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Comparative larvicidal potential of different plant parts of *Withania somnifera* against vector mosquitoes in the semi-arid region of Rajasthan

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Abstract

Larvicidal potential of the extracts from different parts *viz.* green and red fruits, seeds, fruit without seeds, leaves and roots of *Withania somnifera* in different solvents was evaluated against larvae of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*, the important disease vectors prevalent in the semi-arid region. Experiments were carried out on late 3rd or early 4th instar larvae of these mosquitoes using standard WHO technique. 24 and 48 hr LC_{50} values along with their 95% confidence limits, regression equation, chi-square ($\chi 2$)/ heterogeneity of the response have been determined by log probit regression analysis. The 24 hr LC_{50} values as observed for whole green fruits in water, methanol and petroleum ether were 350.9, 372.4, 576.9; 115.0, 197.1, 554.6; 154.9, 312.0, 1085.0 while corresponding values for red fruits were 473.5, 406.4, 445.2; 94.7, 94.5, 1013.0; 241.8, 535.0, 893.3 mg l^{-1} for *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* respectively showing that methanol extracts were more effective against anophelines as compared to culicines when whole fruits were taken. The 24 hr LC_{50} values as observed for seeds in acetone, methanol and petroleum ether were 188.1, 777.5, 822.5; 245.5, 769.0, 1169.0; 140.3, 822.9, 778.4 and for fruit without seeds were 80.2, 97.6, 146.6; 88.4, 404.4, 1030.0; 30.0, 44.5, 54.2 mg l^{-1} for the above mosquito species respectively showing that extract of fruit without seeds were most effective in petroleum ether followed by acetone and methanol extracts. However, experiments conducted with methanol extracts of leaves and roots of this plant species did not show any appreciable larvicidal activity and a 20-40% mortality was observed up to 500 mg l^{-1} of the extracts. Overall larvae of anophelines were found more susceptible as compared to culicines to all the extracts tested. Petroleum ether extract of fruit without seeds was found most effective against all the mosquito species showing that active ingredient might be present in this part of the plant species. The study would be of great importance while planning vector control strategy based on alternative plant derived insecticides.

Key words

Larvicidal potential, *Withania somnifera*, Mosquito, Semi-arid region, Vector control

Introduction

Over and injudicious use of synthetic insecticides in vector control has resulted into environmental hazards through persistence and accumulation of non-biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms. These developments prompted researchers to look for other alternative insecticidal agents with high biocontrol potentiality but with least or no harmful effects to environment and human health. One such possibility is the rational utilization of plant-derived derivatives that inhibit growth (Sharma *et al*., 2006), development and metamorphosis of insects (Mwangi and Rembold, 1986; Sukumar *et al*., 1991). There is an ever-increasing demand for botanical insecticides as they are easily available at competitive prices, non-toxic, biodegradable and show broad-spectrum target specific activities. Unlike conventional synthetic insecticides that are based on single active ingredient, botanical derivatives comprise blends of secondary metabolites, which act concertedly on both behavioral and physiological processes. Thus the chances of resistance development in insect pests to these phytochemicals are meager.

Anopheles stephensi is the important vector of malaria while *Culex quinquefasciatus* and *Aedes aegypti* are the vectors of filaria and dengue/DHF respectively. All these mosquito species have been identified as the primary vectors in this region of Rajasthan (Bansal and Singh, 1993; Bansal *et al*., 1994). Hence, their control either by chemical or biological means is the basic requirement for planning an effective vector control strategy. Several studies have been done on the insecticide susceptibility status of adult (Bansal and Singh, 1995;1996) and larval (Bansal and Singh, 2004;2006) mosquitoes in this region. The major objectives of the present investigation are to explore the mosquito larvicidal/repellant potential of different plant parts of *Withania somnifera* after extraction in aqueous and other organic solvents and Identification of the active constituents present their in.'Ashwagandha', *Withania somnifera, a* plant of solanaceae or nightshade family is widely distributed throughout the arid region. Fruits, leaves and seeds of this plant have traditionally been used for the Ayurvedic system as aphrodisiacs, diuretics and for treating memory loss (Scareezini and Speroni, 2000).

Materials and Methods

W. somnifera was collected from different habitats in and around Jodhpur and help from Botanical Survey of India (BSI), Jodhpur was taken for identification after depositing the sample specimen in the herbarium. The samples of roots, leaves and fruits were washed several times with distilled water, chopped and shade dried separately at a temperature between 30-40°C for 15 days. Dried plant material was then powdered separately and stored at a temperature range of 15-20°C. Seeds were taken out from the dried red fruits and powdered separately. 100 g of the powdered plant material each from roots, leaves, whole green and red fruits, seeds and fruit without seeds was extracted separately using 400 ml of distilled water/ acetone/ methanol/ petroleum ether at room temperature for 2 days with constant stirring and finally filtered, air dried and weighed. The percent yield for green and red fruits were 10.3, 11.2, 5.6 and 13.4, 12.7, 6.1% for the aqueous, methanol and petroleum ether extracts while for fruits without seeds and seeds it was 8.5, 14.5, 5.1 and 10.8, 5.9, 5.5% for acetone methanol and petroleum ether extracts respectively. The yield for roots and leaves in methanol was 4.8 and 14.7% respectively. Stock solutions from the residual extracts (5 g 50 ml^{-1} of solvent) were prepared in distilled water for aqueous extracts while in ethanol for other organic solvents. Test concentrations from 10-500 mg l^{-1} for aqueous and other organic solvent extracts were used during the experimentation.

Efficacy experiments with various solvent extracts were carried out on different mosquito larvae as per protocol described by WHO (1981). Different larval stages from 1st to 4th instar were reared in the laboratory and used for the tests. Serial dilutions of the stock solution were prepared and added to 249 ml of tap water in a 500 ml beaker to obtain the test concentrations. Controls with the same amount of solvent (1 ml Ethanol) were kept side by side. 20-25 healthy late 3rd or early 4th instar larvae were kept in different test concentrations and mortality noted after 24-48 hr. All treatments were replicated 4-5 times and carried out at a controlled room temperature of 28±2°C and RH 75±5%. The percent-corrected mortality was calculated using Abbott's formula (1925) if mortality was between 5-20% in control experiments. The LC_{50} and LC_{90} values were computed using probit regression analysis (Finney, 1971).

Results and Discussion

The results of the relative susceptibility of larvae of all the three mosquito vectors *viz*. *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* to the aqueous and other organic solvent extracts from different parts of *W. somnifera* are given in Tables 1-3. Tests carried out with aqueous extracts of both green and red fruits showed that these were less effective to all the three mosquito species, the 24 hr LC_{50}being 350.9, 372.4, 576.9 mg l^{-1} for the green fruits and 473.5, 406.4, 445.2 mg l^{-1} for the red fruits respectively (Table 1). However, with Methanol and petroleum ether extracts the LC_{50}'s were 115.0, 197.1, 554.6; 154.9, 312.0, 1085.0 mg l^{-1} for the green fruits and 94.7, 94.5, 1013.0; 241.8, 535.0, 893.3 mg l^{-1} for the red fruits respectively (Table 1) showing that methanol extracts of fruits were very much effective followed by petroleum ether and aqueous extracts to all the mosquito species. *An. stephensi* was found to be more susceptible followed by *Ae. aegypti* and *Cx. quinquefasciatus.* Bansal *et al*. (2009a,b) also showed that methanol extracts from green and yellow ripe fruits of *Solanum xanthocarpum* were very much effective against the larvae of *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus.* Singh and Bansal (2003) also observed that extracts from fresh green fruits of *S. xanthocarpum* were very much effective to the vectors of malaria and dengue/ DHF. Mohan *et al*. (2005) also observed that the fruits of *S. xanthocarpum* were very effective against the larvae of *An. stephensi* (24 hr LC_{50} of CCl_4 extract being 5.1 ppm) and *Cx. quinquefasciatus* (24 hr LC_{50} of petroleum ether extract being 62.2 ppm). 48 hr LC_{50} values were much less as compared to the 24 hr values showing that plant derived phytochemicals show delayed action when compared with conventional synthetic insecticides.

Experiments were also carried out with acetone, methanol and petroleum ether extracts of seeds (Table 2) and LC_{50}'s were 188.1, 777.5, 822.5; 245.5, 769.0, 1169.0 and 140.3, 822.9, 778.4 mg l^{-1} for the three mosquito species with the above solvents respectively. However, when the seeds were removed from the whole red fruits (fruit without seeds - Table 2) the LC_{50}'s were 80.2, 97.6, 146.6; 88.4, 404.4, 1030.0 and 30.0, 44.5, 54.2 mg l^{-1} for the above solvents and three mosquito species respectively, showing that extracts from fruits without seeds were most effective as compared to the extracts from seeds with all the three solvents. Petroleum ether extracts were most effective to all the three mosquito species followed by acetone and methanol extracts. Petroleum ether and carbon tetrachloride extracts of some plant species have also been reported to be quite effective against larvae of *An. stephensi and Cx. quinquefasciatus* (Srivastava *et al.*, 2008; Maurya *et al*., 2008). *An. stephensi* was found more susceptible to all the extracts in different solvents followed by *Ae. aegypti* and *Cx. quinquefasciatus*. However, methanol extracts of leaves and roots show only up to

Table - 1: Efficacy of green and red fruits of *Withania somnifera* against different mosquito species in different organic solvents

Mosquito species	Solvent used	Green fruits		Red fruits	
		24 hr LC_{50} (95% C. limits)	48 hr LC_{50} (95% C. limits)	24 hr LC_{50} (95% C. limits)	48 hr LC_{50} (95% C. limits)
An. stephensi	Water	350.9 (239.1-514.7)	238.2 (151.0-375.8)	473.5 (306.8-628.5)	324.8 (184.1-564.7)
Ae. aegypti		372.4 (249.2-556.5)	261.4 (165.0-414.4)	406.4 (267.9-1033.3)	303.6 (113.2-735.6)
Cx. quinquefasciatus		576.9 (314.4-1059.0)	290.7 (188.9-313.4)	445.2 (214.0-845.9)	266.2 (129.0-313.4)
An. stephensi	Methanol	115.0 (88.6-149.3)	77.9 (**267.5**)* (60.4-100.4)	94.7 (**282.3**) (74.6-120.2)	57.2 (**169.6**) (44.8-73.0)
Ae. aegypti		197.1 (153.8-252.4)	106.3 (60.2-187.6)	94.5 (**269.2**) (70.3-127.1)	53.5 (**161.4**) (41.3-69.3)
Cx. quinquefasciatus		554.6 (324.6-947.1)	318.7 (221.9-457.6)	1013.0 (439.4-2333)	790.5 (353.6-1767)
An. stephensi	Petroleum ether	154.9 (107.2-224.0)	86.7 (**629.6**) (61.3-122.7)	241.8 (155.2-432.7)	146.4 (130.5-164.1)
Ae. aegypti		312.0 (212.2-458.7)	239.6 (166.8-344.3)	535.0 (476.5-622.5)	367.9 (231.6-781.9)
Cx. quinquefasciatus		1085.0 (692.6-1418.5)	964.5 (405.5-2294.0)	893.3 (692.6-1418.5)	695.6 (565.2-977.0)

All values of 24 and 48 hr LC_{50} along with their 95% Confidence limits are in mgl^{-1}, * = Bold values in parentheses are the LC_{90} values for that species (Indicated only at places where LC_{50} is <100)

Table - 2: Efficacy of seeds and fruit without seeds of *Withania somnifera* against different mosquito species in different organic solvents

Mosquito species	Solvent used	Fruit without seeds		Seeds	
		24 hr LC_{50} (95% C. limits)	48 hr LC_{50} (95% C. limits)	24 hr LC_{50} (95% C. limits)	48 hr LC_{50} (95% C. limits)
An. stephensi	Acetone	80.2 (**384.0**)* (59.9-107.3)	60.8 (**270.5**) (47.1-78.5)	188.1 (138.1-256.4)	101.6 (79.6-129.9)
Ae. aegypti		97.6 (**475.8**) (70.8-134.6)	53.5 (**228.5**) (39.4-72.6)	777.5 (625.2-1122.7)	650.2 (463.1-987.0)
Cx. quinquefasciatus		146.6 (108.1-198.9)	87.7 (**425.6**) (64.9-118.7)	822.5 (541.4-1275.5)	718.9 (501.8-1217.0)
An. stephensi	Methanol	88.4 (**349.3**) (66.1-118.2)	54.9 (**215.6**) (41.1-73.5)	245.5 (140.2-429.9)	141.6 (92.4-226.6)
Ae. aegypti		404.4 (226.3-722.8)	238.6 (157.5-361.6)	769.0 (369.4-1601.0)	402.6 (256.3-632.5)
Cx. quinquefasciatus		1030.0 (437.7-2426)	474.7 (292.3-771.1)	1169.0 (566.9-2409.0)	609.5 (347.0-1070.0)
An. stephensi	Petroleum Ether	30.0 (**127.7**) (22.4-40.2)	22.4 (**71.6**) (17.4-28.7)	140.3 (103.5-190.3)	81.6 (**224.8**) (65.2-102.2)
Ae. aegypti		44.5 (**225.3**) (32.7-60.7)	27.8 (**176.7**) (19.5-39.8)	822.9 (640.2-1296.0)	765.8 (595.7-1211.3)
Cx. quinquefasciatus		54.2 (**197.7**) (41.8-70.4)	33.0 (**107.4**) (24.7-44.1)	778.4 (414.0-1463.0)	668.9 (371.6-1214.0)

All values of 24 and 48hr LC_{50} along with their 95% Confidence limits are in $mg\ l^{-1}$, * Bold values in parentheses are the LC_{90} values for that species (Indicated only at places where LC_{50} is <100)

Table - 3: Efficacy of methanol extracts of leaves and roots of *Withania somnifera* on larvae of different mosquito vectors after 24 and 48hr

Mosquito species/ Concentrations (mg l^{-1})	Percent mortality with methanol leaves extract					Percent mortality with methanol root extract				
	No. exposed	No. dead		% Exptl. mortality		No. exposed	No. dead		% Exptl. mortality	
		24	48	24	48		24	48	24	48
An. stephensi										
Control	100	1	2	1.0	2.0	98	2	3	2.0	3.1
50	99	5	10	5.1	10.1	99	4	6	4.0	6.0
100	99	10	15	10.1	15.1	95	7	10	7.4	10.5
200	100	15	20	15.0	20.0	99	13	16	13.1	16.2
400	98	25	30	25.5	30.6	96	20	24	20.8	25.0
500	97	30	35	30.9	36.1	98	28	38	28.6	38.8
Ae. aegypti										
Control	80	0	0	0.0	0.0	100	1	1	1.0	1.0
50	80	0	4	0.0	5.0	100	3	4	3.0	4.0
100	80	4	8	5.0	10.0	98	5	7	5.1	7.1
200	80	8	12	10.0	15.0	98	10	13	10.2	13.3
400	79	12	16	15.2	20.3	97	15	18	15.5	18.6
500	80	20	24	25.0	30.0	98	20	28	20.4	28.6
Cx. quinquefasciatus										
Control	80	2	4	2.5	5.0	99	2	4	2.0	4.0
50	79	2	3	2.5	3.8	100	7	9	7.0	9.0
100	80	6	11	7.5	13.8	98	9	12	9.2	12.2
200	80	18	21	22.5	26.3	97	10	14	10.3	14.4
400	80	24	28	30.0	35.0	99	12	18	12.1	18.2
500	78	29	32	37.2	41.0	100	15	20	15.0	20.0

20-40% mortality against any of the mosquito species when tested with 50-500 mg l^{-1} of the extracts (Table 3) suggesting that active larvicidal ingredients may be present only in the fruits. However, petroleum ether root extracts of *S. xanthocarpum* showed synergistic effects with cypermethrin when evaluated against the larvae of *Cx. quinquefasciatus* (Mohan *et al*., 2006) and *An. stephensi* (Mohan *et al*., 2007). An increase in synergistic efficacy over the individual oil cakes of Neem (*Azadirachta indica*) and Karanja (*Pongamia glabra*) have also been shown by Shanmugasundaram *et al*. (2008) against these three mosquito species.

Results of the present study with the extracts from different parts of *W. somnifera* have exhibited variable efficacy to all the three vector species that warrants further investigations. Sukumar *et al*. (1991) have also stated the existence of variations in the efficacies of phytochemical compounds on target species *vis-à-vis* plant parts from which they are extracted, responses in species and developmental stages of species to the specified extract, solvent of extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract, effect on growth (Sharma *et al*., 2006) and reproduction. Keeping in view the above variations, it will be of importance to study the variations in efficacies of extracts and also to characterize the responsible active ingredients present in different tropical, sub tropical and temperate plants. Tropical plants are of great promise from the point of view of discovering and developing new botanical insecticides (Berenbaum, 1989).

Members of different plant families *viz.* Asteraceae, Canellaceae, Labiatae, Meliaceae, Rutaceae and Solanaceae seem to possess the most promising botanicals for use at the present (Jacobson, 1989). Many promising, economical and environmental friendly botanical larvicides have also been reported from the families *viz*. Apiaceae, Araceae, Magnoliaceae, Piperaceae, Rutaceae (Sivagnaname and Kalyanasundaram, 2004), Annonaceae and Zingiberaceae. Several phytochemicals like alkaloids; phenolics and terpenoids exit in plants (Wink, 1993) which may jointly or independently contribute to the generation of mosquito larvicidal activities (Hostettmann and Potterat (1997).

The results of the present study suggest that chemical composition of extracts from different parts of the same or different plants may be different and require thorough understanding of the active ingredients present in these plants. For successful application of these phytochemical's ingredients in insect bio-control, it is obligatory to understand the mechanisms of their action in the target insects as well as the spectrum of insects affected by them. Further work on these plant-derived derivatives is needed for developing them into effective formulations to be utilized in integrated vector control and in exploration of their multiple medicinal properties inherited by these plants. Further research is in progress to identify the biologically active constituents present in the fruits of this plant species.

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