International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2(4): 19-23

Received: 04-10-2015
Accepted: 05-11-2015

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Nicotiana tabacum a prospective mosquitocide in the management of *Anopheles gambiae* (Giles)

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Abstract
The mosquitocidal potential of leaf and seed extract of *Nicotiana tabacum* in the management of larvae, pupae and adults of *Anopheles gambiae* was assessed at five different concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) at ambient temperature (28±2 ^{0}C) and relative humidity (75±5%). Larvicidal and pupacidal activity of both extracts was assessed after 24 h while adulticidal activity was recorded at 3 h post-treatment. Results showed that both extracts of *N. tabacum* elicited 100% mortality in larvae, pupae and adults of *An. gambiae* at the highest experimental concentration. Regardless of the stage of *An. gambiae*, LC_{50} values revealed that leaf extract showed more toxicity than the seed extract. The LC_{50} values of leaf and seed extract of *N. tabacum* also increased with the developmental stages of the mosquitoes with the lowest and highest observed in larval (leaf: 0.153µg/ml; seed: 0.188µg/ml) and adult (leaf: 0.219 µg/ml; seed: 0.290 µg/ml) stage respectively. Median LC_{50} values were however observed in pupae of *An. gambiae* (leaf: 0.176 µg/ml; seed: 0.213µg/ml). Various results obtained from this study therefore showed that *N. tabacum* extract could serve as a means of controlling mosquitoes causing malaria disease in Nigeria.

Keywords: Mosquitocidal, *Anopheles gambiae*, *Nicotiana tabacum*, larvicidal, pupacidal, adulticidal, toxicity

1. Introduction
Mosquitoes have for years remained a causative agent of various diseases in both rural and urban settlements of the world. They are particularly of high prevalence in more than 100 countries, infecting over 700 million people every year globally [3, 21]. Presently, there are over three hundred species in the world grouped in 39 genera and 135 subgenera [22]. *Anopheles gambiae* is one of the most prevalent disease vectors, transmitting the pathogen causing malaria disease in an estimated 3.3 billion people in 97 countries with around 1.2 billion people at high risk [23, 31]. Malaria is known to be highly concentrated in most developing countries killing more people than any other killer disease. In fact, its high prevalence in most African countries has been attributed to poverty and underdevelopment [4, 16]. Nigeria being the most populated country in Africa is at high risk of malaria infection as the disease accounts for 25% of infant mortality and 30% of childhood mortality [8].
Over the years, researchers have shifted their attention towards providing an effective means of controlling the vector associated with the transmission of *Plasmodium* parasite causing malaria disease. Although, the use of synthetic insecticides remained the most effective mode of control; myriads of problems associated with their usage has posited the need for an alternative form of control which is cheap, richly available and eco-friendly. Consequently, the use of botanicals has gained prominence as an alternative to most synthetic insecticides in the control of mosquito species. Thus, the entomotoxic nature of several botanicals in the control of various developmental stages of several mosquito species have been extensively studied [18, 3, 15, 24]. However, due to high abundance of several insecticidal plant species in most African countries particularly Nigeria, there is need to search for more botanicals with mosquitocidal properties.
Nicotiana tabacum, also known as Tobacco is one of the annual herbs chiefly available in Nigeria and it is about 9 to 12cm tall [14, 15]. Although, several researchers have investigated the mosquitocidal activity of the leaves of *N. tabacum* against several mosquitoes species [29, 15, 21, 6]; the authors of this work are not aware of any previous work investigating the efficacy of both seeds and leaves of *N. tabacum* against various developmental stages of Females *An. gambiae* (Giles). Hence, this work aimed at exploring the potential of seed and leaf extracts of

N. tabacum as a future mosquitocide against various developmental stages of *An. gambiae* transmitting plasmodium parasite causing malaria infection.

2. Materials and methods

The experiment was carried out during November 2013 to April 2014 at Research Laboratory, Department of Animal and Environmental Biology, Adekunle Ajasin University Akungba-Akoko, Ondo State, Nigeria.

2.1. Collection of *N. tabacum*

The leaves and seeds of *N. tabacum* were collected from Ogbagi-Akoko, Ondo State, Nigeria. The plant was authenticated in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

2.2. Extraction of plant materials

The leaves and seeds of *N. tabacum* were air dried in the laboratory. When they were crisp dry, the materials were grounded into powders using an electric blender (Binatone Model BLG 400). The powders were further sieved to pass through 1mm^2 perforations before they were stored in separate plastic containers with tight lids and stored in a refrigerator at 4^{o}C prior to use. Acetone extracts of *N. tabacum* were prepared using cold extraction method. About 100g of each of the powder was soaked separately in an extraction bottle containing 300ml of acetone. The mixtures were stirred occasionally with a glass rod and extraction was terminated after 72 hours. Filtration was carried out using a double layer of Whatman No. 1 filter papers and acetone evaporated using a rotary evaporator at 30 to 40^{o}C with rotary speed of 3 to 6 rpm for 8 hours [11]. The resulting extract was air dried in order to remove traces of solvent. The crude extract (semi- solid paste) was kept in a dark bottle and preserved in the refrigerator till further use [2].

2.3. Collection and rearing of mosquito larvae and pupae

Mosquitoes baits consisting of shallow containers with a large surface area and which are opaque in colour were established in the Hatchery Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba Akoko, Ondo state, Nigeria. The container was filled with rain water so as to mimic natural breeding environment and also to attract adult mosquitoes for oviposition. An opaque container was preferred because it encourages oviposition in adult mosquitoes which may not occur when transparent container are used as a mosquitoes bait. Small quantity of industrial yeast was sprinkled on the surface of the water and allowed to decompose slowly. The yeast was added into the medium to nourish the developing larva. Wild mosquitoes were allowed to freely visit the bait and to lay eggs. Afterward, the container bearing mosquito larvae and pupae were transferred to the laboratory. The insect were identified at larval stage through the absence of respiratory siphon and the presence of spiracles located only on the 8th abdominal segment which makes them position themselves to the surface of water for breathing in contrast to larvae of other mosquitoes [10]. Further identification was done with some of the emerged adults. Features such as speckles on the legs, 3rd preapical dark area with a pale interruption on vein 1 and conspicuous pale bands on tersi 1-4 were used to further confirm emerged adults as *An. gambiae* [9]. Larvae, pupae and emerged adults were maintained in the laboratory at ambient temperature (28±2 ^{0}C) and relative humidity (75±5%).

2.4. Effect of the leaves and seeds of *N. tabacum* on larvae and pupae of *An. gambiae*

Larvicidal and pupacidal activity of the plant extracts were carried out at different concentration by preparing the required stock solutions following the standard procedure [32]. The desired concentrations were achieved by adding 1.0µg of crude extract of any of the two plant parts to 100ml of de-chlorinated water. From this, five concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) of each plant extract were prepared. The extracts were mixed with water in a beaker at the desired concentration in the presence of small amount of yeast powder to serve as food source for the larva. Then, 20 larvae or pupae of *An. gambiae* were introduced into the beaker. Control (water only) beakers were similarly infested. There were four replicates for each concentration and the control. Mortality was observed over 24 hours after which the larvae and pupae were introduced into distilled water to notice recovery; a recovery time of 5 minutes was allowed [32]. Larvae and pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe [26].

2.5. Fumigant effect of plant extracts on adult *An. gambiae*

The efficacy of the seeds and leaves of *N. tabacum* extracts against the adults of *An. gambiae* was assessed using the methods described by Akinkurolere *et al*. [3] with little modification. Ten adults were introduced into a test-tube which was later plugged with cotton wool. Strips of Whatman's No.1 filter papers (90 mm in diameter) soaked in varying concentrations of extracts were suspended in the test-tube. Each treatment and the control were replicated four times. Mortality was recorded after 3 hours of application.

2.6. Analysis of Data

Data were subjected to analysis of variance (ANOVA), and means were separated using the New Duncan's Multiple Range test. Data were also subjected to probit analysis to determine the concentration of seeds and leaves of *N. tabacum* lethal to 50% (LC_{50}) of larvae, pupae and adult of *An. gambiae* [7]. All data were analysed using Statistical Package for Social Sciences (SPSS) 16.0 software [27].

3. Results

3.1. Effect of extracts of *N. tabacum* on the mortality of larvae and pupae of *An. gambiae*

The percentage mortality of *An. gambiae* exposed to different concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) of leaf and seed extract of *N. tabacum* after 24 h post-treatment is shown in Table 1 to 5. Generally, the mortality values of extract treated larvae and pupae was significantly higher (p>0.05) than those of the untreated larvae and pupae regardless of the concentration of plant parts. Similarly, there was no significant different (p>0.05) between the mortality of leaf and seed treated larvae and pupae of *N. tabacum* at 0.1 and 0.5% (Table 1 and 5). Both extracts however elicited 100% mortality in larvae and pupae of *An. gambiae* at 0.5% only (Table 5). At 0.2 and 0.3% of both extracts, the mortality of larvae treated with the leaf extract was significantly higher (p<0.05) than those treated with seed extract while there was no significant different (p>0.05) between the pupae treated with leaf and seed extracts (Table 2 and 3). Also, the mortality of *An. gambiae* pupae exposed to the leaf extract at 0.4% was significantly higher (p<0.05) than those treated with seed extract (Table 4).

3.2. Effect of extracts of *N. tabacum* on the mortality of *An. gambiae* adults

The fumigant effect of leaf and seed extracts of *N. tabacum* on the mortality of *An. gambiae* adults at 3 h post-treatment is presented in Table 6. In general, *An. gambiae* adults exposed to extracts of *N. tabacum* showed significantly higher ($p<0.05$) mortality values than untreated adults. Likewise, there was no significant different ($p>0.05$) between the leaf and seed treated adults of *An. gambiae* at 0.1, 0.2 and 0.3% respectively. The fumigant effect of leaf extract of *N. tabacum* on adult mosquitoes at 0.4 and 0.5% was also significantly higher ($p<0.05$) than those exposed to seed extract. However, complete mortality (100%) was only observed at 0.5% of leaf extract of *N. tabacum*.

3.3. Effect of lethal concentrations (LC_{50}) of extracts of *N. tabacum* on the mortality of developmental stages of *An. gambiae*

Table 7 shows the concentration of leaf and seed extract of *N. tabacum* required to kill 50% of larvae and pupae (after 24 h) as well as adults of *An. gambiae* (after 3 h). Generally, the concentration of leaf extract required to achieve 50% mortality in larvae, pupae and adults of *An. gambiae* is lower than that of seed extract. Similarly, the LC_{50} values of leaf and seed extract of *N. tabacum* increased with the developmental stages of the mosquitoes with the lowest and highest observed in larval (leaf:0.153µg/ml; seed: 0.188 µg/ml) and adult stage (leaf: 0.219 µg/ml; seed: 0.290 µg/ml) respectively.

4. Discussion

Botanicals have been suggested as one of the greatest weapon in the control of different insect species both in small and large scale control [17]. The high abundance of most botanicals, low cost of production of crude extract and their high efficacy has led to the adoption of crude extract in most developing nations as a substitute to most purified compounds in the control of mosquitoes [3, 21]. The efficacy of botanicals on target species however differs, depending on the part of the plant from which they have been extracted, plant species and mosquito species among other factors [30, 28].

In this study, the mosquitocidal effect of seed and leaf extract of *N. tabacum* on the mortality of different developmental stages of *An. gambiae* was therefore investigated. Regardless of the plant part used, increasing concentrations of both extracts resulted in increasing mortality of the larvae, pupae and adults of *An. gambiae*. This shows that extracts of this plant parts possess a protective properties against *An. gambiae* which further corroborates findings from Akinkurolere *et al*. [3], Olofintoye *et al*. [15], Rahuman [21] and Edriss *et al*. [6]. Considerable reduction in the population of *An. gambiae* due to both extracts could be linked to the ability of the oil to block oxygen supply to the developmental stages in water or blockage of the spiracles which will later leads to suffocation and death [1].

However, highest mortality was observed in larvae, pupae and adults treated with leaf extract of *N. tabacum* when compared with those treated with seed extract. This could be linked to the phytochemical content of this plant. In a study carried out by Mittai *et al*. [13], they discovered that *N. tabacum* contains nicotine, tar and carbon monoxide as the main components which vary in concentration in different parts of the plant. Of all these components, nicotine has been proposed as an effective insecticide as it is completely biodegradable and effective in controlling insects [19]. The concentration of nicotine usually increases with the age of the plant with mature plant having about 64% nicotine in leaves, 18% in stem, 13% in root, 5% in flowers and 0% in seeds [15]. The high concentration of nicotine in the leaves of *N. tabacum* may be responsible for its high mosquitocidal effect against the developmental stages of *An. gambiae*. Also, the presence of carbon monoxide as one of the active components of *N. tabacum* could suggest the reason for the high mortality of both seed and leaf extract as this gas leads to suffocation in animals.

Of all the developmental stages, LC_{50} values shows that the larva of *An. gambiae* was the most susceptible of all the stages while the adult was the most tolerant irrespective of plant part. The larval stage is the most active stage where the insect feed voraciously. This might have led to the ingestion of dosage of *N. tabacum* lethal enough to cause stomach poisoning and high mortality observed in the larvae of *An. gambiae* used in this study. Similar observations have been reported by other researchers [12, 20, 25]. The leaf extract might have also led to extensive damage and shrunken of the cuticle of the anal papillae in *An. gambiae* larvae [5]. Higher tolerance observed in pupae of *An. gambiae* when compared to larvae may be attributed to the inactive nature of pupae as they do not feed which reduces their chances of ingesting poison into their system. Highest concentration of *N. tabacum* extracts was however needed in controlling the adult stage of *An. gambiae*. Higher LC_{50} values of *N. tabacum* extracts are normal and acceptable, considering that they are generally more biodegradable, have lower non-target toxicity and eco-friendly.

Table 1: Percentage mortality of *An. gambiae* at 24 hours post treatment with 0.1% extracts of *N. tabacum*

Extracts of *N. tabacum*	Developmental stages		
	Larvae		Pupae
Leaf	30.00±4.08^{b}		25.00±2.89^{b}
Seed	25.00±2.89^{b}		17.50±2.50^{b}
Untreated	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 Range Test.

Table 2: Percentage mortality of *An. gambiae* at 24 hours post treatment with 0.2% extracts of *N. tabacum.*

Extracts of *N. tabacum*		Developmental stages		
		Larvae		Pupae
Leaf		55.00±2.89^{c}		45.00±2.89^{b}
Seed		40.00±4.08^{b}		32.50±7.50^{b}
Untreated		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 Range Test.

Table 3: Percentage mortality of An. gambiae at 24 hours post treatment with 0.3% extracts of N. tabacum

Extracts of *N. tabacum*	Developmental stages	
	Larvae	Pupae
Leaf	85.00±2.89^{c}	72.50±7.50^{b}
Seed	70.00±4.08^{b}	67.50±2.50^{b}
Untreated	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 Range Test.

Table 4: Percentage mortality of *An. gambiae* at 24 hours post treatment with 0.4% extracts of *N. tabacum*.

Extracts of *N. tabacum*	Developmental stages	
	Larvae	Pupae
Leaf	100.00±0.00[b]	100.00±0.00[c]
Seed	90.00±4.08[b]	85.00±2.89[b]
Untreated	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 Range Test.

Table 5: Percentage mortality of *An. gambiae* at 24 hours post treatment with 0.5% extracts of *N. tabacum*.

Extracts of *N. tabacum*	Developmental stages	
	Larvae	Pupae
Leaf	100.00±0.00[b]	100.00±0.00[b]
Seed	100.00±0.00[b]	100.00±0.00[b]
Untreated	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 6: Fumigant effect of *N. tabacum* extracts on the mortality of *An. gambiae* adults at 3 hours post-treatment.

Extracts of *N. tabacum*	Concentration (%)				
	0.1	0.2	0.3	0.4	0.5
Leaf	20.00±2.01[b]	37.50±2.50[b]	50.00±5.79[b]	85.00±2.89[c]	100.00±0.00[c]
Seed	12.50±2.31[b]	27.50±2.50[b]	42.50±7.50[b]	65.00±2.89[b]	85.00±2.89[b]
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 7: Lethal concentration (LC_{50}) (µg/ml) of leaf and seed extracts of *N. tabacum* obtained from the mortality test of developmental stages of *An. Gambiae*

Developmental stages	Extracts of *N. tabacum*	Slope (±S.E)	Intercept (±S.E)	χ^2	LC_{50} (95% F.L)
Larva	Leaf	4.05 (±0.33)	3.30 (±0.24)	18.56	0.153 (0.076-0.214)
	Seed	3.56 (±0.29)	2.59 (±0.20)	22.78	0.188 (0.081-0.280)
Pupa	Leaf	4.00 (±0.31)	3.02 (±0.22)	31.96	0.176 (0.049-0.281)
	Seed	3.90 (±0.30)	2.62 (±0.20)	22.28	0.213 (0.114-0.309)
Adult	Leaf	3.47 (±0.29)	2.29 (±0.18)	36.29	0.219 (0.027-0.437)
	Seed	3.02 (±0.28)	1.62 (±0.17)	10.97	0.290 (0.207-0.417)

χ^2: Chi-square; SE: Standard error; FL: Fiducial limits.

5. Conclusion

Various results obtained from this study shows that leaf and seed extracts of *N. tabacum* led to considerable reduction in larvae, pupae and adult population of *An. gambiae*. The leaf extract of *N. tabacum* was however more effective than the seed extract in controlling each of the stages. *N. tabacum* could therefore be suggested as a prospective insecticide for the control of *An. gambiae*, an important vector of malaria pathogen.

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