EVALUATION OF *MELIA VOLKENSII* EXTRACT FRACTIONS AS MOSQUITO LARVICIDES[1]

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ABSTRACT. A standardized fraction of *Melia volkensii* fruit kernel extract was tested against *Anopheles arabiensis* mosquito larvae. The LC_{50} in 48 hr was 5.4 μg/ml. At low concentrations this fraction had growth inhibiting activity producing prolonged larval instars, and lethal effects during ecdysis. Further fractionation of the standardised fraction yielded seven bands on preparative Thin Layer Chromatography. The two most lipophilic bands had acute toxic effects on the larvae, the next two bands had growth inhibiting effects, while the three trailing bands had no effect at all. The acute toxicity and growth inhibiting effects were destroyed by heat during the drying of the fruits.

INTRODUCTION

Co-evolution of plants and insects has led to the production of secondary plant compounds that have insect growth inhibitory and feeding deterrent properties. Plant parts containing such compounds are sometimes used in indigenous methods of insect control. Plants of the family Meliaceae were first reported to contain insecticidal properties by Chopra in 1928. Until recently, much of the work reported on Meliaceae insect growth inhibitory and feeding deterrency properties concentrated mainly on the extracts of the neem tree, *Azadirachta indica* A. Juss, and the chinaberry tree, *Melia azedarach* Linn., whose main bioactive compounds are azadirachtin (Butterworth and Morgan 1968, Zanno et al., 1975, Broughton et al., 1986), salannin (Henderson et al. 1964) and meliantriol (Lavie et al. 1967). Besides the use of such compounds as agricultural insect pest control agents, their use in mosquito larvae control is an interesting prospective. The possibility has previously been investigated by Chavan et al. (1979), Attri and Prasad (1980) and Chavan (1984). However, the toxicological data obtained on testing the neem seed kernel extracts on the larvae of *Aedes aegypti* (Linn.) (Zebitz 1984) were not promising since a 20 ppm methanolic extract only caused 2.6% larval mortality over the whole of the 4th instar period. Further work by Zebitz (1986) has shown that larvae of *Aedes togoi* (Theobald) and *Anopheles stephensi* Liston are more susceptible to neem seed kernel extracts than *Ae. aegypti.*

An azadirachtin free fraction from seed kernel extracts of *Melia volkensii* has been shown to have greater acute toxic and growth inhibitor effects on *Ae. aegypti* larvae (Mwangi and Rembold 1987, 1988) than an azadirachtin containing fraction from neem seed kernel extracts (Zebitz 1984, 1986). The lethal effects for larvae exposed to *M. volkensii* extracts also occurred earlier than for larvae exposed to neem seed kernel extracts. The purpose of this study was to fractionate the active *M. volkensii* fraction further and assess its effects on the larvae of *Anopheles arabiensis* Patton, a major malaria vector in Kenya. This potential, new insect growth inhibitor is readily available, inexpensive, and environmentally safe in Kenya, especially in rural areas where this plant often grows naturally.

MATERIALS AND METHODS

Larvae of *Anopheles arabiensis* were reared at 28°C. The larval food consisted of 5.4 gm TetraMin® and 2.6 gm yeast suspended in 400 ml of water. Three drops of the food suspension was administered daily to the test larvae. All tests were performed in 40 ml of distilled water contained in 200 ml glass jars. The test material was dissolved in 100% ethanol so that the final volume did not exceed 20 μl. Control larvae received 20 μl of alcohol. Test larvae were exposed continuously to test material in water (Hsieh and Steelman 1974). Mortality was recorded daily and dead larvae or adults removed. The percent mortality was corrected for control mortality using Abbott's formula (Finney 1971), and the results plotted on log/probability paper. Drowning malformed adults were recorded as dead. For adults, mortality was recorded 24 hr post-ecdysis.

Fresh ripe fruits of *M. volkensii* were obtained from Embu, Kenya, approximately 150 km north of Nairobi. The whole fruits were dried at 40°C until there was no more change in weight. For the temperature effect experiment a range of temperatures were used. Dried fruits were pulverized to a fine powder by means of a hammer-mill, until the powder passed through a 1 mm mesh sieve. The powder was extracted three

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times with equal volumes of methanol:water 80:20 (v/v). Each round of extraction consisted of ratios of 1:1, powder:liquid (wt/v). The procedure described by Mwangi and Rembold (1987) was used in concentrating the active fraction to a standard extract. In brief the solution was filtered and evaporated to dryness in a Rotavapor® under vacuum at 40°C. The residue was extracted with cold methanol, followed by acetone. The acetone supernatant was adsorbed on silica gel. The sample was applied on a silica gel column and eluted with hexane:ethyl acetate 1:1 (v/v) (Mwangi and Rembold 1987). The dry ex-silica gel fraction was defined as the standard extract.

The eluent was dried and the residue dissolved in ethyl acetate and applied on a preparative Thin Layer Chromatography (TLC) plate coated with silica gel, Kieselgel 60. The sample was eluted with a solvent system of chloroform:acetone 7:3. The separated UV-visible bands were individually scrapped off and fractions extracted with ethyl acetate. The solvent was evaporated and the weight of the residue from each band taken. A similar TLC plate without the extract sample was similarly treated and scrappings extracted and assayed as control.

RESULTS

Results (Table 1) show that the lethal and growth inhibiting compounds occurring in *M. volkensii* fruits are temperature labile. Extracts obtained from fruits dried at 60, 80 and 100°C were less effective than those dried at 30 and 40°C where all larvae died within 48 hours. A dose of 50 μg/ml of extract obtained from fruits dried at 50°C had some residual lethal effects which would account for a value between 10 and 15 μg/ml of an extract dried at 40°C (Table 1, cf Table 2). The amount of active material however was enough to eliminate all larvae before attaining adulthood. Extracts from fruits dried at 60°C had enough activity to cause both mortality and clearly demonstrated growth inhibiting activity, but very little of such activity was evident in extracts from fruits dried at 80 and 100°C.

Table 1. The effect of 50 μg/ml *Melia volkensii* extract dried at various temperatures on the survival and growth inhibition of *Anopheles arabiensis* (n = 50).

Drying temperature (°C)	% survival (48 hr)	% adult emergence	Mean ± SE days for adult emergence
Control	100	92	12 ± 1.2
30	0	0	—
40	0	0	—
50	14	0	—
60	82	22	35 ± 2.7
80	100	90	14 ± 3.3
100	96	86	13 ± 1.9

Table 2. Percent survival of 50 *Anopheles arabiensis* 2nd instar larvae treated with various concentrations of *M. volkensii* standard extract (n = 50).

Days posttreatment	Concentration (μg/ml)						
	0	1	5	10	15	20	25
1	100	98	86	44	38	24	0
2	100	98	70	28	10	06	0
3	100	96	76	16	06	02	0
4	98	94	60	12	06	02	0
5	98	86	40	12	02	0	0
6	98	84	32	10	02	0	0

When the standard extract obtained from fruits dried at 40°C was tested on *An. arabiensis* larvae at different concentrations, there was no survival even after 24 hr of treatment with 25 μg/ml (Table 5). However, a dose of 1 μg/ml had very little effect even up to the 6th day of treatment. From Fig. 1, the LC_{50} of this fraction in 24 hr is 9.5 μg/ml, 5.4 μg/ml, in 48 hr, 4.0 μg/ml in 96 hr, and 2.9 μg/ml in 144 hours. Larvae treated with 20 μg/ml or above were found to be inactive and moved only sluggishly when agitated; unlike the controls which dashed about in water when agitated. The larvae also rested in twisted S-shaped postures.

Fractionation of the standard extract on TLC using chloroform:acetone 7:3 yielded seven bands, including one at the origin (Table 3). The bulk of the material was in fraction III with an Rf value of 0.77. The Rf values obtained in this solvent system give an index of the lipophilic nature of the fractions; the higher the Rf value the more lipophilic the fraction. Fractions I and II produced the highest acute lethal effects at a dose down to 0.5 μg/ml (Table 4). Fractions III and IV had less acute toxicity but were effective at 50 and 100 μg/ml, respectively. Fractions V and VII did not seem to have acute toxicity even at 100 μg/ml. The log concentration/probit mortality regression lines for some of these fractions are shown in Fig. 2.

Table 5 shows that it was not possible to produce healthy adult *An. arabiensis* when larvae were reared in water containing as little as 1 μg/ml of fractions I and II. Rearing of larvae with low concentration of fractions III and IV produced some healthy adults. However, the lifespan of the larvae giving rise to those adults was greatly prolonged. Most larvae with extended longevity were smaller in comparison to controls in the corresponding instar. Few of them ever attained adulthood, and such adults normally drowned in water since they were unable to expand their wings. Many of the growth inhibited larvae died during ecdysis. Some of the

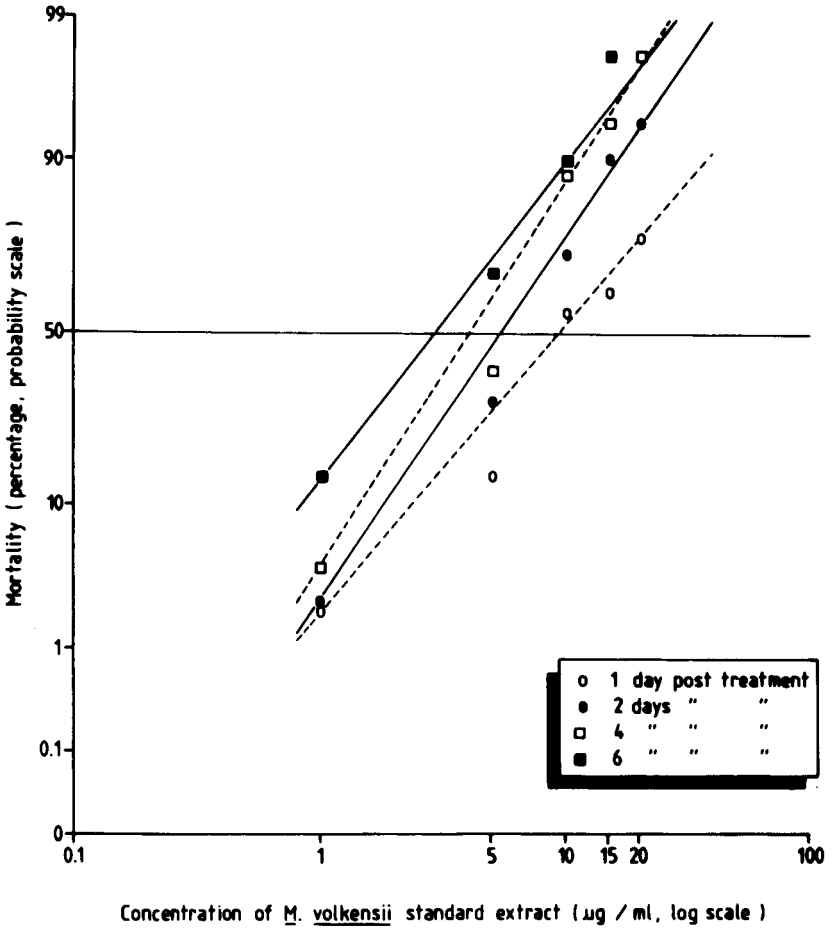

Fig. 1. Log concentrations/probit mortality regression lines for *An. arabiensis* second instar larvae treated with various concentrations of *M. volkensii* extract.

Table 3. TLC fractions of silica gel column-fractionated material (standard extract) of *Melia volkensii*. The solvent system was chloroform: acetone 7:3. The weight of the sample applied on TLC was 300 mg in each case (n = 10).

Designation of fraction	Rf value	Mean (± SE) weight of fraction (mg)	Mean % of sample recovered
I	0.90	18 ± 1.3	6.0
II	0.85	35 ± 2.4	11.7
III	0.77	123 ± 9.8	41.0
IV	0.64	41 ± 3.6	13.7
V	0.41	37 ± 3.0	12.3
VI	0.13	19 ± 2.1	6.3
VII	0.00	15 ± 1.1	5.0

larvae that molted successfully died owing to failure of melanization. Surprisingly, the pupal instar was never prolonged in all cases, and deaths were also not recorded during the pupal stage. However, many 4th instar larvae failed to ecdyse to perfect pupae, producing larval-pupal intermediates which were shortlived.

DISCUSSION

The identities of the insect growth inhibiting compounds in *M. volkensii* have not yet been determined. The presence of four active fractions after TLC separation of the standard extract would indicate that a plethora of limonoids closely related to those found in *A. indica* may be present in *M. volkensii*. The structural configuration of the compounds present seems to be easily disturbed by temperatures above 50°C. It is therefore possible that heavy loss of growth inhibiting activity may be taking place even during processing at 40°C.

It is apparent from the data presented that two major effects are present in the standard extract. At high concentrations larvae experience an acute toxic effect. However, at low concentration the standard extract is growth inhib-

Table 4. Percent survival of 50 *Anopheles arabiensis* 2nd instar larvae exposed to different fractions of *M. volkensii* extract for 48 hr.

Concentration (μg/ml)	Fraction								
	I	II	III	IV	V	VI	VII	Standard extract	Controls
0.05	100	98	100	98	100	94	98	100	100
0.5	72	56	100	100	100	100	100	98	98
1.0	0	26	92	90	94	100	98	100	94
5.0	0	0	94	94	98	96	100	92	100
10.0	0	0	86	92	100	98	98	10	100
20.0	0	0	80	86	100	100	96	06	92
50.0	0	0	34	88	92	100	100	0	98
100.0	0	0	06	74	100	92	100	0	100

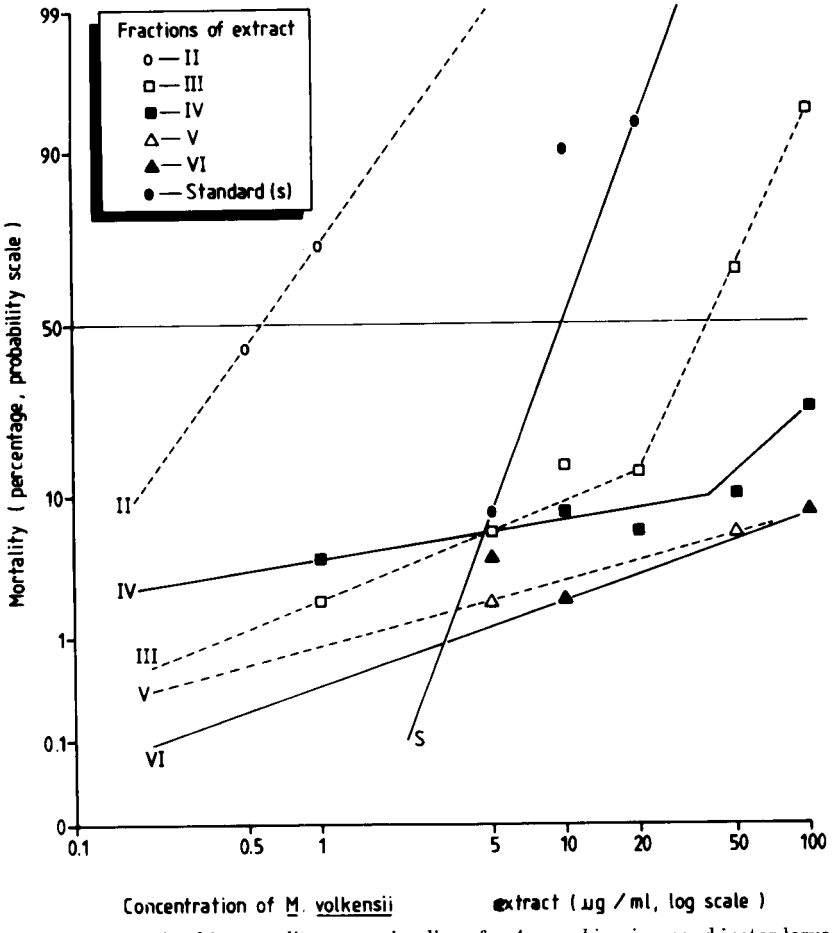

Fig. 2. Log concentration/probit mortality regression lines for *An. arabiensis* second instar larvae exposed to different fractions of *M. volkensii* extract for 48 hours.

iting. Similar effects have been described for *Ae. aegypti*, (Mwangi and Rembold 1988) using a similar extract, but only growth inhibiting effects have been described in *A. indica* extracts (Zebitz 1984, 1986). However, the LC_{50} for larval mortality reported in *Ae. aegypti* is 50 μg/ml in 48 hr, with the lowest growth inhibiting dose being 2 μg/ml (Mwangi and Rembold 1988). The LC_{50} for acute toxicity reported in this study for *An. arabiensis* using the same extract is 5.4 μg/ml (Fig. 1). It would appear that *An. arabiensis* larvae are much more susceptible to the *M. volkensii* extract compared to *Ae. aegypti* larvae. A comparable observation on delayed lethal ef-

Table 5. Mean % of healthy adult *Anopheles arabiensis* emerging from 2nd instar larvae continuously exposed to different concentrations of *Melia volkensii* extract fractions (n = 200).

Fraction	Concentration (μg/ml)					
	Control	1	5	10	20	50
I	96.0	0	0	0	0	0
II	96.0	0	0	0	0	0
III	96.0	26.5	3.0	0	0	0
IV	96.0	78.0	12.5	0.5	0	0
V	96.0	94.0	88.0	84.5	62.5	55.0
VI	96.0	98.5	88.0	84.0	86.5	72.5
VII	96.0	90.5	92.0	94.5	90.5	94.5
Standard extract	96.0	94.5	56.5	0	0	0

fects has been made between *Ae. togoi* and *An. stephensi* when compared to *Ae. aegypti* using neem seed kernel extract and pure azadirachtin (Zebitz 1986). The sluggish movement and peculiar coiling of treated larvae seem to suggest some neural or muscular disturbance by some active principle; which may be causing the acute lethal effect. This type of effect is not observed on larvae treated with superlethal doses of neem seed kernel (Zebitz 1986).

The delayed lethal effect of the extract however, is more likely to be caused by a disturbance of the endocrine mechanisms that regulate moulting and metamorphosis. This mechanism of action has been postulated previously for neem seed kernel extracts (Rembold 1984, Zebitz 1986). Failure for experimental larvae to ecdyse, melanize, and the production of larval-pupal intermediates and adults with malformed wings, would seem to support a similar postulation for *M. volkensii* extract.

Previous studies (Mwangi and Rembold 1988) concluded that the active compound in *M. volkensii* has acute toxicity at high doses, and growth inhibiting activity at lower doses. However, results from this research indicates a potential for two types of compounds; those in fractions I and II having an acute lethal toxic effect and those in fractions III and IV having a growth regulating activity. Since the acute toxicity effects are not observed in neem seed kernel extract, it is possible that this group of compounds may be absent in the neem seed kernel, or at undetectable quantities. This group of compounds may be of great interest since they seem to act in lower concentration than the growth inhibiting group of compounds found in fractions III and IV.

These compounds based on the results of this study offer great potential as new control agents against *An. arabiensis*. Further research into their mode of action, effect on nontarget organisms, and field evaluation are presently under investigation.

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