A Study of the Effects of Seed Extracts of Some Plants on Adult Oviposition of Eggs of *Aedes Aegypti* L.

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***Abstract*—Insects are of importance from both public health and agricultural points of view, as they transmit diseases, while others are pests of field crops and stored products. Among species of insects, mosquitoes are very important because they transmit several diseases such as malaria, filariasis, dengue, Japanese encephalitis. *A. aegypti* is the primary carrier for viruses that cause dengue fever, dengue haemorrhagic fever, yellow fever, Zika virus, and chikungunya fever. Many insects have developed resistance to insecticides, especially most of the synthetic insecticides. Synthetic insecticides are the most commonly used insecticides in controlling insects, but after facing several problems due to over-application of synthetic insecticides in nature, there was renewed interest in phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms. The efficacy of phytochemicals against mosquito larvae varies significantly depending on plant species, plant parts used, age of plant parts, and solvent used during extraction. Hence, this study aims to determine the effects of boiled and dissolved seed extracts of *Moringa oleifera, Citrus aurantifolia,* and *Carica papaya* on oviposition of *A. aegypti.* The powdered seeds of *Moringa oleifera, Citrus aurantifolia,* and *Carica papaya* were measured out in 4, 8, and 12 g and were dissolved in 2 L of distilled cold water for 24, 48, and 72 hours, respectively. The same weights of powdered seeds were also put in 2 L of distilled cold water and boiled for 5, 10, and 15 mins respectively. The effects of boiled extracts of *M. oleifera, C. aurantifolia,* and *C. papaya* were concentration dependent and also increased with an increase in the period of boiling from 5 to 15 mins. The number of eggs laid decreased with an increase in periods of boiling and duration of dissolved extracts. The number of eggs laid by the gravid female was also concentration dependent in the three plant seed extracts from 4 - 12 gl^{-1} respectively. The eggs deposited in the dissolved extracts were higher than those deposited in the boiled extracts. *M. oleifera* had the lowest number of eggs in 12 gL^{-1} at 48 hours, dissolved extract with 9.00 ± 1.68 eggs and 6.33 ± 3.33 eggs in 12gL^{-1} of 15 mins boiled extract compared with *C. aurantifolia* (55.75 ± 4.00 and 19.67 ± 2.61 eggs) and *C. Papaya* (78.33 ± 4.67 and 44.67 ± 1.33 eggs). There was a reduction in oviposition of gravid females of *A. aegypti* in the boiled and dissolved seed extracts.**

Index Terms—*Aedes aegypti,* extract, *Moringa oleifera, Citrus aurantifolia and Carica papaya,* oviposition.

I. INTRODUCTION

Aedes aegypti is the primary vector of yellow fever, a disease that is prevalent in tropical parts of the world. They are container-inhabiting mosquitoes and will often proliferate in artificial containers in urban habitats with diurnal mating and host-seeking behavior (Gubler, 2011). Females produce 100-200 eggs after taking a complete blood meal. Male and female adults feed on the nectar of plants; however, females feed primarily on blood in order to produce eggs, and are active in the daytime. Females are larger than males, and can be distinguished by small palps tipped with silver or white scales. Males have plumose antennae, whereas females have sparse, short hairs.

After a blood meal, timing for an ovipositional flight is determined by the optimal combination of rainfall, humidity, temperature, and wind speeds (Gloria-Soria *et al.,* 2016). Once flight has been initiated, mosquitoes locate and evaluate potential sites using a combination of olfactory and visual cues (Rose *et al.,* 2023). As a female approaches a potential site, several factors determine whether the site is acceptable or not. These factors vary between species, and can potentially vary between individuals. Temperature, humidity, salinity, and oxygen content are all important components, as well as volatiles emitted from the water (Rose *et al.,* 2023). Some mosquitoes detect oviposition pheromones released by previous females or the presence of eggs, and are often attracted to water that contains conspecific larvae (Furtami *et al.,* 2020).

A. aegypti follow a slightly different behavior pattern when selecting an oviposition substrate. Clements (1999) outlined *A. aegypti* oviposition behavior, beginning with erratic flight patterns over the substrate until a female found a place to settle. Once a female had landed, she rested on all six legs. Females walked to the edge of the water and walked along the waterline until a suitable location was determined (Clement *et al.,* 2013). Once an appropriate location was found, she would orient herself facing away from the substrate and raise the meta-thoracic legs. *A. aegypti* then appears to become inactive. After inactivity, the female would raise and lower her legs synchronously, and the abdomen was arched downward to release an egg, and can produce an average of 100 to 200 eggs.

The number of eggs produced is dependent on the size of the blood meal. Females can produce up to five batches of eggs during their lifetime (Dick *et al.,* 2012). A smaller blood meal produces fewer eggs. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers, and are laid singly, rather than in masses. Not all the eggs are laid at once, but can be spread out over hours or days, depending on the availability of suitable substrates. Most often, eggs will be placed at varying distances above the water line, and a female will not lay the entire clutch at a single site, but rather spread out the eggs over two or more sites (Iwamura *et al.,* 2020). This behavior increases their survival rate and population, aside from the facts that they are developing resistance to many synthetic insecticides reported (Bhatt *et al*., 2013). Hence, the study focuses on the use of phytochemicals as insecticides due to its ecofriendly properties and easy accessibility for usage.

II. MATERIALS AND METHODS

The fruits of *Moringa oleifera, Citrus aurantifolia,* and *Carica papaya* were purchased from the Ile-Ife local market and were identified and authenticated at the Department of Botany of Obafemi Awolowo University. The fruits of *C. aurantifolia* and *C. papaya* were sliced open, and the seeds were collected, washed, and dried. *M. oleifera* dried pods were also split open, and the seeds were collected and stored. Each of the seeds was ground into powder with a blender and stored in labelled glass bottles.

Preparation of Plant Extracts: The powdered seeds of *M. oleifera, C. aurantifolia,* and *C. papaya* were measured out in 4, 8, and 12 g and were dissolved in 2 L of distilled cold water for 24, 48, and 72 hr respectively. The same weights of powder seeds were put in 2 L of distilled water and boiled for 5, 10, and 15 min respectively. Each of the mixtures was filtered using muslin cloth to obtain clear solutions. The solutions were put in capped plastic bottles, labelled appropriately, and subsequently used for the various experiments (HSS, 2020).

Rearing of *Aedes aegypti*: Eggs of *A. aegypti* were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The eggs were put in water and allowed to hatch into larvae (Adeleke *et al.,* 2008). They were reared in cages measuring 40x30x30 cm^3 in the Department of Zoology insectary to obtain eggs, which were reared and used for the experiment. The larvae collected were initially kept in a cage (60×30×30 cm^3 net- screened wooden cage) and were fed with dried edible yeast until they emerged as adults. The adults were fed on 0.5 % of glucose solution and fowl blood to obtain eggs used for the subsequent experiments. The eggs were laid on filter papers placed in a slanted form inside various transparent plastic containers containing water in the cage. The eggs laid on the filter paper were submerged in water in a transparent plastic cup (200 ml capacity) until hatching and were fed on dried edible yeast for the subsequent emergence of adults. The adult females were fed on fowl. Absorbent cotton wool wrapped with lint in a petri dish and soaked with 0.5 % concentration of glucose solution was put in the cage to feed the adult males (Hashmat, 2002). A transparent plastic cup (200 ml capacity) filled with 100 ml of water with a filter paper (9 cm in diameter) slanted in it to serve as an oviposition substrate was placed in the cage. The filter paper laden with eggs was removed and submerged in water in a transparent cup (200 ml) for the hatching of the eggs. The larvae obtained were used for the various experiments, which were replicated three times. The larvae were fed dried edible yeast and transferred into bioassay cups using a camel-hair brush (Hashmat, 2002; Asahina, 1964).

Larval Development: Twenty 2nd instar larvae were put into 100 ml of each of the plant dissolved and boiled extract solutions in transparent plastic cups, and twenty 2nd instar larvae were also put in transparent plastic cups containing water to serve as a control. The cups were placed in a mosquito rearing cage

(60×30×30 cm^3). The larvae were provided with edible yeast powder (15 mg) every 24 hours. The larvae were daily monitored for development from the 2nd instar to the adult stage (HSS, 2020).

Oviposition: 100 ml of each of the aqueous dissolved and boiled extract solutions was measured and put in different labelled transparent cups, and a filter paper (9 cm in diameter) was slanted in each of the cups to serve as oviposition medium. The cups were placed in the cage containing a pair of 10 adult females and males, and they were fed on glucose and blood meal. 100 ml of distilled water was also put in a labelled transparent cup, and a filter paper (9 cm in diameter) was slanted in the cup to serve as oviposition medium for the control experiment. The cups placed inside the cages were observed for eggs after 96 hours of the commencement of the experiment (Hashmat, 2002; Asahina, 1964). The total number of eggs laid was counted under a magnifying lens and recorded. Oviposition was determined using the mean number of eggs deposited. The percentage of inhibition was expressed as Antioviposition Index (*I*) using Antiovipositin formula by Abivard and Benz, (1989).

$$I = 100 \text{ X } \frac{\text{Emax} - \text{Eextr.}}{\text{Emax}}$$

Where Emax is the maximum number of mosquito eggs laid in the extract and Eextr. denotes the number of mosquito eggs laid in the other extract. Statistical analysis was carried out using descriptive and inferential statistics.

III. RESULTS

Effects of different concentrations of dissolved water extracts for 24, 48, and 72 hrs on oviposition by mated females are shown in Table 1. The number of eggs deposited in the extract dissolved for 24 hours decreased with an increase in the concentration of the extract. *M. oleifera* had the least oviposition of 24.00 ± 1.17, 23.11 ± 1.93, and 11.00 ± 0.98 at 4, 8, and 12 gL^{-1}, respectively, followed by *C. papaya* with the maximum number of eggs in 4 gL^{-1} extracts. A similar trend was obtained in extracts dissolved for 48 hrs at different concentrations. Oviposition in 4 gL^{-1} in *M. oleifera, C. papaya,* and *C. aurantifolia* was 30, 106, and 170 eggs, respectively. These further decreased at 8 and 12 gL^{-1} extracts. The number of eggs deposited in extracts dissolved for 72 hrs were the lowest at different concentrations of extracts. There was a significant difference ($F = 7.55$, $df = 2$, $p \leq 0.05$; $F = 5.53$, $df = 2$, $p \leq 0.05$) at different concentrations of 4, 8, and 12 gL^{-1}. Eggs laid in *M. oleifera* at different concentrations were significantly lower than eggs laid in *C. aurantifolia* at different concentrations. *C. papaya* extracts have the highest number of eggs at different concentrations of dissolved extracts at different concentrations of 4, 8, and 12 gL^{-1}.

As shown in Table 2, the boiled extract of *M. oleifera* had the minimum number of eggs followed by *C. aurantifolia* and *C. papaya* with the maximum number of eggs at the different concentrations of the extracts. Oviposition followed similar trend in all the extracts boiled for 5, 10 and 15 min with the maximum number of eggs in 4 gL^{-1} and minimum in 12 gL^{-1} extracts. The three plant extracts boiled for 15 min recorded the lowest number of eggs compared with those boiled for 5 and 10 min. Oviposition in 4 gL^{-1} *M. oleifera* boiled for 15 min was 17.56 ± 2.67 eggs compared with 31.11 ± 6.51 eggs in 4 gL^{-1} extract boiled for 5 min. Similarly, oviposition in 12gL^{-1} extract of *C. papaya* boiled for 15 min was 44.67 ± 1.33 eggs in comparison with 137.22 ± 10.92 eggs in the same extract boiled for 5 min. Extracts boiled for 15 min recorded the minimum number of eggs in the three extracts and at different concentrations when compared with the same extracts boiled for 5 and 10 mins. Oviposition indices in the hot water extracts were also inversely proportional to the number of eggs laid.

Table 1: Effects of different concentrations of dissolved plant extracts on oviposition of females *A. aegypti*

Dissolved Extract (Hours)	Plant extracts	Conc. (g/L)	Number of eggs laid (X±S.D)	Anti-oviposition index (%)
24	*M. oleifera*	4	24.22±1.17[a]	86.09
		8	23.11±1.93[a]	86.73
		12	11.00±0.98[b]	93.68
	C. aurantifolia	4	160.22±9.93[a]	8.03
		8	150.00±13.61[a]	12.75
		12	118.89±11.59[a]	31.75

	C. papaya	4	162.67±9.72[a]	6.62
		8	152.00±8.89[a]	13.90
		12	149.22±4.70[a]	14.34
	Control	0	174.22±3.84[a]	0.00
48	*M. oleifera*	4	30.00±2.18[a]	82.92
		8	18.33±2.00[a]	89.56
		12	9.00±1.86[b]	94.87
	C. aurantifolia	4	106.67±13.00[a]	39.27
		8	89.89±0.57[a]	48.83
		12	55.78±4.00[a]	68.24
	C. papaya	4	170.67±5.54[a]	2.84
		8	166.00±10.53[a]	5.50
		12	78.33±4.67[b]	55.41
	Control	0	175.67±40.21[a]	0.00
72	*M. oleifera*	4	22.33±1.17[a]	87.36
		8	22.00±1.73[a]	87.54
		12	9.67±1.39[a]	94.52
	C. aurantifolia	4	58.44±2.40[a]	66.92
		8	55.56±5.81[a]	68.55
		12	30.56±2.84[b]	82.70
	C. papaya	4	140.45±2.33[a]	20.50
		8	102.67±5.05[a]	41.88
		12	91.00±6.19[a]	48.49
	Control	0	176.67±7.20[a]	0.00

Means in each column followed by the same letter(s) are not significantly different at the 5 % level of probability by Turkey's HSD Test

Table 2: Effects of different concentrations of boiled plant extracts on oviposition of females of A. aegypti

Boiled Extracts (minutes)	Plant extracts	Conc. (g/L)	Number of eggs laid (X±S.D)	Anti-oviposition index (%)
5	*M. oleifera*	4	31.11±6.51[a]	82.50
		8	12.00±2.47[ab]	93.25
		12	8.00±2.58[b]	95.50
	C. aurantifolia	4	136.22±4.96[a]	23.37
		8	81.33±6.89[b]	54.25
		12	80.33±5.89[b]	54.76
	C. papaya	4	174.00±18.09[a]	2.12
		8	137.33±6.93[a]	22.75
		12	137.22±10.96[a]	22.75
	Control	0	177.78±10.92[a]	0.00
10	*M. oleifera*	4	21.77±1.81[a]	88.03
		8	20.22±1.81[a]	88.89
		12	8.00±2.58[b]	95.60
	C. aurantifolia	4	97.33±8.29[a]	46.48
		8	90.44±5.30[a]	50.27
		12	51.67±7.60[a]	71.59
	C. papaya	4	157.67±1.69[a]	13.31
		8	147.00±8.34[a]	19.18
		12	96.00±4.40[a]	47.22
	Control	0	181.89±6.24[a]	0.00
15	*M. oleifera*	4	17.56±2.67[a]	90.74
		8	14.33±2.67[a]	92.44

		12	6.33±3.33[a]	96.67
	C. aurantifolia	4	77.78±1.17[a]	59.00
		8	42.67±0.96[b]	77.51
		12	19.67±2.61[c]	89.63
	C. papaya	4	146.00±15.60[a]	23.05
		8	80.67±2.90[b]	57.48
		12	44.67±1.33[c]	76.45
	Control	0	189.74±7.86[a]	0.00

Means in each column followed by the same letter(s) are not significantly different at the 5 % level of probability by Turkey's HSD Test

IV. DISCUSSION

Oviposition of female *A. aegypti* in *M. oleifera* was rather low at different concentrations of extracts but highest in different concentrations of *C. papaya,* suggesting that *M. oleifera* might contain some components that deter egg laying in preference for *C. Papaya.* There were variations in the number of eggs laid in each extract of *C. papaya, C. aurantifolia,* and *M. oleifera,* with the minimum number of eggs laid in *M. oleifera* and the highest number of eggs in *C. papaya* in both dissolved and boiled extracts, indicating deterrence by *M. oleifera* extracts. According to Mutebi *et al.* (2022), in the presence of deterrents, gravid females seek and lay upon a site, assessing site quality, but lay few or no eggs before flying away. A significant reduction in the number and viability of eggs laid on *Corcya cephalonica* was observed when the insect pest was exposed to the vapour of neem oil, and anti-oviposition preference has been reported for turbid distilled and rooted aqueous extracts of *Petiveria alliaceae* tested on *Culex pipens* and *A. aegypti* (Wondwosen *et al.,* 2021). *Aedes* mosquitoes were found to access the water surface, but fly away afterwards without demonstrating any egg laying position. El-gendy and Shaalan, (2012) stated that oviposition deterrence may be due to degradation of various chemical compounds of the plant product in the oviposition medium, which then produces secondary metabolites that act independently or synergistically to inhibit mosquitoes from laying eggs.

V. CONLUSION

The result of the study shows prospects in using dissolved and boiled extracts of *M. oleifera, C. Papaya* and *C. aurantifolia* as larvicidal agent in *A. aegypti* control programme. Boiled extracts of *M. oleifera, C. aurantifolia and C. papaya* at different concentrations were more effective than the dissolved extracts at the same concentrations against the survival of *Aedes* larvae *M. oleifera* either in boiled or dissolved form, was the most effective among the three plants. These three plant extracts could be used to reduce the population of *A. aegypti* in the environment with assurance of eco-friendly positive results.

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