International Journal of Tropical Diseases

ORIGINAL RESEARCH ARTICLE

Insecticidal Activities of *Chromolaena odorata* and *Vernonia amygdalina* leaf extracts against *Anopheles gambiae* [Diptera: Culicidae]

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Abstract

Female *Anopheles* mosquitoes are the vectors of human malaria. The use of chemical insecticides for vector control has hampered with environmental pollution and insect. This suggests the need for the development of more potent and environment-friendly insecticides for effective control of malaria.This research investigated the larvicidal, pupicidal and adulticidal activities of *Chromolaena odorata* and *Vernonia amygdalina* leaf extracts against, *An. gambiae* in the laboratory at ambient temperature of 28 ± 2 °C and 75 ± 5% relative humidity. Different concentrations of 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L were prepared and these aqueous solutions were used for the experiments. Larval, pupal and adult mortality of *An. gambiae* were tested after 24 hours of exposure. Results showed that there were significant differences ($P < 0.05$) in toxicity level of the two plant extracts on *An. gambiae* larvae, pupae and adults. *Vernonia amygdalina* extract was the most toxic to *An. gambiae* larvae at all tested concentrations of 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L causing 47.5%, 82.5%, 100%, 100% and 100% mortality after 24 hours of treatment, respectively. *Chromolaena odorata* extract caused 32.5%, 60%, 82.5%, 92.5% and 100% mortality of *An. gambiae* larvae after 24 hours of treatment at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L, respectively. *Vernonia amygdalina* extract was the most lethal to *An. gambiae* pupae and adults which caused 55% mortality of adult *An. gambiae* at concentration 160 ml/L. The concentration of *C. odorata* and *V. amygdalina* leaves extracts required to evoke 50% death of *An. gambiae* adult were 296.20 mg/L and 147.98 mg/L respectively. The LC_{90} of *C. odorata* extract was 3107.55 mg/L while *V. amygdalina* extract was 2221.05 mg/L for mosquito adults. The plant extracts were not as effective against adults compared to larva and pupa of *An. gambiae*. This study showed that *C. odorata* and *V. amygdalina* were toxic to malaria vector with *V. amygdalina* being more potent. This suggest that *V. amygdalina* extracts could serve as an alternative method to synthetic chemical control of malaria vectors.

Keywords

Insecticidal activities, *Anopheles*, *Chromolaena odorata*, *Vernonia amygdalina*, Malaria

Introduction

Several diseases are transmitted by arthropod vectors [1] of which mosquito is one of them. Mosquitoes are responsible for the transmission of diseases such as malaria, lymphatic filariasis, and dengue fever among others [2] most especially in the tropical regions of the world. Out of these diseases transmitted by mosquitoes, malaria is the most devastating in terms of the number of incidence, prevalence, morbidity and mortality [2]. It is transmitted by the mosquitoes of the genus *Anopheles* and it is caused by the protozoa of the *Plasmodium* genus [3]. Examples of *Anopheles* species include *Anopheles gambiae, Anopheles culicifacies, Anopheles stephensi, Anopheles fluviatilis, Anopheles minimus, Anopheles dirus* and *Anopheles sundaicus* among others with *Anopheles gambiae* being the prominent one among them all [4,5]. Five species of *Plasmodium* namely *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium* ovale and *Plasmodium*

Citation: Ileke KD, Olabimi IO (2019) Insecticidal Activities of *Chromolaena odorata* and *Vernonia amygdalina* leaf extracts against *Anopheles gambiae* [Diptera: Culicidae]. Int J Trop Dis 2:018

Accepted: March 16, 2019; **Published:** March 18, 2019

knowlesi are responsible for human malaria [6] but *P. falciparum and P. vivax* are most notorious in terms of the number of cases they are responsible for [3].

They were an estimated 219 million cases of malaria with 435,000 of them resulting in death in 2017 [3]. In order to stop or reduce the transmission of malaria parasites, there is need to control the vector population. Several methods such as habitat modification [7], the use of synthetic chemical insecticides [4] and the use of biological means such as employing natural predators, parasites and parasitoids to control the populations of mosquitoes [8,9]. The major method that have been employed in the control of mosquitoes is the use of synthethic insecticides such as chlorodane, aldrin, Dieldrin, dichlorodiphenyl trichoroethane (DDT) among others [4].

However, these chemical have been shown to have negative effect in the dynamics of the earth's ecosystem resulting in resistance of mosquito species to the chemicals, environmental pollution and toxicity to human and other non-targeted organisms [10]. Hence, there have been clamor by various quarters to develop a safe means to combat the proliferation of insect vector species [11]. This has led to the focus on developing insecticides from botanical sources that are safe to use in the environment due to the fact that they are easily degradable and less toxic to humans and non-targeted organisms [12,13].

Several plant materials such as *Alstonia boonei, Curuma longa, Cymbopogon winterianus, Ocimum americanum, Nepeta cataria, Viola odorata, Melaleuca quinquenervia, Azadirachta indica, Citrus medica, Nicotiana tabacum, Anacardium occidentale*, *Aframomum melegueta*, *Garcinia kola*, *Citrus sinensis* and *Murraya koenigii* have been used to control mosquitoes [14-22].

Chromolaena odorata is a fast-growing perennial herb which belongs to the family Asteraceae [23]. The plants are known for its medicinal value in many areas as well as pests control purposes [23]. *Chromolaena odorata* contains carcinogenic pyrrolizidine alkaloids [24]. It is not habitat specific and nature of soil, it grows generally in the wild (abandoned land) [25]. The plantis acknowledged for its high proliferation and one of the world worst tropical weeds [26].

Vernonia amygdalina belong to the family Asteraceae and grows in the tropical regions of Africa [23]. The plant is a shrub, evergreen in colour and can grow between 2-5 m in height with petiolate leaf of about 6 mm in diameter [27]. The plant is bitter due to the characteristic odour and bitter taste of the leaves owing to the presence of anti-nutritional factors such as alkaloids, saponins, tannins and glycosides [28,29]. The aim of this research study isto investigate the anti-mosquito activities of *C. odorata* and *V. amygdalina* leaf extracts on the larvae, pupae and adults of malaria vector, *An. gambiae*.

Materials and Methods

Place and duration of study

The research was conducted at the Storage Entomology Postgraduate Research Laboratory, Department of Biology, Federal University of Technology, Akure, Nigeria between August and November, 2018.

Collection and rearing of larva, pupa and adult mosquito

Mosquito bait was established under a partial shade in an open field by filling a white bucket with rain water. Yeast was sprinkled on the surface of the water to serves as source of foods for the nourishment of larvae. Wild mosquitoes were allowed to freely visit the baits and to lay eggs. This was monitored for 4-6 days for the development of eggs and first instar larvae. Subsequently, the larvae were harvested and taken into the laboratory for identification into species levels [30]. *Anopheles gambiae* larvae were transferred into another plastic container containing rain water. Some of the larvae were used for the larvicidal tests while the remaining larvae nurtured to pupae for 4-6 days for pupicidal tests. Adult mosquito that emerged from pupae cultured were fed with sucrose solution [31].

Collection of plant materials

Chromolaena odorata and *V. amygdalina* leaves that have not be sprayed with chemical insecticides (based on the information obtained from the farmer) were obtained in fresh form a farm at Ogbese, Ondo State, Nigeria. They were taken to the Biology Department, Federal University of Technology, Akure, Ondo State for authentication.

Extraction of plant materials

Chromolaena odorata and *V. amygdalina* leaves were washed separately with distilled water, shade dried, cut into small pieces and air dried for 14 days in the laboratory before pulverized into fine powders using an industrial electric pulverizing machine at the Department of Animal Production and Health Laboratory, Federal University of Technology, Akure. The powders were further sieved to pass through mm^2 perforations and kept in an air-tight plastic containers for storage before use at ambient temperature (28 ± 2) °C.

About 300 g of *C. odorata* and *V. amygdalina* powders were soaked separately in an extraction bottle containing 500 ml of absolute methanol for 72 hours. The mixture was stirred occasionally with a glass rod and extraction was terminated after three 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 three to six rpm for eight hours [32]. The resulting extracts were air dried in order to remove traces of methanol. The extracts were kept in labeled plastic bottles till when needed.

Standard stock solutions were prepared by dissolving 3 g of the crude extracts in 1 Litre of water. From these stock solutions, different concentrations of 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L were prepared and these aqueous solutions were used for the various experiments.

Anti-larval activity of *C. odorata* and *V. amygdalina* extracts

Twenty (20) 3rd/4th larval instar of *An. gambiae* were introduced into *C. odorata* and *V. amygdalina* extracts separately at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L with a set of control containing rain water. All tested concentrations were replicated four times. The number of dead larvae were counted and recorded accordingly after 24 hours of exposure. Dead larvae were those unable of rising to the surface or without the characteristic diving reaction when the water was disturbed [33].

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

Percentage larvae mortality was corrected using Abbott [25] formula thus:

$$P_T = \frac{P_o - P_c}{100 - P_o} \text{ x } \frac{100}{1}$$

Where P_T = corrected mortality (%)

P_O = observed mortality (%)

P_C = control mortality (%)

Anti-pupal activity of *C. odorata* and *V. amygdalina* extracts

Twenty-two days old pupae of *An. gambiae* were introduced into *C. odorata* and *V. amygdalina* extracts separately at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L with a set of controls containing rain water. All tested concentrations were replicated four times. The number of dead pupae were counted and recorded accordingly after 24 hours of treatment. Percentage pupal mortality were calculated using standard method and % pupae mortality was corrected using Abbott formula [34].

Anti-adult fumigant efficacy of *C. odorata* and *V. amygdalina* extracts

Twenty adults were introduced into a test-tube covered with cotton wool [9]. Strips of Whatman's No.1 filter papers soaked with 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L of *C. odorata* and *V. amygdalina* extracts were suspended in the test tube separately in four replicates. Mortality of adult insect was evaluated after 24 hours of treatment using standard method. Percentage adult mortality was corrected using Abbott formula as illustrated above.

Statistical analysis of data

Data were subjected to analysis of variance and means were separated using the new Duncan's multiple range test. The log-Probit model analysis was carried out on larvicidal, pupicidal and adulticidal bioassay results to assess the 50% lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}).

Result

Anti-larval activity of *C. odorata* and *V. amygdalina* extracts on *An. gambiae* larvae

There was a significant difference ($P < 0.05$) in toxicity level of *C. odorata* and *V. amygdalina* extracts on *An. gambiae* larvae at all tested concentrations (Table 1). *Vernonia amygdalina* extract was the most toxic to *An. gambiae* at all tested concentrations of 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L which evoked 47.5%, 82.5%, 100%, 100% and 100% mortality after 24 hours of treatment, respectively. *Chromolaena odorata* extract caused 32.5%, 60%, 82.5%, 92.5% and 100% mortality of *An. gambiae* larvae after 24 hours of treatment at rates 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L, respectively.

Anti-pupal activity of *C. odorata* and *V. amygdalina* extracts on *An. gambiae* pupae

Anti-pupal activity of *C. odorata* and *V. amygdalina* extracts on percentage mortality of *An. gambiae* larvae is presented in Table 2. Generally, pupae mortality occurred in a dosage-dependent manner. There

Table 1: Toxicity of *C. odorata* and *V. amygdalina* Leaves Extracts on % mortality of *An. gambiae* Larvae after 24 hours of exposure.

Plant Extracts	Concentration (mg/L)				
	20	40	80	120	160
C. odorata	32.50 ± 2.55^b	60.00 ± 2.04^b	82.50 ± 2.36^b	92.50 ± 3.55^b	100.00 ± 0.0^b
V. amygdalina	47.50 ± 2.36^c	82.50 ± 3.55^c	100.00 ± 3.36^c	100.00 ± 0.00^b	100.00 ± 0.00^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

All values are means of four replicates followed by ± standard error of the mean. Mean 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: Toxicity of *C. odorata* and *V. amygdalina* extracts on % mortality of *An. gambiae* pupae after 24 hours of exposure.

Plant Extracts	Concentration (mg/L)				
	20	40	80	120	160
C. odorata	20.00 ± 2.04[b]	37.50 ± 2.36[b]	50.00 ± 2.04[b]	77.50 ± 3.36[b]	100.00 ± 0.0[b]
V. amygdalina	32.50 ± 2.55[c]	50.00 ± 2.04[c]	77.50 ± 3.36[c]	100.00 ± 0.00[c]	100.00 ± 0.00[c]
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]

All values are means of four replicate followed by ± standard error of the mean. Mean 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: Fumigant toxicity of *C. odorata* and *V. amygdalina* on *An. gambiae* adults.

Extracts of plants	Concentration (mg/L)				
	20	40	80	120	160
C. odorata	7.50 ± 0.36[b]	15.00 ± 1.39[b]	20.00 ± 2.04[b]	30.00 ± 2.04[b]	40.00 ± 2.04[c]
V. amygdalina	17.50 ± 1.36[c]	27.50 ± 2.36[c]	37.50 ± 2.36[c]	42.50 ± 2.22[c]	55.00 ± 2.39[c]
Untreated	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]

Each value is a mean ± standard error of four 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 4: 50% and 90% Lethal Concentration of *C. odorata* and *V. amygdalina* Leaves Extracts on *An. gambiae* larvae, pupae and adults.

Mosquito	Plant Extracts	Concentration (mg/L)	
		LC_{50} (Lower - Upper Limit)	LC_{90} (Lower - Upper Limit)
Larvae	*C. odorata*	31.33 (26.81 - 35.69)	95.73 (82.33 - 115.99)
	V. amygdalina	21.42 (18.51 - 23.99)	44.56 (39.24 - 53.18)
Pupae	*C. odorata*	53.61 (12.03 - 118.45)	117.66 (91.21 - 144.11)
	V. amygdalina	33.93 (11.88 - 54.82)	92.29 (56.78 - 127.80)
Adults	*C. odorata*	296.20 (200.66 - 613.94)	3107.55 (1204.61 - 5010.49)
	V. amygdalina	147.98 (111.29 - 236.73)	2221.05 (912.69 - 3529.41)

was a significant difference ($P < 0.05$) in toxicity level of the two plant extracts on *An. gambiae* pupae at concentrations. *Vernonia amygdalina* extract was the most lethal to *An. gambiae* pupae which evoked 32.5%, 50%, 77.5%, 100% and 100% mortality after 24 hours of treatment at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L, respectively. *Chromolaena odorata* extract caused 20%, 37.5%, 50%, 77.5% and 100% mortality of *An. gambiae* larvae after 24 hours of treatment at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L, respectively. The higher the concentration, the more the mortality rate of pupae mosquito.

Anti-adult fumigant efficacy of *C. odorata* and *V. amygdalina* leaves extracts on *An. gambiae* adults

Fumigant toxicity of *C. odorata* and *V. amygdalina* extracts on percentage mortality of *An. gambiae* adults is presented in Table 3. Adult mortality occurred in a dosage-dependent manner. There was a significant difference ($P < 0.05$) in toxicity level of the two plant extracts on *An. gambiae* adults at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L. *Vernonia amygdalina* caused 17.5%, 27.5%, 37.5%, 42.5% and 55% mortality of adult *An. gambiae* after 24 hours of treatment at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L, respectively. *Chromolaena odorata* extract caused 7.5%, 15%, 20%, 30% and 40% mortality of *An. gambiae* larvae after 24 hours of treatment at concentrations 20 mg/L, 40 mg/L, 80 mg/L, 120 mg/L and 160 mg/L, respectively. The higher the concentration, the more the mortality rate of adult *An. gambiae*.

LC_{50} and LC_{90} values calculated for *C. odorata* and *V. amygdalina* leaves extracts on *An. gambiae* larvae, pupae and adults based on the log-probit regression analysis

The 50% lethal concentration of *C. odorata* extract was 31.33 mg/L while *V. amygdalina* extract was 21.42 mg/L for *An. gambiae* larvae. The concentration of *C. odorata* and *V. amygdalina* leaf extracts to cause 90% death of *An. gambiae* larvae were 95.73 mg/L and 44.56 mg/L respectively (Table 4). *Chromolaena odorata* extract required more concentration than *V. amygdalina* leaf extracts to cause 50% and 90% death of *An. gambiae* larvae. The concentration of *C. odorata* and *V. amygdalina* leaf extracts to cause 50% death of mosquito pupae were 53.61 mg/L and 33.93 mg/L

respectively. The concentration of *C. odorata* and *V. amygdalina* leaf extracts to cause 90% death of *An. gambiae* pupae were 117.66 mg/L and 92.29 mg/L respectively. *Vernonia amygdalina* extract required less concentration than *C. odorata* leaves extracts to cause 50% and 90% death of *An. gambiae* pupae. The concentration of *C. odorata* and *V. amygdalina* leaves extracts required to evoke 50% death of *An. gambiae* adult were 296.20 mg/L and 147.98 mg/L respectively. The LC_{90} of *C. odorata* extract was 3107.55 mg/L while *V. amygdalina* extract was 2221.05 mg/L for mosquito adults. The plant extracts were not as effective against adults compared to larva and pupa of *An. gambiae.*

Discussion

The use of plants as mosquitocides in lieu of synthetic chemical insecticides for the management of malaria vectors is gaining attention in developing nations such as Nigeria [17,20]. Utilization of *C. odorata* and *V. amygdalina* in the biocontrol of insect pest of cereals, legumes and vectors of malaria have been previously reported [35-42].

The result obtained in this study showed that *C. odorata* and *V. amygdalina* leaves extracts were effective in the control of larva, pupa and adult stages of *An. gambiae*. The larvicidal, pupicidal and adulticidal potentials of *C. odorata* and *V. amygdalina* extracts were concentration dependent. The higher the concentration, the higher the mortality rate of larva, pupa and adult mosquito. Larvae were more susceptible to *C. odorata* and *V. amygdalina* extracts than pupa and adults stages. This may be due to the swimming ability of the pupae, picking the lethal dose in the process of searching for food [18,21,43]. The extract also reduces dissolved oxygen which could have caused their death [18,43].

Vernonia amygdalina leaf extract was the most toxic to all stages of *An. Gambiae*in this study. The biocontrol of *V. amygdalina* against stored product pest have been reported [35-39,42]. Adedire and Lajide [35] reported 100% mortality of maize weevil, *Sitophilus zeamais* when treated with *V. amygdalina* Musa, et al. [36] also reported the efficacy of *V. amygdalina* in the management of cowpea beetle, *Callosobruchus maculatus*. Moses and Dorathy [37] reported that bitter leaf gave the best protection against cowpea weevil when compared with garlic and ginger. Akunne, et al. [39] reported on efficacy of mixed application of leaf powders of *V. amygdalina* and *Azadirachta indica* against adult *C. maculatus.* Ileke [42] reported on entomotoxicant potential of bitter leaf, *V. amygdalina* powder in the control of Cowpea bruchid, *C. maculatus* infesting stored cow peaseeds. The larvicidal and pupicidal bioefficacies of *V. amygdalina* extract on the malaria vector developmental stages could be linked to the presence of some chemical compounds like sesquiterpene lactones containing vernodalin, vernodalol and 11, 13-dihydrovernodalin which act as insect feeding deterrent [44].

Chromolaena odorata extract also effectively control larva and pupa stages of malaria vector. This agreed with many earlier researchers on the use of botanicals against suppression of vectors malaria [38,39]. Ahiati, et al. [38] reported on the insecticidal effects of *C. Odorata* Oil extracts on the Larvae and adults of mosquitoes (Family Culicidae). Jagruti, et al. [39] reported on the larvicidal activity of methanolic leaf extracts of plant, *C. odorata* L. (Asteraceae) against vector mosquito. *Chromolaena odorata* extract had been utilized against a household pests such as cockroaches. Udebuani, et al. [41] reported on the effectiveness of *C. odorata* against adult stage of American cockroach, *Periplaneta americana.* Insecticidal property of *C. odorata* could be linked to it chemical constituents such as tannin, saponin, flavonoid and alkaloids. The presence of these phytochemical alters some biochemical functions of organisms. Man [45] reported that increase mortality of *An. gambiae* rate which was reported in thin study could be attributed to phytochemical content of the leaf extract. Studies have shown that high dose of flavonoid alters the normal body functioning of insects [46,47]. Kelm and Nair [48] also reported the presence of flavonoid, tannin, saponin in leaf extract of *C. odorata*. Saponin are a class of steroidal or triterpenoid secondary plant metabolite with diverse biological properties, such as antifeeding [47,49] and growth inhibitory activities [47,50]. *Chromolaena odorata* and *V. amygdalina* leaves were less toxic to the adult mosquito compared with larvae and pupae of *An. gambiae*. The major limitation of this study is that we did not identify the active ingredients in the leaf extracts. However, we propose, in the future, to characterize active ingredients in the *C. odorata* and *V. amygdalina* leaf extracts with a view to developing a potent insecticide against all the life stages of *An. gambiae*.

Conclusion

Anopheles gambiae larvae were more susceptible to leaf extracts tested than their pupae and adults at all tested concentrations. In addition, *V. amygdalina* extracts required less concentration than *C. odorata* to cause 50% and 90% death of malaria vector, *An. gambiae* larvae, pupae and adults. The test plants can be incorporated into a control strategy or intervention programmes on the management of malaria vector, *An. gambiae.*

Acknowledgments

We appreciate Dr. Foluso Akindele Ologundudu, (Plant Physiologist/Taxonomist) of the Department of Biology, Federal University of Technology, Akure, Nigeria who assisted in the authentication of two plants assessed in this study.

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