THE TOXICITY OF HEXANOLIC EXTRACT OF *XYLOPIA AETHIOPICA* TO LABORATORY REARED LARVAE OF *CX. P. QUIUEFASCIATUS.*

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ABSTRACT

The hexanolic extract of *Xylopia aethiopica (*Ethiopean pepper*)* was tested for acute toxicity on the larva of *Culex p. quiquefasciatus* reared in the laboratory of the Department of Biological Sciences University of Agriculture Abeokuta, Nigeria. Five concentrations: (50ppm, 100ppm, 2000ppm, 300ppm and 400ppm) were evaluated for acute toxicity on the larvae and total percentage mortalities recorded at intervals of 1, 12, 24, 48 and 96 hours in each test. Effects of sunlight and ultra violet radiation on stability of the extract's potency at 2,4, and 8 hours respectively were equally examined. The mean lethal concentration LC50 was 257ppm. Toxicity of *Xylopia aethiopica* on *Culex p. quiquefasciatus* larvae was gradual and persisted throughout the test period. Sunlight exposure has no significant effect on the potency of *Xylopia* oil while ultra-violet radiation activated the larvicidal properties.

Key words: *Xylopia aethiopica*, acute- toxicity, larvae, *Cx.p.quiquefasciatus*, Lethal concentration, LC50 and potency.

INTRODUCTION

Insects have attacked plants since the Devonian period. During this prolong interaction many plant taxa evolved higher sophisticated defence system, largely a complex array of defence chemical produced by the plant themselves. (Hedin, 1991). Renewed interest in botanical pesticides is motivated by three major objectives: (i) to encourage traditional use of simple formulation of locally available plant materials in pest control .To identify sources of new botanical pesticides for commercial extraction. (ii)To elucidate the chemical structure of active components of botanicals. This was based on the fact that novel metabolism may serve as model for the chemical synthesis of new pestcides with more desirable properties, for example the known structure of natural pyrethroids have provided a basis for the development of a range of insecticides of great practical value (Elliott *et al.*, 1978).

However, in spite of the fact that a variety of secondary metabolites of plant origin afford protection against insects, only a few of these have been developed into commercial insecticides. These include margosan-0, developed from Neem, (Larson, 1987). Black leaf 40" produced from the by-product of tobacco industry (Merck index 1989), Ryanex or Ryaniacide obtained from *Ryania speciosa.* Other bioactive compounds of plant origin that has been in use in pest control include rotenone from *Derris guinolizidine,* alkaloid from many leguminosae such as Lupinus, Quassin and Pyrethrum.

It is likely that systematic survey of plant materials with the broad range of bioassay using different test species would lead to the discovery of new active compounds although one may not always come across compounds possessing toxicity against insect pests comparable to the very potent currently available insecticides. Hence there is need for continuous search for new compounds with desirable properties.

Studies on the dried fruit of *Xylopia aethiopica* have led to the isolation of a new diterpene acid, Xylopic acid (Ekong and Organ, 1968) and five other kaurane diterpenes (Ekong, 1968). Xylopia essential oil obtained by direct steam distillation of the derived and crushed fruits gave a strong positive reason for aldelydes with Tollens reagent. Oji *et al.*, (1991) reported that laboratory treatment of maize grain with dust or ethanolic extracts of *P. guineense* and *X. aethiopica* caused significant mortality of adult *Sitophilus zeamais* infesting grain. *X. aethiopica* has not been tested on insect's such as mosquitoes. Majority of the work earlier done on *Xylopia* had been on stored products insects the present study aims at investigating the insecticidal properties of *Xylopia aethiopica* on larvae of *Cx.P. quiquefasciatus* in the laboratory.

MATERIALS AND METHODS

Plant extract

Dry fruits of *Xylopia aethiopica* (Ethiopian pepper) were bought from a local market in Ile-Ogbo Osun State Nigeria. . The dried fruit were washed and later sundried. The dried fruit were ground to a fine powder using electric grinder and later exhaustively extracted with n-hexane using Soxhlet apparatus .The extract was then concentrated with rotary evaporator which removed the hexane component leaving behind a viscous oil required for the analysis. Preparation of volume /volume stock solution of *Xylopia aethiopica* was by measuring out 1ml of the extract, emulsify it in about 0.003ml or 3 drops of Tween –80. The emulsified extract is then added up to 1 litre to form 100ppm stock solution. From the stock serial concentrations of; 50ppm, 100ppm, 200ppm, 300ppm and 400pm were prepared.

Insect material.

Sands and debris from breeding sites of *Cx.P. quiquefasciatus* were collected at the peak of dry season (January) and soaked in water. After a day, hatching of viable eggs commenced. Hatched out larvae were then removed in to cleaner water inside brightly coloured enamel plates using a pipette. Active swimming non feeding pupae were thereafter collected into open bottles and placed in a cage where they were left to emerge.

Mosquito cages of about 40 by 40cm were made of light wooden frame with sides of mosquito netting and base of wooding board. One side of the cage is provided with sleeve for taking material in and out of the cage. Adult mosquitos were allowed to feed on rabbits kept in cages after they have been starved for one to two days. Oviposition commenced after three days of the first blood meal and lasted for about 7 days. Eggs were collected dried and stored away in an incubator at 31± 2 ^{0}c. All the third instars larvae used in the bioassay were hatched out from the stored eggs.

Evaluation of the effect of *Xylopia aethiopica* fruit oil on *Cx. P. quiquefasciatus* larvae.

From each concentration 250ml solution was measured and introduced into separate labelled 500ml specimen bottles. Forty larvae of *Cx.P. quiquefasciatus* were then introduced into each. The bottles were then covered mesh of 1 mm for aeration but prevents entry and exit of any insect. Each treatment was replicated five times. Effects of physicochemical parameters such as sunlight and ultraviolet irradiation were tested on the extract using the method described by Adewumi and Marquis (1980). A stock solution of the *X. aethiopica* was exposed to sunlight and a U.V lamp (Gallenkamp LH 530) with peak output at 366nm for 2, 4 and 8 hours. For the U.V light, stock solution was placed at a distance of about 30cm from the light source.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 the 96 hours mortality of the different concentrations were used from these data using probit regression line.Data obtained from processes described above were then subjected to analysis of variance at 5% level of significance and where there was a difference, Duncan's T test was used to determine whether there were significant differences between treated and untreated means.

RESULTS

The acute LC_{50} of *Xylopia aethiopica* oil extract was 257 ppm (Table 1) very few deaths were recorded in the first 6 hours, percentage mortality increased with increase in time. The highest concentration of 400 ppm killed only 5% of the population in 1 hour test period, 3% in 6 hours and 75% in 24 hours test period (Tables 2a and 2b) Toxicity of *X. aethiopica* was observed to be gradual and lasted throughout the test period.

Table 1: LC50 at 96 hours of *Xylopia aethiopica* applied to *Cx.P. quiquefasciatus larvae*

Treatments	Concentration
Untreated	257ppm
2 hrs Sunlight	200ppm
4 hours Sunlight	199.53
8 hours Sunlight	316.23ppm
2 hours U.V	63.1ppm
4 hours U.V	95.5ppm
8 hours U.V	63.1ppm

Duncan's test revealed that *X. aethiopica* oil extract exposed to sunlight showed no significant difference between the untreated X. *aethiopica* oil extract and 2, 4, and 8 hours sunlight exposure (Table 2a). The comparative LC_{50} value presented in Table 1 revealed little differences between the treated and untreated extracts except for 8 hours treatment.

Within the first 1 hour of treatment no larvae death was recorded. At 2 hours, 60%, mortality was recorded while 47%, and 63% were recorded at 4 and 8 hours exposure respectively, compared to 75% mortality value recorded for the unexposed *X. aethiopica* oil extract. While considering the effect of ultraviolet irradiation on the potency of *X. aethiopica,* result reveals that at 1 hour test period, 40%, 60% and 45% mortalities were recorded for 2, 4 and 8 hours *exposure* period respectively. Compared to 5% mortality recorded for unexposed X. *aethiopica oil extract (Table 2b).*

Duncan's test showed significant variation in their means ($P > 0.05$) (Table 3). The treated *Xylopia aethiopica* oil extract was significantly different from the untreated. Variation of the LC_{50} showed an activation of the potency of *Xylopia aethiopica* on irradiation but without a definite trend.

DISCUSSIONS

Toxicity of *Xylopia aethiopica* hexane extract was gradual and persisted throughout the test period of 96 hours. The toxicity and persistence of *X. aethiopica* hexane extract is very reassuring in the control of *Cx.P. quiquefasciatus* in the field since they lay their eggs on a daily basis when and where breeding exist. The persistence will reduce the need for a daily repeated application. The toxicity of *Xylopia aethiopica* extract to larvae of *Cx.P. quiquefasciatus* corroborate the report of Oji *et al* (1991) that *X aethiopica* showed significant mortality effects on adults *Sitophilus zeamais.*

Ekong and Ogan (1968) isolated a deterpen acid, Xylopic acid, which could be the major insecticide present in *X. aethiopica.* However the mode of action of xylopic acid is still obscure. Sunlight exposure has no significant effect on the potency of *X. aethiopica* oil extract this stability was a deviation from Wink's (1993) reported that pesticide principles of plant origin are unstable under sunlight. Ultraviolet (U.V) portion of the sunlight was observed to activate the larvicidal properties of *X. aethiopica.* The findings in this study is in line with observations of Graham *et al.,* 1980,. Wat *et al.,(* 1979) and Arnason *et al.,* (1981) works where sunlight was found to significantly affect the potency of various botanical pesticides.

Since *X. aethiopica* is very common in the local market, it could be a very cheap source of a ne pesticide however further research is necessary to identify the active ingredient in *Xylopia aethiopica* as we as its mode of action.

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

Sunlight exposure	Untreated	4.600a
	2 hours	5.250a
	4 hours	4.950a
	8 hours	6.050a
	LSD	2.289
U.V	Untreated	4.600a
	2 hours	6.600a
	4 hours	4.650b
	8 hours	3.35b
	LSD	1.7467

*Means followed by the same letter are not significantly different.

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