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Do traditional mosquito repellent plants work as mosquito larvicides?

N LUKWA

SUMMARY

Plant derived larvicides were evaluated in Kamhororo, an area of Zimbabwe. Twenty five third and fourth instar *An. gambiae* s.s mosquito larvae were used per test according to the method of WHO. All larvicides were effective against the *An. gambiae* s.s mosquito larvae and were comparable to studies done in Tanzania using Orange peel extracts. The extracts of the plant *Ocimum canum* (LC_{50}=54,94 x 10^3 ug/ml) were more effective in killing the larvae than *Lippia javanica* (LC_{50}=125,34mg x 10^3 ug/ml). These concentrations are higher when considering commercial larvicides.

INTRODUCTION

Malaria is a serious health problem in many African countries, including Zimbabwe[1] and the concept that malaria transmission can be interrupted by the use of residual insecticides has long been the basis for malaria control programmes. In Zimbabwe, mosquitoes are widely controlled by the use of residual spraying in rural areas and this is paid for the government.[2] This puts pressure on scarce resources to sustain the mosquito control programme, hence there is need to evaluate indigenous plants as mosquito larvicides.

Of all the plants mentioned as mosquito repellents, *Lippia javanica* and *Ocimum canum* were very common and therefore were evaluated as larvicides. The Bulawayo City council used Termephos "Abate®" to control nuisance mosquitoes[10] and 300 litres of used oil to treat defective septic tanks. Coopex Larvicide® containing 2 pc permethrin was evaluated in Gokwe[11] and results suggest that the larvicide has no effect on the pupa but the emerging adults die later on.

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Studies have been done elsewhere to evaluate the effectiveness of natural plants as mosquito larvicides.[5,7,8] Many plants produce chemicals with anti-feedant or insecticidal properties. These could potentially be exploited to provide pesticides which are safer and considerably less environmentally damaging than synthetic chemicals.[3] Zebitz[5] exposed fourth instar larvae of *Ae. aegypti* to water extracts of the neem tree and this resulted in disruption of growth. Alternatively, exposure of the first instar larvae caused a prolongation of the larval period and eventually about 90 pc mortality. However, organo-phosphorous compounds have been used world wide and larvicide resistance has been reported in some countries.[4]

Crude extracts have natural slow release formulations containing a range of chemicals with differing modes of action and insects would need to develop a wide range of enzymes to become resistant to all of them. Thus, the likelihood of resistance developing appears to be remote.[8]

Mwaiko[6] evaluated the efficacy of citrus oil extracts as mosquito larvae insecticides. Results revealed that lemon peel oil was more effective than orange peel oil, with a LC_{50} = 28,84 X 10^3 ug and LC_{95} = 89,12 x 10^3 ug. Therefore, preliminary studies were done to evaluate the effectiveness of the plants *Lippia javanica* and *Ocimum canum* as larvicides.

MATERIALS AND METHOD

1. Study area: Kamhororo Communal Area is in Gokwe district, Midlands province and is located at grid reference 18° 20'S x 30° E. The altitude is 300m above sea level. The area has several water bodies which are ideal for mosquito breeding all year round.

2. Preparation of the larvicides: Leaves of the plants *Lippia javanica* and *Ocimum canum* had been collected from previous field trips and therefore they were already dry when they were used. Each batch of leaves was pounded to powder using a laboratory homogenizer and the homogenate was separated using a fine sieve. One kilogramme of each repellent was dissolved in one litre of water and then 10ml of this preparation was pipetted and made up to 50ml with 70pc ethanol. Stock solutions of one litre of each repellent were prepared. The *Ocimum canum* preparation had a blue/green colouration whilst the *Lippia javanica* preparation had a brown colouration.

3. Collection of larvae: *Anopheles gambiae* s.s mosquito larvae were collected a day before the start of each experiment. After collection, they were trans-

ferred to clean water and left to acclimatize until the following morning.

4. Susceptibility tests: The tests were carried out according to the standard method described by WHO.[9] Twenty five third and fourth instar mosquito larvae were placed in 500ml yellow plastic containers containing 200ml of water at a depth of 7cm and the water temperature was noted to be 23°C. The mosquito larvae were left to acclimatize for 15 minutes. After that, 1 ml of each plant preparation, at different concentrations, was placed in a new set of plastic containers and 1 ml of 70pc ethanol was used in the control tests. The contents of the first set of containers containing the larvae were tipped into the new set of containers which had been serially labelled. Finally, the test containers were placed in a tent which was being used as the field insectary. All tests were done in duplicate and mortality counts were done after 24 hours.

RESULTS

The effectiveness of *Lippia javanica* as a mosquito larvicide: Higher dosages of the plant extracts were used as compared to the commercial larvicides (Abate 2 x 10^3 ug/ml). It was possible to reach a 100 pc mortality rate with a concentration of 240 x 103 ug/ml. Mwaiko[6] found that 79,9 x 10^3 ug/ml of orange peel extracts were suffcient to cause a mortality rate of 79,34 pc as compared to almost the same concentration which caused a mortality rate of 16 pc. A preparation[6] of the orange peel extracts (39,95 x 103 ug/ml) was sufficient to cause a 28,57 pc mortality as compared to *L. javanica* of a 40 x 10^3 ug/ml (12 pc) [Table I].

For computing the LC_{50} and LC_{95}, the package SPSS.PC programme was used. The LC_{50} of the plant *Lippia javanica* was 125,34 x 10^3 ug/ml as compared to Bitter orange extracts[6] (50,11 x 10^3 ug/ml) whilst the LC_{95} was 215,27 x 10^3 ug/ml (165,95 x 10^3 ug/ml for Bitter orange. The mortalities increased with the increase of the concentrations of the larvicides.

The effectiveness of *Ocimum canum* as a mosquito larvicide: The results show that 95 x 10^3 ug/ml was enough to give a 100 pc mortality rate (Table II). There was a 8 pc mortality rate when the plant extract was used at a concentration of 19 x 10^3 ug/ml as compared to orange extracts[6] (5,46 pc for 19,97 x 10^3 ug/ml) and the efficacy is not very different. When the plant extract was used at a concentration of 95 x 10^3 ug/ml, there was a 100 pc mortality rate as compared to the Bitter orange[6] preparation (79,6 for 90 x 10^3 ug/ml).

The LC_{50} of this larvicide was 58,94 x 10^3 ug/ml and that of Bitter orange was 50,11 x 10^3 ug/ml, the order was almost similar. The LC_{50} of *Ocimum canum* was 92,18 x 10^3 ug/ml as compared to Bitter orange (165,95 x 10^3 ug/ml).

*Table I: Evaluation of the plant **Lippia javanica** as a mosquito larvicide.*

Concentration (ug/ml x 10^3)	Test No. 1	2	3	4	Average pc Mortalities
	W:X:Y:Z	W:X:Y:Z	W:X:Y:Z	W:X:Y:Z	12
40	1:2:22:0	1:0:24:0	3:1:21:0	2:1:22:0	
80	2:3:20:0	2:2:21:0	2:1:22:0	1:3:21:0	16
120	10:1:14:0	10:0:15:0	9:0:16:0	7:2:16:0	60
160	17:0:8:0	14:1:10:0	17:2:6:0	15:2:6:0	68
200	21:1:3:0	24:0:1:0	20:0:5:0	22:0:3:0	88
240	25:0:0:0	25:0:0:0	25:0:0:0	25:0:0:0	100
Control	0:0:25:0	1:24:0:0	2:0:23:0	0:0:25:0	4

Key: W = Dead larva; X = Moribund larvae; Y = Alive larvae; Z = Emerged pupa

*Table II: Evaluation of the plant **Ocimum canum** as a mosquito larvicide.*

Concentration (ug/ml x 10^3)	Test No. 1	2	3	4	Average pc Mortalities
	W:X:Y:Z	W:X:Y:Z	W:X:Y:Z	W:X:Y:Z	8
19	1:2:22:0	1:1:23:0	0:2:23:0	2:0:23:0	
38	3:0:22:0	1:1:23:0	2:2:21:0	2:1:22:0	16
57	6:0:19:0	5:2:18:0	5:1:19:0	6:0:19:0	36
76	24:0:1:0	23:1:1:0	20:4:1:0	21:3:1:0	96
95	25:0:0:0	25:0:0:0	25:0:0:0	25:0:0:0	100
110	25:0:0:0	25:0:0:0	25:0:0:0	25:0:0:0	100
Control	2:0:23:0	2:1:22:0	1:0:24:0	2:0:23:0	8

Key: W = Dead larva; X = Moribund larvae; Y = Alive larvae; Z = Emerged pupa

The average number of larvae per experiment was taken and the figures rounded off to the nearest whole number. The average mortality rates were not corrected using Abbots's formula because control mortality reates never exceeded the maximum required 20 pc. All dead and moribund larvae were counted as dead. There were no larve which pupated during the experiments.

DISCUSSION

The trials were done in order to demonstrate the effectiveness of the plant derived larvicides and therefore were carried out in laboratory similated conditions. Higher dosages of the plant extracts were used as compared to the commercial larvicides (Abate 2 x 10^3 ug/ml). Mwaiko[6] found that 79,9 x 10^3 ug/ml of orange peel extracts were sufficient to cause a mortality rate of 79,34 pc as compared to almost the same concentration of *Lippia javanica* which caused a mortality rate of 16 pc.

The LC_{50} of the plant *Lippia javanica* was 125,34 x 10^3 ug/ml as compared to Bitter orange extracts[6] (50,11 x 10^3 ug/ml) whilst the LC_{95} of the plant was 215,27 x 10^3 ug/ml (165,95 x 10^3 ug/ml for Bitter orange). The mortalities increased with the increase of the concentrations of the larvicides. *Lippia javanica* is not as effective as the orange preparations.[6]

When *Ocimum canum* extracts were used at a concentration of 95 x 10^3 ug/ml, there was a 100 pc mortality rate as compared to the Bitter orange[6] preparation (79,6 pc for 90 x 10^3 ug/ml) and the plant appeared to be a better candidate. The LC_{50} of this larvicide was 58,94 x 10^3 ug/ml and that of Bitter orange was 50,11 x 10^3 ug/ml, the order was almost similar. The LC_{95} of *Ocimum canum* was 92,18 x 10^3 ug/ml as compared to Bitter orange (165,95 x 10^3 ug/ml).

The use of alcohol (ethanol) as a solvent had no impact on larvae mortalities as evidenced by the low control mortalities. Twenty four hour mortality rates were reasonably high as the concentration of the larvicides were increased. Plants can be used as larvicides at higher dosages as compared to commerial larvicides. When plants are compared to other natural larvicides as orange peels, the efficacy is not very different. However, it is not possible to use the extracts as they are at mass level unless they have been purified and the active ingredients concentracted.

This investigation represents preliminary observations on the susceptibility of *An. gambiae* mosquito larvae to plant derived larvicides. Further investigation will concentrate on the identification of the active ingredients of these plants.

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