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Short Communication

Larvicidal activity of the saponin fractions of *Chlorophytum borivilianum santapau* and *Fernandes*

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The present communication deals with the laboratory studies carried out to ascertain the larvicidal properties of *Chlorophytum borivilianum* Sant. and Fernand. saponin extracts for the mosquito species *Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti*. Methanolic extract (ME), crude saponin extract (CSE) and purified saponin fractions (PSF) were used as test solutions. Concentration to kill 50% larvae and concentration to inhibit emergence of 50% adult that is LC_{50} and EC_{50} values respectively were calculated. All extracts found to be larvicidal but PSF was found more effective.

Key words: Larvicidal, *Chlorophytum borivilianum*, saponin.

INTRODUCTION

Chlorophytum borivilianum Sant. and Fernand. belonging to family Liliaceae is a very well known plant for its aphrodisiac as well as immunomodulatory properties (Oudhia, 2001). Roots of the plant are used both in Ayurveda and Unani system to treat oligospermia, arthritis, diabetes and dysuria (Wealth of India 1996). In earlier studies, Antiviral (Siddiqui YM, 2005), Anticancer (Arif JM, 2005), immunomodulatory (Singh et al., 2004), anti-diabetic (Govindrajan et al., 2005), antistess (Gopalkrishna et al., 2006), aphrodisiac (Thakur et al., 2006), antimicrobial (Deore et al., 2007) and anti-inflammatory (Deore et al., 2008) activities of root extracts have been evaluated. Roots of this plant contain carbohydrates, phenolic compounds, saponins and alkaloids (Deore et al., 2008).

As the saponins reported to have insecticidal and larvicidal actions (Sparg et al., 2004), the present study was conducted to ascertain the larvicidal properties against larvae of three species of mosquito (*Anopheles stephensi*), *Culex quinquefasciatus* and *Aedes aegypti*. The most common mosquito larvicides used currently are organophosphates, insect growth regulators and microbial larvicides. Current research focuses on microbials such as *Bacillus thuringiensis* and *Bacillus sphaericus* as well as herbal larvicidal, oviposition inhibiting, repellent or insect growth regulatory effects. Such products contain a multitude of active ingredients with different modes of action, which lessens the chance of resistance developing in mosquito populations (Rajkumar et al., 2005).

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MATERIALS AND METHODS

Plant materials, extraction and isolation

C. borivilianum Sant. and Fernand. roots were purchased from local cultivator and a specimen sample was deposited at Department of Botany, Vidarbh institute of Science and Humanities, Amravati. The roots were washed dried and powdered and defatted by petroleum ether. Thereafter, extracted with methanol for 3 h with mild heating. Methanol extract was concentrated and methanol extract (ME) was obtained. In order to get the crude saponins, extract was again dissolved in methanol and acetone was added (1:5 v/v) to precipitate the saponins as described by Yan et al. (1996). The precipitate was dried under vacuum. The whitish amorphous powder, thus obtained is named as a crude saponin extract (CSE). To get the pure saponin fraction (PSF), certain amount of CSE was fractionated by applying to silica gel-60 (230 400) mesh. Column chromatography and eluted successfully with chloroform-methanol-water (70:30:10) as described by Favel et al. (2005). Eluted fractions combined to give PSF.

Test mosquitoes

Laboratory-reared III instars mosquito larvae of *A. stephensi, C. quinquefasciatus* and *A. aegypti* was provided by the Head of Zoology Department, Vidarbh Institute of Science and Humanities, Amravati.

Table 1. Mean LC_{50} values of different fractions of *C. borivilianum Sant.* and *Fernand.* tuber extracts.

Mosquito species	LC_{50} (PPM)			
	ME	CME	PSF	Malathion
A. stephensi	8066.67	5300	4000	0.00547
C. quinquefasciatus	6300	4833.33	3850	0.00477
A. aegypti	5733.33	4250	3916.67	0.00503

Table 2. Mean EC_{50} values of different fractions of *C. borivilianum* Sant. and Fernand. tuber extracts.

Mosquito species	EC_{50} (PPM)			
	ME	CME	PSF	Malathion
A. stephensi	7219	4305	4872	0.0024
C. quinquefasciatus	5201	3988	2981	0.0036
A. aegypti	4721	3201	2290	0.0022

METHODOLOGY

The larvicidal bioassay followed the World Health Organization (WHO) standard protocols (World Health Organization (1981). Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC, 81:807.) with slight modifications. Each of the concentrations of different extracts of *C. borivilianum* (0.1 - 0.5%) was transferred into sterile glass Petri dishes (9 cm diameter/150 ml capacity). 10 third instar larval form of *S. aegypti* were separately introduced into different Petri dishes containing graded concentrations and the mortality was recorded for 48 h of the exposure period. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated three times and conducted under laboratory conditions at 25 - 30℃ and 80 - 90% relative humidity. Similar types of bioassay were conducted with different concentration of each extracts of the *C. borivilianum* and with a chemical insecticide, Malathion, on third instar larval forms of selected mosquito larvae. A negative control was run in tap water. 6 replicates were run under the same microclimatic conditions. The lethal concentration was determined and compared with Malathion.

The effects of the treatments were monitored by counting the number of dead larvae each day. For the LC_{50} the data of 48 h was uses because till that time no pupa was observed even in control treatments. During the course of experiment, a food based on the baby food was provided to the larvae. In another series of experiments, observations on the emergence and larval duration of larvae that were reared at sublethal doses of the active fractions of the treatments were made and the emergence of the 50% of the test larvae (EC_{50}values) was determined. Experiment was carried out in triplicate and results are expressed Table 1 and 2.

RESULTS AND DISCUSSION

Mosquitoes transmit several public health problems, such as malaria, filariasis, dengue and Japanese encephalitis; causing millions of deaths every year (World Health Organization, 1981). Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat. The use of conventional chemical pesticides has resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health cosncerns (Vatandoost et al., 2001, Severini et al., 1993). Plants are rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance (World Health Organization, 1970) and easily biodegradable (Forget, 1989).

The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities. Most studies report active compounds as steroidal saponins (Hostettmann et al., 1995). Saponins are freely soluble in both organic solvents and water, and they work by interacting with the cuticle membrane of the larvae, ultimately disarranging the membrane, which is the most probable reason for larval death (Wiesman et al., 2005).

In 48 h experimental period, the crude extracts of tuber of *C. borivilianum* Sant. and Fernand. has been found to possess larvicidal and adult emergence inhibition activity against the mosquito *A. stephensi, C. quinquefasciatus* and *A. aegypti*. Among the three mos-quito species tested, A. aegypti was the most sensitive followed by *C. quinquefasciatus* and *A. stephensi* in case of all extracts. The biological activity of the extracts might be due to the saponins and alkaloids exist in plants, these compounds may jointly or independently contribute to produce larvici-dal and adult emergence inhibition activity. The larvicidal efficacy of *C. borivilianum* Sant. and Fernand. is not comparable to well established insecticidal plant species or synthetic insecticides such as Malathion but it can be suggested that its use for control of this mosquitoes. Future scope needs to isolate responsible single constituent/s should be identified and utilized, if possible, in preparing a commercial product / formulation to be used as a mosquito repellent.

Conclusion

In conclusion, *Chlorophytum borivilianum* Sant. and Fernand offers promised as a potential bio control agent against A. *Aegypti*, *C. quinquefasciatus* and *A. stephensi* particularly in its markedly larvicidal effect. The extract or isolated bioactive phytochemical from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes.

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