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Short communication

LARVICIDAL PROPERTIES OF FOUR ETHIOPIAN MEDICINAL PLANTS AGAINST *CULEX QUINQUEFASCIATUS*

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ABSTRACT: The emergence and spread of insecticide resistant mosquitoes necessitated the search for plant derived insecticides. A study was conducted to investigate the larvicidal properties of four Ethiopian medicinal plants against second instars of laboratory reared *Culex quinquefasciatus*. Finely ground powders of *Chrysanthemum coronarium* inflorescence, *Melia azadirach* seeds, *Azadiractha indica* seeds and *Phytolacca dodecandra* berries were tested for their larvicidal effect. The tests were conducted by exposing 20 mosquito larvae per cup to plant materials diluted in distilled water at different test concentrations (400, 200, 100, 50 and 25 mg/ml). *P. dodecandra* (LC_{50}=90.21 mg/lit) and *C. coronarium* (LC_{50} = 96.21 mg/lit) showed better lethal effect compared to *M. azadirach* (LC_{50}= 203.06 mg/lit) and *A. indica* (LC_{50}= 229.86 mg/lit). Larvicidal properties of these plants decreased in the order *P. dodecandra* berries, *C. coronarium* inflorescence, *M. azadirach* seeds and *A. indica* seeds.

Key words/phrases: *Culex quinquefasciatus*, Ethiopian medicinal plants, larvicidal activity

INTRODUCTION

The continued use of synthetic insecticides resulted in the emergence and spread of physiological resistance in mosquitoes. Most insecticides have been non-selective and their extensive use has led to health and environmental problems. Many cases of lethal and sub-lethal pesticide poisoning of humans have occurred (Goulding, 1988; Forget, 1989). The problems of resistance and high cost to the available insecticides revived interest in the search and development of biodegradable, low cost, easy to apply and indigenous products for vector control (Grainge and Ahmed, 1988; Sukumar *et al.*, 1991).

Herbal products with proven insecticidal or repellent potential can play an important role in the control of mosquito borne diseases. Studies revealed that several plants such as *Tagetes minuta* (Perich *et al.*, 1995), *Dennettia tripetala* (Anyaele and Amusan, 2003), *Atlantia monophylla* (Sivagnaname and Kalyanasundaram, 2004) and *Momordica charantia* (Singh *et al.*, 2006) have larvicidal activities. Although Ethiopia is rich in its plant biodiversity, little is done if any, concerning their mosquitocidal and repellency properties. Preliminary screening and validation of the diverse plant resources may result in having a more effective and safe insecticide that can alleviate the problem of cost and resistance to synthetic chemicals. The present study reveals larvicidal properties of four Ethiopian plants against laboratory bred second instar larvae of *Culex quinquefasciatus*.

MATERIALS AND METHODS

Plant species

Four different plant samples were collected for the study. These were: *Melia azadirach* seeds from Dire Dawa (500 km east of Addis Ababa), *Phytolacca dodecandra* berries from Aklilu Lemma Institute of Pathobiology, *Chrysanthemum coronarium* inflorescence from Technology Faculty South, near Aklilu Lemma Institute of Pathobiology and *Azadiractha indica* seeds, from Afar regional state. They were air dried at Aklilu Lemma Institute of Pathobiology and finely ground to powder by pestle and mortar. The powder of each sample was sieved through 0.5mm^2 pore size sieve, weighed and kept in refrigerator until use.

Bioassay tests

A total of 20 second instar larvae of laboratory reared *Culex quinquefasciatus*, per cup containing 150 ml distilled water, were arranged into groups of six in duplicates and exposed for each plant sample with concentrations of 400, 200, 100, 50 and 25 mg/lit. The control (sixth) group for each test contained no plant sample. The larvicidal property of each plant sample was tested twice. The number of dead larvae was counted every 24 and 48 hours by probing the tip of the abdomen using a thin metal rod. Larvae that did not move when their siphons were touched with the rod were considered dead, while those moving were considered alive.

Statistical analysis

The results for the 48 hours mortality were subjected to probit analysis, and the LC_{50} (lethal concentration that kills 50% of the total population) values were obtained at 95% confidence limits. The heterogeneity was also tested by Chi-square. When control mortality exceeded 5%, the mortality rates of the treated groups were corrected according to Abbott's formula (Swaroop, 1966). Test results with control mortality greater than 20% were discarded and repeated.

RESULTS

All the four plants showed lethal effects on second instar larvae of *Culex quinquefasciatus* at different concentrations tested (shown in Table 1). The LC_{50} (48 hours) values of *C. coronarium*, *M. azadirach*, *A. indica* and *P. dodecandra* were 96.21, 203.06, 229.86 and 90.21 mg/lit respectively. *P. dodecandra* and *C. coronarium* showed better larvicidal activities. The other two plant species also showed larvicidal activities although at higher concentrations. The average mortality after treatment with *M. azadirach* at the concentration of 400 mg/lit in the two replicates was about 18 (90%), which is approximately equal to the number of dead larvae when treated with 200 mg/lit of *C. coronarium*. On the other hand the average larval mortality, 48 hours post exposure to *A. indica*, at the concentration of 400 mg/lit was 14 (70%). This was the least mortality recorded among the four plant sample treatments at the highest concentration. In general, the larvicidal activity of these plants against second instar larvae of *Cx. quinquefasciatus* decreased in the order *P. dodecandra* berries, *C. coronarium* inflorescence, *M. azadirach* seeds, and *A. indica* seeds.

Table 1. Larvicidal efficacy of *Chrysanthemum coronarium* inflorescence, *Melia azadirach* seeds, *Azadirachta indica* seeds and *Phytolacca dodecandra* berries against second instar larvae of *Culex quinquefasciatus*.

Plant species	Concentration (mg/lit)	Number Dead Replicate I 24 hr	Number Dead Replicate I 48hr	Number Dead Replicate II 24hr	Number Dead Replicate II 48hr	Corrected Mean (%) Kill (48hrs)	LC50 (48hrs)	95% CL LCL[a]	95% CL UCL[b]	X^2(df=3)
C. coronarium	400	17	19	19	20	95.94	96.21	72.93	126.62	0.76
	200	13	16	18	19	85.14				
	100	7	9	7	12	47.3				
	50	4	5	6	8	25.67				
	25	2	3	1	2	4.05				
	0	1	2	0	1	7.5				
M. azadirach	400	20	20	12	16	87.14	203.06	145.78	322.43	4.38
	200	8	12	5	6	37.14				
	100	5	6	3	6	18.57				
	50	4	7	2	4	15.71				
	25	4	4	2	4	7.14				
	0	1	3	0	2	12.5				
A. indica	400	11	15	9	14	64.71	229.86	148.14	507.28	0.99
	200	8	10	7	11	41.17				
	100	8	10	5	8	33.82				
	50	6	9	3	5	22.06				
	25	3	4	3	3	2.94				
	0	1	3	1	3	15				
P. dodecandra	400	5	14	4	16	70.83	90.21	58.74	177.49	2.09
	200	6	9	4	12	45.83				
	100	4	5	3	13	38.9				
	50	2	3	4	12	27.8				
	25	1	2	4	2	2.8				
	0	0	1	0	3	10				

Note: a, lower confidence limit; b, upper confidence limit; mg/lit, milligram per litre; 20 second instar larvae were used per cup.

DISCUSSIONS AND CONCLUSION

Plant derived insecticides may serve as alternatives to synthetic insecticides as they are relatively safe and available in many parts of the world (Sivagnaname and Kalyanasundaram, 2004). Though several plant species have been reported for their mosquitocidal activities, only few are applied for practical purposes. The plants are light and heat instable compared to synthetic insecticides (Green *et al.*, 1991), and thus frequent application will be needed, necessitating adequate agronomic supply. This is often a big challenge.

P. dodecandra berries, *C. coronarium* inflorescence, *M. azadirach* seeds, and *A. indica* seeds showed lethal effects against second instar larvae *Cx. quinquefasciatus*. *P. dodecandra* berries showed the strongest larvicidal effect compared to the other three. Previously, it has been used for the control of schistosome transmitting snails (Aklilu Lemma, 1965) and its butanol extract was found to be toxic to second- and third instar larvae of *Culex*, *Aedes* and *Anopheles* mosquitoes (Spielman and Aklilu Lemma, 1973). The plant is used for washing clothes and to treat different diseases of public and veterinary importance.

C. coronarium is a small flowering plant, which is mainly cultivated for ornamental purposes. Further insecticidal evaluation and proper characterization of the plant regarding its impact on non-target organisms, in aquatic environments, and its possible toxic effects on humans is essential for large-scale use in malaria control programs. Flowers of *C. cinerefolium*, a different species of the same genus, have been used as insecticides (Jacobson and Crosby, 1971) and as sources of pyrethroid insecticides.

M. azadirach and *A. indica* were found to have lower lethal effect (LC_{50} = 203.06 mg/lit and 229.86 mg/lit, respectively) against the larvae compared to *P. dodecandra* and *C. coronarium* (LC50 = 90.21 mg/lit and 96.21 mg/lit, respectively). However, *A. indica* contains azadirachtin that have antifeedant, ovipositional deterrence, repellency and larvicidal action against insects (Schmutterer, 1990). Aqueous extract from de-oiled tree seed kernels exhibited toxic and growth regulating activities against *Cx. quinquefaciatus* larvae with a 100% larval mortality during the first and second instars (Sagar and Sehgal, 1996). The lower larvicidal property of this plant in our study could be due to the differences in the amount of the active components in different cultivars of the same species. Its oil is also reported to have strong larvicidal activity against *Cx. quinquefasciatus* (Mittal and Subbarao, 2003); and various concentrations of its oil when mixed with coconut oil is reported to protect humans from the bite of *Anopheles* mosquitoes (Mishra *et al.*, 1995). Further validation of insecticidal or repellent property of different extracts of these plants is needed. Furthermore, it will be necessary to show if *P. dodecandra* berries and *C. coronarium* inflorescence can be applied on small water bodies for the control of immature stages of mosquitoes.

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