LARVICIDAL EFFICACY OF *MONODORA MYRSITICA* ON LABORATORY REARED THIRD INSTAR LARVAE OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI (L).*

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ABSTRACT

Laboratory studies were conducted to evaluate the effectiveness of *Monodora myristica* in controlling the 3rd instar larvae of *Aedes aegypti*. *Monodora myistica* significantly reduced the populations of *Aedes aegypti* larvae (P<0.05). Application of *Monodora myristica* hexanolic extract at 200ppm gave 70% mortality within 1 hour of application and 100% mortality within 24 hours of test period. There was a great reduction in potency of the extract when exposed to sunlight and U.V radiations.

Key words: *Monodora myristica* hexanolic extract, larvicide, potency, *Aedes aegypti* 3rd instars larvae.

INTRODUCTION

Aedes aegypti is the known principal vector of urban yellow fever, dengue fever and zika viruses. (Theiler and Downs, 1978). *Aedes aegypti* was typically a breeder in domestic containers and its breeding habits kept it in intimate association with man (Surtees, 1958). Surtees (1958) revealed that *A. aegypti* eggs are laid singly and scattered individually on the moist surface immediately above the water level or less commonly on the actual water surface. The length of larval life of *Aedes aegypti* varies with such factors as the water temperature and availability of food. Although vaccine against yellow fever is available the disease has tragically continued to infect and kill thousands of unimmunized people every year. Between 1988-90 global total cases of 8685 and 2643 deaths due to yellow fever were recorded .Records for these three years represents the greatest number reported to WHO since 1948 (WHO 1992).

Man had always resulted to use of chemicals to control insects and other pests however most insecticides developed after 1,1,1 trichloro2,2 bis 4-chlorophenyl ethane (DDT) and Hexachlorocyclohexane (Gamma H.C.H) generally have been synthetic non-selective poisonous chemicals. Pesticides have effectively controlled various pest species but their extensive use has led to serious social and environmental repercussions.

Many cases of lethal and sub lethal pesticide poisoning of human have occurred (Forget, 1989; Goulding, 1988). Repeated application of chemical control also results in an unintended artificial selection of those mutants within the pest population, equally insecticides cause ecological imbalance manifested by pollution of the environment and destruction of non-target organisms. Hence, the need for the development of environmentally friendly pesticide. Plant extracts offer a safer and environmentally friendly but effective pesticide. (Berenbaum, 1989; Amusan *et al* 2002; Anyaele *et al* 2002). *Monodora myristica* is one of such plants. *M. myristica* (African nutmeg or false nutmeg) it is a fair sized tree usually found in moist forest. The fruit contains several seeds used as a condiment in soup and as flavouring agent to other medicines and snuff. The seeds are chewed and rubbed on the fore head to treat headache. A reddish brown fixed oil is also extracted from the seeds.

However, work has not been done on the insecticide properties of extract of *M.myristica.* Olaifa *et al.*, (1987) reported the protecting ability of *Monodora tenuifolia* along side with other plants against storage pests. *M. tenuifolia* belong to the same genus as *Monodora myristica* therefore the present work aims at investigating the insecticide properties of Monodora myristica hexanolic extract on *Aedes aegypti* 3rd instars larvae with a view to use it to control the vector.

MATERIALS AND METHODS

Collection and rearing of Aedes aegypti larvae

In February 2004, at the peak of the dry season when the breeding sites of mosquito had dried up sands and debris were collected from disused tyres found in the car park of the University of Agriculture Abeokuta library and soaked in water. After a day, hatching of viable eggs commenced. Hatched out larvae were removed into cleaner water using collecting pipette, into brightly coloured enamel plates for easy visibility. The larvae were fed with dry straw. Active, swimming non-feeding pupae were collected into open bottles and placed in a cage where they were left to emerge as adults.The cage was about 40 by 40cm dimension made of light wooden frame with sides of mosquito netting. (Black for easy visibility). The base was made of wooden board. One side of the cage is provided with a sleeve for taking materials in and out of the cage.

Adult female mosquitoes were allowed to feed on blood of rabbit immobilized by using wire gauze of large mesh to encage the animal before putting them into the mosquito cages. Drinking water and cotton soaked sugar solution was provided for the males for feeding. Oviposition of eggs commenced after three days of the first blood meal and last for about 7 days. Eggs were laid singly and scattered on moist filter paper placed round the sides of a 500ml beaker. Eggs were collected, dried and stored away in an incubator at 31 ± 2^{0}C. All the 3rd instars larvae required for the study was obtained from this procedure.

Preparation of plant extracts

Dry fruits of *M. myristica* were bought from a local market (Oje market) in Ibadan, Nigeria.The fruits were washed and dried in the sun. The dried fruits were ground to a fine powder using electric blender. The dried ground samples were then extracted with n-hexane using Soxhlet extractor. The extract was then concentrated with Rotary evaporator, which removed the hexane component leaving behind viscous oil.

Preparation of *M. myristica* concentrations

Preparation of the volume/volume stock solution of *M. myristica* was done by measuring out line of the extract and emulsified with about 0.003ml or 3 drops of Tween -80 from a needle tip. The emulsified extract is then added up to 1 litre to form 1000ppm stock solution. From this stock solution the following serial concentrations were prepared 30ppm, 50ppm, 70ppm, 100ppm, 150ppm and 200ppm.

Evaluation of *M. myristica on Aedes aegypti larvae*

Bioassay of the extract

The tests were carried out in two categories. The first category referred to as untreated test was done using the serial graded concentrations of the extract. The second category of test evaluated the effects of physico-chemical factors such as sunlight and U.V irradiation exposure on the Larvicidal property of the extract. Four replicates were used for each concentrations and the average number of larvae in a test bottle was forty.

From each graded concentrations was measured 250ml solution and transferred into separate labeled 500ml specimen bottles. Forty third instars larvae of *Aedes aegypti* were then introduced into each. The bottles were then covered with mesh of 1mm for aeration but prevent entry and exit of any insect. Each treatment was replicated four times.

Mortalities were recorded at intervals of 1, 12, 24, 48 and 96 hours. To estimate the 96-hour median lethal concentration (LC 50) of the extract on the 3rd instars larvae cumulative mortalities were recorded at intervals of 1, 12, 24, 48 and 96 hours. From these data the 96-hour mortality of the different concentrations were used to estimate the LC50 using probit regression line. Bioassay data were subjected to analysis of variance at 5% level of significance where there was a difference; Duncan test was used to determine whether there were significant differences between the means.

RESULTS AND DISCUSSION

The LC50 of *Monodora myristica* was 70ppm and 75.86ppm when exposed to sunlight for 2 hours and 229ppm when exposed to sunlight for 8 hours. The LC_{50} was 112.2ppm when exposed to U.V light for 2 hours and 91.2ppm when exposed to U.V light for 8 hours (Table1).

Table 1: LC_{50} at 96 hours treatment with *M. myristica*

Treatments	Concentrations
Untreated	70ppm
2 hours sunlight	75.86ppm
4 hours sunlight	194. 98ppm
8 hours sunlight	229.09ppm
2 hours U.V	112.2ppm
4 hours U.V	131.83ppm
8 hours U.V	91.20ppm

The highest concentration of 200ppm killed 70% of the population within 1 hour of test period and reached 100% mortality in 24 hours test period *Aedes aegypti* larvae is very susceptible to the larvicidal properties of *M.myristica h*exane extract. After 24 hours the mortality rate decreased drastically showing virtually no activity. Hence the toxicity does not persist up to 96 hours test period.

Results of toxicity of *Monodora myristica* to *Aedes aegypti* larvae after exposure of the extract to sunlight shows that 200ppm concentration achieved a 90% mortality at 2 hours in 24 hours test period and increased to 100% mortality at 2 hours in 48 hours test period. (Table 2a). Duncan's test with results of bioassay exposure to 2,4 and 8 hours sunlight (at $P > 0.05$) showed no significant difference between the means of the 2 hours sunlight exposure and the unexposed extract (Table 3) there was a significant difference between the means of the 4 and 8 hours sunlight exposure and unexposed extract probit regression lines revealed the different LC_{50} of 70ppm, 76ppm, 195ppm and 227ppm for the unexposed, 2 hours, 4 hours and 8 hours sunlight treatment respectively.

When exposed to U.V light Duncan's test reveals that there was no significant difference between the means of the 2 hours U.V treated extract and that of the untreated extract ($P > 0.05$) but a significant difference exist between the means of 4 and 8 hours U.V treated extract and the untreated extract. The comparative LC50 values at 96 hours presented in Table 1 showed a decrease in potencies of the extract. Table 2b showed a 12.5%, 12.5% and 7.5% mortality for 2 hours, 4 hours and 8 hours irradiated *Monodora myristica* hexanolic extract respectively at 1 hour test period, while at 24 hour test period 79%, 52% and 72% mortalities were recorded for 2, 4, and 8 hours respectively. Generally there was reduction in potency of the extract when irradiated with Ultra violet radiations, a deviation from the reports of Arnason *et al.,* (1989) and Graham *et al.*, (1980).

There was a great reduction in potency of the extract when exposed to sunlight, the reduction increases with increase in sunlight exposure period of the *Monodora myristica* hexanolic extract, similar observations were made by Wink's (1993), that pesticide principles are degraded by sunlight exposure

Monodora myristica has not been tested on insects before the present study, however Schul (1988), had worked on *Monodora tenuifolia* and reported that the essential oil of *M. tenuifolia* was a potential protector of stored products against storage pest. The present study therefore support that the genus *Monodora* possesses insecticide properties of yet unknown identity and mode of action. However *M. myristica* appears to be quite promising in the control of larvae of mosquitoes.

Table 2a: Effect of sunlight exposure on % Mean Mortality in Time of *Monodora myristica* hexane extract treated Ae. aegypti.

	1 hour				12 hours				24 hours				48 hours					
Conc.	N	S^2	S^4	S^8	n	S^2	S^4	S^8	N	S^2	S^4	S^8	n	S^2	S^4	S^8	n	S^2
200ppm	32.5	0	2.5	2.5	80	35	22.5	20	100	90	37.5	25	100	97.5	42.5	45	100	52.5
150ppm	0	0	0	0	62.5	20	15	12.5	87.5	62.5	22.5	18	87.5	75	37.5	22.5	90	90
100ppm	0	0	0	0	15	2.5	10	5	42.5	22.5	12.5	25	7.5	75	55	25	75	70
70ppm	0	0	0	0	5	12.2	5	25	37.5	75	25	0	50	12.5	20	25	50	37.5
50ppm	0	0	0	0	10	25	25	0	0	25	0	0	40	12.5	25	0	40	20
30ppm	0	0	0	0	0	25	0	0	0	0	0	0	0	25	0	0	0	25
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2b: Effect of U.V. radiation on % mean mortality in time of *Monodora myristica hexane* extract treated A. *aegypti*.

	1 hour				12 hours				24 hours				48 hours					
Conc.	N	U^2	U^4	U^8	n	u^2	U^4	U^8	n	U^2	U^4	U^8	n	U^2	U^4	U^8	n	U^2
200ppm	32.5	51.2	51.2	7.5	80	32.5	20	35	100	50	47.5	72.5	100	70	52.5	82.5	100	75
150ppm	0	0	0	0	62.5	15	2.5	20	87.5	20	35	62.5	87.5	35	50	75	90	60
100ppm	0	0	0	0	15	2.5	7.5	75	72.5	35	20	35	75	37.5	25	50	75	45
70ppm	0	0	0	0	5	2.5	12.5	25	42.5	75	75	20	50	12.5	17.5	22.5	50	20
50ppm	0	0	0	2.5	10	12.5	2.5	25	37.5	12.5	25	75	40	17.5	5	17.5	40	22
30ppm	0	0	0	0	0	2.5	0	25	0	2.5	0	2.5	0	2.5	0	2.5	0	5

Table 3: Comparison of treatment mean (Duncan's test)

Treatment	
U.V Radiation	
Untreated	6.292a
2hours	5.333a
4 hours	2. 667a
8-hour	1.458b
LSD	(1.6694)
Sunlight exposure	
Untreated	6.292a
2 hours	3. 792b
4 hours	3.208b
8 hours	4.875ab
LSD	(1.8466)

* Means with the same letter are not significantly different

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