COMMUNICABLE DISEASES

1.5 Synergistic efficacies of some plant extracts with and without some insecticidal formulations against vectors of malaria and dengue in North-Western Rajasthan

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OBJECTIVES

1. Determination of the larvicidal activity of different parts of the plant *Withania somnifera* against urban mosquito vector species.
2. Determination of joint actions, synergistic/antagonistic of *W. somnifera* with both synthetic and plant-origin insecticides in view to develop the most promising combination(s) for inclusion in the IDVC programmes.

PROGRESS

Individual plant extracts are active only at higher concentration, which makes them uneconomical for field use. Therefore, in the present study synergistic, antagonistic and additive toxic effects of binary mixtures involving phytochemicals alone and in combination with other synthetic insecticides and microbial control agents are studied for their potential use in vector control.

Studies are being carried out on *W. somnifera*, also known as Ashwagandha, a plant in Solanaceae or nightshade family. Fruits, leaves and seeds of this plant have been traditionally used for the Ayurvedic system as aphrodisiacs, diuretics and for treating memory loss. We have already evaluated the insecticidal properties of *Solanum xanthocarpum,* the Indian nightshade commonly known as 'baigan kateli'. It is found throughout the country but more abundantly in the arid areas and used widely for a variety of ailments in public health. Extracts prepared from fruits of these plant species have been screened for their synergistic larvicidal potential against larvae of different mosquito vectors present in this area.

Susceptibility tests were carried out with larvae of two mosquito species viz. *Aedes aegypti* and *Culex quinquefasciatus*. For this purpose larvae of all the three mosquito species were collected from different areas of Jodhpur city and reared in the laboratory for further generations under controlled conditions. The different parts of the plant differ in their active constituents when extracted in different solvents. Ripe fruits of *S. xanthocarpum* and *W. somnifera* were shade dried between 30-40°C for 10-15 days. Dried plant material was powdered separately and dissolved in methanol and stock solutions and serial

dilutions were made as per requirement. Third or early fourth instar larvae of these mosquito species were tested as per standard WHO method for determining the baseline data on their susceptibility status. Experiments were carried out in 500 ml beakers containing 249 ml of water by using 20-25 larvae of each mosquito species. Mortality was noted after 24 hr and corrected by using Abbott's formula. Average of four observations was taken and data subjected to log probit regression analysis.

Observations on the results of the larval susceptibility to methanol extracts of yellow ripe fruits of *S. xanthocarpum* are given in Table 1. 24 hr LC_{50} and LC_{90} values along with their 95% fiducial limits, regression equation and chi-square were calculated. 24hr LC_{50} values as observed for *Ae. aegypti* and *Cx. quinquefasciatus* were 121.6 & 142.8 mg/l while the corresponding 24 hr LC_{90} values were 1261.5 & 1003.9 mg/l respectively. The results of the larval susceptibility to methanol extracts of red fruits of *W. somnifera* are given in Table 1. 24 hr LC_{50} values as observed for *Ae. aegypti* and *Cx. quinquefasciatus* were 135.1 & 1092.1 mg/l while the corresponding 24 hr LC_{90} values were 710.7 & 2159.4 mg/l respectively.

The results of the synergistic studies on larvae of *Ae. aegypti* and *Cx. quinquefasciatus* to the methanol extracts from yellow ripe fruits of *S. xanthocarpum* and red fruits of *W. somnifera* are given in table 1. Tests were carried out at concentrations from 5, 10, 25, 50, 100,200, 400 and 500 mg/l with both the mosquito species and with different binary pairs. In the first test series the volume of *W. somnifera* was fixed and the proportion of *S. xanthocarpum* was increased and three different binary combinations (*viz.*WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3)) were prepared for both the species (Table-1). A dose response curve was established to determine the 24 hr LC_{50} and LC_{90} values along with their 95% confidence limits, regression equation, chi-square (χ^2)/ heterogeneity of the response by log probit regression analysis. In the second test series the volume of *S. xanthocarpum* was fixed and the proportion of the *W. somnifera* was increased and three different binary combinations (*viz*. SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) were again prepared for both the species (Table-1).

The 24h LC_{50} values as determined for the three binary mixtures *viz*. WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3) were 43.2, 31.1 & 25.8 mg/l and the 24hr LC_{90} were 380.2,250.2 & 239.2mg/l for *Ae. aegypti* respectively, while the corresponding 24 hr LC_{50} values for these above three pairs were 87.2, 46.2 & 39.2 mg/l and the 24hr LC_{90} values were 281.2, 230.1 & 192.2mg/l for *Cx. quinquefasciatus* respectively (Table 1). From the results it is clear that both the species were very much susceptible to the binary mixture WS:SX (1:3) as compared to the binary pairs WS:SX (1:2) and WS:SX (1:1) or in other words the efficacy goes up with the increase in the proportion of *S. xanthocarpum* extract.

The 24h LC_{50} values as determined for other three binary mixtures *viz*. SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) were 43.2, 76.7 & 103.6 mg/l and the 24hr LC_{90} were 380.2, 476.6 & 513.4 mg/l for *Ae. aegypti* respectively, while the corresponding 24 hr LC_{50} values for these above three pairs were 87.2, 160.9 & 220.6 mg/l and the 24hr LC_{90} values were 281.2, 971.1 & 1057.6 mg/l for *Cx. quinquefasciatus* respectively (Table 1). From the results it is clear that both the species were very much susceptible to the binary mixture SX:WS (1:1) as compared to the binary pairs SX:WS (1:2) and SX:WS (1:3) or in other words the efficacy goes down with the increase in the proportion of *W. somnifera* extract. Synergistic Factor (SF) and Co-toxicity Coefficient (CTC) were also determined and it was observed that when the conc. of WS was fixed and the conc. of SX was increased from 1:1 to 1:3, the Binary pair 1:3 was found most effective synergist pair (Table 1). However, a mixture of 1:1 was found most effective when the conc. of SX was fixed and the conc. of WS was increased from 1:1 to 1:3 (Table 1). However, with respect to 24hr LC_{50} value, the pairs SX:WS (1:2) and SX:WS (1:3) were found antagonistic (SF- 0.9 and 0.7) while with respect to 24hr LC_{90}, the pair SX:WS (1:2) was additive (SF-1.0) for *Cx. quinquefasciatus*.

Table 1. Synergistic and antagonistic effects on larvae of *Ae. aegypti and Cx. quinquefasciatus* when exposed to different proportions of methanol extracts (Fruits) of *S. xanthocarpum* (SX) and *W. somnifera* (WS)

Treatment Ratios	Mosquito Species	Regression Equation	Chi Sq. (χ^2)	24 hr LC_{50} ± SE Fiducial limits (mgl^{-1})	CTC	SF	Response	24 hr LC_{90} ± SE Fiducial limits (mgl^{-1})	CTC	SF	Response
SX	*Aedes aegypti*	Y= 1.26x +2.37	0.11	121.6 (81.7-180.9)	-	-	-	1261.5 (396.6-4012.4)	-	-	-
WS		Y= 1.78x +1.22	0.35	135.1 (98.9-184.5)	-	-	-	710.7 (339.2-1489.2)	-	-	-
WS:SX (1:1)		Y= 1.36x +2.78	1.01	43.2 (29.2-63.9)	312.7	3.1	S	380.2 (125.0-1155.9)	186.9	1.9	S
WS:SX (1:2)		Y= 1.41x +2.89	1.46	31.1 (20.0-48.1)	434.4	4.3	S	250.2 (79.0-791.9)	284.1	2.8	S
WS:SX (1:3)		Y= 1.32x +2.13	1.68	25.8 (16.4-40.7)	523.6	5.2	S	239.2 (64.4-888.5)	297.1	3.0	S
SX:WS (1:1)		Y= 1.78x +1.22	1.01	43.2 (29.2-63.9)	281.5	2.8	S	380.2 (125.0-1155.9)	331.8	3.3	S
SX:WS (1:2)		Y= 1.85x +1.51	0.50	76.7 (56.6-104.0)	158.5	1.6	S	476.6 (176.2-805.0)	264.7	2.7	S
SX:WS (1:3)		Y= 1.84x +1.29	0.63	103.6 (77.0-139.4)	117.4	1.2	S	513.4 (246.6-1068.7)	245.7	2.5	S
SX	*Culex quinquefasciatus*	Y= 1.76x +1.20	1.91	142.8 (101.4-201.0)	-	-	-	1003.9 (319.7-3804.1))	-	-	-
WS		Y= 1.22x +1.28	1.36	1092.1 (470.0-2537.2)	-	-	-	2159.4 (1640.5-9012.0)	-	-	-
WS:SX (1:1)		Y= 2.52x +0.12	0.29	87.2 (68.5-110.9)	1252	12.5	S	281.2 (171.5-461.3)	767.9	7.7	S
WS:SX (1:2)		Y= 1.84x +1.94	0.87	46.2 (34.0-62.9)	2363	23.6	S	230.1 (109.2-484.6)	938.5	9.4	S
WS:SX (1:3)		Y= 1.85x +2.05	0.56	39.2 (28.3-54.1)	2786	27.9	S	192.2 (94.2-392.2)	1123	11.2	S
SX:WS (1:1)		Y= 2.52x +0.12	0.29	87.2 (68.5-110.9)	163.8	1.6	S	281.2 (171.5-461.3)	357.0	3.6	S
SX:WS (1:2)		Y= 1.64x +1.38	0.64	160.9 (118.0-219.3)	88.7	0.9	A	971.1 (418.7-2252.1)	103.4	1.0	D
SX:WS (1:3)		Y= 2.39x -0.59	0.80	220.6 (172.0-282.9)	64.7	0.7	A	1057.6 (439.5-1306.0)	94.9	0.9	A

SF - Synergistic factor; CTC - Co-Toxicity Coefficient
S- Synergistic; A- Antagonistic; D- Additive