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BIO-EFFICACY OF GROUNDED *SESAMUM RADIATUM* AND *PSORALEA CORYLITOLIA* ON EMERGENCE OF *ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Mosquito larvae are highly distributed in tropical regions where breeding sites are diverse and causing a number of public health diseases, when they emerge. This study tested the efficacy of two grounded insecticidal plant powders against the emergence of *Anopheles* and *Culex* species. Larvae of mosquitoes were collected using 350mL deep ladles and scooping spoon from Ethiope East LGA, Delta State, Nigeria. The mosquito larvae were exposed to 0.5, 1.0, and 1.5g of *Psoralea corylitolia* and *Sesamum radiatum* in 100mL of water. This corresponded to 0.005, 0.01, and 0.015g/mL concentrations for the experiment, respectively. Mosquitoes were exposed to these treatments in 20 larvae per concentration in quadruplicates. Mean mortality and time of mortality were recorded. Probit model was used to determine LT_{50} and LT_{95}. Mortality of larvae increased as concentration and time increased. Highest mortality was recorded in *Culex* mosquitoes exposed to 1.5g of *Psoralea corylitolia* and was closely followed by *Anopheles* mosquitoes exposed to same concentration of *Sesamum radiatum.* The differences between the mortalities were highly significant (P<0.00001). Emergence depreciated as the concentration increased with high mortality especially with 0.5g of *Psoralea corylitolia* exposed to *Anopheles* mosquito larvae. The insecticidal test plants did not determine the sex of mosquitoes and it was not significant ($F_{\text{sex ratio}}$=1.127; P=0.357). Physicochemical parameters of water may have influenced mortality of mosquitoes exposed to *Sesamum radiatum* for 30min (P<0.05). Lethal time for 50% of mosquitoes ranged from 31 to 5858min whereas LT_{95} ranged from 1435 to 10218min for the two insecticidal test plants respectively. There is need for more study with the trial of oil of *Sesamum radiatum* and the determination of the gas chromatograph of this novel plant to ascertain the active components.

Keywords: Bio-efficacy, emergence, mosquitoes, *Sesamum radiatum*, *Psoralea corylitolia*.

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1. INTRODUCTION

Mosquitoes are highly abundant vectors that cause several diseases of increasing public health concern in humans and animals globally (WHO 2017). Unlike vectors of other infectious diseases, mosquitoes have been spotted as the most important vector due to the role they play in disease transmissions (Becker et al. 2020). Some of these diseases include dengue fever, malaria, filariasis, arboviruses, west Nile virus, Japanese encephalitis, yellow fever, chikungunya and so numerous to mention many of them (Chancey et al. 2015; Dad et al. 2019). Amongst these diseases, malaria stands out as the major human disease in Africa and Nigeria, with about 97% of Nigerian population at risk (Okorie et al. 2014; Nwaneli et al. 2020). Apart from the fact that mosquitoes transmit several diseases, their bites can in rare cases cause associated syndrome with swellings, sore, reddish spots that are itchy and presented with pain (Lopez 2022). Approximately 3500 species of mosquitoes are known globally with the exception of the polar regions (CDC 2020). Mosquitoes are spread in various parts of the world. There are about 100 mosquito species native to Europe, over 870 species to South-east Asia, and Africa is considered home for diversities of species due to the warm climate that favors life stage development (Steinbrink et al. 2018; Lai et al. 2018; Kampen and Walther 2018; Trájer and Padisák 2019; Steinbrink et al. 2022). The prominent species causing mosquito-borne diseases in Nigeria are common in three genera, i.e., *Anopheles*, *Aedes* and *Culex*. Mosquito-biting activities and diseases cause economic losses amounting to over one billion dollars in vector and disease management (Narladkar 2018; Hamlet et al. 2022).

In order to reduce the burdens of continuous budgeting for disease management, it is important to control mosquitoes through the use of insecticides in the form of larvicides or adulticides, manipulation of physicochemical characteristics of breeding habitat, physical controls in the use of traps, biological control in the use of *Bacillius thuringiensis* (BTI), larvivorous fishes and plant materials. The use of insecticides, either chemical or plant-extracted, is recommended in the forms of treated bed nets, treated clothing, personal protection by rubbing repellents and spraying indoor of houses (Kimani et al. 2006; Farag et al. 2021). Insecticide treated nets and materials confer 75% protection against mosquito bites and guaranteed the reduction of deaths in vulnerable children under the age of 5 by 20% (CDC 2019). The spraying of insecticide indoors accounted for 60% of insecticide usage in vector control. The issues of resistance in adult vectors have diverted attention towards the use of growth regulators, bacterial and plant extracts in the control of immature stages of mosquitoes in their breeding habitats (Mahran 2022). Oil film made by the oil extracts of *Azadirachta indica* and many other plants was successful in killing immature stages of *Anopheles*, *Culex*, and *Aedes* mosquitoes (Ayinde et al. 2020). Plant-based oil has been proven to be environmentally friendly, effective and poses lesser threat to human health and the environment. This is for their active components that are target specific on the vectors. There is no information on the biological efficacy of these plants on the emergence of *Anopheles* and *Culex* mosquitoes. Therefore, this study was conducted.

2. MATERIALS AND METHODS

2.1. Mosquito Collection

Immature stages of mosquito for this study were collected using 350mL deep ladles and scooping spoons from potential breeding habitat including ponds, tire marks, and puddles in Ethiope East Local Government Area, Delta State. These immature stages were transferred to the Entomology Laboratory in transparent buckets. The larvae of *Anopheles* were distinguished from *Culex* using the resting position on water surfaces. *Anopheles* larvae lie 180^{o} to the water surface and *Culex*, at 45^{o}.

2.2. Plant Source and Preparation

The plants, *Psoralea corilifolia* and *Sesamum radiatum*, for this experiment were sourced from the environment. These plants were air dried at room temperature and grounded into powder and stored in airtight containers which were well labelled.

2.3. Experimental Design

Complete randomized exposure was used in this study. Twenty (20) larval stage three (L_3) of *Anopheles* and *Culex* were sorted into 1000mL capacity glass exposure vails and assigned to four replicates to various concentrations of 0.5, 1.0, and 1.5g, respectively in 100mL of water. Readings for acute and chronic toxicity were taken. Acute toxicity was taken at 10, 20, 30, 40, 50, and 60mins. Chronic toxicity was taken from 6-72hrs. Plastic pipette was used to prickle the larvae in the case where larvae show sign of death. Larvae in various experimental set up were fed biscuit and yeast powder.

2.4. Physicochemical Analysis

Multiplex water analysis and pH device was used to take readings for pH, water conductivity, salinity, and temperature of water in the set up.

2.5. Data Analysis

Data were entered into Microsoft Excel Spreadsheet and checked thoroughly. Descriptive statistics were used in result presentation. Analysis of Variance (ANOVA) test was used to test significance at P=0.05. Mortality was correlated with physicochemical parameters of water to check for correspondence. Probit analysis was used to model the lethal time for 50% and 95%.

3. RESULTS

3.1. Mean Mortality of Mosquitoes

Mean mortality of mosquito larvae exposed to two insecticidal test plants in Ethiope East LGA, Delta State, Nigeria is shown in Table 1. The mortality of larvae increased as concentration increased, but a slight decline was observed in *Culex* exposed to 1.0g of *Psoralea corylitolia*. The highest mortality was recorded in *Culex* mosquitoes exposed to 1.5g of *Psoralea corylitolia*. This was closely followed by *Anopheles* mosquitos exposed to same concentration of *Sesamum radiatum*. The differences between the mortalities of mosquito larvae were highly significant (P=0.0001).

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3.2. Time Mortality

The percentage time mortality of *Anopheles* and *Culex* mosquitoes to two insecticidal test plants is shown in Fig. 1 and 2. Generally, mortality increased with time as concentration increased. 1.5g of *Psoralea corylitolia* caused higher mortality in *Culex* mosquito larvae than in other concentrations and mosquito. *Psoralea corylitolia* did not cause acute toxicity in less than 1 hour of exposure. The optimum concentration (1.5g) of *Sesamum radiatum* caused high chronic mortality in both *Anopheles* and *Culex*. Other concentrations did too. There was significant mortalities with the use of insecticidal plants against the emergence of *Anopheles* and *Culex*.

Table 1: Mean mortality of mosquito larvae exposed to two insecticidal test plants in Ethiope East LGA, Delta State, Nigeria

Plants	Mosquito	Conc. (gram)	Mean±SE	F-ANOVA	P-value
Psoralea corylitolia	*Anopheles*	0.5	5.64±3.65		
	Culex	0.5	22.55±4.02		
	Anopheles	1.0	7.64±4.59		
	Culex	1.0	20.73±4.43		
	Anopheles	1.5	10.36±5.86		
	Culex	1.5	47.46±7.78	35.98	0.00000
Sesamum radiatum	*Anopheles*	0.5	22.91±7.82		
	Culex	0.5	26.91±9.62		
	Anopheles	1.0	31.46±8.50		
	Culex	1.0	33.27±8.50		
	Anopheles	1.5	45.64±9.18		
	Culex	1.5	43.46±7.63	11.15	0.000000

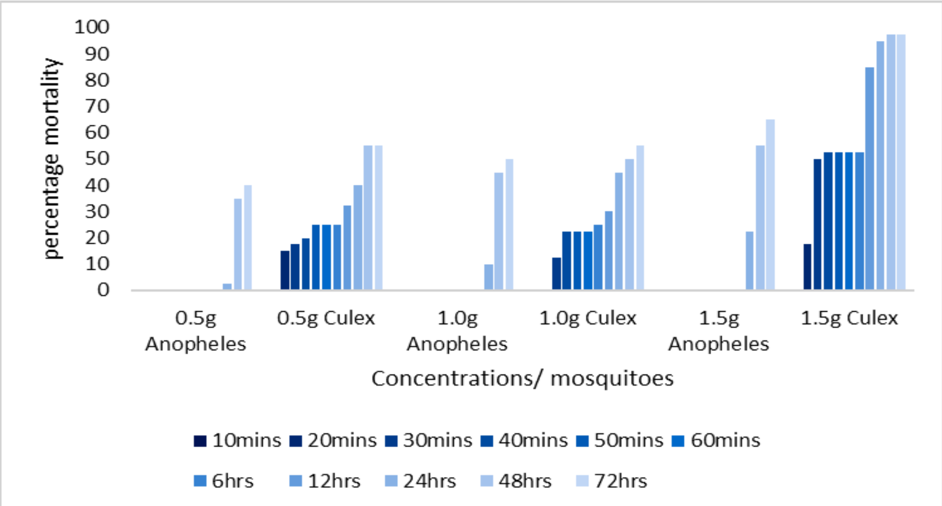

Fig. 1: Percentage time mortality of *Anopheles* and *Culex* mosquitoes to *Psoralea corylitolia* (F=19.57; P=0.000001).

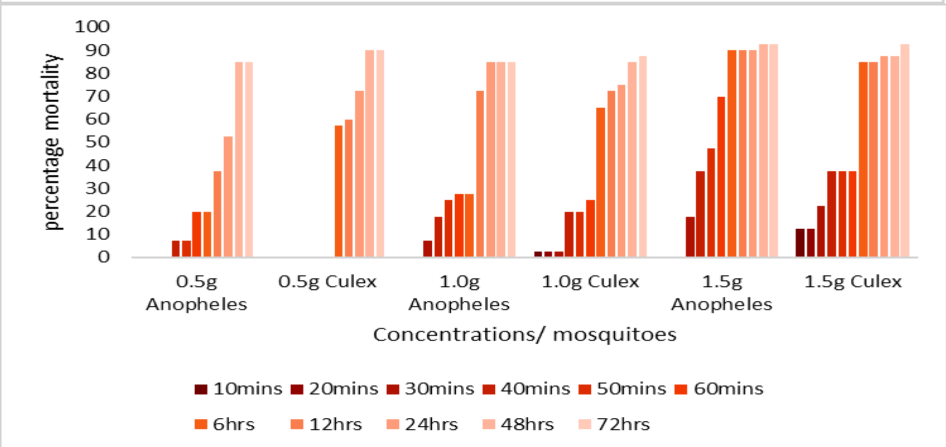

Fig. 2: Percentage time mortality of *Anopheles* and *Culex* mosquitoes to *Sesamum radiatum* (F=56.35; P=0.00001).

Some mosquitoes emerged in various concentrations. The percentage emergence of *Anopheles* and *Culex* mosquitoes to two insecticidal test plants is shown in Fig. 3 and 4. Emergence depreciated as the concentration increased with high mortality. The highest emergence in the *Psoralea corylitolia* was recorded with 0.5g of

Psoralea corylitolia exposed to *Anopheles* mosquito larvae. Emergence in the *Sesamum radiatum* was higher in 0.5 and 1.0g of the plant exposed to *Anopheles* and *Culex* mosquitoes. There was non-significance in the emergence of *Anopheles* and *Culex* mosquitoes to the insecticidal plants (P>0.05).

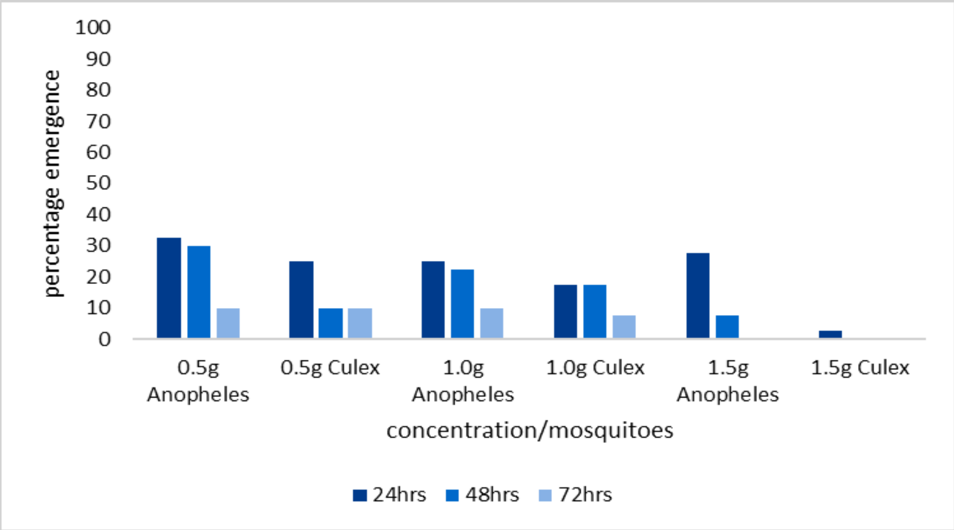

Fig. 3. Percentage emergence of *Anopheles* and *Culex* mosquitoes to *Psoralea corylitolia* (F=2.107; P=0.135).

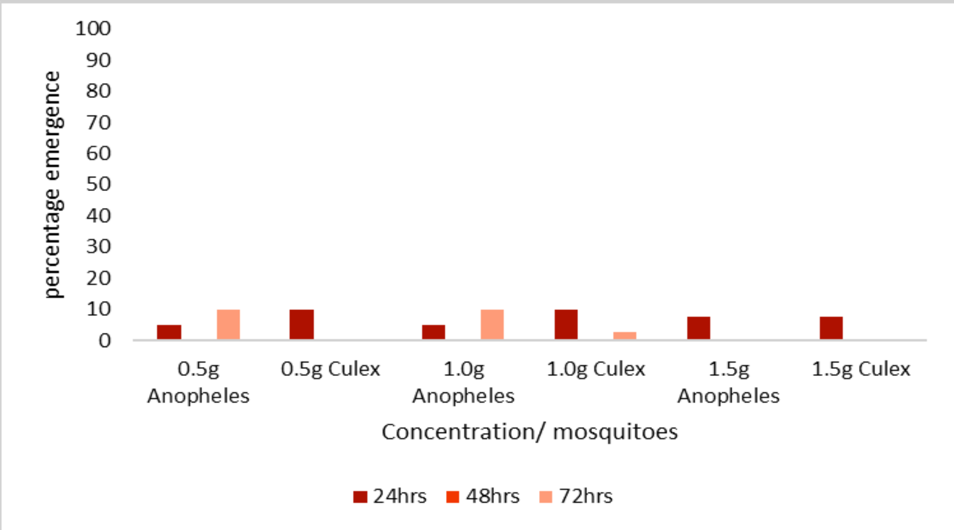

Fig. 4: Percentage emergence of *Anopheles* and *Culex* mosquitoes to *Sesamum radiatum* (F=0.161; P=0.972).

3.3. Sex Ratio of Emerged Mosquitoes

Sex ratio of mosquito emergence after exposure to two insecticidal test plants with time is shown in Table 2. The insecticidal test plants did not determine the sex of mosquitoes. There were more emerged female mosquitoes (n=146) than males (n=98). Male and female *Anopheles* were 66 and 84, respectively after exposure to plants. Similarly, male and female *Culex* were 32 and 62, respectively after exposure to plants. The difference in sex of mosquitoes exposed to the plants was not significant ($F_{sex\ ratio}$=1.127; P=0.357).

3.4. Correspondence Analysis

Correspondence analysis between physicochemical parameters and mortality of mosquito larvae to two insecticidal test plants in Ethiope East LGA, Delta State, Nigeria is shown Fig. 5. It appeared that all parameters except salt supported the mortality of *Culex* mosquito larvae more than in *Anopheles* mosquito larvae when exposed to powder of *Psoralea corylitolia*. Similarly, all parameters supported the mortality of both larvae exposed to *Sesamum radiatum* from 30 minutes to the end of the post experimental exposure.

3.5. Lethal Time Toxicity

Lethal time model of mosquito larvae exposed to two insecticidal test plants in Ethiope East LGA, Delta State, Nigeria is shown in Table 3. Lethal time for 50% of mosquitoes ranged from 31 to 5858min whereas LT_{95} ranged

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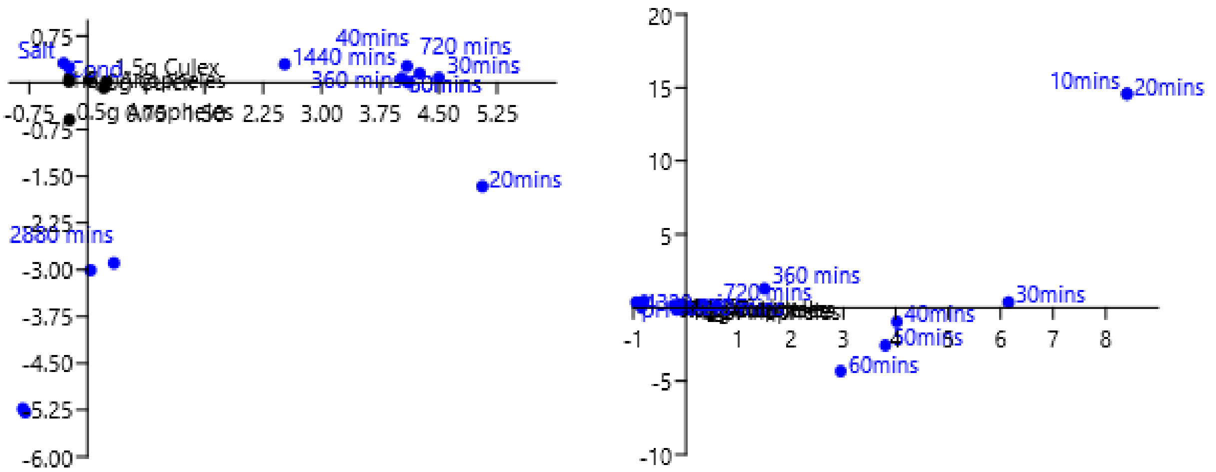
from 1435 to 10218min for the two insecticidal test plants respectively. *Culex* mosquitoes exposed to 1.5g of *Psoralea corylitolia* recorded the lowest lethal time and this followed by *Anopheles* mosquitoes exposed to same concentration of *Sesamum radiatum*. The lethal time model of the mosquitoes followed a well fitted curve as observed in the Pearson goodness of fit and r^2 (Table 3).

Table 2: Sex ratio of mosquito emergence after exposure to two insecticidal test plants with time.

Plant	Mosquito	Conc. (g)	Sex	24hrs (%)	48hrs (%)	72hrs (%)
Psoralea corylitolia	*Anopheles*	0.5	♂	14 (17.5)	6 (7.5)	6 (7.5)
			♀	12 (15.0)	8 (10.0)	2 (2.5)
		1.0	♂	10 (12.5)	4 (5.0)	2 (2.5)
		0.5	♀	10 (12.5)	8 (10.0)	6 (7.5)
			♂	14 (17.5)	0 (0.0)	0 (0.0)
		1.0	♀	8 (10.0)	6 (7.5)	0 (0.0)
	Culex	0.5	♂	12 (15.0)	0 (0.0)	2 (2.5)
			♀	8 (10.0)	8 (10.0)	6 (7.5)
		1.0	♂	2 (2.5)	6 (7.5)	0 (0.0)
		0.5	♀	12 (15.0)	8 (10.0)	0 (0.0)
			♂	2 (2.5)	0 (0.0)	0 (0.0)
		1.0	♀	0 (0.0)	0 (0.0)	0 (0.0)
Sesamum radiatum	*Anopheles*	0.5	♂	0 (0.0)	0 (0.0)	2 (2.5)
			♀	4 (5.0)	0 (0.0)	6 (7.5)
		1.0	♂	2 (2.5)	4 (5.0)	0 (0.0)
		0.5	♀	2 (2.5)	4 (5.0)	0 (0.0)
			♂	4 (5.0)	0 (0.0)	0 (0.0)
		1.0	♀	2 (2.5)	6 (7.5)	0 (0.0)
	Culex	0.5	♂	4 (5.0)	0 (0.0)	0 (0.0)
			♀	4 (5.0)	0 (0.0)	0 (0.0)
		1.0	♂	2 (2.5)	0 (0.0)	0 (0.0)
		0.5	♀	6 (7.5)	4 (5.0)	2 (2.5)
			♂	2 (2.5)	0 (0.0)	0 (0.0)
		1.0	♀	4 (5.0)	0 (0.0)	0 (0.0)



A. *Psoralea corylitolia* B. *Sesamum radiatum*

Fig. 5: Correspondence analysis between physiochemical parameters and mortality of mosquito larvae to two insecticidal test plants in Ethiope East LGA, Delta State, Nigeria.

4. DISCUSSION

This study determined the toxicity of two insecticidal test plants against the emergence of *Anopheles* and *Culex* in Ethiope East LGA, Delta State, Nigeria. This was done to ascertain their biological potency due to the urgent need for toxic materials with ecological and environmentally friendly potential. Different plant substances have been tested for their insecticidal potentials on various insects (Ojianwuna et al. 2011; Ojianwuna and Umoru 2011; Ojianwuna and Enwemiwe 2020b). Even some pyrethroids have been tried on adult mosquitoes (Ojianwuna and Enwemiwe 2020a). Most larcivicidal agents of plant origins are actively toxic against the three major mosquitoes causing disease transmission in Nigeria (Ojianwuna et al. 2021). Amongst these are the effects of leaf extracts from

Table 3: Lethal time model of mosquito larvae exposed to two insecticidal test plants in Ethiope East LGA, Delta State, Nigeria

Plant Powders	Mosquito Larvae	Conc. (g)	Regression Line	Pearson χ^2 Goodness of Fit (r^2)	LT_{50} (mins)	LT_{95} (mins)
Psoralea corylitolia	*Anopheles*	0.5	y= 0.010x-1.98	9.362 (0.907)	5858	10218
		1.0	y= 0.013x-2.01	11.56 (0.937)	4318	7200
		1.5	y= 0.017x-1.99	14.762 (0.960)	2715	6341
	Culex	0.5	y= 0.010x-19.01	5.496 (0.770)	1536	5759
		1.0	y= 0.011x+16.17	4.763 (0.716)	2859	4312
		1.5	y= 0.017x+44.53	3.222 (0.536)	31	1435
Sesamum radiatum	*Anopheles*	0.5	y= 0.021x+9.50	8.387 (0.887)	1348	4398
		1.0	y= 0.020x+21.46	4.110 (0.653)	419	4358
		1.5	y= 0.016x+42.46	2.317 (0.374)	56	4327
	Culex	0.5	y=0.023x+12.63	4.640 (0.705)	295	4331
		1.0	y= 0.019x+24.30	3.773 (0.613)	196	4328
		1.5	y=0.016x+39.65	3.010 (0.502)	199	4329

Note: Lethal time of 50% and 95% (LT_{50} and LT_{95}) are in minutes; P>0.05 suggests a well-fitting model, P<0.05 suggests an invalid model population.

three asteraceous plants which caused complete or partial mortality. Petroleum products have equally been tried against these mosquitoes (Ekedo et al. 2019; Ojianwuna et al. 2021b). Plants have been applied as topical treatment for other insect causing disease lesions either as extracted oils or grounded with positive results (Enwemiwe et al. 2020, Enwemiwe et al. 2021). Alam et al. (2018) revealed the toxic potential of *Psoralea corylifolia* in insect studies. Apart from this, plants have several potentials in life applications. Even the toxicity of *Sesamum indicum*, a relative of the plant under study has been tried on *Culex* species.

In this present study, the mortality of larvae increased as concentration increased with the two mosquitoes, but a slight decline was observed in *Culex* exposed to 1.0g of *Psoralea corylitolia*. Mortality was highly recorded in *Culex* mosquitoes exposed to 1.5g of *Psoralea corylitolia* and this was closely followed by *Anopheles* mosquitoes exposed to same concentration of *Sesamum radiatum.* The result of this study corresponded with the findings of Ojianwuna et al. (2021) where complete mortality was recorded with optimum concentration of *Bidens pilosa*, and *Spilanthes filicaulis*. The mortality was not in agreement with those reported in other insects (Ojianwuna et al. 2011; Ojianwuna and Enwemiwe 2020b; Ojianwuna and Enwemiwe 2021).

Mortality equally increased with time as concentrations of test plants increased. *Psoralea corylitolia* in 1.5g caused higher mortality in *Culex* mosquito larvae than in other concentrations and mosquito. They did not cause acute toxicity in less than 1 hour of exposure. The optimum concentration (1.5g) of *Sesamum radiatum* caused higher chronic mortality in both *Anopheles* and *Culex*. In the findings of the present study, plant powders did not cause acute toxicity in reduced time. It took longer time than possible as compared to the study of Ojianwuna et al. (2021). Emergence depreciated as the concentration increased with high mortality. The highest emergence in the *Psoralea corylitolia* was recorded with 0.5g of *Psoralea corylitolia* exposed to *Anopheles* mosquito larvae. Emergence in the *Sesamum radiatum* was higher in 0.5 and 1.0g of the plant exposed to *Anopheles* and *Culex* mosquitoes. Emergence inhibition was not determined in this study. To considerable extent, the plant powders did not suppress adult mosquito emergence as reported in the study of Ojianwuna and Enwemiwe (2022). Insecticidal test plants under study did not determine the sex of mosquitoes. There were more emerged female mosquitoes than males. Most studies do not consider the sex of mosquitoes after emergence unlike in the study of Ojianwuna and Enwemiwe (2020) where adult mosquitoes emerged and died due to the inhibitory effect of floating naphthalene in water.

In this study, it appeared that all parameters except salt supported the mortality of *Culex* mosquito larvae more than in *Anopheles* mosquito larvae when exposed to powder of *Psoralea corylitolia.* Similarly, all parameters supported the mortality of both larvae exposed to *Sesamum radiatum* from 30 minutes to the end of the post experimental exposure. Physicochemical parameters of mosquito breeding sites and termitarium show that it affects the abundance and distribution of species as reported in the study of Ojianwuna et al. (2021) and Akpan et al. (2020). Similar observation was reported in the study of Kipyab et al. (2015) as physical and chemical variables of the environment influenced the distribution of malaria vector in the field. Moreover, Emidi et al. (2017) revealed that high conductivity and saltiness of water encourage the abundance of *Anopheles* larvae. The plant powders may have influenced the salt and conductivity, thus causing variable mortalities. Lethal time for 50% of mosquitoes ranged from 31 to 5858min whereas LT_{95} ranged from 1435 to 10218min for the two insecticidal test plants respectively. *Culex* mosquitoes exposed to 1.5g of *Psoralea corylitolia* recorded the lowest lethal time and this followed by *Anopheles* mosquitoes exposed to same concentration of *Sesamum radiatum*. The lethal time recorded in this study is higher than those reported in other studies where plant materials were tried in other insects (Ojianwuna et al. 2018; 2021).

5. Conclusion

This study forms the baseline for the trial of *Sesamum radiatum* and dust of *Psoralea corylitolia* on the mortality of these mosquitoes. It has demonstrated that these two plants did not cause complete mortality on the mosquito larvae. Proper care is required in the interpretation of the result of this study as oil extraction of these plants could cause complete mortality in the mosquitoes. Optimum concentration of *Sesamum radiatum* on both mosquitoes and *Psoralea corylitolia* on *Culex* mosquitoes suppressed the emergence of larvae. More studies on the gas chromatogram of these plants are required to check for the active components.

Authors' Contributions

CC and VN designed the study, VN, MG and CC carried out the experiment, collected, analyzed field data, and interpreted the data. CC, MG, ET and VN wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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