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Original article

TOXICITY OF HEXANOLIC EXTRACT OF DENNETTIA TRIPETALA (G. BAXER) ON LARVAE OF AEDES AEGYPTI (L)

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The hexanolic extract of Dennellia tripetala was tested for acute toxicity on 3rd Instar larvae of Aedes aegypti reared in the Laboratory at the Department of Zoology, University of Ibadan,. Six sets of graded concentrations 30pprn, 50ppm 60pprn 70pprn, 80ppm and 90ppm were tested for acute toxicity on the 3rd instar larvae of Aedes agypti and total percentage mortalities recorded at intervals of 12, 24, 48, 72 and 96 hours in each test. Effects of sunlight and ultraviolet radiation were tested on the potency of the extract, at 2, 4 and 8 hours respectively. The mean lethal concentration LC_{5O} was 44.7 $\pm$ 0.82ppm. The toxicity of the extract does not persist for a longtime; the potency of the extract was slightly affected by sunlight while the potency was activated by ultraviolet radiation.

Keywords: Dennetia tripeta acute toxicity. 3rd instar larvae, Aedes aegvpti Median Lethal Concentration. LC_{50}

INTRODUCTION

Most insecticides developed after DDT and Gamma H.C.H generally have been synthetic, non-selective poisonous chemicals although they effectively controlled some pest species, 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; and Goulding (1988).

Also, repeated application of chemical control results in an unintended artificial selection of those mutants within the pest population. Furthermore insecticides causes ecological imbalance in the ecosystem by causing destruction to even non-target organisms, which is not only uneconomical but aggravate the problems (Kumar, 1984).

The demand for more food and adequate maintenance of public and animal health will not permit significant elimination of broad-spectrum synthetic pesticides a problem that has led to an increased interest in the discovery of new chemicals. The botanicals, which are less likely to cause ecological damage, have been in use longer than any other group of insecticides. Klocke (1987) observed that plant extract have been used as insecticides by human before the time of the Roman Empire. Only a small percentage of plants have been screened for insecticidal activities (Granige and Ahmed 1988).

Dennettia tripetala fruit is red when ripe and has a pungent spicy taste. Agbakwuru *et al* (1978) isolated some oil using steam distillation treatment of Dennetti fruit and observed that the oil contained substantial quantities of B-Phenylnitroethane.

Dennettia oil had been reported by Agbakwuru *et al* (1978) to have protectant ability on cowpea against storage insect pest. However, Dennettia oil or any of its active components have never been tested against insect pests than those of stored food products except for the work of Iwuala (1981) on *Periplanata americana* and *Zonoccus varriegatus*.

Therefore the present work aims at evaluating the toxicity of hexanolic extract of *D. tripetalla* oil on 3rd Instar larvae of *Aedes aegypti*.

MATERIALS AND METHODS

Collection of *Aedes aegypti* larvae

Mosquito cages of about 40 by 40cm dimension were made from light wooden frame with sides of black mosquito netting. The base of the cage was made of wood and one side of the cage was provided with sleeve for taking materials

into and out of the cage. Mosquito eggs were collected at the peak of the dry season in January 1995 by collecting sands and debris from the breeding sites of *Aedes aegypti* in disused tyres in the maintenance Unit, University of Ibadan and soaked in water. Hatching of viable eggs commenced after a day. The larvae were then transferred into cleaner water for easy visibility in bright coloured enamel plates using a collecting pipette.

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. Rabbits were used to feed the mosquitoes. The feeding mosquitoes were first starved for a day or two for proper biting. 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°C (conditioning). The 3rd instar larvae required for this study were obtained from the eggs stored using the above procedures.

Plant Material

Dennettia tripetala which normally fruit around March-April were bought fresh from the Eastern part of Nigeria, washed and dried in the sun and later ground into a fine powder consistently using a thoroughly cleaned electric grinder. The extraction with n-hexane was done using Soxhiet extractor. The extract was then concentrated with Rotary evaporator, which remove the hexane component leaving behind viscous oil required for the analysis.

Volume/volume stock solution of *Dennettia tripetala* was prepared by measuring out 1ml of the extract and emulsify with Tween-80 of about 0.003m1 or 3 drops from a needle tip. The emulsify extract is then added up to 1 litre to form l000ppm stock solution. From the stock solution serial concentration of 30ppm, 60ppm, 90ppm, 120ppm and 150ppm were prepared.
From each concentration 250ml was measured and introduced into separate labeled 500ml specimen bottles. Forty 3rd instar larvae of *A aegypti* were then introduced into each bottle. Each treatment had four replicates.

Mortalities were recorded at intervals of 12, 24, 48, 72 and 96 hours. To estimate the 96-hour median lethal concentration (LC_{50}) of the extract, 96-hour mortality of the different concentrations were used for the probit regression graph.

Data obtained from the processes described above were then subjected to analysis of variance at 5% level of significance and where there was a difference,

Duncan test was applied to determine whether there were significant differences between treated and untreated means.

Effects of physicochemical parameters such as sunlight and ultraviolet irradiation were tested on the oil extract using the method described by Adewumi and Marquis, (1980). Stock solutions of the *D. tripetala* was exposed to sunlight and U-V lamp (Gallen Kanip LH 530) with a peak output at 366nm) for 2, 4 and 8 hour. In the case of U-V light stock solutions were placed at a distance of about 30cm from the light source.

To estimate 96 hour median lethal concentration LC_{5O} of Dennettia oil on the 3rd instar larvae of *A aegypti*, cumulative mortalities were recorded at interval of 12, 24, 48, 72 and 96 hours.

The data were also 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 treatment means.

RESULTS

Dennetlia tripetala hexane extract was found to be toxic to *A. eagypti* larvae. The acute LC_{50} at 96 hours was 447ppm. The toxicity of the extract was gradual and persisted throughout the 96-hour test period. At 24-hour test period, a 75% mean mortality was recorded for 9Oppm treatment while 100% mean mortality was achieved using the same concentration after 96 hours when stock solution was exposed to U-V Radiation (see Table 1).

Table 1:
Effects of U-V radiation (% mortality in time) on hexane extract of D. tripetala activity against *A. aegypti* larvae

Conc	12 hrs				24 hrs				48 hrs				72 hrs				96 hrs			
ppm	n	U2	U4	U8	n	U2	U4	U8	n	U2	U4	U8	n	U2	U4	U8	n	U2	U4	U8
90	37.5	35	12.5	82.5	75	90	77.5	100	92.5	7.5	95	100	97.5	100	100	100	100	100	100	100
80	20	75	75	65	50	85	40	90	67.5	92.5	90	97.5	90	100	95	100	92.5	100	100	100
70	10	17.5	10	40	37.5	82.5	57.5	60	50	87.5	85	85	77.5	100	90	95	80	100	10	100
60	7.5	7.5	7.5	27.5	12.5	65	37.5	47	35	82.5	75	65	62.5	90	80	85	70	100	85	87.5
50	7.5	12.5	12.5	17.5	12.5	57.5	30	17.5	27.5	77.5	50	55	37.5	85	50	60	40	100	70	67.5
30	0	0	0	0	0	0	20	0	0	45	10	35	5	47	10	50	17.5	50	12.5	60
ctrl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key: n = No treatment; U2 = 2 hrs U-V; U4 = 4 hrs U-V; U8 = 8 hrs U-V

Table 2:
Effects of Sunlight (% mortality in time) on hexane extract of D. tripetala activity against *A. aegypti* larvae

Conc	12 hrs				24 hrs				48 hrs				72 hrs				96 hrs			
ppm	n	S2	S4	S8	n	S2	S4	S8	n	S2	S4	S8	n	S2	S4	S8	n	S2	S4	S8
90	37.5	20	32.5	27.5	75	60	67.5	40	25	85	80	72.5	97.5	95	92.5	80	100	100	100	90
80	20	12.5	20	10	50	25	45	27.5	67.5	60	70	60	90	90	82.5	75	92.5	97.5	90	80
70	10	25	12.5	12.5	37.5	52.5	45	20	50	60	55	52.5	77.5	80	80	60	80	90	85	70
60	75	75	10	75	12.5	50	37.5	10	35	55	40	37.5	62.5	72.5	65	52.5	70	77.5	70	55
50	75	0	5	0	12.5	10	22.5	10	27	30	30	10	37.5	30	47.5	10	40	32.5	62	10
30	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	17.5	0	10	0
ctrl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key: n = No treatment; S2 = 2 hrs sunlight ; S4 = 4 hrs sunlight; S8 = 8 hrs sunlight

Table 3
Comparison of treatment means (Duncan's test) for U-V and sunlight exposures

Treatments	Means
U-V radiation	
Untreated	6.667b
2 hrs	9.167a
4 hrs	8.583a
8 hrs	7.792ab
LSD	1.516b
Sunlight exposure	
Untreated	6.667a
2 hrs	6.792a
4 hrs	5.958a
8 hrs	6.167a
LSD	1.73a

**means with the same letter are not significant*

Duncan's test showed a significant difference in the means of the treated and untreated (P>0.05). The comparative LC_{50} values at 96 hour, presented in Table 2, reveal 447ppm, 44.7ppm and 24ppm for the untreated, 2 hours 4 hours and 8 hours treatments respectively. The comparative trend of mortality over time show that at 1-hour test period, 20% mortality was observed for 8 hours U-V irradiated Dennettia oil and no death was recorded in other treatments. At 24 hours, 75%, 90%, 78% and 100% were recorded for untreated, 2 hours; 4 hours and 8 hours U-V irradiated Dennettia oil respectively.

On the effect of sunlight on D. tripe/ala oil at 90ppm treatment, 100% mean mortality was recorded at 4 hours sunlight while no death was recorded in the control set up as shown in Table 3. Duncan's test of the data showed no significant difference between the untreated Dennettia oil and various sunlight exposure of the extract (Table 4). The comparative LC5O presented in Table 2 at 96 hours shows only a slight variation. Sunlight exposure did not show any significant effect on the potency of Dennettia oil.

DISCUSSION

The persistence of Dennettia oil toxicity over 96 hours test period is promising in the control of mosquito larvae. The susceptibility of *Aedes aegypti* larvae to the toxic principle of Dennellia oil in this study corroborates the insecticidal activity of D. tripetala repoiled by Agbakwuru et al (1978) and Iwuala et al (1981). The toxic activity of Dennettia oil probably relate to phenylnitroethane, a natural nitro-compound which forms about 80% of Denneitia oil as reported by Okogun and Ekong (1969).

Sunlight exposure has no significant effect on the potency of Denneitia oil. The extract is virtually stable under s exposure. This i co to the report of Wink (1993) and D and Sturrock (1983) that pesticidal principles of plant oligin are unstable under sunlight.

U-V Irradiation activated the potency of Denneitia oil. The U-V activation of the larvicidal properties of Denneiria oil in this report, is in line with findings of Graham *et al* (1980) and Arnason et al (1981). *Dennettia tripetala* hexanolic extract has a high larvicidal properties on mosquito larvae and is environmentally tolerable and non persistent in the environment.

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