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ARTICLE

Evaluation of larvicidal efficacy of indigenous botanicals from Nigeria against larval stages of *Aedes aegypti* and *Culex quinquefasciatus*

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Resumen

Evaluación de la eficacia de productos botánicos autóctonos frente a estadios larvales de Aedes aegypti *y* Culex quinquefasciatus

El uso continuado de productos quimicos para eliminar o reducir las poblaciones de mosquitos a un nivel tolerable desarrolla resistencias con el tiempo. Se exploran extractos de plantas como alternativas para mitigar su incidencia en el medio ambiente. El estudio comparó las eficacias larvicidas de cinco productos botánicos autóctonos frente a los estadios larvales de *Aedes aegypti* y *Culex quinquefasciatus*. Grupos de treinta larvas de de estadio 1 a 3 de *Ae. aegypti* y *Cx. quinquefasciatus* se expusieron a 5, 10 y 15 mg/100 ml de los extractos de los productos botánicos analizándose en laboratorio su actividad larvicida a las 24, 48 y 72 horas. Los extractos de *Morinda lucida* y *Vernonia amygdalina* fueron los más efectivos frente a los estadios larvarios 1 a 3 de *Ae. aegypti* y *Cx. quinquefasciatus*.

Palabras clave: Insecticidas botánicos; Control vectorial de larvas; *Morinda lucida*; *Vernonia amygdalina*.

Abstract

Mosquitoes are known to develop resistance over time while using chemicals to eliminate or reduce their population to tolerable level. Plant extracts are explored as alternatives to mitigate their incidence in the environment. The study compared the larvicidal efficacies of five different indigenous botanicals against the larval stages of *Aedes aegypti* and *Culex quinquefasciatus*. Batches of thirty 1st-3rd instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to 5, 10 and 15mg/100ml of the crude extracts of the botanicals respectively and they were assayed in the laboratory for larvicidal activities for 24, 48, and 72hrs post exposure. *Morinda lucida* and *Vernonia amygdalina* extracts were the most effective against 1st-3rd instars of *Ae. aegypti* and *Cx. quinquefasciatus*.

Key words: Botanical insecticides; Vector larval control; *Morinda lucida*; *Vernonia amygdalina*.

Introduction

Aedes aegypti (Linnaeus, 1762) and *Cx. quinquefasciatus* Say, 1823 are vectors of parasites and pathogens for human and animals (WHO 2011). In tropical and subtropical countries, about two billion human population are at risk of mosquito-borne diseases such as dengue fever, haemorrhagic fever, filariasis or malaria (WHO 2017). Generally, *Aedes* spp., *Anopheles* spp. and *Culex* spp. are identified as major vectors involved in transmission of certain diseases to animals and humans which are of public health importance across the globe specifically in Asia and Africa (WHO 2015). *Aedes aegypti* and *Cx. quinquefasciatus* are two of these mosquito species that act as vector of Dengue, Chikungunya, Zika, or West Nile Virus (Arensburger *et al.* 2010, Powell *et al.* 2018). Their ecological habitat includes flood areas, rice farms, stagnant water, bore holes' casings, used tires, uncovered water storage tanks, abandoned drainages etc. which serve as suitable breeding sites.

Although WHO (2017) recommended integrated vector control management strategies, but there is persistent use of chemical insecticides such as organochlorine pesticide families [DDT, Endosulfan – persistent organic compound] which are of great environmental concern around the world for their chronic mammalian toxicity, persistence and bioaccumulation. Organophosphates such as Malathion, Dibrom, Chlorpyrifos, Temephos, Diazinon and Terbufos are less toxic to mammals but toxic to target organisms and carbamates which are esters of N-methyl carbamic acid include Aldicarb, Carbaryl, Propoxur, Oxamyl and Terbucar. However, these chemical interventions are compromised overtime by insecticide resistant development in the mosquito vectors which are of public health concern (Chouaibou *et al.* 2012, Ruikar *et al.* 2012). One of the measures recommended by WHO to reduce malaria incidence include the distribution and use of LLINs (Long Lasting Insecticide treated Nets) acting as barrier in several African countries. This method was successful at a time but recorded retrogressive achievement lately due to insecticide resistant development, abuse of use, high cost of acquiring and acceptability challenge (Promsiri *et al.* 2006, Fasasi *et al.* 2020). There are several published reports on the control of mosquito larvae and adults using plant extracts and their essential oils (Maheswaran *et al.* 2008, Benelli 2015, Govindarajan & Rajeswary 2015, Fasasi *et al.* 2019) as alternatives to synthetic chemical insecticides. This study is focused on the larvicidal activities of indigenous botanicals from Nigeria against specific larval stages of *Ae. aegypti* and *Cx. quinquefasciatus*.

Materials and methods

Collection and preparation of extract of selected indigenous plants

The leaves of five types of botanical plants were sourced and collected from the biological garden managed and coordinated by Departments of Plant Biology and Zoology of Osun State University, Osogbo Campus, Osun State, Nigeria. The botanical plants used include *Azadirachta indica* (Maliaceae) (Neem, Dongoyaro), *Morinda lucida* (Rubiaceae) (Brimstone tree, Oruwo), *Vernonia amygdalina* (Asteraceae) (Bitter leaf, Ewuro), *Ocimum gratissimum* (Lamiaceae) (Clove basil or Scent leave, Efinrin) and *Hyptis suaveolens* (Lamiaceae) (Pignut or Chan, Arunfofo) which were identified by plant taxonomist at Department of Plant Biology, Osun State University, Osogbo, Osun State. The leaves of the five botanicals were washed lightly and separately with distilled water to remove all the dust particles and impurities and shade dried at laboratory temperature (29 ± 3 ºC) and 70-90% RH for 24hrs. About 100 g of leaves of each botanical was blended with 100 ml of distilled water using electric QASA Commercial Blender (Pro Heavy Duty -1500 w) for 5 minutes. Thereafter, aqueous leaf extract of each plant was sieved using net mesh (1.41 mm), bottled and stored at 4 ºC for less than 24 hrs before larvicidal bioassay. This process was repeated for each of the botanicals.

Collection and Maintenance of *Ae. aegypti* and *Cx. quinquefasciatus* larvae

Larval samples of *Ae. aegypti* and *Cx. quinquefasciatus* were collected separately during wet season (April-September) from stagnant water pool in drainages and tires using scoop. The collection period was between 6.30 am and 9.30 am (early morning collections) and 4.30 pm and 6.30 pm (evening collections) within Osogbo metropolis specifically at Ajegunle (7º46'47''N, 4º33'8''E) and Iyana Abesu (7º45'9''N, 4º34'49''E), Osun

State, Nigeria. Collected larvae were taken to the laboratory and maintained in water sourced from the larval habitat. They were fed with mixture of dog biscuits and yeast (3:1) powder. The set ups were maintained at 29 ± 3 ºC and 70-90% RH under a photoperiod of 14:10 h (light/ dark) cycles until the larvicidal bioassay is conducted during which the larvae are sorted out into specific L_1 to L_3 for the bioassay.

Larvicidal bioassays

The concentration of each of the botanicals were prepared as 5, 10 and 15 mg/100 ml for the bioassays against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Transparent plastic containers (200 ml) were used for the bioassays in the laboratory. There are four replicates per each concentration per botanical and a control containing distilled water. Thirty larvae of *Ae. aegypti* or *Cx. quinquefasciatus* were introduced into each replicate. After introducing *Ae. aegypti* larvae or *Cx. quinquefasciatus* the set-ups were maintained at 29±3 ºC and 70–90% RH. Mortality and survival rate of the larvae were recorded after 24, 48 and 72 hours respectively for each concentration. Based on the WHO (2005) protocol no food was offered to avoid the difference in mortality. The moribund and dead larvae in each of the replicate at different concentrations were combined and expressed as a percentage of larval mortality for each concentration. Dead larvae were identified when they failed to move after probing with a sharp object in the cervical region or the siphon. The moribund larvae were those that cannot rise to the surface within reasonable period. Dead larvae were removed as soon as possible to prevent decomposition, which may cause rapid death of the remaining larvae.

Statistical Analysis

Data gathered were input into excel sheet for descriptive statistics and subjected to Probit analysis to include linear, logit curves and Probit equations for each of the botanicals to determine LC_{50} and LC_{90} of each botanical. Two-way ANOVA analysis was used to determine the mean difference between parameters. The level of significance was 95%.

Results

The study generally observed that the five botanicals have larvicidal effects on both the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* at 5, 10 and 15 mg/100 ml respectively at 24, 48 and 72 hr exposures respectively at varying degree of toxicity and lethality (Figs. 1a-c & 2a-c). The Probit equations in each of the linear, logarithm and exponential graphs were used to determine the LC_{50} and LC_{90} of each the botanicals (Table 1). From the two-way ANOVA analyses, the effects of botanical extracts, concentration levels, and hours of exposure are significantly contributing to the mosquitoes' mortality rate (p= 0.02447*) (Table 1). However, the effects of the botanical extracts at the same concentration levels and hours of exposure varies significantly across *Ae. aegypti* (p= 0.04402*) and *Cx. quinquefasciatus* (p= 0.01680*).

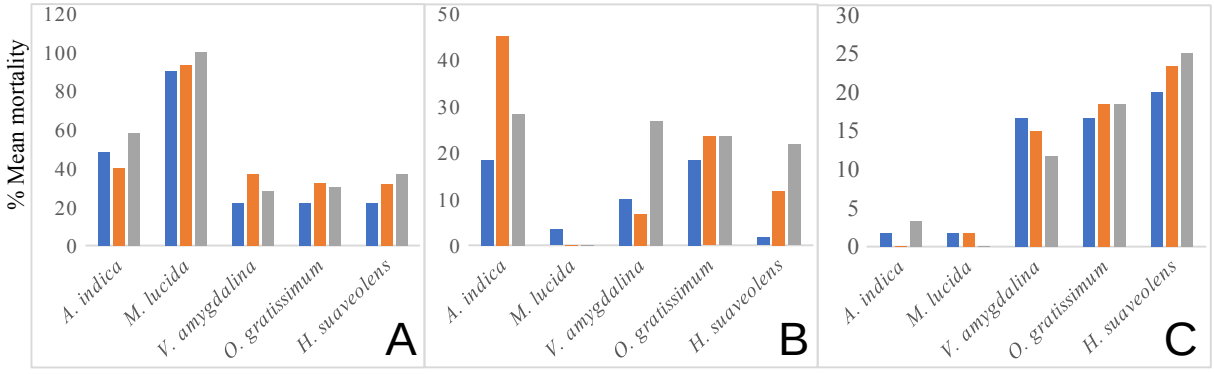

Figura 1. Efectos comparativos de cinco productos botánicos autóctonos sobre *Cx. quinquefasciatus* a distintos tiempos de exposición. **A:** 24 h; **B:** 48 h; **C:** 72 h. Extractos botánicos a 5, 10 y 15mg/100ml.

Figure 1. Comparative effects of five indigenous botanicals on *Cx. quinquefasciatus* at different exposure times. **A:** 24 hr; **B:** 48 hr; **C:** 72 hr. Botanicals at 5, 10 and 15mg/100ml.

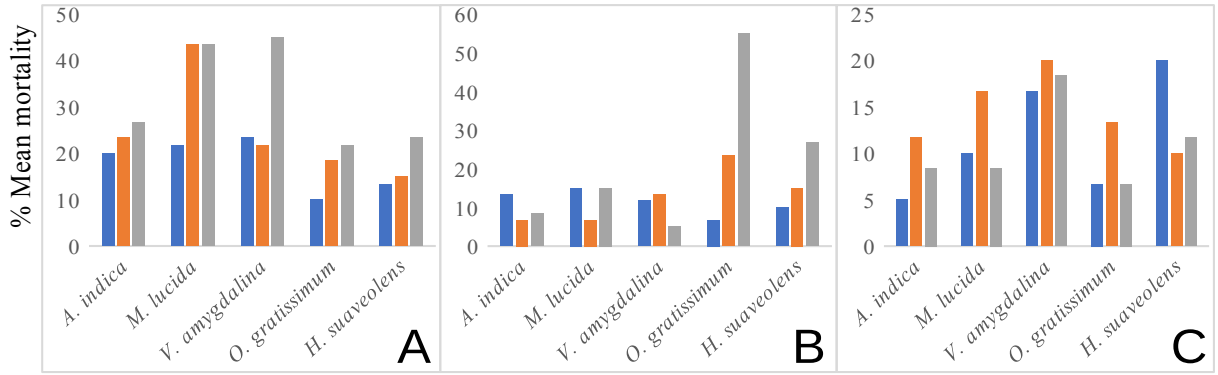

Figura 2. Efectos comparativos de cinco productos botánicos autóctonos sobre *Ae. aegypti* a distintos tiempos de exposición. **A:** 24 h; **B:** 48 h; **C:** 72 h. Extractos botánicos a 5, 10 y 15mg/100ml.

Figure 2. Comparative effects of five indigenous botanicals on *Ae. aegypti* at different exposure times. **A:** 24 hr; **B:** 48 hr; **C:** 72 hr. Botanicals at 5, 10 and 15mg/100ml.

Time	Indigenous botanicals	LC_{50} / LC_{90}				
		A. indica	*M. lucida*	*V. amygdalina*	*O. gratissimum*	*H. suaveolens*
24 hr	*Cx. quinquefasciatus*	11.44 / 27.15	1.77 / 10.36	23.45 / 47.00	33.17 / 75.81	25.48 / 54.70
	Ae. aegypti	41.24 / 87.53	18.00 / 42.60	17.35 / 31.24	16.80 / 33.37	104.9 / 224.64
48 hr	*Cx. quinquefasciatus*	27.38 / 62.71	* / *	56.41 / 111.05	36.56 / 72.85	36.64 / 63.36
	Ae. aegyti	* / *	82.92 / 158.40	* / *	15.62 / 27.29	33.05 / 61.58
72 hr	*Cx. quinquefasciatus*	* / *	-70.25 / -136.92	89.04 / 174.15	49.71 / 97.45	32.46 / 64.01
	Ae. aegyti	318.13 / 616.64	175.09 / 344.58	52.49 / 104.57	130.00 / 253.04	137.45 / 271.68

Tabla 1. Concentraciones letales (mg/100ml) de los productos botánicos autóctonos esperadas para que alcancen una mortalidad del 50 % y el 90 % sobre *A. aegypti* y *Cx. quinquefasciatus* a las exposiciones de 24, 48 y 72 h, respectivamente. * No se alcanzaron dosis letales.

Table 1. Lethal concentrations (mg/100ml) of indigenous botanicals expected to achieve 50% and 90% mortality of *A. aegypti* and *Cx. quinquefasciatus* at 24, 48 and 72 hours exposure, respectively. * Lethal doses were not achieved.

Discussion

Various authors showed that indigenous botanicals can be used as alternatives insecticides against the vectors of Malaria, filariasis, dengue fever etc (Promsiri *et al.* 2009, Ruikar *et al.* 2012, Fasasi *et al.* 2019, Sukumaran & Maheswaran, 2020). They are eco-friendly, less expensive, and highly efficacious for the control of mosquito larvae. This study observed that *A. indica*, *M. lucida*, *V. amygdalina*, *O. gratissimum* and *H. suaveolens* are all effective against the larval stages of the *Cx. quinquefasciatus* and *Ae. aegypti* after 24hr exposure at 5, 10 and 15 mg/100 ml, respectively. This agrees with Alouani *et al.* (2009), who reported that *A. indica* reduces the fecundity of adult female *Cx. pipiens* and causes 93.70% mortality of the larval stages (1^{st}-3^{rd}) of the mosquitoes. Also, Sukumaran & Maheswaran (2020) evaluated leaf powder of *Elytraria acaulis* (L.f.) Lindau against late 3^{rd} or early 4^{th} instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* with LC_{50} values of 116.07 mg/100 ml and 124.25 mg/100 ml, respectively. Partially purified plant extracts are less expensive and efficacious for the control of mosquito larvae rather than the purified compounds or extract (Jang *et al.* 2002, Cavalcanti *et al.* 2004) as observed in this study. The leaves of *H. suaveolens* contains 'β-pinene, sabinene, 1,8-cineol, β-caryophyllene, limonene, α-pinene and bergamotene conferring a repellent effect over mosquitoes (Jaenson *et al.* 2006, Vongsombath *et al.* 2012) as well as larvicidal potency as observed in this study. The leaves of *O. gratissimum* contained high percentage of tannin (Fasasi *et al.* 2019, Otabor *et al.* 2019) which may be responsible for the larvicidal activity of the plant. *Vernonia amygdalina,* which has more alkaloids and flavonoids than *O. gratissimum*, achieve higher larvicidal activity against *Ae. aegypti* (Alexander 2016, Unachukwu *et al.* 2016).

This study clearly revealed that *M. lucida* was the most effective and potent botanical larvicide against *Cx. quinquefasciatus*. Nweze *et al.* (2004) and Akinyemi *et al.* (2005) reported that the leaves of *M. lucida* contains alkaloids, tannins, anthraquinone and steroids which may be responsible for the high larvicidal activities observed in the study.

The study revealed that these indigenous botanicals, from *A. indica*, *M. lucida*, *V. amygdalina*, *O. gratissimum* and *H. suaveolens*, can be

used as an ecofriendly, biodegradable, and economical larvicides in sustainable integrated mosquito control program. It is recommended that the application of these indigenous potential botanicals against mosquito larvae may be used as alternatives to synthetic chemical insecticides appropriately as part of interventions for the control of these vectors to reduce vector-borne diseases without any harm to the environment. Although the effect of the botanical extracts varies significantly at different concentrations and hours of exposure of *Ae. aegyti* and *Cx. quinquefasciatus*. However, certain lethal doses were not achieved at some concentrations. Hence, the need for further research in future.

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