambiae* pupae at concentrations tested (62.5mg/L, 125mg/L, 250mg/L, 500mg/L and 1000mg/L). *Clerodendrum capitatum* caused 30%, 35%, 57.5%, 72.5% and 97.5% mortality of *An. gambiae* larvae after 24 hours of exposure at concentrations 62.5mg/L, 125mg/L, 250mg/L, 500mg/L and 1000mg/L respectively. *Bridelia machrantha* extract at concentrations 62.5mg/L, 125mg/L, 250mg/L, 500mg/L and 1000mg/L caused 15%, 30%, 47.5%, 65% and 90% mortality of *An. gambiae* larvae after 24 hours of exposure, respectively. Generally, *An. gambiae* pupae mortality increased with increase in hours of exposure.

LC₅₀ and LC₉₀ of *C. capitatum* and *B. machrantha* Extracts on *An. gambiae* larvae and pupae

LC₅₀ and LC₉₀ of *C. capitatum* and *B. machrantha* Extracts on larvae and pupae of *An. gambiae* is shown on table 3. *C. capitatum* had lower LC₅₀ and LC₉₀ recorded when compared to *B. machrantha* that had the higher value on both larvae and pupae. LC₅₀ and LC₉₀ of *C. capitatum* on larvae were 50.14 mg/L and 172.30 mg/L respectively while that of *B. machrantha* was 69.27 mg/L and 268.94 mg/L respectively. On pupae, LC₅₀ and LC₉₀ of *C. capitatum* was 190.87 mg/L and 854.02 mg/L respectively while *B. machrantha* had 253.76 mg/L and 1305.47 mg/L respectively.

Phytochemicals screening of *C. capitatum* and *B. machrantha* Leaves

Table 4 presented the result of the phytochemical screening of the methanol and aqueous extracts of *C. capitatum* and *B. machrantha* leaves.

machrantha leaves. The phytochemicals present in then-hexane, and aqueous extracts of *C. capitatum* and *B. machrantha* were identical. Alkaloids, saponins, tannins, phlobatannins, anthraquinones, flavonoids and cardiac glycosides were detected in both aqueous and n-hexane extracts of *C. capitatum* and *B. machrantha* leaves.

Discussion

This study revealed that botanicals extract can be alternatives to the use of synthetic insecticide in the control

Table 1: Effect of *C. capitatum* and *B. machrantha* Extracts on % mortality of Larvae *An. gambiae* after 24 hours of exposure.

Plant Extracts	Concentration (mg/L)				
	25	50	100	150	200
<i>C. capitatum</i>	30.00±2.22 ^b	47.50±2.50 ^b	60.00±2.22 ^b	90.00±2.22 ^b	100.00±0.0 ^b
<i>B. machrantha</i>	22.50±2.50 ^b	35.00±2.89 ^b	50.00±2.22 ^b	77.50±2.50 ^b	92.50±2.50 ^b
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

Mean ± Standard error followed by the same letters, superscript at the end of each value, down the column are not significantly different ($p > 0.05$) from one another using New Duncan's Multiple Range Test.

Table 2: Effect of *C. capitatum* and *B. machrantha* Extracts on % mortality of Pupae *An. gambiae* after 24 hours of exposure.

Plant Extracts	Concentration (mg/L)				
	62.5	125.0	250.0	500.0	1000.0
<i>C. capitatum</i>	20.00 ± 2.22 ^c	35.00 ± 2.25 ^c	57.50 ± 2.50 ^c	72.50 ± 2.50 ^b	97.50 ± 2.50 ^c
<i>B. machrantha</i>	15.00 ± 1.29 ^b	30.00 ± 2.22 ^b	47.50 ± 2.50 ^b	65.00 ± 2.25 ^b	90.00 ± 2.25 ^b
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

Mean ± Standard error followed by the same letters, superscript at the end of each value, down the column are not significantly different ($p > 0.05$) from one another using New Duncan's Multiple Range Test.

Table 3: LC₅₀ and LC₉₀ of *C. capitatum* and *B. machrantha* Extracts on *An. gambiae* larvae and pupae.

Plant Extracts	Plant Extract	Concentration (mg/L)	
		LC ₅₀ (Lower – Upper Limit)	LC ₉₀ (Lower – Upper Limit)
Larvae	<i>C. capitatum</i>	50.14 (6.86 – 95.33)	172.30 (9.74 – 334.86)
	<i>B. machrantha</i>	69.27 (31.86 – 119.87)	268.94 (144.69 – 393.13)
Pupae	<i>C. capitatum</i>	190.87 (120.81 – 283.49)	854.02 (505.09 – 1202.96)
	<i>B. machrantha</i>	253.76 (216.87 – 297.2 9)	1305.47 (983.16 – 1919.19)

Table 4: Phytochemicals in different extracts of *C. capitatum* and *B. machrantha* Leaves.

Phytochemicals	n-hexane extract of <i>C. capitatum</i>	Aqueous extract of <i>C. capitatum</i>	n-hexane extract of <i>B. machrantha</i>	Aqueous extract of <i>B. machrantha</i>
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phlobatannins	-	-	-	-
Anthraquinones	-	-	-	-
Flavonoids	+	+	+	+
Cardiac glycosides	+	+	+	+

Key: -Absent ; + Present.

of insect vector. The two plants assessed had proven high insecticide ability against mosquito and this was in agreement with Ileke *et al.* [6], who reported the insecticidal potential of botanicals like *Anacardium occidentale*, *Aframomum melegueta*, *Garcinia kola* and *Citrus sinensis* plants against malaria vector, mosquito, *Anopheles gambiae*. Akinkulere *et al.* [15] found plant oils to be effective against mosquito larvae and pupae.

The high toxicity of these plants on mosquito larvae and pupae may be attributed to the active compounds present in these plants. The preliminary phytochemical analysis of extracts from the plants leaves was found to have presence of alkaloids, flavonoids, cardiac glycosides, tannins, anthraquinones and saponins. Flavonoid was present in the two plants tested which may be responsible for its high larvicidal and pupicidal potentials [5-7]. Also, the swimming ability of the mosquito's larvae and pupae could have been affected, thereby hinder their swimming to the surface for oxygen [5,25].

Nevertheless, *C. capitatum* caused high mortality than *B. machrantha* leaves on *Anopheles* mosquito larvae and pupae. This result is in agreement with the findings of Morya *et al.*, [26] who tested the powder leaf of some plants including *Clerodendrum* against *Corcyra cephalonica* and among the plants, *Clerodendrum* sp was reported to have the highest biopesticide ability. Adesina *et al.* [27] on Bio-efficacy of *Clerodendrum capitatum* against *Dermestes maculatus* larvae infestation on smoked catfish *Claria gariepinus*, found that the plant powder cause significantly ($p < 0.05$) larval mortality compared to the untreated fish at 24 h, 48 h and 72 h post infestation. *Clerodendrum* genus had being widely study for its bioactivities toward medicinal benefits as well as its insecticidal ability [28].

It took more concentration for both plants to achieved higher mortality on pupae than on larvae. This means that larvae is more susceptible to these plants than pupae and this could be attributed to the active feeding stage of the larvae since pupae stages of the insect are not feeding [5,29,30]. Related observations were reported by Akinkulere *et al.* [15], who obtained a 75.35% and 47% mortality of *Anopheles gambiae* larvae and pupae respectively. Also supported by Ileke and Ogungbite [5], who worked on the effectiveness of *Altonia boonei* on malaria vector. The strong choky odour of the plants may have also disrupted respiratory activity of the insect [31], blocking the spiracles thereby hinder the larvae breathe [32]. This could result into asphyxiation and death of the larvae [5,33,34] (Akinkulere *et al.*, 2006, Ileke and Oni, 2011; Ileke and Ogungbite, 2015).

Conclusion

Ultimately, due to the efficiency of *C. capitatum* and *B. machrantha* leaves extracts against *Anopheles* mosquitoes in the study, natural insecticide proved to be an alternative approach to the control of mosquito and to the use of synthetic insecticides whose cost, phenomenon of resistance developed in mosquitoes treated and proven action on non-target organisms are challenges in malaria controls.

References

- (2017) World malaria report. Geneva: World Health Organization (WHO). [Link: https://goo.gl/sGZUvK](https://goo.gl/sGZUvK)
- (2016) WHO - World malaria report. WHO Press, Geneva, Switzerland. [Link: https://goo.gl/RxaV4w](https://goo.gl/RxaV4w)
- Becker N, Petrić D, Boase C, Lane J, Zgomba M, et al. (2010) Mosquitoes And Their Control. Springer, Berlin. [Link: https://goo.gl/Lg8zcf](https://goo.gl/Lg8zcf)
- Adesina JM, Jose AR, Rajashekar Y, Afolabi LA (2015) Entomotoxicity of *Xylopia aethiopica* and *Aframomum melegueta* in suppressing oviposition and adult emergence of *Callasobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) infesting stored cowpea seeds. *Jordan Journal of Biological Sciences* 8: 263-268. [Link: https://goo.gl/q2BfF4](https://goo.gl/q2BfF4)
- Ileke KD, Ogungbite OC (2015) *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. *Journal of Coast Life Medicine* 3: 557-563. [Link: https://goo.gl/9dpGHe](https://goo.gl/9dpGHe)
- Ileke KD, Afolabi OJ, Ogungbite OC, Olagunju JO, Akanbi OM (2014) Mosquitocidal activity of *Anacardium occidentale*, *Aframomum melegueta*, *Garcinia kola* and *Citrus sinensis* against the developmental stages of mosquito, *Anopheles gambiae* Giles. *Journal of Mosquito Research* 4: 21-26. [Link: https://goo.gl/HHLRbb](https://goo.gl/HHLRbb)
- Ileke KD, Oyeniyi EA, Ogungbite CO, Adesina JM (2015) Nicotianatobacum: a prospective mosquitocide in the management of *Anopheles gambiae* (Giles). *International Journal of Mosquitoes Research* 2: 19-23. [Link: https://goo.gl/HfbSQ6](https://goo.gl/HfbSQ6)
- Ileke KD, Adesina JM, Okunola OG (2017) Larvicidal and Pupicidal Potential of *Aframomum melegueta* K. Schum Extracts Against Mosquito, *Anopheles* Species. *Entomol. Res Soc* 19: 121-127. [Link: https://goo.gl/RJckHw](https://goo.gl/RJckHw)
- Afolabi OJ, Simon-Oke I, Adepeju EO, Oniya MO (2018) Adulticidal and repellent activities of some botanical oils against malaria mosquito *Anopheles gambiae* (Diptera: Culicidae). *Beni-Suef University Journal of Basic and Applied Sciences* 7: 134-138. [Link: https://goo.gl/z7um5N](https://goo.gl/z7um5N)
- Awosolu O, Adesina F, Iweagu M (2018) Larvicidal effects of croton (*Codiaeum variegatum*) and Neem (*Azadirachta indica*) aqueous extract against *Culex* mosquito. *International Journal of Mosquito Research* 5: 15-18. [Link: https://goo.gl/gNn5PP](https://goo.gl/gNn5PP)
- Georghiou GP (2012) Pest Resistance to Pesticides. Springer Science and Business Media, Berlin. [Link: https://goo.gl/Nyr6Nc](https://goo.gl/Nyr6Nc)
- Edwards C (2013) Environmental Pollution by Pesticides. Vol. 3. Springer Science and Business Media, Berlin.
- Shayeghi M, Vatandoost H, Gorouhi A, Sanei-Dehkordi AR, Salim-Abadi Y, et al. (2014) Biodiversity of aquatic insects of ZayandehRoud River and its branches, Isfahan Province, Iranian *Journal of Arthropod Borne Disease* 8: 197-203. [Link: https://goo.gl/gMaF4s](https://goo.gl/gMaF4s)
- Isman MB (2000) Plant essential oils for pest and disease management. *Crop Protection* 19: 603-608. [Link: https://goo.gl/1Fb3QH](https://goo.gl/1Fb3QH)
- Akinkulere RO, Adedire CO, Odeyemi OO, Raji J, Owoeye JA (2011) Bioefficacy of Extracts of some Indigenous Nigerian Plants on the developmental stages of mosquito (*Anopheles gambiae*). *Jordan Journal of Biological Science* 4: 237-242. [Link: https://goo.gl/h56EpE](https://goo.gl/h56EpE)
- Arivoli S, Ravindran KJ, Tennyson S (2012) Larvicidal Efficacy of Plant Extracts against the Malarial Vector *Anopheles stephensi* Liston (Diptera: Culicidae). *World Journal of Medical Sciences* 7: 77-80. [Link: https://goo.gl/F3akMR](https://goo.gl/F3akMR)
- Kehail MAA, Bashir NHH, Abdelrahman EE, Abdelrahim AM (2017) Larvicidal activity of three plants powders and aqueous extracts on *Anopheles* and *Culex* mosquito larvae (Diptera: Culicidae). *International Journal of Mosquito Research* 4:37-41. [Link: https://goo.gl/RRDEYr](https://goo.gl/RRDEYr)

18. Sanei-Dehkordi A, Gholami S, Abai MR, Sedaghat MM (2018) Essential Oil Composition and Larvicidal Evaluation of *Platyclus orientalis* against Two Mosquito Vectors, *Anopheles stephensi* and *Culex pipiens*, *Journal of Arthropod-Borne Diseases* 12: 101-107. [Link: https://goo.gl/jWTM2Z](https://goo.gl/jWTM2Z)
19. Gillies MT, De Meillon B (1968) The anophelinae of Africa South of the Sahara. *South African Institute for Medicinal Research* 54: 1-343. [Link: https://goo.gl/ScQhwY](https://goo.gl/ScQhwY)
20. Udo IO (2011) Potentials of *Zanthoxylum xanthoxyloides* (LAM.) for the control of stored product insect pests. *Journal Stored Products and Postharvest Research* 2: 40-44. [Link: https://goo.gl/11zmqn](https://goo.gl/11zmqn)
21. (2005) World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP/2005.13. [Link: https://goo.gl/QNj62U](https://goo.gl/QNj62U)
22. Harborne JB (1973) *Phytochemical methods*. Chapman and Hall, London. 113. [Link: https://goo.gl/3teYqB](https://goo.gl/3teYqB)
23. Trease GE, Evans WC (1989) *Pharmacognosy: a physicians guide to herbal medicine*, 13th ed. Bailliere Tindall, London 176-180.
24. Sofowora A (1993) *Medicinal plants and traditional medicine in Africa*. Spectrum books Ltd. Ibadan, Nigerian 289-289. [Link: https://goo.gl/tcPxx4](https://goo.gl/tcPxx4)
25. Bhattacharya K, Chandra G (2014) Phagodeterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata* L. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus* (Diptera: Culicidae), *Asian. Pac. J. of Trop. Dis* 4: 226-232. [Link: https://goo.gl/aPDtTx](https://goo.gl/aPDtTx)
26. Morya K, Pillai S, Patel P (2010) Effect of powdered leaves of *Lantana camara*, *Clerodendrum inerme* and *Citrus lemon* on the rice moth. *Corcyra cephalonica*. *Bull Insectology* 63:183-189. [Link: https://goo.gl/4EdcrV](https://goo.gl/4EdcrV)
27. Adesina JM, Jose AR, Rajashekar Y (2016) Bio-efficacy of *Clerodendrum capitatum* (Willd) Schumacher. Thonn. (Lamiales: Verbenaceae) against *Dermestes maculatus* De Geer (1774) (Coleoptera: Dermestidae) larvae infestation on smoked catfish *Claria gariepinus* (Burchell, 1822) (Siluriformes: Clariidae). *Brazilian Journal of Biological Sciences* 3: 37-44. [Link: https://goo.gl/ZJVqH9](https://goo.gl/ZJVqH9)
28. Shakeel AK, Sammia S, Waqar A, Sami U (2017) Pharmacological Importance of *Clerodendrum* Genus: A Current Review. *International Journal of Pharmaceutical Science and Research* 2: 22-30. [Link: https://goo.gl/ARFK15](https://goo.gl/ARFK15)
29. El – Bokl MM (2003) Latent toxicity of azadirachtin treatment on *C. pipiens* (Diptera: Culicidae). *J. Egypt. Acad. Soc. Environ. Develop* 3: 63-74.
30. Nathan SS, Kalaivani K, Murugan k (2005) Effects of neem limonoids on the malaria vector *An. stephensi* Liston (Diptera: Culicidae). *Acta Trop.* 96: 47-55. [Link: https://goo.gl/p9voA2](https://goo.gl/p9voA2)
31. Ileke KD, Odeyemi OO, Ashamo MO (2013) Response of Cowpea Bruchid, *Callosobruchus aculatus* (Fab r.) [Coleoptera: Chrysomelidae] to Cheese Wood, *Alstonia boonei* De Wild Stem Bark oil extracted with different solvents. *Archives Phytopathol. Plant Protect* 46: 1357-1370. [Link: https://goo.gl/t8ZNMk](https://goo.gl/t8ZNMk)
32. Kaufmann C, Briegel H (2004) Flight performance of the malaria vectors *Anopheles gambiae* and *Anopheles atroparvus*. *Journal of Vector Ecology* 29:140-53. [Link: https://goo.gl/6VKP0r](https://goo.gl/6VKP0r)
33. Akinkulore RO, Adedire CO, Odeyemi OO (2006) Laboratory evaluation of the toxic properties of forest *Anchomanes*, *Anchomanes difformis* against pulse beetles *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Insect Sci* 13: 25-29. [Link: https://goo.gl/se2SXR](https://goo.gl/se2SXR)
34. Ileke KD, Oni MO (2011) Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored wheat grains, *African Journal of Agricultural Research* 6: 3043-3048. [Link: https://goo.gl/27kx9S](https://goo.gl/27kx9S)