Ife Journal of Science vol.8, no. 1 (2006)

BIOLOGICAL EFFECT OF SOME PLANT EXTRACTS ON THE REPRODUCTIVE DEVELOPMENT OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI* LINN.

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(*Submitted:* 23 May 2005; *Accepted:* 08 August 2005)

Abstract

Aqueous leaf extracts of ten plant species were tested for their biological effect on the reproductive development of the *mosquito Aedes aegypti*, responsible for the transmission of dengue and yellow fever in tropical Africa. Results showed that Oviposition was least in *Momordica charantia* with Antioviposition index *(I)* of 87.7%, compared with other extracts, indicating that the plant significantly repelled gravid females from laying their eggs. Among the six plant materials that showed similar effect, extracts of *Momordica charantia,* and *Tithonia diversifolia* were the most effective in delaying hatchability (each delaying hatching for about 40 hours). Extracts of *Momordica charantia, Newbouldia laevis*, and *Tithonia diversifolia* accelerated larval growth resulting in premature pupal emergence while that of *Vernonia amygdalina* caused a delay in larval development. The result of this study revealed *M. charantia, N. laevis* and *T. diversifolia* as potential materials for bio-insecticides preparations against *Ae. aegypti* because of their antiovipositional effect and significant biological effect on the development of the mosquito larvae.

Keywords: Leaf extract, Antioviposition, *Aedes aegypti, Momordica charantia,Newbouldia laevis, Tithonia diversifolia.*

1. Introduction

Much effort had been made in the past to control vector–borne diseases using chemotherapeutic methods and synthetic insecticides, which are often considered to be the most potent means of control for insects. However, there are many serious drawbacks in the use of synthetic insecticides for vector control and alternative means of control are therefore needed. Besides the adverse environmental effects, high cost and mammalian toxicity attributable to the use of certain insecticides, most vectors have become physiologically resistant to the use of these compounds (Zebitz, 1986). The economic constraints in most developing countries also militate against national mosquito control eradication programme. These factors have therefore created the need for the use of plant products, which are environmentally safe, degradable and target-specific bio-insecticides against mosquitoes.

Several workers who have reported their investigations into the insecticidal activities of plant natural products have established insecticidal properties for a number of plant constituents including essential oils and bioactive compounds have been isolated from promising plants. The leaf, fruit and seed kernels of neem, *Azadirachta indica* (A. Juss) for example has been reported to contain azadirachtin, salamin and other active principles that possess repellent, antifeedant and growth disruptive properties against various species of insects (Jacobson *et al.*, 1971; Schmutterer *et al.*, 1981). Insecticidal activities have been described for certain pharmaceutical formulations of neem extract and *Hemizygia welwitschi* oil in Nigeria (Olaifa *et al.* 1993; Oyedele, 1995). Insecticidal activities of certain other plants against mosquitoes and other insect pests have also been described (Adebayo *et al.* 1999). Sofowora (1993) established that much is known about the effects of plants as growth inhibiting agents as well as antifeedants. A variety of insects have been controlled by derived plant extracts without adverse effect on most non-target organisms (Saxena, 1989). Futhermore, the toxicity of plant extracts to agricultural pests has been documented (Gbolade and Adebayo, 1993).

In continuation of the search for potent mosquito larvicides and the need to have a data bank on biodegradable insecticides of plant origin, the present study examines the effect of some indigenous plants on the reproductive development of *Aedes. aegypti,* a mosquito responsible for the transmission of dengue and yellow fever in tropical Africa.

2. Materials and Methods

(a) Rearing of *Aedes aegypti*

Larvae of the mosquito, *Ae. aegypti* were bred in the insectary of the Department of Zoology,Obafemi Awolowo University, Ile-Ife at a temperature of 28 ± 2 °C and relative humidity of 75-85%. The initial

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eggs were obtained from the Department of Pharmacognosy of the same institution.

(b) Plant Materials

The leaves of plant materials such as *Cyathula achranthoides* (Amaranthaceae), *Pandiaka heudelotii* (Amaranthaceae), *Annona muricata* (Annonaceae), *Aspilia africana* (Asteraceae), *Bidens pilosa* (Asteraceae), *Tithonia diversifolia* (Asteraceae), *Vernonia amygdalina* (Asteraceae), *Newbouldia laevis* (Bignoniaceae), *Momordica charantia* (Cucurbitaceae) and *Senna alata* (Fabaceae) used for this investigation were collected from Obafemi Awolowo University and its environs. The leaves were air-dried and ground to fine powder with a Moulinex blender.

Aqueous extracts were prepared by adding 1.5 g of the fine powder of each plant material to 1 litre of water (0.15% concentration), and then boiled to a temperature of 100 °C for 30 minutes. The mixture was cooled and sieved twice through a muslin cloth to obtain a clear filtrate. The clear filtrate was put in labeled capped plastic containers and subsequently used for the various experiments.

(c) Effect of plant extracts on Oviposition of *Ae. aegypti*

An aliquot of 100 ml of aqueous extract of each of the ten plants was put in a labeled transparent cup and a filter paper (9 cm diameter) was slanted in each of the cups to serve as oviposition medium. The cups were placed in the cage containing adult fed mosquitoes and observed for eggs 24 hours later. The total number of eggs laid were counted using a magnifying lens and recorded. Each extract had 3 replicates. The mean number of eggs obtained was used to determine preference for oviposition. Percentage of inhibition of oviposition for each plant extract was expressed as Antioviposition Index (*I*) according to Abivardi and Benz (1986), with modification.

$$I = 100\,x\,\frac{E\max. - Eextr.}{E\max}$$

where E*max* is the maximum number of eggs laid in all extracts

E*extr*. is the number of eggs laid in each extract.

(d) Effect of plant extracts on hatching of *Ae. aegypti*

The transparent cups containing the filter paper laden with eggs from above were removed from the cage. The filter papers laden with eggs were then fully submerged in each of the plant extracts contained in the cups. The time for hatching was observed in each case and recorded accordingly. In the control set-up, the filter paper laden with eggs was submerged in matured water.

(e) Effect of plant extracts on larval development of *Ae. aegypti*

Twenty 5-day-old larvae (2nd instar) were put into 100 ml of each of the plant extracts contained in transparent plastic cups while water was used in the control set-up. There were three replicates for each set-up. The cups were then placed in a rearing cage made of cotton mosquito netting material (22 x 22 x 22 cm^3) and suspended on a wooden frame (40 x 30 x 30 cm^3). The larvae were provided with edible yeast powder (25 mg) every 48 hours. They were daily monitored and the number of dead larvae, emerging pupae and emerging adults were recorded until emergence ceased.

(f) Statistical analysis

The data obtained were analyzed for variance using the SAS (1997) Statistical Package, and where significant differences existed at 5 % level of probability, the New Duncan's Multiple Range Test was used to separate the means.

3. Results

All the plant materials used supported oviposition with the presence of eggs in all the extracts 24 hours after exposure to mated females (Table 1). The number of eggs laid by gravid female *Ae. aegypti* varied with the different plant extracts. The mean number of eggs laid was highest in *Senna alata* with 91.7 ± 40.3 while the least number of eggs deposited was observed in *Momordica charantia* extract with 3.7 ± 1.5. The antioviposition index (*I*) ranged between 0 to 87.7% in *Senna alata* and *Momordica charantia* respectively. The extract of *Momordica charantia* demonstrated strong oviposition deterrent activity against mated females of *Ae. aegypti*. There were significant differences in the mean number of eggs produced.

Eggs hatched into larvae in the control medium twenty-four hours after oviposition and in four other plant extracts which are *Cyathula achranthoides, Bidens pilosa, Vernonia amygdalina* and *Newbouldia laevis*. The hatching of eggs was however delayed up to 40 ± 6.5 hours in *Tithonia diversifolia* and *Momordica charantia* (Table 2).

Survival of larvae was over 80 % at day 2 of exposure in all the plant extracts as well as in the control experiment (Table 3). At day 3 of development, survival was 52.7 ± 2.9 % in the control but ranged from 68.7-100 % in all the plant extracts. Larvae died on day 7 in *Newbouldia laevis* and day 8 in both *Tithonia diversifolia* and *Momordica charantia*. Survival of larvae at 7 day post-treatment was high in *Bidens pilosa* with 55.4 ± 12.7 %.

In the control set-up (i.e. water medium), the maximum longevity of larvae was 10 days with mean

Table 1: Effect of aqueous plant extracts on oviposition by mated females of *Ae. aegypti*

S/N	Plant extract	Mean number of eggs laid (± S.E)	Antioviposition index (*I*)
1.	*Senna alata*	91.7 ± 40.3^{a}	0
2.	*Annona muricata*	70.0 ± 15.0^{ab}	15.3
3.	*Bidens pilosa*	49.7 ± 18.7^{abc}	37.5
4.	*Vernonia amygdalina*	49.0 ± 12.1^{abc}	38.2
5.	*Tithonia diversifolia*	37.7 ± 21.0^{abc}	50.6
6.	*Pandiaka heudelotii*	23.0 ± 8.1^{bc}	66.6
7.	*Cyathula achranthoides*	19.0 ± 12.3^{bc}	70.9
8.	*Newbouldia laevis*	16.7 ± 5.5^{bc}	73.5
9.	*Aspilia africana*	12.0 ± 5.8^{bc}	78.6
10.	*Momordica charantia*	3.7 ± 1.5^{c}	87.7

Means followed by same alphabet(s) are not significantly different at $P < 0.05$ by Duncan's New Multiple Range Test (DMRT).

Table 2: Effect of aqueous plant extracts on egg hatching in *Ae. aegypti*

S/N	Plant Extract	Mean time of hatching (hr) (X ± S.E)
1.	*Bidens pilosa*	24.0 ± 0.0
2.	*Cyathula achranthoides*	24.0 ± 0.0
3.	*Newbouldia laevis*	24.0 ± 0.0
4.	*Vernonia amygdalina*	24.0 ± 0.0
5.	*Annona muricata*	32.0 ± 6.5
6.	*Aspilia africana*	32.0 ± 6.5
7.	*Senna alata*	32.0 ± 6.5
8.	*Pandiaka heudelotii*	32.0 ± 6.5
9.	*Momordica charantia*	40.0 ± 6.5
10.	*Tithonia diversifolia*	40.0 ± 6.5
11.	Control	24.0 ± 0.0

survival time of 9.3 ± 0.5 days. This was comparable with those larvae maintained in extracts of *Annona muricata*, *Aspilia africana*, *Cyathula achranthoides*, and *Vernonia amygdalina* with mean survival time ranging between 9.7 ± 2.2 and 10.7 ± 0.3. Maximum longevity and mean survival time were lowest in *Newbouldia laevis* with 7 and 6.3 ± 0.3 days respectively. There was significant difference in the mean survival time of larvae maintained in the different plant extracts (Table 4).

4. Discussion

Egg laying by female *Ae. aegypti* in each extract of the 10 different plants within 24 hours of their exposure is an indication of the indiscriminate manner with which *Ae. aegypti* oviposit in aquatic media, irrespective of the odour, colour or type of water (Richards and Davies, 1977). Zebitz (1986) reported that gravid *Ae. aegypti* females laid eggs on filter paper treated with neem oil but oviposition of female *Culex* on filter paper treated similarly was deterred considerably. *Ae. aegypti* showed preference for oviposition by laying varying number of eggs in each of the aqueous plant extracts. *Senna alata* and *Annona muricata* extracts mostly supported oviposition to a larger extent while extracts of *Momordica charantia*, *Aspilia africana*, *Newbouldia laevis*, and *Cyathula achranthoides* inhibited oviposition considerably. *Momordica charantia* extract had the least number of eggs and thus the greatest antioviposition activity, but none of the plant extracts totally deterred oviposition of gravid *Ae. aegypti*. Adedire and Akinneye (2004) also observed significant reduction in the number of eggs laid by *Callosobruchus maculatus* in cowpea seeds treated with powder and ethanol extract of *T. diversifolia*. Aqueous extracts of *Annona muricata*, *Aspilia africana*, *Pandiaka heudelotii*, *Momordica charantia*, *Senna alata* and *Tithonia diversifolia* variously delayed egg hatching in *Ae. aegypti* in comparison with the control medium (i.e. water). Among these plants, *Momordica charantia*, and *Tithonia diversifolia* were the most effective, but these extracts did not prevent larval hatch.

The maximum developmental period of larvae was comparatively shorter in extract of *Newbouldia laevis* with 7 days and 8 days in both extracts of Momordica *charantia* and *Tithonia diversifolia*. The larvae, which should ordinarily feed more in water before pupating in order to produce viable offspring,

Table 3: Percentage Mean Survival of 5 - day old larvae of *Ae. aegypti* in different aqueous crude boiled extracts and the control

Day	*Am*	*Mc*	*Sea*	*Nl*	*Va*	*Td*	*Bp*	*Aa*	*Ca*	*Ph*	Cont.
1	100	98.7	100	98.0	98.7	99.4	100	100	100	100	97.4
	±0.0	±0.5	±0.0	±0.9	±0.5	±0.5	±0.0	±0.0	±0.0	±0.0	±1.4
2	99.4	96.0	96.0	84.7	95.4	88.7	100	100	100	100	84.0
	±0.5	±0.9	±1.6	±2.4	±2.4	±0.5	±0.0	±0.0	±0.0	±0.0	±0.9
3	98.7	85.4	92.0	68.7	90.0	78.0	100	94.0	97.4	96.7	52.7
	±0.5	±2.0	±0.9	±2.2	±2.4	±0.0	±0.0	±1.9	±1.4	±2.0	±2.9
4	86.7	68.0	68.7	45.4	78.0	62.7	94.0	89.4	89.3	89.4	36.7
	±5.2	±2.8	±7.0	±6.8	±4.7	±1.1	±0.9	±1.1	±2.2	±0.5	±4.3
5	74.7	44.0	50.0	31.4	56.0	48.0	83.4	74.7	77.4	76.0	32.7
	±7.0	±2.8	±5.3	±6.6	±3.4	±4.3	±4.5	±3.9	±2.9	±2.5	±5.5
6	71.4	28.0	23.4	20.7	34.0	36.0	65.4	56.7	55.4	60.7	21.4
	±6.3	±5.0	±4.5	±2.8	±5.9	±6.6	±9.2	±4.5	±2.4	±4.4	±3.0
7	44.7	10.7	8.7	4.7	18.7	14.0	55.4	45.4	42.7	54.0	12.7
	±7.1	±4.8	±5.2	±3.8	±9.5	±2.8	±12.7	±6.3	±1.1	±5.0	±3.0
8	26.7	3.4	3.4	0.0	14.0	0.7	36.0	25.4	20.0	30.0	10.0
	±4.0	±2.0	±2.7		±9.1	±0.5	±16.4	±6.7	±2.5	±4.3	±2.5
9	15.1	0.0	0.7		7.4	0.0	4.7	12.7	2.0	8.7	6.0
	±3.9		±0.5		±6.0		±3.8	±3.6	±0.9	±3.8	±2.5
10	9.4		0.0		6.0		0.0	2.7	0.0	0.0	2.7
	±4.5				±4.9			±1.4			±1.4
11	2.0				4.7			0.0			0.0
	±0.9				±3.8						
12	0.0				2.0						
					±1.6						
13					2.0						
					±1.6						
14					0.7						
					±0.5						
15					0.7						
					±0.5						
16					0.0						

Am-Annona muricata, Aa-Aspilia africana, Ca-Cyathula achranthoides, Ph –Pandiaka heudelotii, Bp -Bidens pilosa,, Mc-Momordica charantia, ,Sea-Senna alata, Nl– Newbouldia laevis, , Va – Vernonia amygdalina, Td-Tithonia diversifolia ,Cont.-Control

Table 4: Survival data in different plant extracts and control for 5–day old larvae (2nd instar) of *Ae. aegypti.*

Plant extract	Above 50% Survival Time in days (X±S.E)	Mean Survival Time in days (X ± S. E)	Survival Time Range in days
Annona muricata	6.3 ± 0.3^{ab}	10.7 ± 0.3^{bac}	10 - 11
Aspilia africana	6.3 ± 0.5^{ab}	9.7 ± 0.3^{bdac}	9 – 10
Bidens pilosa	6.7 ± 0.7^{a}	8.3 ± 0.3^{bcde}	8 – 9
Vernonia amygdalina	5.0 ± 0.0^{bcde}	9.7 ± 2.2^{abcd}	6 – 15
Control	2.7 ± 0.3^{h}	9.3 ± 0.5^{abcd}	8 – 10
Cyathula achyranthoides	6.3 ± 0.5^{ab}	9.7 ± 0.3^{abcd}	9 – 10
Momordica charantia	4.3 ± 0.3^{defg}	7.3 ± 0.5^{defg}	6 – 8
Newboldia laevis	3.3 ± 0.3^{fgh}	6.3 ± 0.3^{efg}	6 – 7
Pandiaka heudelotii	6.7 ± 0.3^{a}	8.7 ± 0.3^{bcde}	8 – 9
Senna alata	4.3 ± 0.3^{defg}	7.3 ± 0.7^{defg}	6 – 9
Tithonia diversifolia	4.7 ± 0.5^{cdef}	7.3 ± 0.3^{defg}	7 – 8

Means followed by same alphabet(s) are not significantly different at $P< 0.05$ by Duncan's New Multiple Range Test (DMRT).

showed accelerated but abnormal growth in these extracts. Some died as white slightly melanized pupae with adult structure visible through the skin, while most of those that eventually emerged as adult have malformed wins and shortened abdomen. This could be an indication that these extracts are not favourable to the normal growth of the larvae. Similarly, Haasler (1984) reported that the larvae of tobacco hornworm, *Manduca sexta* when treated with neem seed extract, metamorphosed into malformed pupae. The extracts of *Vernonia amygdalina* however prolonged longevity of larvae suggesting the presence of growth regulating substances. Consistently, the boiled extract of neem leaf (*Azardirachta indica*) was reported to have inhibited the growth of larvae and caused a delay in pupation of *Anopheles gambiae* (Muse *et al*., 1996). Jotwani and Sicar (1965) reported insecticidal properties and growth retarding effect of neem seed on stored grain insects.

This study has revealed that *Momordica charantia, Newbouldia laevis, and Tithonia diversifolia* significantly affected development of larvae of *Aedes aegypti* as well as promoted premature pupal emergence. Also, aqueous extract of *Momordica charantia* significantly reduced oviposition by gravid females. Furthermore, extracts of *Momordica charantia* and *Tithonia diversifolia* delayed egg hatching considerably.

From the fore going, these three indigenous plants (i.e. *Momordica charantia, Newbouldia laevis* and *Tithonia diversifolia)* are potential candidates for bio-insecticide preparations against *Ae. aegypti* because of their antiovipositional effect and significant effect on the biology of the mosquito larvae and could therefore be used to reduce the population of *Ae. aegypti* in a pilot programme. They are recommended for further investigation at different concentrations.

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