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Synergistic efficacy of *Solanum xanthocarpum* and *Withania somnifera* on larvae of mosquito vector species

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Abstract

Synergistic larvicidal efficacy of methanol extracts from yellow ripe fruits of *Solanum xanthocarpum* (Schrad and Wendl.) and red fruits of *Withania somnifera* (Dunal) **(**Fam. Solanaceae**)** was evaluated against late 3[rd] or early 4[th] stage larvae of *Anopheles stephensi* (Liston), *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say), using standard WHO technique. Experiments were carried out with methanol extracts of fruits of *S. xanthocarpum* (SX) (24hr LC_{50}: 77.7, 121.6, 142.8) and *W. somnifera* (WS) (108.4, 135.1, 1092.1 mg l^{-1}) which revealed that extracts from *S. xanthocarpum* were more effective as compared to *W. somnifera* with all the three mosquito species, respectively. For synergistic studies on *An. stephensi* six different binary pairs viz. WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3); SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) were prepared and a dose response curve was established to determine the 24 and 48 hr LC_{50} and LC_{90} values, along with their 95% confidence limits, regression equation, chi-square (χ^2)/ heterogeneity of response by log probit regression analysis. The 24 and 48hr LC_{50} values as determined for three binary pairs *viz.* WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3) were 32.7, 30.6 and 22.2 and 22.9, 16.3 and 12.9 mg l^{-1} while the values for other three pairs *viz.* SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) were 32.7, 47.5 and 63.8 and 22.9, 29.1 and 50.2 mg l^{-1} respectively. From the results it is clear that *An. stephensi* was very much susceptible to the binary pair WS:SX (1:3) as compared to the binary pair SX:WS (1:3) or in other words, the efficacy went up with increase in proportion of *S. xanthocarpum* and went down with increase in proportion of *W. somnifera* extract. Experiments were done by mixing different fractions *viz.* 1/2, 1/3, 1/4[th] of LC_{50} each of SX and WS and it was observed that synergistic effect was observed even up to 1/4[th] of LC_{50} of these two plant extracts. Synergistic Factor (SF) and Co-toxicity Coefficient (CTC) were also determined to show the antagonistic and synergistic effects. The study would be of great importance while formulating vector control strategy based on utilization of the most effective synergist pair.

Key words

Arid region, Desert plants, Larvicidal, Mosquitoes, Syngistic

Introduction

Mosquito-borne diseases such as malaria, filariasis, dengue and Japanese encephalitis contribute significantly to disease burden in tropical countries. Among these diseases, malaria and dengue continue to be the major public health problems. However, over and injudicious application of synthetic insecticides in controlling the vectors of above diseases has resulted in development of insecticide resistance among vector species, environmental hazards through persistence and accumulation of non-biodegradable chemicals in ecosystem, biological magnification through food chains and toxic effects on human health (Rawani *et al.*, 2009). Hence, there is an ever-increasing demand for plant-based insecticides as they are non-toxic, easily available and biodegradable. However, individual plant extracts are active only at higher concentration, which makes them uneconomical for field use.

Over the past decade, phytochemicals have received progressively more attention as potential mosquito larvicides (Ghosh *et al.*, 2012). Sukumar *et al.* (1991) listed and discussed 344 plant species that only exhibited mosquitocidal activity. Most synergistic studies involving phytochemicals have been conducted on agricultural pests rather than on vectors of diseases. Few studies on mosquitocidal activity have investigated the combined effects of phytochemicals alone or involving insecticides, insect growth regulators or microbial control agents (George and Vincent, 2005). Synergism between synthetic insecticides and phytochemicals alone or in combination appears to be more common (Mansour *et al*., 2000) for a better vector control. Shaalan *et al.* (2005a, b, c) reviewed the mosquitocidal potential of several phyto-chemicals and advocated that the use of combinations of insecticides and phytochemicals is suitable for mosquito control. Mohan *et al.* (2006, 2007) also evaluated the synergistic efficacy of Cypermethrin and *Solanum xanthocarpum* and found that mixtures were much more synergistic in nature as compared to individual alone. Thangam and Kathiresan (1997) evaluated the larvicidal efficacy of *Rhizophora apiculata*, a mangrove plant, and observed that it has a synergistic effect when tested with pyrethrum against *Culex quinquefasciatus*. Similarly, Madhu and Vijayan (2010) found that *Piper longum* and *Curcuma aromatica,* when used together showed synergistic activity against *C. quinquefasciatus*.

Although work on larvicidal efficacy of *S. xanthocarpum* and *W. somnifera* (Fam. Solanaceae) has been carried out in this arid region (Bansal *et al*., 2009a, b; 2011, 2012 and 2014), but studies on synergistic potential of these plants is meagre. Therefore, in the present study, synergistic and antagonistic effects of above plant extracts alone and in combination were tried on *An. stephensi* in order to find suitable synergists which could be used for vector control.

Materials and Methods

S. xanthocarpum and *W. somnifera* **(**Fam. Solanaceae**)** were collected from different habitats in and around the city and help from Botanical Survey of India (BSI) was taken for identification of the above species. The ripe fruits of both plants were washed several times with distilled water and shade dried separately at 30-35ºC for 15 days. Dried fruits were then powdered separately and stored at a temperature range of 15-20ºC. One hundred gm of fruit powder was extracted separately using 400 ml of methanol on a hot plate at 40±2ºC for 2 days with constant stirring and finally filtered, air dried, weighed and stored in glass vials in a refrigerator till further use. The percent yield for fruits of *S. xanthocarpum* and *W. somnifera* in methanol was 10.2 and 11.4 % respectively. Stock solutions from the residual extracts (5g /50 ml of solvent) were prepared in ethanol. Test concentrations ranging from 05-500 mgl^{-1} were used during experimentation. Susceptibility experiments with fruit extracts from both plants individually and their extracts mixed in different proportions viz. WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3); SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) were carried out on different mosquito larvae as per protocol described by WHO (1983). Different mosquito larvae were reared in laboratory and used for tests. Serial dilutions of stock solution were prepared and added to 249 ml of tap water in a 500 ml beaker to obtain the desired 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 stage larvae were kept in different test concentrations and mortality was noted after 24 and 48hr. All treatments were replicated four times and carried out at room temperature of 28±2ºC and RH 75±5%. The percent corrected mortality was calculated using Abbott's 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). Co-toxicity Coefficient (CTC) (Sarup *et al*., 1980) and synergistic factor (SF) (Kalyanasundaram and Das 1985) for mixed formulations were calculated using LC_{50}, LC_{90} for each combination. A value of SF >1 indicated synergism and SF<1 indicated antagonism.

Results and Discussion

Experiments carried out with methanol extracts from fruits of *S. xanthocarpum* showed that these were more effective, the 24 hr LC_{50} being 77.7, 121.6, 142.8 mg l^{-1} as compared to extracts from *W. somnifera,* the 24 hr LC_{50} being 108.4, 135.1, 1092.1 mg l^{-1} with all the three mosquito species respectively (Table 1). Singh and Bansal (2003), Mohan *et al*. (2006,2007) and Bansal *et al*. (2009a, 2009b; 2011) also showed that organic solvent extracts from fruits of *S. xanthocarpum* and *W. somnifera* were highly effective against the larvae of *An. stephensi* followed by *Ae. aegypti* and *Cx. quinquefasciatus.* Organic solvent extracts of some plant species and their oil cakes have also been reported to be quite effective against larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* (Srivastava *et al.*, 2008; Maurya *et al*., 2008; Shanmugasundaram *et al*., 2008).

The 24 and 48 hr LC_{50} values for *An. stephensi* with three binary mixtures *viz*. WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3) were 32.7, 30.6 and 22.2 mgl^{-1}; 22.9, 16.3 and 12.9 mgl^{-1} respectively. From the results it is clear that *An. stephensi* was highly susceptible to binary mixture WS:SX (1:3) as compared to WS:SX (1:2) and WS:SX (1:1) or, in other words, the efficacy went up with increase in proportion of *S. xanthocarpum* extract (Table 3). The 24 and 48hr LC_{50} values as determined for the other three binary mixtures *viz*. SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) were 32.7, 47.5 and 63.8 mgl^{-1}; 22.9, 29.1 and 50.2 mgl^{-1} respectively. From the results it is clear that *An. stephensi* was highly susceptible to binary mixture SX:WS (1:1) as compared to SX:WS (1:2) and SX:WS (1:3) or, in other words, the efficacy went down with increase in proportion of *W. somnifera* extract (Table 4). Synergistic Factor (SF) and Co-toxicity Coefficient (CTC) were also determined and it was observed that all the pairs showed synergistic response (Table 3) except for pair SX:WS (1:3) which behaved antagonistically with 48hrs LC_{50} (Table 4). Experiments have also been done on different fractions *viz*. 1/2, 1/3, 1/4th of LC_{50} values of SX and WS and it was observed that synergistic effect was observed even up to 1/4th of LC_{50} of these two plant extracts (Table 2).

Table 1 : Efficacy of methanol extracts of ripe *S. xanthocarpum* and *W. somnifera* fruits on larvae of different mosquito vectors

Mosquito species/ Conc. (mg^{-1})	**(%) Corrected mortality 24hr**	**48hr**	**24 hr LC_{50} and LC_{90} (Fiducial limits) mgl^{-1}**	**48 hr LC_{50} and LC_{90} (Fiducial limits) mgl^{-1}**
Solanum xanthocarpum				
An. stephensi				
Control	-	-	77.7± 1.17'	48.4± 1.16*
25	18.4	28.6	(56.9-106.0)	(36.1-65.0)
50	40.4	49.5		
100	62.5	75.0	465.6±1.48**	218.2±1.41**
200	73.2	86.6	(216.5-1001.3)	(110.7-430.0)
400	83.7	96.9		
Ae. aegypti				
Control	-	-	121.6±1.22	71.1±1.22
25	18.4	28.6	(81.7-180.9)	(48.4-104.4)
50	33.0	41.2		
100	45.5	55.6	1261.5±1.80	687.3±1.76
200	60.6	72.7	(396.6-4012.4)	(226.4-2086.3)
400	73.2	84.5		
Cx. quinque-fasciatus				
Control	-	-	142.8±1.19	115.4±1.21
25	11.2	20.4	(101.4-201.0)	(79.1-168.3)
50	20.6	28.9		
100	36.0	41.2	1003.9±1.55	948.4±1.75
200	57.3	62.5	(319.7-3804.1)	(282.9-2517.9)
400	93.8	86.7		
Withania somnifera				
An. stephensi				
Control	-	-	108.4 ± 1.16*	66.3±1.13*
25	11.2	12.8	(80.7-45.5)	(52.2-84.2)
50	28.3	45.0		
100	45.5	66.4	523.1±1.42**	226.4±1.29**
200	71.8	84.8	(263.1-1040.1)	(137.7-372.3)
400	84.4	95.5		
Ae. aegypti				
Control	-	-	135.1±1.17	79.0±1.17
25	8.3	20.8	(98.9 – 184.5)	(57.8 – 107.9)
50	25.0	37.5		
100	41.2	51.5	710.7±1.46	455.0±1.15
200	61.2	75.5	(339.2-1489.2)	(202.3 – 1023.3)
400	79.2	93.8		
Cx. quinque-fasciatus				
Control	-	-	1092.1±1.54	456.0±1.23
50	3.3	3.4	(470.0-2537.2)	(306.5 – 679.5)
100	14.1	17.7		
200	19.8	35.0	2159.4±2.78	1850.0±1.68
400	27.0	43.4	(1640.5-9012.0)	(1031.8-4872.9)
500	34.1	49.6		

*Values are 24 and 48hr LC_{50} respectively; ** -Values are 24 and 48hr LC_{90} respectively; a. The values of Slope, Reg. Equation, Chi-Sq. and Intercept are for 24hr; b. The values of Slope, Reg. Equation, Chi-Sq. and Intercept are for 48hr

Grzybowski *et al.* (2012) also determined the combined action of *Annona muricata* and *Piper nigrum* and found that combinations behaved synergistically. In the present study, it was also observed that all combinations showed synergistic effects, except for pair SX:WS (1:3) which showed antagonistic effect. Sakthivadivel *et al.* (2012) also evaluated the toxic effects of petroleum ether leaf extracts of many plants individually and in combination against larvae of *Cx. quinquefasciatus* and showed that efficacy of *Argemone mexicana* (Individual LC_{50} 48.9 mgl^{-1}) increased (LC_{50} 28.6 mgl^{-1}) when mixed (1:1) with the extract from *Clausena dentata,* indicating synergistic action of *A. mexicana*. In the present study, it was also observed that the 24hr LC_{50} of *S. xanthocarpum* (77.7 mgl^{-1}) became 32.7 mgl^{-1} when the combination of SX:WS (1:1) was used. However, when combination was increased to 1:2 and 1:3, the LC_{50} value increased from 47.5 (SX:WS-1:2) to 63.8 mgl^{-1} (SX:WS-1:3). These results indicated that when the proportion of less efficacious component (*W. somnifera*) was increased, the efficacy went down though the synergistic effect remained.

Yongkhamcha and Indrapichate (2012) studied the individual and combined insecticidal efficacy of ethanolic seed extracts of *Hyptis suaveolens, Pachyrhizus erosus* and *Apium gravelling* on *Ae. aegypti* larvae and demonstrated that efficacy was much better when the extracts were used jointly instead of individually. Shanmugasundaram *et al.* (2008) also studied the effect of neem (*Azadirachta indica*) and karanja (*Pongamia glabra*) oil cakes (individuals and combination) on larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* and observed that combination of neem and karanja oil cakes in equal proportion showed better effects than individual treatments. Increase in efficacy was found to range about 4 to 10 fold in terms of LC_{50}. In the present study, increase in efficacy of combined treatment over individuals on larvae of *An. stephensi* was also found to range from 1.22 to 5.14 fold in terms of 24hr $LC_{50.}$ George and Vincent (2005) also evaluated the combined efficacy of *Annona squamosa* and *Pongamia glabra* to *A. indica* against larvae of *Cx. quinquefasciatus* and found that 1:1 mixture of the plants was significantly more effective than 100% *Az. indica* extract alone.

Singha *et al.* (2011) investigated the synergistic effect of crude and solvent extract of *Croton caudatus* (fruits) and *Tiliacora acuminata* (flowers) against *C. quinquefasciatus* larvae and showed 100% mortality after 24hr, instead when individual treatment was given. Maurya *et al.* (2012) also evaluated the synergistic larvicidal activities of different combinations of synthetic nicotinoid insecticide, imidacloprid with *Ocimum basilicum* against *An. stephensi* larvae using crude petroleum ether leaves extract. They showed that 1:1 ratio of all binary mixtures was most effective as compared to 1:2 and 1:4 and concluded that the tested combination was more effective than its individual constituents and more cost-effective and eco-friendly to the aquatic fauna. However, Chenniappan *et al.* (2011) while

Table 2 : Efficacy of methanol extracts of ripe *S. xanthocarpum* and *W. somnifera* fruits when tested at different fractions of LC_{50} each against *An. stephensi*

Treatment combinations	No. Exp.	No. dead after %		Exptl. mortality after		% Corrected mortality after	
		24h	48h	24h	48h	24h	48h
Control	100	1	5	1.0	5.0	-	-
SX (LC_{50})	98	51	79	52.0	80.6	52.0	79.6
WS (LC_{50})	99	48	68	48.5	68.7	48.5	45.8
SX+WS **(LC_{50} each)**	100	100	100	100.0	100.0	100.0	100.0
Control	98	1	2	1.0	2.1	-	-
SX (1/2 LC_{50})	98	23	48	23.5	49.0	23.5	49.0
WS (1/2 LC_{50})	99	20	41	20.2	41.4	20.2	41.4
SX+WS **(1/2 LC_{50} each)**	97	97	97	100.0	100.0	100.0	100.0
Control	100	0	0	0.0	0.0	-	-
SX (1/3 LC_{50})	100	16	28	16.0	28.0	16.0	28.0
WS (1/3 LC_{50})	100	12	22	12.0	22.0	12.0	22.0
SX+WS **(1/3 LC_{50} each)**	98	67	80	68.4	81.6	68.4	81.6
Control	97	2	2	2.1	2.1	-	-
SX (1/4 LC_{50})	99	11	19	11.1	19.2	11.1	19.2
WS (1/4 LC_{50})	99	8	16	8.1	16.2	8.1	16.2
SX+WS **(1/4 LC_{50} each)**	100	44	56	44.0	56.0	44.0	56.0

Table 3 : Synergistic and antagonistic effects on larvae of *An. stephensi* when exposed to different proportions of methanol extracts of *W. somnifera* (WS) and *S. xanthocarpum* (SX) fruits

Treatment ratios (mgl^{-1})	Exp. time	Regression equation	Chi Sq.	LC_{50} ± SE Fiducial limits (mgl^{-1})	CTC	SF	Type of response	LC_{90} ± SE Fiducial limits	CTC	SF	Type of response
WS	24	Y=1.87x +1.19	0.15	108.4±1.16 (80.7-45.5)	-	-	-	523.1±1.42 (263.1-940.1)	-	-	
-	48	Y= 2.40x+0.63	0.87	66.3±1.13 (52.2-84.2)	-	-	-	226.4±1.29 (137.7-372.3)	-	-	-
WS:SX (1:1)	24	Y=1.94x+2.06	0.22	32.7±1.16 (24.5-43.6)	331.5	3.31	Synergistic	149.4±1.40 (77.6-287.8)	350.1	3.50	Synergistic
	48	Y=1.88x+2.44	0.98	22.9±1.17 (16.8-31.4)	289.5	2.90	Synergistic	109.8±1.43 (54.2-222.6)	206.2	2.06	Synergistic
WS:SX (1:2)	24	Y=1.48x+2.80	2.26	30.6±1.20 (21.3–43.8)	354.2	3.54	Synergistic	152.6 ± 1.56 (93.4–530.3)	342.8	3.43	Synergistic
	48	Y=1.54x+3.13	0.64	16.3±1.20 (11.5-23.1)	406.7	4.07	Synergistic	110.0±1.54 (47.2–256.4)	205.8	2.06	Synergistic
WS:SX (1:3)	24	Y= 1.62x+2.81	1.34	22.2±1.18 (16.1-30.8)	488.3	4.88	Synergistic	136.8±1.45 (66.0–283.4)	382.4	3.82	Synergistic
	48	Y=1.84x+2.96	0.29	12.9±1.17 (9.4–17.6)	514.0	5.14	Synergistic	64.0±1.43 (31.9–128.4)	353.8	3.54	Synergistic

SF–Synergistic factor; CTC - Co-Toxicity Coefficient; All values of LC_{50} and LC_{90} are in mgl^{-1}

evaluating the synergistic activity of ethanolic extracts of *Andrographis paniculata* with *Bacillus thuringiensis* against *An. stephensi* observed that 1:4 ratio of Bti and extract was more effective (51.6 fold) than 1:2 and 1:1 ratios. Mohan *et al.* (2006, 2007 and 2010) also evaluated the efficacy of Cypermethrin, temephos and fenthion and crude petroleum ether extracts of *S. xanthocarpum* against *C. quinquefasciatus* and *An. stephensi* larvae alone and in combination with each other in 1:1, 1:2 and 1:4 ratio and found that 1:1 ratio to be more efficient than other combinations, indicating synergism. The present results also showed synergistic activities at 1:1 ratio with both combined pairs (SX:WS and WS:SX) against *An. stephensi* and 5.14 fold increase in the efficacy was observed at 24hr LC_{50} value of binary pair (WS:SX-1:3). Shaalan *et al.* (2005a, b, c) in their bioassays with *Ae. aegypti* and *Cx. annulirostris* found that binary mixtures of phytochemicals, with or without synthetic insecticides, produced promising results when each was applied at LC_{25} dose. All mixtures resulted in 100% mortality against *Cx. annulirostris*

Table 4 : Synergistic and antagonistic effects on larvae of *An. stephensi* when exposed to different proportions of methanol extracts of *S. xanthocarpum* (SX) and *W. somnifera* (WS) fruits

Treatment ratios	Exp. time	Regression equation	Chi Sq. (x^2)	LC_{50}±SE Fiducial limits (mgl^{-1})	CTC	SF	Type of response	LC_{90}±SE Fiducial limits (mgl^{-1})	CTC	SF	Type of response
SX	24	Y=1.65x+0.90	0.90	77.7±1.1 (56.9-106.0)	-	-	-	465.6± 1.48 (216.5-1001.3)	-	-	-
	48	Y=1.96x+1.70	0.10	48.4±1.16 (36.1-65.0)	-	-	-	218.2±1.41 (110.7-430.0)	-	-	-
SX:WS (1:1)	24	Y=1.94x + 2.06	0.22	32.7±1.16 (24.5-43.6)	237.6	2.38	Synergistic	149.4±1.40 (77.6-287.8)	311.6	3.12	Synergistic
	48	Y=1.88x+2.44	0.98	22.9±1.17 (16.8-31.4)	211.4	2.11	Synergistic	109.8±1.43 (54.2-222.6)	198.7	1.99	Synergistic
SX:WS (1:2)	24	Y= 1.55x+2.40	0.57	47.5±1.20 (33.4-67.6)	163.6	1.64	Synergistic	316.8±1.59 (128.1–783.57)	147.0	1.47	Synergistic
	48	Y =1.51x+2.78	1.48	29.1±1.20 (20.3–41.5)	166.3	1.66	Synergistic	203.2±1.63 (77.9–529.8)	107.4	1.07	Synergistic
SX:WS (1:3)	24	Y = 1.47x+2.34	0.25	63.8±1.21 (44.2-92.1)	121.8	1.22	Synergistic	471.7±1.65 (175.8–1258.4)	99.7	0.99	Antagonistic
	48	Y =1.49x+2.46	1.73	50.2±1.21 (34.5–73.1)	96.4	0.96	Antagonistic	361.9 ± 1.74 (122.6 – 1068.1)	60.3	0.60	Antagonistic

SF–Synergistic factor; CTC-Co-Toxicity Coefficient; All values of LC_{50} and LC_{90} are in mgl^{-1}

larvae within 24 hrs rather than the expected mortality of 50%. All mixtures acted synergistically against *Ae. aegypti* larvae and concluded that mixtures can have profound effect on growth and development.

Hence, from the present study it can be concluded that mixtures were more effective than phytochemicals alone and that synergism enabled a reduced dose to be applied for vector control potentially leading to improved resistance management and reduced costs.

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