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Larvicidal activity of the extracts from different parts of the plant *Solanum xanthocarpum* against important mosquito vectors in the arid region

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Abstract: *Larvicidal efficacy of the aqueous and methanol extracts from green unripe and yellow ripe fruits, seeds, leaves and roots of the plant Solanum xanthocarpum was evaluated against Anopheles culicifacies, Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus, the important mosquito vectors prevalent in the arid region. Studies were carried out on late 3rd or early 4th instar larvae of these species using standard WHO technique. Based on concentration mortality data LC_{50} and LC_{90} values along with their fiducial limits, regression equation, chi-square (λ^2)/ heterogeneity of the response have been determined by log probit regression analysis. The 24 hr LC_{50} values as observed for aqueous extracts for green unripe and yellow ripe fruits were 112.7, 498.2 and 846.3 mgl^{-1} and 104.7, 267.7 and 832.2 mg l^{-1} for Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus respectively. However, the 24 hr LC_{50} values for methanol extracts of yellow ripe fruits and seeds were 51.6, 52.2, 118.3 and 157.1 mg l^{-1} and 66.9, 73.7, 123.8 and 154.9 mg l^{-1} for the above four vector species respectively. The results showed that larvae of anophelines were much more susceptible as compared to culicines to both the aqueous and methanol extracts from fruits and seeds of the plant tested. The methanol extracts were found 2-5 times more effective as compared to the aqueous extracts. The chi-square values calculated during the analysis did not show any heterogeneity of the response. Experiments conducted with aqueous and methanol extracts from leaves and roots of this plant species didn't show any larvicidal activity against any of the mosquito species tested. The study would be of great importance while formulating vector control strategy based on alternative plant based insecticides.*

Key words: *Larvicidal, Solanum xanthocarpum, Vector mosquitoes, Arid region*
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Introduction

Mosquitoes are responsible for more diseases than any other group of arthropods. Mosquito-borne diseases, such as malaria, filariasis, dengue/DHF, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries. Among these diseases, malaria continues to be a major public health problem in most tropical countries. In 1992, WHO reported that 2.2 billion people in 90 countries were annually exposed to malarial infections, causing an estimated 1.4-2.6 million deaths (WHO, 1992). However, control of malarial and other mosquito-borne diseases is becoming increasingly difficult because the effectiveness of vector control has declined due to the development of resistance in mosquitoes against currently used insecticides (Chandre *et al.*, 1998). It is also important to recognize that only a limited number of insecticides are available for use in public health (Rozendaal, 1997; Casida and Quistad, 2000). Secondly, the synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. Therefore, an effort should be made to find alternative insecticides. Plants may be an alternative source of mosquito-control agents because they constitute a rich source of bioactive chemicals, which inhibit growth (Sharma *et al.*, 2006), development and metamorphosis of insects (Mwangi and Rembold, 1986; Arnason *et al.*, 1989; Sukumar *et al.*, 1991; Shanmugasundaram *et al.,* 2008). Much effort has been focused on phytochemicals and their essential oils (Dharmagadda *et al.*, 2005) as potential sources of mosquito-control agents as they are relatively safe, degradable, cost effective and readily available with least mammalian toxicity.

Among the anophelines, *Anopheles stephensi* and *An. culicifacies* are the important vectors of malaria while among the culicines, *Culex quinquefasciatus* and *Aedes aegypti* are the vectors of filaria and dengue/DHF respectively. All these mosquito species have been identified as primary vectors of the above communicable diseases in this region of Rajasthan (Bansal and Singh, 1993; Bansal *et al.*, 1994). Hence, their control either by biological or chemical 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, 2007) mosquitoes in this region. *Solanum xanthocarpum / S.surrattense / S. virginianum,* the Indian nightshade commonly known as 'baigan kateli', belonging to the family Solanaceae is widely distributed throughout the country but more abundantly in the desert areas and is wild in nature. The plant is known to have multiple medicinal properties (Govindan *et al.*, 1999; Gupta *et al.*, 1966) and the extracts from various parts have been used against agricultural pests as repellants (Husain, 1995), contact poison and as molluscicide (Wei *et al.*, 2002) in public health. The different parts of this plant differ in their active constituents when extracted through different extraction procedures using different solvents. The level of susceptibility of the larval stages to different phyto-derivatives is not yet assessed in this arid region.

Materials and Methods

Plant material 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

Table - 1: Efficacy of aqueous extracts of *S. xanthocarpum* (green unripe fruits) on larvae of different mosquito vectors

Mosquito species/ conc. (mg l^{-1})	No. exposed	No. dead	(%) Expected mortality	(%) Corrected mortality	Regression coefficient (Slope)	Regression equation	Chi-square (df)	24 hr LC_{50} (mg l^{-1})	24 hr LC_{90} (mg l^{-1})
An. stephensi									
Control	100	03	03.0	-	2.19	Y=0.50+2.19x	0.16	112.7	431.7
25	99	08	08.1	08.1			(2)	(85.5-148.6)	(236.4-788.4)
50	98	22	22.4	22.4					
100	97	43	44.3	44.3					
250	99	76	76.8	76.8					
500	100	95	95.0	95.0					
Ae. aegypti									
Control	97	06	06.2	-	2.83	Y=-2.62+2.83x	4.20	498.2	1414.0
100	97	12	12.4	06.6			(2)	(403.5-615.0)	(870.0-2296.0)
250	95	20	21.1	15.9					
500	93	47	50.5	47.2					
750	98	70	71.4	69.5					
1000	100	96	96.0	95.7					
Cx. quinque-fasciatus									
Control	117	06	05.1	-	3.04	Y=-3.91+3.04x	1.11	846.3	2228.0
250	116	13	11.2	06.4			(2)	(743.9-962.7)	(1828.0-2716.0)
500	113	32	28.3	24.4					
1000	112	65	58.0	55.7					
1500	114	90	78.9	77.8					
2000	118	114	96.6	96.4					

Table - 2: Efficacy of aqueous extracts of *S. xanthocarpum* (yellow ripe fruits) on larvae of different mosquito vectors

Mosquito species/ conc. (mg l^{-1})	No. exposed	No. dead	(%) Expected mortality	(%) Corrected mortality	Regression coefficient (Slope)	Regression equation	Chi-square (df)	24 hr LC_{50} (mg l^{-1})	24 hr LC_{90} (mg l^{-1})
An. stephensi									
Control	100	06	06.0	-	1.91	Y=1.14+1.91x	1.00	104.7	490.4
25	98	16	16.3	11.0			(2)	(77.64-141.3)	(248.2-968.7)
50	99	32	32.3	28.0					
100	97	52	53.6	50.6					
250	99	72	72.7	71.0					
500	100	96	96.0	95.7					
Ae. aegypti									
Control	96	04	04.2	-	2.07	Y=-0.03+2.07x	1.00	267.7	1111.0
100	97	20	20.6	20.6			(2)	(202.5-353.9)	(598.2-2062.0)
250	99	44	44.4	44.4					
500	100	68	68.0	68.0					
750	99	86	86.9	86.9					
1000	100	94	94.0	94.0					
Cx. quinque-fasciatus									
Control	120	07	05.8	-	2.71	Y=-2.91+2.71x	1.80	832.2	2741.0
250	120	16	13.3	08.0			(2)	(684.1-1013.0)	(1584.0-3852.0)
500	118	39	33.1	29.0					
1000	116	64	55.2	52.4					
1500	116	93	80.2	79.0					
2000	120	113	94.2	93.8					

Table - 3: Efficacy of methanol extracts of *S. xanthocarpum* (yellow ripe fruits) on larvae of different mosquito vectors

Mosquito species/ conc. (mg l^{-1})	No. exposed	No. dead	(%) Expected mortality	(%) Corrected mortality	Regression coefficient (Slope)	Regression equation	Chi-square (df)	24 hr	
								LC_{50} (mg l^{-1})	LC_{90} (mg l^{-1})
An. culicifacies									
Control	80	04	05.0	-	2.62	Y=0.51+2.62x	0.25	51.6	159.0
25	80	19	23.7	19.7				(40.1-66.4)	93.3-270.6
50	78	42	53.7	51.3					
100	78	59	75.6	74.3					
150	79	71	89.9	89.4					
200	80	77	96.3	96.1					
An. stephensi									
Control	100	07	07.0	-	2.41	Y=0.86+2.41x	1.26	52.2	177.3
25	99	29	29.3	24.0			(2)	(40.1-66.4)	(101.6-309.4)
50	98	48	49.0	45.2					
100	98	72	73.4	72.5					
150	99	87	87.9	87.0					
200	100	99	99.0	98.9					
Ae. aegypti									
Control	100	02	02.0	-	4.35	Y=-4.01+4.35x	2.69	118.3	233.1
50	99	09	09.3	09.3			(2)	(102.5-136.4)	(171.4-317.0)
100	98	31	31.3	31.3					
150	98	67	69.1	69.1					
200	99	89	89.0	89.0					
250	100	98	98.0	98.0					
Cx. quinque-fasciatus									
Control	99	06	06.1	-	2.43	Y=-0.33+2.43x	1.42	157.1	529.4
50	99	13	13.1	07.5			(2)	(121.3-203.4)	(283.1-990.0)
100	100	38	38.0	34.0					
200	98	57	58.2	55.5					
300	99	78	78.8	77.4					
400	100	95	95.0	94.7					

specimen in the herbarium. The samples of roots, leaves and fruits were washed 2-3 times with distilled water, chopped and shade dried separately at a temperature between 30-40°C for 10-15 days. Dried plant material was then powdered separately and stored at a temperature range of 15-20°C. 100 g of the powdered plant material each from roots, leaves, fruits and seeds was extracted separately using 400 ml of distilled water/ methanol at room temperature for 2 days with constant stirring and finally filtered, air dried and weighed. The percent yield for roots, leaves, fruits and seeds were 5.9, 10.4, 14.7, 5.2% and 5.6, 10.2, 10.5 and 7.5% for the aqueous and methanol extraction respectively. Stock solutions from the residual extracts (5 g / 50 ml) were prepared in their respective solvents and test concentrations from 25- 2000 mgl^{-1} for aqueous while 25-400 mgl^{-1} for methanol extracts as given in tables 1-5 were used during the experimentation.

Bioassays of the extracts on different mosquito larvae were carried out as per the method described by WHO (1981). 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. Different larval stages from 1st to 4th instar were reared in the laboratory and used for the tests. 25 healthy late 3rd or early 4th instar larvae were kept in different test concentrations and mortality noted after 24 hr. Controls with the same amount of solvent were kept side by side. 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 (1925) formula 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 four important mosquito vectors *viz. An. culicifacies*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* to the aqueous and methanol extracts from different parts of *S. xanthocarpum* are given in Tables 1-5. Experiments carried out with aqueous extracts of both green and yellow fruits showed that these were quite effective to three mosquito species *viz*., *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus,* the LC_{50} being 112.7, 498.2 and 846.3 mg l^{-1} for the green unripe fruits and 104.7, 267.7 and 832.2 mg l^{-1} for the yellow ripe fruits respectively Table 1, 2). *An. stephensi* was found

Table - 4: Efficacy of methanol extracts of *S. xanthocarpum* (seeds) on larvae of different mosquito vectors

Mosquito species/ sonc. (mg l^{-1})	No. exposed	No. dead	(%) Expected mortality	(%) Corrected mortality	Regression coefficient (Slope)	Regression equation	Chi-square (df)	24 hr LC_{50} (mg l^{-1})	24 hr LC_{90} (mg l^{-1})
An. culicifacies									
Control	80	05	06.3	-	2.77	Y=-0.05+2.77x	0.95	66.9	194.2
25	79	12	15.2	09.5			(2)	(52.4-85.4)	(121.0-311.7)
50	79	36	45.6	41.9					
100	80	56	70.0	68.0					
150	78	67	85.9	85.0					
200	77	76	98.7	98.6					
An. stephensi									
Control	100	06	06.0	-	2.48	Y=0.35+2.48x	0.99	73.7	241.1
25	99	16	16.2	10.9			(2)	(58.3-93.3)	(1461.1-397.7)
50	99	41	41.4	37.7					
100	98	61	62.2	59.8					
150	99	83	83.8	82.8					
200	97	95	97.9	97.8					
Ae. aegypti									
Control	89	04	04.5	-	2.73	Y=-0.72+2.73x	0.68	123.8	364.0
50	88	12	13.6	13.6			(2)	(98.6-155.4)	(224.6-590.0)
100	90	38	42.2	31.3					
150	84	55	65.5	69.1					
200	83	73	88.0	89.0					
250	88	85	96.6	98.0					
Cx. quinque-fasciatus									
Control	99	05	05.1	-	2.51	Y=-0.50+2.51x	3.11	154.9	501.2
50	100	18	18.0	14.0			(2)	(122.0-196.7)	(280.3-896.0)
100	98	31	31.6	27.9					
200	98	57	58.2	56.0					
300	99	81	81.8	80.8					
400	99	95	96.9	96.7					

to be the most susceptible followed by *Ae. aegypti* and *Cx. quinquefasciatus.* Experiments were also carried out with methanol extracts of yellow ripe fruits and seeds of this plant species and it was observed that these extracts were 2-5 times more effective as compared to the aqueous extracts, the LC_{50} being 51.6, 52.2, 118.3 and 157.1 mg l^{-1} for the ripe fruits and 66.9, 73.7, 123.8 and 154.9 mg l^{-1} for the seeds to all the four mosquito species respectively (Table 3, 4). The LC_{50} and LC_{90} values obtained with methanol extracts of green unripe fruits were 62.8, 75.4, 180.1 and 309.3 mg l^{-1} and 192.2, 224.3, 312.7 and 608.5 mg l^{-1} to all the four mosquito species respectively. Anophelines were found much more susceptible as compared to the culicines, the efficacy being almost double the culicines. However, leaves and roots did not show any appreciable mortality either with aqueous or with methanol extracts to any of the mosquito species tested (Table 5) suggesting that active larvicidal component may be present only in the seeds and fruits. Results of this preliminary study with the extracts from different parts of this plant have exhibited variable toxicity to all the four important disease vector species and warrants further investigations. Singh and Bansal (2003) also observed that extracts from fresh green fruits of this plant were very much effective to the vectors of malaria and dengue/ DHF. Mohan *et al.* (2005) also observed that the fruits of this plant 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). They also observed that the petroleum ether root extracts of this plant showed synergistic effects with cypermethrin when evaluated against larvae of *Cx. quinquefasciatus* (Mohan *et al.*, 2006) and *An. stephensi* (Mohan *et al.*, 2007). Sukumar *et al.* (1991) have also stated the existence of variations in the toxicities of phytochemical compounds on target species *vis-a-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 toxic effects of extracts and also to characterize the active ingredients responsible for the toxicity in

Table - 5: Efficacy of aqueous (A) and methanol (M) extracts of leaves and roots of *S. xanthocarpum* on larvae of different mosquito vectors

Mosquito species/ concentrations (mg l^{-1})	Percent mortality with leaves extract						Percent mortality with root extract					
	No. exposed		No. dead		% Expected mortality		No. exposed		No. dead		% Expected mortality	
	A	M	A	M	A	M	A	M	A	M	A	M
An. culicifacies												
Control	80	80	00	00	0.0	00	60	80	00	00	0.0	0.0
50	-	79	-	00	-	00	-	-	-	-	-	-
100	78	79	00	01	0.0	1.3	60	80	01	02	1.7	2.5
250	79	80	01	01	1.3	1.3	-	-	-	-	-	-
500	79	80	02	02	2.5	2.5	65	80	01	03	1.5	3.8
1000	80	-	02	-	2.5	-	-	-		-		-
An. stephensi												
Control	100	97	01	01	1.0	1.0	75	98	00	01	0.0	1.0
50	-	98	-	01	-	1.0	-	-	-	-	-	-
100	99	98	01	01	1.0	1.0	75	80	00	02	0.0	2.5
250	100	100	00	03	0.0	3.0	-	-	-	-	-	-
500	98	100	01	04	1.2	4.0	74	100	01	04	1.4	4.0
1000	98	-	02	-	2.0	-	-	-		-		-
Ae. aegypti												
Control	97	79	01	00	1.0	0.0	73	80	00	00	0.0	0.0
50	-	79	-	02	-	2.5	-	-	-	-	-	-
100	98	78	02	01	2.0	1.3	75	80	00	01	0.0	1.3
250	100	80	00	02	0.0	2.5	-		-	-	-	-
500	98	80	03	03	3.1	3.8	73	80	01	02	1.4	2.5
1000	99	-	03	-	3.0	-	-	-		-		-
Cx. quinque-fasciatus												
Control	100	98	00	00	0.0	0.0	74	95	01	01	1.4	1.1
50	-	99	-	00	--	0.0	-	-	-	-	-	-
100	100	100	00	02	0.0	2.0	75	95	01	02	1.3	2.1
250	100	99	01	03	1.0	3.0	-	-	-		-	-
500	99	100	03	03	3.0	3.0	73	96	03	02	4.1	2.1
1000	98	-	02	-	2.0	-	-	-		-	-	-

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

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. Further work on these plant-derived derivatives is needed for developing them into effective formulations to be utilized in integrated vector control measures through community participation. *S. xanthocarpum* is easily available to the local people and being an ayurvedic herb with multiple medicinal properties is easily acceptable to them, since during application it would neither cause any toxic effects nor any additional economic burden. Further research is in progress to identify the biologically active constituents present in the seeds and fruits of this plant species.

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