Research Article / Araştırma Makalesi GU J Sci, Part A, 9(2): 87-95 (2022) https://doi.org/10.54287/gujsa.1079339

Gazi University
Journal of Science
PART A: ENGINEERING AND INNOVATION
http://dergipark.org.tr/gujsa

Moringa oleifera (Lam.) and *Momordica charantia* (Lam.¬) as Potential Larvicides and Fumigants of Culex *Mosquitoes*

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Keywords

Larvicide
Fumigant
Moringa
Momordica
Culex

Abstract

Mosquitoes cause life threatening diseases such as yellow fever, malaria, filariasis, encephalitis infection etc. The focus of this research is to evaluate the larvicidal and fumigant properties of Moringa oleifera and Momordica charantia extracts on the larvae and adult mosquitoes. The leaves of both plants were dried and pulverized into fine powder. Rotary evaporator was used to extract the plant oils. The results showed that Momordica charantia was more effective as larvicide than Moringa oleifera as its evoked 100% larval mortality at 20% concentration for 3 hours with LC50 and LC90 of 0.5% and 8.5% respectively. Moringa oleifera produced 100% larval mortality and LC50 (0.75%) and LC90 (10%) at 25% concentration for 4 hours. Similarly, the leaf extract of Momordica charantia is a better fumigant than Moringa oleifera, the former produced LC50 of 0.5% and LC90 of 3.75%, while the latter produced LC50 and LC90 of 1.05% and 4.25% respectively. The significantly higher larvicidal and fumigant activities observed in Momordica charantia is due to the presence of cardiac glycosides only in the plant in addition to saponins, tannins, flavonoids and alkaloids which are common to both plants. Therefore botanicals are advocated to be included in vector control programs. This is because botanicals are relatively safe, cheap and easy to obtain in many parts of the world.

Cite

Afolabi, O. J., & Olonisakin, A. A. (2022). *Moringa oleifera* (Lam.) and *Momordica charantia* (Lam.¬) as Potential Larvicides and Fumigants of Culex *Mosquitoes*. *GU J Sci, Part A*, *9*(2), 87-95.

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Article Process

Submission Date 25.02.2022
Revision Date 26.05.2022
Accepted Date 08.06.2022
Published Date 09.06.2022

1. INTRODUCTION

Mosquitoes are vectors of important parasites such as protozoans and nematodes which are responsible for fatal infections such as malaria, yellow fever and filariasis (Dahmana & Mediannikov, 2020; Bamou et al., 2021). The vectorial capacity of mosquitoes is as a result of their hematophagous attitude, during blood meal, mosquitoes acquire the pathogen from one vertebrate host and transmit to another (Powell, 2019). Highly efficient vectors must live in close association with the hosts and have a relatively long lifespan (Powell, 2019).

Plants are globally useful in protecting people from hematophagous insects and several researches have reported the repellent efficacy of plant oils. These plant oils are easily decomposed, ecologically friendly, popular and generally have low mammalian toxicity. Moreover, the plants are easily obtainable at cheaper cost in most endemic areas. In addition, plant oils contain several important properties. First, they easily penetrate insect cuticles, which increases their bioavailability. These properties could be of useful in reducing insect longevity on treated surface. Secondly, active ingredients in the plant oils may have specific mode of action, which make them good alternative to pyrethroids. Among plants that are useful as protectants are *Moringa* and bitter gourd. *Moringa*, which is the only genus in the family Moringaceae and common called drumstick tree (Milla et al., 2021). *Moringa* is a fast growing plant which is resistant to drought and originated from the southern foothill of Himalayas in northern India, and widely cultivated in

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tropical and subtropical area. It is used in water purification, and herbal medicine. *M. oleifera* leaf powder is also effective as soap for hand washing (Fidrianny et al., 2022). *M. oleifera* could be used as functional food and other industrial food applications (Oyeyinka & Oyeyinka, 2018). Therefore, *M. oleifera* provides nutrients that benefit health, making it a key food for food security in areas with fewer economic resources (Sagona et al., 2020), According to Kumar et al. (2010), the introduction of *Moringa oleifera* seed flour improves the organoleptic properties of different breads and biscuits; In addition, almost all parts of the plant: root, bark, gum, leaf, fruit (pods), flowers, seeds and seed oil, have been used to treat various disease such as skin infections, swelling, anemia, asthma, bronchitis, diarrhea, headache, joint pain, rheumatism, gout, diarrhea, heart problems, fevers, digestive disorders, wounds, diabetes, conjunctivitis, haemorrhoids, goitre, earache, measles and smallpox in the indigenous system of medicine (Thakur & Sharma, 2016). *Momordica charantia* or bitter gourd, is widely grown in Africa, Asia, and the Carribbean as edible fruit, which is extremely bitter. It originated in India subcontinent and introduce into China in the 14th century. Bitter gourd is important in Chinese cooking because of its bitter flavour (Abu-Odeh & Talib, 2021). Especially in soup and herbal teas. *M. charantia* on the other hand is useful as cancer prevention, diabetes treatment, fever, and in reducing blood glucose level (Afolabi et al., 2018). This study explored the larvicidal and fumigant properties of the *Moringa oleifera* and *Momordica charantia* leaf extracts on *Culex* mosquitoes.

2. MATERIALS AND METHOD

2.1. Preparation of the Plant Extracts

The leaves of *Moringa oleifera* and *Momordica charantia* used for this research were obtained from Apatapiti environment of Federal University of Technology Akure. The leaves were mildly washed in a bowl of clean water and subsequently air dried at room temperature (28 ± 2°C) for 25days, at the undergraduate research laboratory of the biology department. The dried leaves were pulverized into powder and blended with the electric blender. The powders were kept separately in labeled containers until when use.

2.2. Insect Culture

Larvae of mosquitoes used for this research were obtained from Sabo environment in Akure, the larvae were harvested and brought to the laboratory. The larvae were transfered to another plastic container containing water, where they were fed with yeast. Ten larvae were used for the larvicidal experiment and the rest were allowed to develop or emerge as adult in the experimental cage. The pupae transformed to adult in 4 days at temperature of 27±2°C, relative humidity of 70±10% and a cycle 14h of light and 10h darkness (Chiroma et al., 2018).

2.3. Preparation of Plant Extracts

The leaf powders of the plants were soaked in absolute ethanol for 72hrs to obtain the extracts. The soaked powders were mixed for 30 minutes every 24hrs to enhance more concentration of the extract. The mixture was filtered using muslin cloth. The filtrates were transfered into rotary evaporator to evaporate solvent at its boiling temperature 70°C. After this stage, the standard extract obtained was stored in a bottle till usage (Fidrianny et al., 2022).

2.4. Phytochemicals Screening

Moringa oleifera and Momordica charantia extracts were screened for the presence of tannin, phlobatannin, cardiac glycosides, saponins, steroids, terpenoids and flavonoids. For tannin, 5 g of each portion of the plant extracts was stirred with 10 ml of distilled water and filtered as described by Gul et al. (2017). Blue black, green, or blue-green precipitates formed following the addition of few drops of 5% ferric chloride were taken as evidence for the presence of tannins. Deposition of a red precipitate when aqueous solutions of leaf extract was boiled with 1% (v/v) HCl was taken as evidence for the presence of phlobatannin (Gul et al., 2017). Salkowski's test, as described by Akinneye and Afolabi (2014), was used to test for cardiac glycosides. Leaf extract (0.5 g) was dissolved in 2 ml of chloroform prior to the careful addition of 1% (v/v) H_2SO_4 to form a lower layer. A reddish-brown colour at the interface was taken as evidence for the cardiac glycoside. Concentration of saponin, steroids and terpernoids were measured adopting the methods of Akinneye and

Afolabi (2014). Plant materials (0.5 g) were mixed with acetic anhydride (2 ml) in the presence of concentrated H_2SO_4 (2 ml) to measure the concentration of steroids. For terpenoids, plant materials (0.5 g) re-suspended in distilled water were mixed with chloroform (2 ml) in the presence of concentrated H_2SO_4 (3 ml). Colour change in the presence of re-suspended plant materials and diluted ammonium solution (5 ml) was used to estimate the concentration of flavonoids.

2.5. Larvicidal Experiment

The stock solutions for the larvicidal experiment was prepared following the standard procedure of Pam et al. (2021). Crude extract of each plants was added to ethanol to achieve the desired concentrations (5%, 10%, 15%, 20%, and 25%). The extracts were mixed with water in a plastic container at the desired concentration in the presence of small amount of sucrose to serve as food source for the larvae. Then 10 mosquito larvae were tranferred into the plastic containers including the control. There were 5 replicates including one control for every concentration. Insect death was recoeded every 1hour for 4hrs, after which the larvae were introduced into distilled water to notice recovery. A recovery time of 5 minutes was allowed (Pam et al., 2021). The larval death in treatments was corrected for the controls (Kalimuthu et al., 2020). Larvae considered to be dead when insensitive to probe (Adedire et al., 2011).

2.6. Fumigant Test

The fumigant test was evaluted by placing ten mosquito adults in a test-tube covered with cotton wool suspended with a muslin cloth. 1ml of 5%, 10%, 15%, 20%, and 25% concentrations of the plant extracts was then injected in the cotton wool on the test-tube. Death rate was determined every 5minutes for 20 minutes.

2.7. Data Analysis

Research data were subjected to one-way analysis of variance and means were separated using Tukey's test at $p \leq 0.05$. All data generated were processed using SPSS version 22.

3. RESULTS

The results presented in Table 1 showed the phytochemical analysis results of *Moringa oleifera and Momordica charantia* leaf extracts. The results revealed the presence of terpenold, saponins, steroid, tannins, flavonoids, alkaloids in both plant extracts. Meanwhile, phlebotannins and cardiac glycosides were absent in the leaf extract of *Moringa oleifera* while only phlebotannins were absent in *Momordica charantia*. In addition, only the leaf extract of *Momordica charantia* was noted to contain cardiac glycosides.

Table 1. *Phytochemical analysis of the Moringa oleifera and Momordica charantia*

Phytochemicals	*Moringa oleifera* Leaves	*Momordica charantia* Leaves
Terpenoids	+	+
Saponins	+	+
Phlebotannins	-	-
Steroids	+	+
Tannins	+	+
Cardiac glycosides	-	+
Flavonoids	+	+
Alkaloids	+	+

The larvicidal effect of the two plant extracts was presented in Table 2 and 3. The results showed that *Moringa oleifera* achieved 73.33% larval mortality at 25% concentration for 1 hour of exposure, the mortality increased to 96.67% at the same concentration for 2 hours of exposure. Meanwhile, the plant recorded 100% larval mortality at 25% concentration for 3 hours of exposure (Table 2). The highest larval mortality evoked by the *Moringa oleifera* was 100% at 3 hours of exposure while the lowest mortality (30%) was recorded at 1 hour of exposure. The larvicidal result of the leaf extract of *Momordica charantia* as presented in Table 3 showed that the plant extract evoked 100% larval mortality at 20% concentration for 3 hours of exposure while the lowest larval mortality (53.33%) was recorded at 5% concentration for 1 hour.

Generally it was observed that the larvicidal effects of both plant extracts were concentration and time dependent. This shows that the larval mortality increased as the concentration and time of exposure increased.

Table 2. *Larvicidal effect of Moringa oleifera leaf extract on Culex mosquitoes*

Concentration (%)	Mortality (%)			
	1 hour	2 hours	3 hours	4 hours
Control	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
5	$30.00^{a} \pm 10.00$	$60.00^{b} \pm 11.67$	$73.00^{b} \pm 3.33$	$83.33^{b} \pm 0.00$
10	$50.00^{a} \pm 10.00$	$80.00^{b} \pm 5.80$	$86.67^{b} \pm 3.33$	$90.00^{b} \pm 5.80$
15	$45.77^{a} \pm 3.33$	$76.67^{b} \pm 8.88$	$83.33^{b} \pm 0.33$	$86.67^{b} \pm 5.80$
20	$70.00^{a} \pm 10.00$	$86.67^{a} \pm 6.67$	$90.00^{a} \pm 0.58$	$90.00^{a} \pm 5.80$
25	$73.33^{a} \pm 3.33$	$96.67^{b} \pm 3.33$	$100.00^{b} \pm 0.00$	$100.00^{b} \pm 0.00$

Table 3. *Larvicidal effect of Momordica charantia leaf extract on Culex mosquitoes*

Concentration (%)	Mortality (%)			
	1 hour	2 hours	3 hours	4 hours
Control	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
5	$53.00^{a} \pm 3.33$	$83.33^{b} \pm 3.33$	$83.33^{b} \pm 3.33$	$83.33^{b} \pm 5.80$
10	$73.33^{a} \pm 6.67$	$93.33^{b} \pm 3.33$	$93.33^{b} \pm 3.33$	$96.67^{b} \pm 5.80$
15	$86.67^{a} \pm 3.33$	$93.33^{b} \pm 3.33$	$93.33^{b} \pm 3.33$	$93.33^{b} \pm 3.33$
20	$90.00^{a} \pm 5.80$	$96.67^{a} \pm 3.33$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$
25	$73.33^{a} \pm 3.33$	$86.67^{a} \pm 6.67$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$

The results as presented in Table 4 and 5 showed that *Moringa oleifera* produced mortalities of 46.67%, 93.33%, 100% and 100% adult mortalities at 25% concentration for 5, 10, 15 and 20 minutes respectively. However, the least adult mortality (10%) of the plant extract was observed at 5% concentration for 5 minutes while the highest mortality (100%) was recorded at 25% concentration for 15 minutes. The fumigant effect of *Momordica charantia* showed that the leaf extract evoked 63.33%, 96.67%, 100% and 100% at 25% concentration for 5, 10, 15 and 20 minutes respectively.

Table 4. *Fumigant effect of Moringa oleifera leaf extract on Culex mosquitoes*

Concentration (%)	Mortality (%)			
	1 hour	2 hours	3 hours	4 hours
Control	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
5	$10.00^{a} \pm 0.00$	$23.33^{a} \pm 5.80$	$90.00^{b} \pm 5.80$	$100.00^{b} \pm 0.00$
10	$13.33^{a} \pm 3.33$	$53.33^{b} \pm 5.80$	$93.33^{c} \pm 6.67$	$100.00^{c} \pm 0.00$
15	$33.33^{a} \pm 3.33$	$66.67^{b} \pm 5.80$	$96.67^{c} \pm 3.33$	$100.00^{c} \pm 0.00$
20	$40.00^{a} \pm 10.00$	$86.67^{b} \pm 5.80$	$96.67^{b} \pm 3.33$	$100.00^{b} \pm 0.00$
25	$46.67^{a} \pm 5.80$	$93.33^{b} \pm 6.67$	$100.00^{b} \pm 0.00$	$100.00^{b} \pm 0.00$

Table 5. *Fumigant effect of Momordica charantia leaf extract on Culex mosquitoes*

Concentration (%)	Mortality (%)			
	1 hour	2 hours	3 hours	4 hours
Control	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
5	$13.33^{a} \pm 3.33$	$46.67^{b} \pm 8.80$	$86.67^{c} \pm 3.33$	$96.67^{c} \pm 3.33$
10	$16.67^{a} \pm 3.33$	$53.33^{b} \pm 5.80$	$96.67^{c} \pm 3.33$	$100.00^{c} \pm 0.00$
15	$50.00^{a} \pm 3.33$	$73.33^{b} \pm 3.33$	$100.00^{c} \pm 0.00$	$100.00^{c} \pm 0.00$
20	$50.00^{a} \pm 5.67$	$80.00^{b} \pm 5.67$	$100.00^{c} \pm 0.00$	$100.00^{b} \pm 0.00$
25	$63.33^{a} \pm 3.33$	$96.67^{b} \pm 3.33$	$100.00^{b} \pm 0.00$	$100.00^{b} \pm 0.00$

The toxicities of the two plant extracts were compared using LC50 and LC90 as shown in Table 6. The results showed that leaf extract of *Momordica charantia* was more toxic as larvicide than that of *Moringa oleifera*. This is because 50% and 90% population of the test animals (*Culex* mosquitoes) died when 0.5% and 8.5% concentrations of *Momordica charantia* were applied to the mosquitoes respectively. Meanwhile 50% and 90% population of *Culex* mosquitoes died when 0.75% and 10% concentrations were applied to the *Culex* mosquitoes. Similarly, leaf extract of *Momordica charantia* was more toxic to the mosquitoes than the leaf extract of *Moringa oleifera*. This implies that at 0.5% and 3.75% concentrations, 50% and 90% *Culex*

mosquito populations were killed by *Moringa oleifera* while the same mortalities were achieved by *Momordica charantia* at 1.05% and 4.25% concentrations respectively (Table 6).

Table 6. *Lethal toxicity of the plant extracts on Culex mosquitoes*

Plant extracts	Concentration (%)	LC50	LC90
Larvicides			
Moringa oleifera	5		
	10		
	15		
	20	0.75	10.0
	25		
Momordica charantia	5		
	10		
	15	0.5	8.5
	20		
	25		
Fumigant			
Moringa oleifera	5		
	10	1.05	4.25
	15		
	20		
	25		
Momordica charantia	5		
	10	0.5	3.75
	15		
	20		
	25		

4. DISCUSSION

The result revealed that *Moringa oleifera* and *Mormodica charantia* extracts tested as larvicide and fumigant had significant effects on larvae and adult *Culex* mosquitoes and were all effective at different concentrations and time intervals. All these plants are relatively safe, cheap and easy to obtain in many parts of the world and their activities is traceable to their active ingredients. This finding agrees with the report of Pam et al. (2021) who elucidated that the toxicity of phytochemicals on target species depends on the plant's parts used. Other factors are the species response and the life stages exxposed to the specific extract, extraction solvent, plant origin, phyto-sensitivity of phytochemicals, species growth and reproduction (Kalimuthu et al., 2020). The plant oils are effective as larvicides, because the oils could block the spiracles, resulting in asphyxiation and death of the larvae. This antioxidant activity of oil has been reported by other authors (Bukar et al., 2010; Adedire et al., 2011). The results also showed *Momordica charantia* leaf to be the most potent of the two plants as there was 100% larval mortality at 20% concentration for 3hours with LC50 and LC90 of 0.5%

and 8.5% respectively, th*is* is followed by *Moringa oleifera* leaf extract with a total mortality of 25% for 3hour and LC50 and LC90 of 0.75% and 10% respectively. This maybe due to the presence of flavonoid present in both plant extracts (Anwari et al., 2007). The research work of Aina et al. (2009) supports the findings. The authors affirmed that *Moringa oleifera* leaf is highly potent antidiabetic plant, due to the presence of flavonoids which inhibit a-amylase activity to regulate the blood glucose which results in mosquito death. The significant higher larvicidal property observed in *Momordica charantia* might be as a result of the presence of cardiac glycosides which are only present in *Momordica charantia* and absent in *Moringa oleifera* leaf extract. It was observed in the experiment that zero mortality was recorded in all the control experiments. This suggests that mosquito death was caused by the plant extracts. *M. oleifera* toxicity has been confirmed, as many studies have found variou phytochemicals to be toxic against insects and lower mammals (Asare et al., 2012). The leaf has a high concentration of saponins, which can be potentially harmful for vegetarians, as their consumption reduces the bioavailability of divalent and trivalent metals such as Zn and Mg (Canett-Romero et al., 2014). Moringin alkaloids, spirochin and the phytochemical benzothiocyanate have been found in the root and bark, toxic substances that predominate in the root and bark; The spirochin has been found to block the insect spiracles and asphyxiate the insects (Chiroma et al., 2018).

In the fumigant experiment, significant death was reported for all experimental concentrations but at different time interval. Adult death (100%) was recorded at 5%, 10%, 15%, 20% and 25% concentrations for 20minutes for *Moringa oleifera* extract. However, the lethal concentration (LC50: 0.5% and LC90: 3.75%) showed that *Momordica charantia* was more effective as fumigant on the adult *Culex* mosquitoes than the *Moringa oleifera* with LC50 of 1.05% and LC90 of 4.25%. The high toxicity of *Momordica charantia* against mosquito adults could be due to its strong pungent smell which blocked insect trachea and killed the insect by asphyxiation. Plants with high pungent smell have been reported to have fumigant effect against insect pests (Aina et al., 2009). It was generally observed for all the experiments that mortality increases as the concentration and time interval increase.

5. CONCLUSION

The two botanicals (*Moringa oleifera and Momordica charantia*) used in this research had showed strong larvicidal and adulticidal efficacies and hence can be used in controlling the mosquito larvae and adults in order to reduce the prevalence of malaria in endemic areas. The phytochemical screening of the plants has also shown that the plants are relatively safe, inexpensive and readily available in many parts of the world.

ACKNOWLEDGEMENT

The authors acknowledged the contribution of Mrs E.T. Ojo in the preparation and extraction of the plant extracts and oils respectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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