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Mosquito larvicidal activity of *Rauvolfia serpentina* L. seeds against *Culex quinquefasciatus* Say

Dipanwita Das, Goutam Chandra*

Mosquito and Microbiology Research Units, Parasitology Laboratory, Department of Zoology, Burdwan University, West Bengal, 713104, India

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ABSTRACT

Objective: To establish the larvicidal activities, if any of solvent extracts of *Rauwolfia serpentina* (*R. serpentina*) L. seeds against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say, 1823 as target species. **Methods:** Seeds of *R. serpentina* were extracted with five solvents graded according to the polarity [viz. petroleum ether, benzene, ethyl acetate, acetone and absolute alcohol] continuing one after another with the same seeds. **Results:** Mortality rate with petroleum ether extract was significantly higher than other extracts. The mortality rates of late 3rd instar larvae were 50.33±5.51, 10.00±1.00, 0.00±0.00, 21.33±1.53 and 0.00±0.00 in 100 ppm concentration of petroleum ether, benzene, ethyl acetate, acetone and absolute alcohol respectively, after 24 h of exposure period. **Conclusions:** Results of this study show that petroleum ether extract of *R. serpentina* seed may be considered as a potent source of mosquito larvicidal agent.

1. Introduction

Mosquito control is facing a threat due to the emergence of resistance in mosquitoes to conventional synthetic insecticides. So, there is need to utilize biological agents to control larval mosquitoes[1]. Synthetic insecticide based method also contributes to environmental hazards[2]. In recent years search for new insecticides remains the top priority-it should be easily biodegradable and without ill effects on non target organisms[3]. Phytochemicals have a major role in mosquito control programme[4-10]. Plant products can be obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, acetone, absolute alcohol, methanol, chloroform, benzene, petroleum ether *etc*.

Rauvolfia serpentina (*R. serpentina*) is an evergreen, perennial, glabrous and erect under shrub grows up to a height of 60 cm (rarely more than it). Roots are tuberous with pale brown cork. Leaves are in whorls of three, elliptic to lanceolate or obovate. Flowers are in many flowered irregular corymbose cymes and are white, often with violet colored tinge. Drupes are green in colour and became purplish black when ripe. Seeds are cream in colour ('*Rauvolfia* L' Germplasm Resource information Network. http//www.ars-grin.gov/cgi-bin/npqs/html/gonus and Little *et al*[11,12]). *R. serpentina* contains a number of bioactive chemicals, including ajmaline,deserpidine, rescinnamine, serpentinine, reserpine. Hindus used this plant as an antidote to the bites of poisonous reptiles like snakes. It was reported that the extract of the plant has been used by Mahatma Gandhi as a tranquilizer during his life time (Time Magazine, November 8, 1954). Reserpine is used to treat high blood pressure and mental disorders including schizophrenia, and was particularly popular for that purpose in the West from 1954 to 1957[13].

The objective of the present study was to examine the mosquito larvicidal activity of the seeds of *R. serpentina* against the larvae of *Culex quinquefasciatus* (*Cx. quinquefasciatus*) and to gather preliminary information about the application of best solvent extract for this plant.

2. Materials and method

2.1. Test mosquitoes

The present study was conducted at Burdwan (23°16′ N, 87°54′ E), West Bengal, India during March-April 2009. Larvae of all the instars of *Cx. quinquefasciatus* were

*Corresponding author: Goutam Chandra, Ph. D, D.Sc. Professor, Department of Zoology, Mosquito and Microbiology Research Units, The University of Burdwan, Golapbag, Burdwan 713104, West Bengal, India.
Tel: +91-9434573881
E-mail address: goutamchandra63@yahoo.co.in

collected from cemented drains surrounding the University campus and kept in plastic buckets (201) with the addition of artificial foods (powdered mixture of dog biscuits and dried yeast powder in the ratio of 3:1).

2.2. Preparation of plant extract in different solvent systems

Mature fruits were harvested; seeds were collected from fruits, rinsed with distilled water and dried in paper towel. The seeds were poured in soxhlet apparatus and the plant extract was prepared according to the method of Tang and Young[15]. The plant extracts were prepared using five solvents graded according to the polarity [viz. petroleum ether, benzene, ethyl acetate, acetone and absolute alcohol (Merck)] continuing one after another with the same seeds. The extracts were collected separately and the column after each type of solvent extraction procedure was washed with 200 cm^3 of water and treated with similar solvent (100 cm^3) as an eluent. The eluted materials were concentrated at 40 ℃ to 10 cm^3 by evaporating in a rotary evaporator (Eyela rotary vaccum evaporator). Then each of the extracts was filtered through Whatman No. 41 filter paper and evaporates to dryness. The solid residue was weighed and dissolved in suitable volume of distilled water to make different concentrations.

2.3. Bioassay experiments

The larvicidal bioassay followed the World Health Organization standard protocols[15] along with a set of controls containing distilled water without any test solution. Required concentrations of different polar and non-polar solvent extracts (concentrations of 50, 75 and 100 ppm) were prepared through mixing stock extract with variable amounts of sterilized distilled water. Each of the earlier prepared concentrations of solvent extracts was transferred into the sterile glass Petri dishes (9 cm diameter; 150 mL capacity). Hundred third instar (in case of all polar and non-polar solvent extracts) and first, second, third, and fourth instar (in case of petroleum ether extract) larval forms of *Cx. quinquefasciatus* were separately introduced into different Petri dishes containing appropriate concentrations. Ten milligrams of larval food (dried yeast powder) was added per petri dish. Mortality rates were recorded after 24, 48 and 72 h of post-exposures. The data of mortality in 48 and 72 h were expressed by the addition of the mortality at 24 h and 48 h, respectively. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated thrice on three different days (n=3) and conducted at 25-30 ℃ and 80%-90% relative humidity.

3. Results

The total yield of each extract in the soxhlet extraction from 250 g of seeds was as follows: petroleum ether extract, 4.19 g; benzene extract, 1.23 g; ethyl acetate extract, 1.28 g; acetone extract, 2.04 g; and absolute alcohol extract, 1.46 g. Present study on the toxicity of polar and non-polar extracts against the 3rd instar larval form of *Cx. quinquefasciatus*, revealed the most active result in petroleum ether extract among all (Table 1). Highest mortality was shown in 100 ppm of petroleum ether extract (61.00±1.00) at 72 h of exposure. The mortality rate in the 100 ppm concentration was higher than 75 ppm and 50 ppm concentration in 24, 48 and 72 h study period. The mortality rates of 1st, 2nd, 3rd and 4th instars were 69.00±1.00, 60.00±1.00, 50.33±5.51 and 43.33 ±1.53 respectively at 100 ppm concentration of petroleum ether extract after 24 h of exposure (Table 2). The mortality rates of 1st, 2nd, 3rd and 4th instars after 24, 48 and 72 h of exposure at 75 ppm concentration were 51.67±1.53, 46.33 ±1.53,

Table 1
Effect of different solvent fractions of *R. serpentina* seeds on late 3rd instars of *Cx. quinquefasciatus*.

Solute used	Concentration (ppm)	Exposure (h)		
		24	48	72
Petroleum ether	100	50.33±5.51	54.33±4.04	61.00±1.00
	75	31.67±1.53	41.00±1.00	42.00±1.00
	50	28.33±1.53	32.00±2.00	37.67±2.52
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Benzene	100	10.00±1.00	10.00±1.00	10.00±1.00
	75	0.00±0.00	0.00±0.00	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Ethyl acetate	100	0.00±0.00	0.00±0.00	0.00±0.00
	75	0.00±0.00	0.00±0.00	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Acetone	100	21.33±1.53	31.67±1.53	34.33±1.55
	75	18.00±2.00	21.67±1.53	23.67±3.21
	50	9.00±1.00	11.00±1.00	19.67±1.53
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Absolute alcohol	100	0.00±0.00	0.00±0.00	0.00±0.00
	75	0.00±0.00	0.00±0.00	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00

Table 2

Susceptibility of *Cx. quinquefasciatus* larvae to petroleum ether solvent extract from seeds of *R. serpentina* (mean ± standard error).

Instar	Concentration (ppm)	Exposure (h)		
		24	48	72
1st	100	69.00±1.00	73.00±1.00	77.67±0.58
	75	51.67±1.53	55.00±1.00	56.67±1.53
	50	41.33±1.53	47.00±1.00	50.33±1.53
	Control	0.00±0.00	0.00±0.00	0.00±0.00
2nd	100	60.00±1.00	61.33±1.15	66.33±1.53
	75	46.33±1.53	48.67±1.53	52.33±2.52
	50	38.00±1.00	39.67±0.58	42.67±1.53
	Control	0.00±0.00	0.00±0.00	0.00±0.00
3rd	100	50.33±5.51	54.33±4.04	61.00±1.00
	75	31.67±1.53	41.00±1.00	42.00±1.00
	50	28.33±1.53	32.00±2.00	37.67±2.52
	Control	0.00±0.00	0.00±0.00	0.00±0.00
4th	100	43.33±1.53	45.67±1.15	49.67±1.53
	75	27.67±2.08	30.33±0.58	31.33±1.00
	50	23.33±1.53	25.33±1.53	28.33±1.53
	Control	0.00±0.00	0.00±0.00	0.00±0.00

31.67±1.53, 27.67±2.08, 55.00±1.00, 48.67±1.53, 41.00 ± 1.00, 30.33±0.58, 56.67±1.53, 52.33±2.52, 2.00±1.00 and 31.33±1.00 respectively. The 100 ppm concentration of petroleum ether extract for late 3rd instar was not found to have any toxic effect to the non-target organisms (*Diplonicus annulatum* and *Gadusia chapra*) after 24, 48 and 72 h of exposure (data not shown as all values comes to be zero).

4. Discussions

Plants could be an unconventional source for controlling larval mosquitoes because they comprise a potential source of bioactive chemicals and are usually free from harmful effects. Application of these plant derivatives in mosquito control as a substitute of synthetic insecticides could diminish the cost of production and environmental pollution. Plant materials were used to control agricultural and veterinary pest long before the introduction of synthetic insecticides[17]. During bioassay experiment the extraction of active photochemical depends upon the polarity of the solvents because polar solvent will extract polar molecules and non-polar solvents will extract non-polar molecules. A review of literature demonstrate that non-polar solvents such as petroleum ether and hexane (polarity index of 0.1) mainly extract essential oil; moderately polar solvents such as chloroform or ethyl acetate (polarity index of 4.1) mainly extract steroids, alkaloids etc; and the strong polar solvents such as absolute alcohol or water, (polarity index of 10.2) extract biochemicals with higher molecular weight such as proteins, glycans, *etc*[16]. In this purpose most of the researchers use non-polar solvents[17-26] or strong polar solvents[27-33]. However, significant results were also reported by applying moderately polar solvents such as chloroform and methanol[4-6,34-35] and ethyl acetate. Senthil Nathan *et al*[36] reported that fourth instar larvae of *Anopheles stephensi* are highly sensitive to the ethyl acetate extract of the leaves of *Dysoxylum malabaricum*. Matasyoh *et al*[37] reported 100% mortality at a concentration of 0.2 mg/mL with a LC_{50} value of 0.11 mg/mL to the ethyl acetate leaves extract of *Aloe turkanensis* against *Anopheles gambiae*. The ethyl acetate leaves extract of *Ocimum sanctum* produced significant mortality against *Aedes aegypti* and *Cx. quinquefasciatus*, with LC_{50} values of 425.94 and 592.60 ppm, respectively[38]. Elango *et al*[39] reported that ethyl acetate extract of the leaves of *Aegle marmelos* (L) exhibited high larvicidal properties against *Anopheles subpictus* and *Culex tritaeniorhynchus*, having LC_{50} values of 167.00 and 99.03 ppm, respectively. During the present study, the solvent extracts of. *R. serpentina* has been well established as a biocontrol agent against *Cx. quinquefasciatus* in the laboratory condition. Among the polar and nonpolar solvent extracts of mature seeds of *R. serpentina*, the highest efficacy as a larvicidal agent against third instar larvae is found in petroleum ether which definitely suggests that any essential oil of the plant is responsible for larval mortality. In recent years, eco-friendly and plant-based insecticides have been used with great importance because these are safe to use in aquatic ecosystem as they do not harm the non-target species and easily biodegradable[3]. The results of the present study with solvent extracts of mature *R. serpentina* seeds clearly indicate that this species can serve as a potent larvicide against *Cx. quinquefasciatus* larvae.

Conflict of interest statement

We declare that we have no conflict of interest.

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