FIELD CONTROL OF PERIDOMESTIC MOSQUITOES OF MEDICAL IMPORTANCE WITH EXTRACTS OF *Petiveria alliacea L.*

T.A. ADEBAYO[1,+], J.I. OLAIFA[1], A.J. AKINTOLA[2] and A.O. OJO[1]

1. Environmental Toxicology Laboratory, Dept. of Agronomy and 2. Dept. of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

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Abstract

The toxicity of the aqueous extract and turbid distillate of roots of *Petiveria alliacea L.* to larvae of *Aedes aegypti. Culex pipiens fatigans* and *Anopheles gambiae* were investigated in the laboratory and in the field. Both extracts were larvicidal to second instars. In laboratory tests, a 10% (v/v) aqueous extract caused 100% mortality in all three species. The turbid distillate was less potent than the aqueous extract under field conditions. The significance of the present findings for mosquito control using locally – an available natural resource is discussed.

Keywords: *Petiveria alliacea*, larvicidal, *Aedes aegypti*, *Culex pipens fatigans*, *Anopheles gambiae*.

1. Introduction

Many plants in the family Phytollacaceae are known to be toxic to mammals when consumed fresh. The toxicity of the Australian species *Phytolacca octandra* to cattle and pigs has been reported by Duncan, (1962). *P. Americana* gives rise to haemorrhagic gastritis, nausea, depression and prostration (Kingsburry, 1964) *P. dodecandra* is lethal to sheep and cattle (Mugera, 1970).

The guinea hen weed *Petiveria alliacea L.* (Phytollacaceae), common in central America (Clarke and Clarke, 1975), is planted in residential areas in Southwestern Nigeria to repel snakes, scorpions and insects (Olaifa and Akingbohungbe, 1987). The plant imparts a garlic-like taint to milk, contains a volatile carbamate-like substance (Blohm, 1962; Ruiz, 1972), antimicrobial trisulphide (Szozcepanski *et al.*, 1972) and has insecticidal properties (Olaifa and Akingbohungbe, 1987). The aqueous extract and turbid distillate have been found to deter oviposition by gravid females of *Anopheles gambiae, Aedes aegypti* and *Culex pipiens fatigans* (Adebayo, 1992).

The current study was undertaken to further explore the bioactive properties of the aqueous extract and turbid distillate of the plant against three species of mosquitoes that are endemic in Nigeria: *Anopheles gambiae* (vector of malaria parasites), *Aedes aegypti* (vector of yellow fever viruses) and *C. pipiens fatigans* the intermediate host of filaria worms *Wuchereria bancrofti* and *Dirofilaria immitis*.

2. Materials and Methods

Preparation of Extracts

Fresh roots of *P. alliacea* were obtained from the medicinal plant reserve of the Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. They were chopped into small pieces and placed in a round bottom flask fitted to a glass extractor. Distilled water was added at ratio 1: 2 (w/v) (weight of roots: volume of water) and heated to boiling on a heating mantle. The first 60ml of the condensed steam (turbid distillate) were collected and stored at 4°C until needed. This served as a stock solution which was later diluted to different concentrations for the bioassays.

In another set up, fresh roots of *P. alliacea* were cut into small pieces and distilled water added at a 1:2 (w/v) ratio. The water plus the pieces of roots were heated for 2hrs at 100°C and then cooled to room temperature. The liquid was later decanted and kept refrigerated until use (i.e. 24h later). This served as a stock solution of the aqueous extract and was diluted in subsequent bioassays.

Culture of insects

Individual colonies of each mosquito species, i.e. *A. aegypti, A. gambiae and C. pipiens fatigans* were obtained as larvae from the malaria and vector control unit of the National Institute for Medical Research, Yaba, Lagos, Nigeria. These larvae were fed with ground dog biscuits until they became pupae and later adults. Both sexes were raised together in the same cage and allowed to mate freely. Adult female mosquitoes have hypodermic mouthparts which enable them to pierce the skin and suck the blood of mammals, birds, reptiles, and other arthropods while adult males which have reduced mouthparts feed on nectar and water. In this study, females were allowed to take a blood meal every morning from a restrained chicken whose feathers had been removed and males were fed on a 10% sugar solution according to methods described earlier (Adebayo and Olaifa, 1994). Larvae hatched from eggs laid, were also fed as described earlier and used for bioassay at the second instar stage.

Bioassay

Laboratory Test: The larvicidal activity of each extract (aqueous extract or turbid distillate) was tested by preparing the following range of dilutions from the respective stock solutions: 0, 1, 3, 5, 7.5 and 10% (v/v) using distilled water to give a final test volume of 200ml in a 250ml beaker. Fifty second instar larvae of *C. pipiens fatigans, A. aegypti or A. gambiae* were placed in each beaker and fed with dog

+ corresponding author

biscuits. Each test was replicated three times. The test containers were placed on tables and held under ambient conditions (25 –30°C, (Relative humidity) 70%). Larval mortality was assessed 12 and 24h after treatment and every 24h thereafter for 5 days (120 h). Mortality data by treatment were corrected according to the control mortality using Abbott's formula (Abbott, 1925) and then subjected to probit analysis (SAS Institute, 1985) and the means ranked using Duncan's multiple range test (Duncan, 1955).

Larvicidal activity of the aqueous extract of P. alliaceae in the field

Tests were conducted in concrete tanks (96 x 60x 50 cm) each containing 30 liters of aqueous extract at the following concentrations: 1, 3, 5 and 10% (v/v). Untreated water served as the control. One hundred and thirty 2nd instar *A. Aegypti* were introduced into each pond and fed with dog biscuit. *C. pipiens fatigans and A. gambiae* larvae were similarly exposed to the extracts. All treatments were replicated three times.

Mosquito net was spread over each pond to prevent emerging adults from escaping and to shield the ponds from feral mosquitoes and predators. The effects of the aqueous extracts on the test mosquito larvae were evaluated by counting the number of adults emerging from each pond from 3 to 11 days after treatment (DAT) at 24 h intervals. All these were done without adjusting water level. Dead adults on the surface of the ponds were also counted as emerged adults. Percentage emergence was determined according to Rathburn *et al.* (1980) as follows:

$$\%(Emergence) = \frac{CS - DA}{CS + PE + DP}$$

where,

CS = Cast pupal skin

DA = Dead adults on water surface

PE = Partially emerged adults

DP = Dead pupae

The % control was calculated by comparison with the 'check' treatment with mortality levels adjusted accordingly, using Abbott's (1925) formula.

Test of the larvicidal activity of the turbid distillate of *P. alliacea*

The experiment was carried out in 15 liter plastic bowls, each containing 4 liters of the turbid distillate at the following concentrations: 0, 1, 3, and 7.5 and 10% (v/v). Ninety, 2nd instar C. *pipiens fatigans* were introduced into each bowl. Each bowl was placed inside a cage (to prevent the escape of emerged adults and to shield the bowl from feral mosquitoes) and set out doors. The treatments were similarly evaluated against *A. aegypti* and *A. gambiae* larvae. Each treatment was replicated 3 times during a single experiment. The percentage emergence and corrected percent control were determined as outlined above.

4. Results

Laboratory assays

A. egypti, C. pipiens fatigans and *A. gambiae* exhibited different levels of susceptibility to the extracts of *P. alliacea* in the laboratory (Fig. 1). After 96h, *A. aegypti* was the most susceptible to the aqueous extract with an LC_{50} of 4.5% (v/v) followed by *C. pipens fatigans* with an LC_{50} 4. 7%. *A. gambiae* was the least susceptible with an LC_{50} of 5.5 %. However, a dose of 10% caused 100% mortality in all of the test larvae.

Experiments with different doses of turbid distillate (Fig 2), revealed that, the LC_{50} for *A. aegypti* and *C. pipiens fatigans* was 4.5% and 5.9% respectively, and 7.1% for *A. gambiae.* A 10% (v/v) concentration of turbid distillate was required to cause 100% mortality in *An. Gambiae* after 96h (Fig. 2). Suggesting that this species is less sensitive to the distillate and that the aqueous extract is more potent than the turbid distillate.

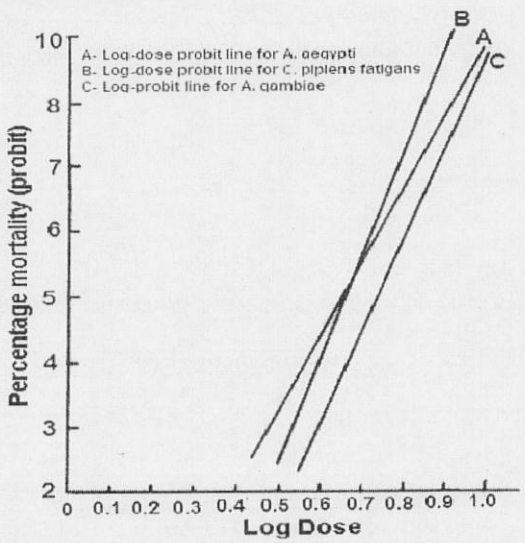

Figure 1: The dose-mortality response of second instar *A. aegypti, C. pipiens fatigans* and *A. gambiae* exposed to the extract of *P. alliacea.*

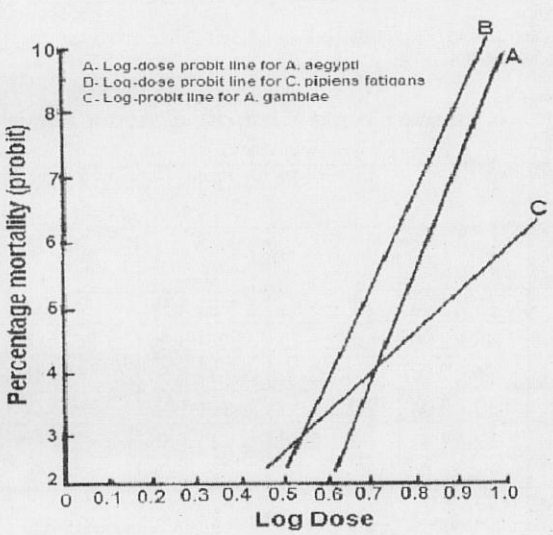

Figure 2: The dose-mortality response of second instar *A. aegypti, C. pipiens fatigans* and *A. gambiae* 96hr after exposure to turbid distillate extract of *P. alliacea.*

Field activity of aqueous extracts and turbid distillate

In all the field tests, adult emergence in all the ponds occurred mostly between 7 and 8 DAT. However, in the ponds containing 7.5% and 10% (v/v) aqueous extracts, foam and a thin layer of colloidal substances covered the surface of the ponds from 3 DAT. No *A. aegypti* adults emerged from ponds containing 10% (v/v) extract (Table1). Larvae and pupae sampled from the ponds 48h after exposure revealed that 2nd instars died while molting to the 3rd instars or during pupal – adult ecdysis. A dose of 7.5 % (v/v), which gave 100% control in the laboratory, caused only 52.8% mortality in the field. Results in Table 2 show that, compared to the untreated check, the 5, 7.5 and 10% (v/v) aqueous extracts reduced *C. Pipiens fatigans* adult emergence by 62.7, 73.1 and 100% respectively.

When larvae of *A. gambiae* were exposed to the aqueous extract or turbid distillate of *P. alliacea* in the field, the aqueous extract provided superior levels of control (Table 3). A 10% (v/v) extract caused 95. 5 % mortality, compared to 47.9% for the turbid distillate. All of the larvae that pupated emerged as adults. Larval mortality caused by the distillate against *C. pipens fatigans and A. aegypti* was not significantly different from the untreated check at the doses tested.

5. Discussion

The results clearly demonstrate that both the aqueous extracts and the turbid distillate of *P alliacea* are toxic to larvae of *A. aegypti, C. pipiens fatigans* and *A. gambiae*. The results of the study corroborated earlier findings on the toxicity of the turbid distillate of *P. alliaceae to C. albiventris* (Edwards) larvae (Olaifa, *unpublished*). Ruiz (1972) reported that the plant contains a volatile carbamate-like substance, which may be the insecticidal component. Carbamates such as propoxur (2–1sopropoxyphenyl methyl carbamate) are popular insecticides for controlling mosquitoes and other household pests.

In the current investigations, the 10% (v/v) aqueous extracts provided operationally feasible levels of control (causing 100% mortality) in all of the tests. The differential response to the turbid distillate shown by *A. aegypti, Cx. pipens fatigans and An. gambiae* could be due to differences in larval physiology that are yet to be identified.

The toxins present in the extracts appear to have neuromuscular activity, as evidenced by the sluggishness and coiling of the larvae following exposure to the preparations. The general symptoms of carbamates in insects are primarily those of poisoning of the central nervous system, since the

Table 1: Field tests of an aqueous extract of *Petiveria alliacea* as a mosquito larvicide against second instar *Aedes aegypti*

	EMERGENCE OF ADULTS/PER DAY (± SD).								
Conc. (%)	1-5	6	7	8	9	10	11	Total Emergence	Corrected Control %
0	0	6.3 ± 1a	63 ± 2.6a	50 ± 1.5a	3 ± 2.1a	3 ± 1.0a	0	125a	-
1	0	3 ± 1ab	59 ± 2.0a	47 ± 2.6a	3 ± 1.5a	0 ± 00b	0	112a	10.4
3	0	5 ± 1.6a	65 ±15.5a	7 ± 2.1c	1 ± 1.0b	3 ± 0.5a	0	79b	36.8
5	0	1 ± 1b	20 ± 2.1b	36 ± 5.0b	1 ± 1.0b	0. 00	0	58c	53.7
7.5	0	0b	12 ± 2.0b	40 ± 2.0b	3 ± 1.0a	0. 00	0	59c	52.8
10	0	0b	0	0	0	-	0	0	100

(Initial larval population 130)
Within columns, means followed by a common letter are not significantly different at $P = 0.05$ Duncan's Multiple Range Test.

Table 2: Field tests of an aqueous extract of *Petiveria alliacea* as a larvicide against second instar *Culex pipiens fatigans*

	EMERGENCE OF ADULTS/PER DAY (± SD).								
Conc. (%)	1-5	6	7	8	9	10	11	Total Emergence	Corrected Control %
0	0	59 ± 5.0a	51 ± 2.6a	8± 1.5b	10a	5 ± 1a	1±1	134a	-
1	0	48 ± 2.0b	32 ± 2c	26± 3.3a	0c	1 ±1b	0	107b	20.1
3	0	26 ± 2.0c	48±2.0b	1± 1.5c	3 ± 1b	0b	1±1	85c	36.6
5	0	19 ± 5.0d	21 ± 2.5d	10± 1.5b	0c	0b	0	50d	62.7
7.5	0	16 ± 4.8d	18 ± 2.0d	1± 1.5b	0c	0b	0	36e	73.1
10	0	0e	0e	0c	0c	-	0	0.0	100

(Initial larval population 130)
Within columns, means followed by a common letter are not significantly different at $P = 0.05$ Duncan's Multiple Range Test.

Table 3: Evaluation of the larvicidal activities of different extracts of *Petiveria alliacea* against second instar *Anopheles gambiae*

	Aqueous extract		Turbid distillate	
Concentration	% Emergence	Corrected	% Emergence	Corrected
0	97.3	-	98.1	-
1	89.0	8.5	90.7	7.54
3	80.4	17.4	84.6	13.75
5	70.1	27.9	77.2	21.30
7.5	36.7	62.3	51.1	47.91
10	4.0	95.9	47.1	51.98
	LSD=25.9	LSD=26.3	LSD=15.8	LSD=16.1

insect neuromuscular junction is not cholinergic. Nerve poisoning, digestive disturbances, muscular atrophy and glomerulonephritis have been reported when cattle were fed with the plant (Clarke and Clarke, 1975). Further studies are needed to elucidate the specific chemical nature of the bioactive compound present in the plant.

The foam and colloidal substances which formed and covered the surface of the treated ponds 3 DAT with 7.5 and 10% (v/v) aqueous extracts in both laboratory and field trials might have contributed to larval mortality. Levy *et al.* (1980) pointed out that such substances can modify the physical properties of the water surface in ways which interfere with the normal behaviour and development of mosquito larvae and pupae and also with the emergence of adults. The surface film formed by the aqueous extract significantly reduced the surface tension of the water and subsequently killed larvae and pupae by inhibiting proper orientation at the air – water interface and/or by increasing the wetting of the tracheal structures. It seems unlikely that both the aqueous extracts and turbid distillate of *P. alliacea* exerted any juvenoid effects as larval development rate was not significantly different in the treated and untreated ponds.

Under field conditions, the turbid distillate was only marginally active compared to the mosquitocidal effect shown in laboratory bioassays. The reduction in activity may have been caused by photodegradation of the active ingredients, a common phenomenon in the botanicals. Similarly the potency of the aqueous extract was lower in the field than the laboratory, 5% and 7.5% (v/v) concentrations caused 100% mortality in most bioassays whereas only the 10% (v/v) preparation effected 100% mortality under field conditions. The lower activity may have been due to the photo – instability of the extracts but in addition many biotic and abiotic factors can influence activity in the field. Further studies are necessary to identify the most important of these in order to refine formulations that will maintain the larvicidal activity of the active compound after application, or keep the active ingredient in the feeding zones of the mosquito larvae.

Both the aqueous extract and turbid distillate of *P. alliacea* are inexpensive, easy to prepare, handle and apply. Furthermore these extract are excellent oviposition deterrents to gravid females of *C. pipens fatigans* and *A. aegypti* at a 10% (v/v) concentration (Adebayo, 1992). Extracts of indigenous plant have significant potential for use in mosquito abatement programmes in the tropics, where they will not only be more economical but more environmentally acceptable than synthetic insecticides. Extracts of *P. alliacea* may thus be one of the first options in response to Gratz's (1985) demand for mosquito control measures that can be "undertaken by individuals and families in and around their homes".

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