Effects Of Selected Plant Seed Extracts on The Hatchability of *Aedes Aegypti* Eggs

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***Abstract*—*Aedes aegypti* is the primary vector of many viral diseases, including dengue fever and dengue hemorrhagic fever. It has also been reported that this mosquito has developed insecticide resistance. Thus, there is needs for effective and environmentally friendly alternatives. This study investigated the effects of selected plant seed extracts (*Moringa Oleifera, Carica papaya, and Citrus aurantifolia)* on the hatching of *Aedes aegypti* eggs. Eggs were obtained from laboratory-reared mosquitoes at the insectary of the Department of Zoology, Obafemi Awolowo University, Ile-Ife, and they were collected into transparent cups. Twenty eggs were exposed to different concentrations of plant seed extract solutions (4, 8, 12 g/L) dissolved for 24, 48, and 72 hrs. and also to different concentrations of extracts (4, 8, and 12 gL^{-1}) boiled for 5, 10, and 15 mins respectively. The results showed that most eggs hatched within 24 hours in dissolved extracts of *C. papaya* and *C. aurantifolia*, whereas a delay was observed in the hatching in *M. oleifera* dissolved extract. The greatest delay in egg hatching was recorded in the boiled extracts of all three plants. Notably, the highest concentration of (12g/L) effectively inhibits hatchability compared to the control group (0%). Morphological examinations indicated a possible alternation in the egg structure, which may have contributed to the delay in the normal hatching process. These findings suggested that plant seed extracts possess ovicidal potential and could be explored as eco-friendly tools for the control of *A. aegypti*.**

***Index Terms*—Aedes aegypti, eggs, *Moringa Oleifera, Carica papaya, and Citrus aurantifolia,* hatchability.**

I. INTRODUCTION

Mosquitoes have been persistent for years through the process of evolution making them to survive under adverse conditions (Manimegalai and Sukanaya, 2014). Aedes aegypti is known to be a vector of dengue viruses worldwide. It is a species that is represented as synanthropic and anthropophilic in nature, and this species can survive in many regions, making it very common in tropical and subtropical places (Weaver *et al.,* 2016). They are extremely common in areas lacking piped water systems and depend greatly on stored water for breeding. Male and female adult feed on the nectar of plants; however, females feed primarily on blood to produce eggs (David *et al.,* 2023). The tropical areas are the most susceptible to parasitic diseases and are also at risk of contracting arthropod-borne diseases due to climate change and the rise in temperature levels worldwide. Dengue and dengue hemorrhagic fever are primarily spread by Aedes mosquitoes, with approximately 50 million cases of dengue hemorrhagic fever annually. The mosquito also transmits zika virus, especially Aedes aegypti and Aedes albopictus (Kean *et al.,* 2014)

The female *A. aegypti,* when ingesting blood, may also inject a virus, thus becoming an arbovirus vector. The infection is transmitted and initiated in a healthier host who receives the infective blood along with the pathogen that was secreted in the mosquito's saliva (Rose *et al.,* 2023). Thus, the females help in transmitting diseases from one person to another. The females produce 100 to 200 eggs after taking a complete blood meal. The eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers, and they are laid singly rather than in masses (Clement 1992). The eggs are long, smooth, ovoid-shaped, and approximately one millimeter long. When the eggs are first laid, they appear white but within minutes turn shiny black. In warm climates such as the tropics, eggs may hatch within two days, whereas in temperate climates, development can take up to a week (Farnesi *et al.,* 2017). The female does not lay all her eggs at once, but can lay them out over hours or days, depending on the availability of suitable substrates. Most eggs are often placed at varying distances above the water line

by the female. This oviposition behavioral pattern and also the egg's ability to survive for longer period of time has make this species of mosquito to thrive better in many parts of the world (Ajayi *et al.*, 2023). The behavioral shift.

Control of mosquitoes have been achieved by the use of insect growth regulators (bacterial larvicides, Methoprene and diflubenzuron), Organophosphorus pesticides (temephos, fenthion and chlorpyrifos) (Dong and Young, 2013), however, this synthetic organic chemical have cause ecosystem degradation and the insects are showing pesticides resistance ability. These pesticides also killed non-target organisms and caused harmful effects on human due to their broad spectrum of activity. To evade this problem, a major emphasis has been placed on the use of non-synthetic means to control insects. Phytochemicals are chemicals that are derived from various parts of plants with fewer side effects and degrade over time. Targeting mosquitoes' larvae and eggs is also more desirable than controlling adults because they are concentrated in a relatively small area. Thus, controlling insects at the early stages, using biological control methods such as plant seed extract, would be a good approach in mosquitoes control program.

II. MATERIAL AND METHOD

Description of site

The study was carried out in the Department of Zoology insectary, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

Data Collection and Methodology

Mosquito eggs used in the present study were those of *Aedes aegypti* collected at the insectary unit.

Preparation of Plant Extracts

Powdered seeds of *M. oleifera, C. aurantifolia*, and *C. papaya* were measured in 4, 8, and 12 g and were dissolved in 2 L of distilled cold water for 24, 48, and 72 hrs respectively. The same weights of powder seeds were put in 2 L of distilled water and boiled for 5, 10, and 15 mins respectively. Each of the mixtures was filtered using a muslin cloth to obtain clear solutions. The solutions were put in capped plastic bottles, labelled appropriately, and subsequently used for the various experiments.

Hatching of Eggs

Egg strips (containing 20 eggs each) of *A. aegypti* were collected at the insectary unit of the Zoology department, Obafemi Awolowo University. The eggs (20) were exposed to each concentration of *M. oleifera, C. aurantifolia,* and *C. papaya* seed extracts. The time of hatching was observed and recorded.

Statistical Analysis

The data obtained were analyzed using the Statistical Analysis Package (SPSS) and Microsoft Office Excel Worksheet.

III. RESULT

The effect of dissolved plant extracts on the hatching time of *A. aegypti* eggs is presented in Table 1. In the control experiment, eggs consistently hatched after 24 hours at all observation periods. Most treatments at the lowest concentration of 4 g/L did not affect egg hatching. However, an increase in concentration resulted in a noticeable delay in hatching. Eggs that are exposed to *Moringa oleifera* and *Citrus aurantifolia* at higher concentrations of 8 and 12 g/L showed a delay of 32 hours to hatch, while *Carica papaya* showed little or no effect at this duration. The longest delay was observed at the highest concentration of 12 g/L, that were dissolved for 72 hrs, Hatching was extended to 40 hours in *M. oleifera* and *C. aurantifolia*, while *C. papaya* delayed hatching to 32 hours. At 72 hrs dissolved extracts, there was a marked delay in egg hatching compared to the control. *Moringa oleifera* produced the strongest effect, with eggs taking up to 64 hours to hatch at concentrations 8 and 12 g/L. Both *Citrus aurantifolia* and *Carica papaya* also delayed hatching, with mean hatching times reaching 40 hours at the highest concentration.

The effect of boiled plant extracts on the hatching time of *Aedes aegypti* eggs is presented in Table 2. Eggs in the control group consistently hatched after 24 hrs, indicating normal development in the absence of treatment. Boiled extracts prepared for 5 mins caused a moderate delay in egg hatching. *Moringa oleifera* delayed hatching to 32 hrs at the lowest concentration and to 40 hrs at higher concentrations. *Citrus aurantifolia* showed delayed hatching only at concentrations 8 and 12 g/L, while *Carica papaya* did not affect egg hatching, as hatching time remained comparable to the control, as represented in Table 2.

At 10 mins boiling duration, a greater inhibitory effect was observed, which showed that *Moringa oleifera* significantly prolonged hatching time, with eggs hatching after 64 hrs at concentrations 8 and 12 g/L. *Citrus aurantifolia* delayed hatching to 64 hrs at the highest concentration, whereas *Carica papaya* produced a mild effect, delaying hatching to 32 h at higher concentrations. The strongest effect was recorded with 15-minutes boiled extracts. *Moringa oleifera* effectively prolonged egg hatching, with mean hatching times of 72 hrs at concentration 8 and 12 g/L. *Citrus aurantifolia* delayed hatching to 64 hrs at the highest concentration, while *Carica papaya* caused a relatively small delay, with hatching occurring at 40 hrs as represented in Table 2.

Table 1: Effect of different concentrations of dissolved plant extracts on the hatching of *A. aegypti* eggs

Dissolved Extract (duration)	Plant extracts	Concentration	Mean time of hatching (X±S.D)
24 hr	*M. oleifera*	4	24.00 ± 0.00
		8	24.00 ± 0.00
		12	32.00 ± 0.00
	C. aurantifolia	4	24.00 ± 0.00
		8	32.00 ± 0.00
		12	32.00 ± 0.00
	C. papaya	4	24.00 ± 0.00
		8	24.00 ± 0.00
	Control	0	24.00 ± 0.00
48 hr	*M. oleifera*	4	24.00 ± 0.00
		8	32.00 ± 0.00
		12	40.00 ± 0.00
	C.aurantifolia	4	24.00 ± 0.00
		8	24.00 ± 0.00
		12	40.00 ± 0.00
	C. papaya	4	24.00 ± 0.00
		8	24.00 ± 0.00
		12	32.00 ± 0.00
	Control	0	24.00 ± 0.00
72 hr	*M. oleifera*	4	32.00 ± 0.00
		8	64.00 ± 0.00
		12	64.00 ± 0.00
	C. aurantifolia	4	32.00 ± 0.00
		8	32.00 ± 0.00
		12	40.00 ± 0.00
	C. papaya	4	32.00 ± 0.00
		8	32.00 ± 0.00
		12	40.00 ± 0.00
	Control	0	24.00 ± 0.00

Table 2: Effect of different concentrations of boiled plant extracts on the hatching of *A. aegypti* eggs

Boiled Extracts (duration)	Plant extract	Concentration	Mean time of hatching (X±S.D)
5 min	*M. oleifera*	4	32.00 ± 0.00
		8	40.00 ± 0.00
		12	40.00 ± 0.00
	C. aurantifolia	4	24.00 ± 0.00
		8	32.00 ± 0.00
		12	32.00 ± 0.00
	C. papaya	0	24.00 ± 0.00
		4	24.00 ± 0.00
		8	24.00 ± 0.00
	Control	12	32.00 ± 0.00
10 min	*M. oleifera*	4	32.00 ± 0.00
		8	64.00 ± 0.00
		12	64.00 ± 8.00
	C. aurantifolia	4	32.00 ± 0.00
		8	32.00 ± 0.00
		12	64.00 ± 0.00
	C. papaya	4	24.00 ± 0.00
		8	32.00 ± 0.00
		12	32.00 ± 0.00
	Control	0	24.00 ± 0.00
15 min	*M. oleifera*	4	72.00 ± 0.00
		8	88.00 ± 0.00
		12	88.00 ± 0.00
	C. aurantifolia	4	32.00 ± 0.00
		8	40.00 ± 0.00
		12	64.00 ± 0.00
	C. papaya	4	32.00 ± 0.00
		8	32.00 ± 0.00
		12	40.00 ± 0.00
	Control	0	24.00 ± 0.00

IV. DISCUSSION

The study showed that both dissolved and boiled plant extracts significantly delayed the hatching of *A. aegypti* eggs, and the effect depends on the plant species, extract concentration, and the method used for the preparation. The control group experiment showed that hatching was not delayed (within 24 hrs), which confirmed that the delay of hatching of eggs exposed to different extracts was due to the plant extract effect. At low concentrations, the effect was minimal, which indicates that there may be a threshold dose required to impact embryonic development compared to high concentrations, with an increase in egg hatching timing. This inhibition of mosquito development by

plant compounds has also been reported in other studies, where higher concentrations generally produced stronger effects on insect development rates (David *et al.,* 2023).

Moringa oleifera consistently produced the greatest delay in hatching time, followed by *Citrus aurantifolia*, while *Carica papaya* had the least effect. The strong effect of *M. oleifera* is supported by previous research, which concluded that the lectins and other in this plant seed exhibit ovicidal activity against *A. aegypti* eggs by disrupting egg structure and preventing normal development (Kean *et al.,* 2014). Moreover, it has been reported that *M. oleifera* seed lectins are shown to reduce hatching rates, indicating that there is a direct toxic effect on the embryos (Dong and Young, 2013). The limited effect of *C. papaya* in delaying egg hatching may be due to its phytochemical composition or it may have weaker penetrating active compounds into the egg since the egg shells are hard (Alam *et al.,* 2010), thus, their ovicidal potential appears to be lower, as observed in the study. Boiled extracts generally exhibited stronger inhibitory effects than dissolved extracts that were used for this study, and this effect increased with longer boiling times, which may be due to increased release of heat-stable bioactive compounds. And also, there may be a breakdown of complex plant constituents into more potent forms. This observation aligns with the research of Couret *et al. (*2014), indicating that heat treatment can enhance the availability of certain bioactive molecules in plant extracts, which in turn may have a significant effect on mosquitoes' survival rate. For extracts boiled for 10 or 15 mins, *M. oleifera* again showed the strongest inhibition of egg hatching, delaying hatching by more than three times that of the control experiment, and *Citrus aurantifolia* showed moderate inhibitory effects at higher concentrations, while *Carica papaya* showed only modest delays.

V. CONCLUSION

The findings of this study show that plant extracts can delay the hatching of *A. aegypti* eggs, and that this effect also depends on the method of extraction used. All the plant seed used showed some level of activity, *Moringa oleifera* consistently caused the greatest delay in egg hatching, followed by *C. aurantifolia*, whereas *C. papaya* had the weakest effect. The result showed that boiled extracts were more effective than dissolved extracts, suggesting that heat enhances the release of active compounds that affect mosquito hatchability. These results indicate that the plant seed extracts study has potential as a natural and environmentally friendly option for controlling mosquito eggs. Further studies are needed to identify the active compounds and to confirm their effectiveness under field conditions.

Declaration

The author affirms that there are no financial or non-financial competing interests.

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