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INSECTICIDAL EFFECTS OF *HYPTIS SPICIGERA* (*LABIATAE*) ON *AEDES AEGYPTI* LARVEA AND ADULTS

[1]Auta, K. I., [1]Naman, K. and [1]Hosea, Y. C.

[1]Department of Biological Sciences, Kaduna State University, Kaduna

ABSTRACT
The insecticidal potency of a local plant *Hyptis spicigera* (Labiatae) was evaluated against *Aedes aegpti* larvae and adults alongside conventional insecticides 0-2,2 dichloroving 0.0 dimethyl phosphate. The air dried leaf extracts obtained using soxehlet extractor were applied to the fourth larvae instar of *Aedes aegypti* larvae obtained from blood fed adults mosquitoes caught from the Jos metropolis. *Hyptis spicigera* produced 81. 33% and 100 % mortality for the larvae and adult respectively at a concentrations of 100mg/l which compete significantly $P<0.05$ with the convention 0-2, 2 dichlorovinyl 0.0 dimethyl phosphate ($p<0.05$).The implication and usefulness of the finding are herein discussed.

Key words: *Hyptis spicigera*, Extract Efficacy, Aedes *aegypti*, Larvae, Adult

INTRODUCTION
The use of pesticides specifically for the control of insects and other arthropods dated back to some three hundred years ago (National Academy of Science, 1969). The toxic nature of arsentcals was known to the Greeks and Chinese during the first century A.D and the use of pest averting sulphur had been recommended a thousand years earlier (Busvine, 1980). Despite the length of time when such chemicals were used, the development of insecticides has been slow probably due to little understanding of insect biology.
During the last hundred years,, there has been steady progress in the development of effective insecticides (Busving, 1980). These insecticides include crude organic chemicals such as arsentcal mercury compounds and plants product such as nicotine, others include natural product from coaltar and petroleum (crude oil). The use of pyrethrum derris and other active plants materials were not feasible as their synthesis proved elusive (Nwankwo, 1983). Botanical pesticides are chemicals toxicant of plant origin and they include chemical compounds such as pyrethrum and their synthetic analogues,. Although both natural pyrethrums and their synthetic analogues are less stable in sunlight, fundamental study of structure activity relationship in the pyrethroids led to the synthesis of permethrim which is stable for several days in sunlight (Ellot *et al.,* 1973). Abate and Hadis (2011) reported that *Anopheles arabiensis* was resistant to an array of insecticides that includes DDT, permethrin, deltamethrin and Malathion However, these achievements may have caused two serious problems. The development of resistance and its toxicity on man, livestock, wildlife and crop-plants (Abate and Hadis 2011).
There has been growing anxiety and concern about the use of synthetic compounds. However, the widespread use of synthetic insecticides has led to many negative consequences (Pavela, 2008), resulting in increasing attention to natural products (Pirali-Kheirabadi and da Silva, 2010). Also critics stressed the harmful effects of these synthetic insecticides especially DDT Aldrin etc. This has led to the ban imposed on such core chlorinated organic insecticides in developed countries like United States of America and Great Britain. Alternatively such ban led to the search for other control measures which led to the discovery of the 3rd and 4th generation insecticides i.e. Juvenile and moulting hormones (Pirali-Kheirabadi and da Silva, 2010). But none of these is able to adequately fill the gap if routine insecticides are withdrawn. The activity of pyrethrum has heightened the search for chemical compound of plant origin, which have little or no effect on non-target organism and the environment generally.

MATERIALS AND METHODS

Collection, Drying and Extraction of Plant Materials
Hyptis spicigera were obtained from three villages, Tammah, Gunki and Marmara all in Nasarawa Local Government Area of Nasarawa State, Nigeria. This plant was authenticated at the Federal College of Forestry, Jos-Nigeria. By the Institute Currator. The fresh plant was obtained during the raining season because it is at this time of the year that the plants are in the bloom. The whole plant was uprooted from the soil. The leaves were carefully plucked from the stems and air dried for one week. The dried leaves were pulverized using mortar and pestle in the laboratory.

Extraction procedure
Sixty four grammes (64g) of powdered leaves of *Hyptis spicigera* were measured into a clean beaker, the powdered materials was wrapped in the thimble of soxhlet extractor. Three hundred and fifty milliliter (350 ml) of absolute ethanol were added into the extraction chamber and allowed for soxhlet refluxing for a period of 72 hours. The extract was then dried using water bath set at the boiling point of the solvent (78.37°C), the ethanol was evaporated until a solid extract was obtained.

Formulation of the extracts
Graded weight volume dilution of each of the test extracts was made using ethanol. In each case, a solution was prepared from each of the different test materials subsequently, dilution along a logarithm gradient of 10.00, 7.50, 5.63, 4.22, 3.17, and 2.38 respectively was made to determine their effective ranges. Small batches (25) each of the different larval stages of mosquito were introduce into the solution.

Source of Insects and Breeding Method
Blood fed *Aedes aegypti* were obtained from residential areas of Apata in Jos North local government area, Plateau State Nigeria. The mosquitoes were reared in a mosquito's cage measuring 40 x 40 mm in the Zoology research Laboratory, University of Jos. The larvae, which hatched from the eggs, were fed two times a week using few splashes of fine yeast suspension. They were reared till they reached the fourth instars stages used for the tests based on their resting position in the water. Some larvae were allowed to

pupate and develop into adult. The adult who emerged thereafter were offered 10% sugar solution in soaked cotton wool pad placed on top of the cage and covered with a plastic cup to avoid being dried. The female *Aedes aegypti* were additionally offered blood from the belly region of a tied up rabbit, which had been shaved clean to allow the mosquitoes suck blood effectively. The mosquitoes were blood-fed in the same manner every three days. The cage were later provided with can ovitrap in form of a Whatman filter paper in plastics container half filter filled with water such that the mosquitoes could lay their eggs attached to the filter paper. The filter paper on water in the ovitrap were removed and replaced with new ones every week. The period between hatching and adult emergency took seven to fourteen days.

TEST METHODS

The following test methods were used: liquid medium exposure techniques: 5 g of the test compound was mixed with 20 ml water to get the desired concentration in the plastic container of 200 ml capacity. Batches of twenty-five (25) fourth stage larvae were introduced after stirring the medium to a uniform solution. A control experiment was set up with only water as the test compound and the lavare were later introduced (WHO 1976).

Test for the Larvae

The liquid medium exposure techniques test methods were used fot the larvae. 5g of the test extract, was mixed with 20ml of distile water to get the desired concentration in the plastic container of 200ml capacity. Batches of twenty-five (25) fourth stage larvae were introduced after stirring the medium to a uniform solution. A control experiment was set up with only distile water as the control and the lavare were later introduced.

Test for the Adult

For the adult test, 5g of the extracts were weighed, dissolved into 200 ml of distilled water. Whatman filter paper was used as the exposure surface. The filter paper was dropped into the solution of the extract to be impregnated. The impregnated paper was air-dried for thirty minutes before being rolled into the WHO killing tube. Mosquitoes in the holding tube were later released to come in contract with the treated surface.

RESULTS

Effect of *Hyptis spicigera* on *Aedes aegypti* larvae

For each test, the twenty-five larvae exposed to the test extract solution for a period of twenty-four to fourty eight hours, using the liquid medium exposure method as described (WHO 1976). The mortality effect of *Hyptis spicigera* extract varied in the different concentrations of the extract. The percentage mortality were; 81.33%, 72.00%; 53.33%; 32.00%; 17.33%; 4.00% for the various concentration in mgl respectively (Table 1).

TABLE 1: Mortality rate of *Aedes aegypti* larvae exposed for 48 hours to different concentrations of *Hyptis spicigera* extract

Con of extract (mgl)	Log of conc	Total No. larvae exposed	Total No. larvae death	Mean No.	% Mortality	Abbot's corrected % morality
10	4.00	(75)	61	20.33	81.33	80.55
7.50	3.73	(75)	54	18.00	72.00	70.83
5.63	3.75	(75)	40	13.33	53.33	51.39
4.22	3.63	(75)	24	8.00	32.00	29.17
3.17	3.50	(75)	13	4.33	17.33	13.89
2.38	3.38	(75)	03	1.00	4.00	0
Control	-	(75)	03	1.00	4.00	0

Mg/l = Miligramme per litre , LogCon + Logarithm of concentration

Effect of 0.2,2 dichlorovinyi 0, 0 dimethyl phosphate on a *Aedes aegypti* larvae

Twenty-five larvae exposed to the graded concentration dichlorovinyl dimethyl phosphate first period of twenty four hours and then four eight hours using the liquid exposure method. The mortality rate at the end of period was evident as it increased with increasing concentration of the toxicant for instance, at a concentration of 2.38 mg/l and 10.00 mg/l the percentage mortality was 1.33% and 100% respectively (Table 2).

TABLE 2: Mortality scores of *Aedes aegypti* larvae exposed for 48 hours to grade concentration of 0-2, 2 dichlorovinyl 0.0 dimethyl phosphate.

Conc of extract mg/l	Log of conc	Total No. larvae exposed	Total No. larvae death	Mean No. dying	% Mortality	Abbot's corrected % morality
10.00	4.00	75	75.00	25.00	100.00	100.00
7.50	3.73	75	60.00	20.00	80.00	79.17
5.63	3.75	75	43.00	14.33	57.33	55.55
4.22	3.63	75	25.00	8.33	33.33	30.55
3.17	3.50	75	15.00	5.00	20.00	16.67
2.38	3.38	75	1.00	0.33	1.33	2.78
Control	-	25	0.00	0	0	0

Mg/l = Miligramme per litre , LogCon + Logarithm of concentration

Effect of *Hyptis spicigera* on adults *Aedes eagypti* after twenty-four and fourty eight hours of exposure

Hyptis spicigera extract on the treated surface exerted high killing action on the adult of *Aedes aegypti* within twenty-four hours of exposure. The mortality was observed to be 100% at a concentrations of 10.00 mg/L, 7.50 mg/L and 5.63 mg/L respectively while at a concentration of 4.22mg/l, 3.17mg/land 2.38mg/l has 77.33, 66.67, 26.67 % mortality respectively (Table 3).

TABLE 3: Percentage mortality of *aedes aegypti* adults exposed for 48 hours to different surfaces treated with *hyptis spicigera extract*

Con of extract In mg/l	Log of conc	Total No. Adult exposed	Total No. death	Mean No. death	% Mortality	Abbot's corrected % morality
10.00	4.00	(75)	75	25.00	100.00	80.55
7.50	3.73	(75)	75	25.00	100.00	70.83
5.63	3.73	(75)	75	25.00	100.00	51.39
4.22	3.63	(75)	58	19.33	77.33	29.17
3.17	3.50	(75)	50	16.67	66.67	13.89
2.38	3.38	(75)	20	6.67	26.67	0.00
Control	-	(75)	09	3.00	12.00	0.00

Mg/l = Miligramme per litre , LogCon + Logarithm of concentration

TABLE 4: Mortality scores of *Aedes aegypti* adult exposed for 48 hours to grade concentration of 0-2, 2 dichlorovinyl 0.0 dimethyl phosphate

Conc of extract mg/l	Log of conc	Total No. larvae exposed	Total No. larvae death	Mean No. dying	% Mortality	Abbot's corrected % morality
10.00	4.00	75	75	25.00	100.00	100.00
7.50	3.73	75	75	25.00	100.00	100.00
5.63	3.75	75	75	25.00	100.00	100.00
4.22	3.63	75	25	25.00	100.00	100.00
3.17	3.50	75	25	25.00	100.00	100.00
2.38	3.38	75	25	25.00	100.00	100.00
Control	-	25	00	0.00	0.00	0.00

Mg/l = Miligramme per litre , LogCon. Logarithm of concentration

DISCUSSION

The test were conducted on different days using liquid medium exposure method for the larvae test. On introduction of the larvae into the formulated solution, they were seen to show excitation and tremors closely followed by decreased movement and eventually death. This happens under an hour of their introduction with the larvae appearing swollen and darkened. All treatment and control test organism were discarded after each Bioassay. Test were obtained on different days, the results were used to calculate the percentage mortality against log of concentration, probit killed value against long concentration. Regression analysis for the Aedes aegypti larvae however showed that there is no significant difference in the mortality effects on the larvae of the test extract and the organo phosphate ($p < 0.05$). There is a relationship between the test components and the conventional insecticide. The result of this work indicates that both the synthetic insecticide and plant extracts have marked lethal effect on both larvae and adult of Aedes aegypti, however, the result suggests that the test extracts were less efficient in toxic activity in comparison with the routine insecticide 0-2,2 dichlorovinyl 0,0 dimethyl phosphate. The mortality effect of the test extracts was gradual, increasing with concentration. Bower *et al*., (1976) showed that insecticides of vegetable origin could be actually toxic to various insects. The toxicity had been traced to the potency of the active component of such extracts (Busvine, 1980). In the present studies however, the active component of the test extract were not identical and therefore their seemingly toxic principles could not be identified. Djam (1983) observed that the mortalities of *Toxorhnchthyes ambionensis* and *Aedes aegypti* larvae increased as the concentration of the toxicant increased. This showing a direct relationship between the larvae and the test extract. The search of insecticides of plant origin have been limited in the Nigerian situation, however, Agbakwu *et al*., (1978), Iwula *et al*., (1981) demonstrated that *Dinnetia iripetala* oil was active against adult nymps of the cockroach, *Periplanatal Americana* and the prasshopper *Zonocerus variegate* as well as insect pest store cowpeas and maize.

It is in further search for the insecticides of plant origin that the present work was undertaken. The full potency of this local plant may not be easily evaluated due perhaps to experimental limitations. However, the result obtained from the work suggest that the plant posses some insecticidal components. *Hyptis spicigera* was found to be more potent against adult mosquitoes.

On the other hand, *Hyptis spicigera* may pose a great hazard to man since it is not edible as such. However, conclusion cannot be reached on the actual potentialities of the extract based on this present work. The plant extracts hold a better promise as insecticides. Further research is needed to determine their actual toxicity level on mosquito larvae or adult and effort made to identify and isolate their active components. They could also be tested for repellency or chemosterilant potentialities or even for systemic poison.

Human toxicity test may as well be carried out, there has been many question of their toxic effects at the moment, this confer a major advantage when we consider the fact that the potential toxicity of the extract to the insect can be accentuated with little or no health risk to man and his animals or crops (Iwula *et al*., 1981)

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