EFFICACY OF *NICOTIANA TABACUM* L. AND *MORINGA OLEIFERA* LAM. LEAF EXTRACTS ON *CULEX QUINQUEFASCIATUS* SAY LARVAE

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Abstract: *Culex* mosquitoes transmit filarial worms, and current control methods heavily use chemical insecticides. Evaluating botanicals for their phyto-constituents and insecticidal properties is vital for greener and more effective mosquito vector control. We then investigated aqueous and ethanolic leaf extracts of *Nicotiana tabacum* and *Moringa oleifera* for their phyto-constituents and larvicidal effects on *Culex quinquefasciatus* larvae at concentrations of 1.0, 1.5, and 2.0 mg/mL over exposure times of 24, 48, and 72 h. Data were analysed using the one way analysis of variance (ANOVA), probit analysis and Chi-square tests. Phytoscreening of extracts revealed various compounds, including flavonoids, tannins, alkaloids, and glycosides. Additionally, the ethanolic extract from *N. tabacum* contained starch and phenols Mortality of mosquito larvae exposed to both extracts of *N. tabacum* varied significantly ($p < 0.05$); specifically at 24 and 48 h for the aqueous extracts and at 48 and 72 h for the ethanolic extracts. Similarly, for the *M. oleifera* extracts, significant differences ($p < 0.05$) in mortality were recorded only at 48 h for the aqueous and at 48 and 72 h for the ethanolic extracts. In all, highest percentage mortality occurred with the highest test concentrations (range: 86.7 – 93.3 %). The Lethal concentration 50 (LC_{50}) values of all the plant extracts, varied significantly ($F_{(2,9)} = 101.0$; $p < 0.01$) across exposure times: highest at 24 h and lowest at 72 h. Easy access to these plants, combined with the potency of both aqueous and ethanolic extracts, positions them as crucial tools for local mosquito vector control.

Keywords: Botanicals, *Culex quinquefasciatus*, larvicides, *Moringa oleifera*, *Nicotiana tabacum*, phytochemicals.

Introduction

Culex quinquefasciatus Say are among the most successful and widespread mosquito species globally (WHO, 2013). This may not be unconnected with the plasticity in their choice of breeding site and environmental condition. They are closely associated with areas and dwellings with inadequate sanitation systems (Service, 2012; WHO, 2013) often characteristic of most third world nations. These mosquitoes serve as one of the important vectors for the filarial worms, *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* which causes Lymphatic filariasis (Medeiros *et al*., 2022). They also act as significant vectors of various diseases affecting both humans and animals including West Nile Virus (WNV), Usutu Virus (USUV), Avian malaria, Japanese Encephalitis virus and a host of other viruses (ECDPC, 2020).

Successful transmission of lymphatic filariasis which is arguably the most commonly associated infection with *Cx. quinquefasciatus*, requires multiple bites by infected mosquitoes. As a result, residents who live in tropical and subtropical regions for extended periods face a higher risk of infection compared to short-term visitors (Al-Tameemi & Kabakli, 2019). Apart from the clinical manifestations of the diseases that could impair the wellbeing of the infected hosts, the long-term disability also inflicted by lymphatic filariasis (Gordon *et al*., 2011) is a cause for concern and gives strong credence for formulation of effective control against them.

Larvicides are among the insecticide types that are used in mosquito control. They are introduced into the larval aquatic habitat to inhibit the emergence of the adult stages which mostly act as the infected and infective life cycle stage of mosquitoes relative to their disease transmission (Rose, 2001). Continuous use of the conventional chemical insecticides approved against mosquitoes have raised concerns over the years as to the unlikely effects it has on organisms and ecosystem which consequently encourages the formulations of eco-compatible insecticides (Russell *et al*., 2009; Service, 2012). However, using botanicals either alone or as supplementary biological control agents in integrated vector management is proving to be a sustainable, safer, and more effective alternative (Ghosh *et al*., 2012).

Moringa oleifera Lam. is an important bio-pesticidal plant (Mamun & Ahmed, 2011), grown globally (Santos *et al*., 2009). It belongs to the family Moringaceae. Its root, leaves, stem-bark and fruit are useful therapeutically against inflammatory diseases, asthma, helmithic, diarrhoea, liver and pancreas disease (Odugbemi & Akinsulire, 2006). *Nicotiana tabacum* commonly called cultivated tobacco, are well known herbaceous medicinal plants grown in most parts of Nigeria. It belongs to the family Solanaceae and its leaves are used traditionally as anthelmintics and also for treatment of ringworm, cold, convulsions, ulcers and nausea (Odugbemi & Akinsulire, 2006). Apart from their medicinal role, nicotine and other alkaloidal constituents of tobacco including anabasine and nornicotines do induce insecticidal activities (Khan *et al*. 2013; Kumar *et al*. 2013).

Previous accounts of use of *M. oleifera* and *N. tabacum* for insecticidal purposes had either shown promise or been efficacious (Ashfaq & Ashfaq, 2012; Omoregie *et al*., 2018; Ogbalu *et al*., 2014; Lamria *et al*., 2019, Afolabi & Olonisakin, 2022). Studies that directly compared their potency against a common mosquito species are either rare or none existent. Taking into account the factors mentioned above, as well as the relatively lower maintenance required for the successful cultivation of *M. oleifera* and *N. tabacum*, and their easy accessibility by locals especially in Nigeria, we initiated an investigation to assess the efficacy of aqueous and ethanol extracts of *M. oleifera* and *N. tabacum* as larvicides against *Cx. quinquefasciatus* larvae.

Materials and Methods

Collection, Identification and Preparation of Plant Extracts

Fresh collections of leaves of *N. tabacum* and *M. oleifera* were made in Benin City, Nigeria. The plant materials were authenticated by a Botanist in the Department of Plant Biology and Biotechnology, University of Benin. After collection, they were thoroughly rinsed with water and air dried for 23 days after which, pulverized to powder with a mechanical blender.

A section of the each ground plant material was further subjected to aqueous extraction and the other, ethanol extraction. To prepare the aqueous extract, 200 g each of the blended *N. tabacum* and *M. oleifera* were separately weighed and dissolved 750 ml of distilled water and left for 24 h. Ethanol extraction of the other section was done by mechanical maceration in 750 ml of ethanol in a glass jar for 72 h. The crude aqueous and ethanolic extracts for each plant materials was then actualized by filtration and utilizing a crucible water bath to concentrate the filtrates to paste level at 70^{O} C.

Qualitative Screening of Phytochemicals

The phytochemical constituents of the extracted plant materials - aqueous and ethanolic extracts of *N. tabacum* and *M. oleifera*, were determined using standard methods. The screened phytochemicals included phenols, carbohydrates, free reducing sugars, flavonoids, alkaloids, starch, glycosides, and tannins. Additionally, terpenoids, saponins, and steroids were also ascertained (Edeoga *et al.*, 2005).

Collection of Mosquito Larvae

Egg rafts of *Cx. quinquefasciatus* mosquitoes were collected from breeding sites around the University of Benin, Ugbowo Campus. The eggs were transferred into bowls and kept in mosquito rearing cages measuring 0.4 m x 0.4 m x 0.4 m (L x B x H) in the Department of Animal and Environmental Biology following guidelines from the World Health Organization (1975) with some modifications. Rearing was carefully maintained at an average room temperature of 28±2°C and a relative humidity of 78±2%.

Preparation of Stock solution of the Extracts

Stock solutions for the different extracts were prepared according to standard WHO (2005) procedure. The concentrations of the solution was 20 mg/mL for each; aqueous and ethanolic extracts of *N. tabacum*, and *M. oleifera*.

Bioassay

To prepare test concentrations, 1.0, 1.5, and 2.0 mg/mL were made from the stock solution by adding 5.0, 7.5, and 10.0 ml of stock solution to each round plastic container, respectively. The volume was then diluted to 100 ml by adding 95.0, 92.5, and 90.0 ml of water, respectively. The control (0.0 mg/mL) was made up of only water (100 ml) without any added extract. Each test concentration was made into triplicates. Ten (10) individuals of *Cx. quinquefasciatus* in their second and third larval instar developmental stage were introduced into each test bowl with the defined concentrations and the control for 72 h. Mortality in the test bowls was observed and recorded at 24, 48, and 72 h post-exposure intervals. Larvae were recorded as dead if they stayed still at the bottom when triggered by external disturbance. No food materials were added to the treatment groups during the exposure periods.

Statistical analysis

Analysis of data was done using Microsoft Excel 2016 and IBM Statistical Package for Social Scientists (SPSS 27.0). Larval mortality data was presented in mean, standard deviation and percentage mortality of the larvae. The one-way Analysis of Variance (ANOVA) test was used to analyse the differences in the larval mortality in relation to exposure time and test concentrations of the extracts. This was followed by the Duncan's Multiple Range test (DMRT) to determine the source of significant difference. Prior to the ANOVA test, data sets were first transformed (Sqrt+0.5). Mortality data was also subjected to Probit Analysis to determine the lethal concentrations (LC_{50}) of each plant extract against the *Cx. quinquefasciatus* larvae at 95 % confidence limits. Differences in the lethal concentrations of extracts within and between the 24, 48 and 72 h exposure times were analysed using the Chi-square and ANOVA tests respectively. Significance in comparisons were set at $p < 0.05$.

Results and Discussion

The phytochemical screening of aqueous and ethanolic extracts from *Nicotiana tabacum* leaves revealed the presence of flavonoids, steroids, tannins, reducing sugars, alkaloids, and glycosides in both extracts. Additionally, starch and phenols were detected exclusively in the ethanolic extract (Table 1). As for *M. oleifera*, only carbohydrates, flavonoids, tannins, amino acids and alkaloids were present in both the aqueous and ethanolic extracts (Table 2). Noteworthy, phytochemicals detected in the ethanolic extracts were more abundant compared to those in the aqueous extracts of both *N. tabacum* and *M. oleifera* (Tables 1 and 2). Alkaloids, flavonoids and tannins were all present in aqueous and ethanolic extracts of the leaves of *M oleifera*. This is partly in line with previous report of the presence of alkaloids, flavonoids, tannins, phenol, glycoside, steroids, triterpenoids, anthraquinones and saponins in the methanolic extracts of the roots of the plant by Omoregie *et al*. (2018). The additional detection of saponins in that study which was absent in our findings may not be unconnected with the difference in the solvent of extraction and plant part. *N. tabacum* had earlier been reported to possess alkaloids, polyphenol, flavonoid and reducing sugars (Leal *et al*., 2023). The observation of phenols, flavonoids and tannins in extracts of *N. tabacum* in the report by Zou *et al.* (2021) is also partially congruent with our findings. Botanical pesticides employ various modes of action against their target pests, including growth restriction, toxicity, repellency, and structural modifications (Rattan, 2010). They interfere with the morphology, physiological processes, biochemical reactions, behaviour, and metabolic pathways of insects (Lengai *et al*., 2020).

Nicotiana tabacum and *M. oleifera* extracts derived from the use of chemicals (ethanol) for their leaf extraction yielded higher concentration of phytochemicals in them than those that were gotten from aqueous extraction in this study. Thus, the solvent selected for extraction is crucial in determining the bioactive compounds present in the plant extract (Ingle *et al*., 2017; Nortjie *et al*., 2022).

Table 1: Phyto-constituents identified in the ethanolic and aqueous extracts of *N. tabacum*

CLASS OF COMPOUNDS	ETHANOLIC EXTRACTS	AQUEOUS EXTRACTS
Starch	+	-
Phenols	++	-
Flavonoids	+++	+
Steroids	+++	+
Tannins	++	+
Reducing sugar	++	±
Alkaloids	+++	+
Glycosides	+++	+

Key: Present (+), Slightly present (++), Obviously present (+++), Trace (±), Absent (-).

Table 2: Phytochemical Result for Aqueous and Ethanolic Extracts of *M. oleifera*

CLASS OF COMPOUNDS	ETHANOLIC EXTRACTS	AQUEOUS EXTRACTS
Carbohydrates	++++	+++
Proteins	_	_
Flavonoids	+++	+
Saponins	_	_
Tannins	++	+
Amino acid	++++	+
Alkaloids	+++	+
Fixed oil and fats	_	_

Key: Present (+), Slightly present (++), Obviously present (+++), Strongly present, Trace (±), Absent (-)

Mortality of the mosquito larvae exposed to the aqueous and ethanolic extracts of *N. tabacum* and *M. oleifera* all varied significantly ($p < 0.05$) at all the exposure periods of 24 h, 48 h and 72 h (Table 3 & 4). Highest percentage larval mortality were recorded in setups with the highest concentration of the extracts at each period of exposure (Table 3 & 4). The lethal concentration 50 (LC_{50}) between the aqueous and ethanolic extracts of each plant showed no significant difference within the 24 h, 48 h and 72 h exposure times ($p > 0.05$). However, the difference in the calculated LC_{50} of the plant extracts was highly significant ($F_{(2,9)} = 101.0$; $p < 0.01$) between the 24 h, 48 h and 72 h exposure time. Those for 24 h were highest and 72 h, the least (Table 5). Mortalities and Lethal concentrations of the *Cx. quinquefasciatus* larvae recorded in this study were a reflection of the concentrations of the extract dilutions they were exposed to and the duration of their exposure. The mortality of the mosquito larvae were all dose dependent. As the concentration of the extract dilutions increased, recorded mortality also increased, and the lethal concentrations decreased with increasing exposure time. This compares with previous reports (Ashfaq & Ashfaq, 2012; Ullah *et al*., 2018).

Table 3: Effect of Aqueous and Ethanolic Extracts of *N. tabacum* on mortality of *Cx. quinquefasciatus* larvae

Name of Plant	Extracting solvent	Concentration (mg/mL)	Mean mortality ± SD (% Mortality)		
			24 h	48 h	72 h
N. tabacum	Water	0.0	0.0 ± 0.0^{c} (0.0)	0.0 ± 0.0^{d} (0.0)	0.0 ± 0.0^{c} (0.0)
		1.0	0.7 ± 0.6^{bc3} (6.7)	2.3 ± 0.6^{c2} (23.3)	7.0 ± 1.0^{b1} (70.0)
		1.5	1.3 ± 0.6^{ab3} (13.3)	4.3 ± 1.2^{b2} (43.3)	7.3 ± 0.6^{b1} (73.3)
		2.0	2.3 ± 0.6^{a2} (23.3)	7.7 ± 0.6^{a1} (76.7)	8.7 ± 0.6^{a1} (86.7)
	Ethanol	0.0	0.0 ± 0.0^{c} (0.0)	0.0 ± 0.0^{c} (0.0)	0.0 ± 0.0^{d} (0.0)
		1.0	1.0 ± 1.0^{bc2} (10.0)	3.7 ± 1.5^{b1} (36.7)	6.0 ± 1.0^{c1} (60.0)
		1.5	2.0 ± 1.0^{ab3} (20.0)	5.0 ± 0.0^{ab2} (50.0)	7.3 ± 0.6^{b1} (73.3)
		2.0	3.3 ± 0.6^{a3} (33.3)	6.3 ± 0.6^{a2} (63.3)	9.3 ± 0.6^{a1} (93.3)

Within each section of the different extracting solvent; means on the same column with different letter superscript are significantly different ($p < 0.05$) and means on the same row with different number superscript are significantly different ($p < 0.05$)

Table 4: Effect of Aqueous and Ethanolic Extracts of *M. oleifera* on mortality of *Cx. quinquefasciatus* larvae

Name of Plant	Extracting solvent	Concentration (mg/mL)	Mean mortality ± SD (% Mortality)		
			24 h	48 h	72 h
M. oleifera	Water	0.0	0.0 ± 0.0^{c} (0.0)	0.0 ± 0.0^{d} (0.0)	0.0 ± 0.0^{c} (0.0)
		1.0	1.0 ± 1.0^{bc3} (10.0)	2.7 ± 0.6^{c2} (26.7)	6.0 ± 1.0^{b1} (60.0)
		1.5	1.3 ± 0.6^{ab3} (13.3)	4.0 ± 1.0^{b2} (40.0)	8.3 ± 1.0^{a1} (83.3)
		2.0	3.0 ± 1.0^{a2} (30.0)	7.0 ± 1.0^{a1} (70.0)	9.0 ± 1.0^{a1} (90.0)
	Ethanol	0.0	0.0 ± 0.0^{c} (0.0)	0.0 ± 0.0^{c} (0.0)	0.0 ± 0.0^{c} (0.0)
		1.0	1.3 ± 0.6^{b3} (13.3)	3.7 ± 0.6^{b2} (36.7)	7.7 ± 0.6^{b1} (76.7)
		1.5	2.3 ± 0.6^{ab3} (23.3)	5.0 ± 1.0^{b2} (50.0)	8.3 ± 0.6^{ab1} (83.3)
		2.0	3.0 ± 1.0^{a2} (30.0)	7.3 ± 1.2^{a1} (73.3)	9.3 ± 0.6^{a1} (93.3)

Within each section of the different extracting solvent; means on the same column with different letter superscript are significantly different ($p < 0.05$) and means on the same row with different number superscript are significantly different ($p < 0.05$)

Table 5: Lethal concentrations of *N. tabacum* and *M. oleifera* Extracts against *Cx. quinquefasciatus* larvae

Plant Extract	Lethal Concentration 50 (mg/mL)		
	24 h	48 h	72 h
Aqueous *N. tabacum*	3.9	1.5	0.5
Ethanol *N. tabacum*	2.9	1.4	0.9
Aqueous *M. oleifera*	3.4	1.6	0.8
Ethanol *M. oleifera*	3.7	1.3	0.5

In our study, similar to the aqueous and ethanol extracts of *M. oleifera* leaves that resulted in over 90 % larval mortality in *Cx. quinquefasciatus* exposed to 2 mg/mL (highest dose) of the extracts after 72 h, the highest dose rate of aqueous extract from *M. oleifera* seeds (120 mg/L) also caused the highest mortality of 98.89 ± 0.54 % after 24 h of treatment, as reported by Ashfaq & Ashfaq (2012). In a separate study, the exposure of *Culex* mosquito larvae to *M. oleifera* led to complete (100 %) mortality within just 3 hours (Afolabi & Olonisakin, 2022); a significantly shorter time frame compared to our findings. Although the observed rapid larvicidal effect observed in that study was influenced by the concentration of the plant material, it does underscore the potency of *M. oleifera* extracts against the mosquito larvae. The concentration of phytochemicals obtained from plant extracts and their corresponding potency are influenced by several factors, including the extraction method (Belokurov *et al*., 2019), and the specific plant parts used for extraction (Fotsing Yannick Stéphane *et al*., 2022).

All test botanicals, including both aqueous and ethanolic extracts, demonstrated similar potency against the test organisms in our study. This similarity was evident in the LC_{50} values calculated for all plant extracts at 24 h, 48 h, and 72 h of exposure. Additionally, the activities and mortalities induced by all distinct extracts on the *Cx. quinquefasciatus* larvae exhibited a comparable pattern. This finding contradicts the reports by Fafioye *et al*. (2004) and Aina *et al*. (2009). The toxicity studies of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles revealed greater potency from the ethanolic extracts than the aqueous'. Also, Aina *et al*. (2009) found that the ethanolic extract of *Piper guineense* and the aqueous extract of *Jatropha curcas* were the most and least effective in controlling *An. gambiae* larvae, respectively.

Conclusion

This study reaffirms the potency of the investigated plant materials – *M. oleifera* and *N. tabacum* – and strongly suggests their significance as tools for mosquito control. The nearly equal mortality rates induced by both aqueous and ethanolic extracts of the same plant materials are advantageous for controlling *Cx. quinquefasciatus* larvae. This finding removes any compulsion to use ethanol for plant material extraction when water is equally effective. What's advantageous about the plants extracts is their easy availability in this region. Coupled with the potency of their aqueous extracts, they can play a crucial role in local mosquito vector control.

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