

1.7 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

RATIONALE

Mosquito-borne diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease load in tropical countries. Among these diseases, malaria and dengue continues to be the major public health problem in most tropical countries. However, over and injudicious application of synthetic insecticides in controlling the vectors of above diseases has resulted in to development of insecticide resistance among vector species, environmental hazards through persistence and accumulation of non-biodegradable chemicals in ecosystem, biological magnification through the food chains and toxic effects on human health. These developments demand that a renewed effort be made to register alternative insecticidal agents with high bio-control potentiality but causes little or no harmful effect to environment and human health. One possible strategy is the exploitation of bioactive products from phytochemicals by systematically exploring the local desert medicinal plants. There is an ever-increasing demand for plant-based insecticides as they are non-toxic, easily available at affordable prices, biodegradable and show broad-spectrum target-specific activities against different species of vector mosquitoes. Furthermore, unlike conventional commercial insecticides that are based on single active ingredient, plant-derived insecticides comprise botanical blends of secondary metabolites, which act jointly on both behavioural and physiological processes. Thus, the chances of pests developing resistance to such substances are meager. However, efficacy observed for botanicals over 24hr, render many of them unusable by economic standards.

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 would be tried in order to find suitable synergists or additives which can be used for vector control.

PROGRESS

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 for the larvae of three mosquito species viz. *Anopheles stephensi*, *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 and 48 hr LC₅₀ and LC₉₀ values along with their 95% fiducial limits, regression equation and chi-square were calculated. 24 and 48h LC₅₀ values as observed for *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were 77.7, 121.6 & 142.8 and 48.4, 71.1 & 115.4 mg/l while the corresponding 24 and 48h LC₉₀ values were 465.6, 1261.5 & 759.5 and 218.2, 687.3 & 844.0 mg/l respectively. The results of the larval susceptibility to methanol extracts of red fruits of *W. somnifera* are given in Table 2. 24 and 48h LC₅₀ values as observed for *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were 108.4, 135.1 & 1092.1 and 66.3, 79.0 & 456.0 mg/l while the corresponding 24 and 48h LC₉₀ values were 523.1, 710.7 & 2159.4 and 226.4, 455.0 & 1850.0 mg/l respectively.

The results of the synergistic studies on larvae of *An. stephensi* to the methanol extracts from yellow ripe fruits of *S. xanthocarpum* and red fruits of *W. somnifera* are given in tables 3 and 4. In the first test series the volume of *W. somnifera* was fixed

Table 1. Efficacy of methanol extracts of *S. xanthocarpum* (Yellow ripe fruits) on larvae of different mosquito vectors

Mosquito Species/ Conc. (mg ⁻¹)	(%) Corrected Mortality		Regression Equation	Chi-Sq. (df)	24 hr LC ₅₀ & LC ₉₀ (Fiducial limits) mg ⁻¹	48 hr LC ₅₀ & LC ₉₀ (Fiducial limits) mg ⁻¹
	24h	48h				
<i>An. stephensi</i>						
Control	-	-	Y=1.65x+1.89 ^a	0.90 ^a (3)	77.7± 1.17* (56.9-106.0)	48.4± 1.16* (36.1-65.0)
25	18.4	28.6	Y=1.96x+1.70 ^b	0.10 ^b (3)	465.6± 1.48** (216.5-1001.3)	218.2±1.41** (110.7-430.0)
50	40.4	49.5				
100	62.5	75.0				
200	73.2	86.6				
400	83.7	96.9				
<i>Ae. aegypti</i>						
Control	-	-	Y=1.26x+2.37	0.11 (3)	121.6±1.22 (81.7-180.9)	71.1±1.22 (48.4-104.4)
25	18.4	28.6	Y=1.30x+2.59	0.11 (3)	1261.5±1.80 (396.6-4012.4)	687.3±1.76 (226.4-2086.3)
50	33.0	41.2				
100	45.5	55.6				
200	60.6	72.7				
400	73.2	84.5				
<i>Cx. quinque-fasciatus</i>						
Control	0.0	0.0	Y=1.76x+1.20	1.91 (3)	142.8±1.19 (101.4-201.0)	115.4±1.21 (79.1-168.3)
25	11.2	20.4	Y=1.48x+1.94	2.30 (3)	1003.9±1.55 (319.7-3804.1)	948.4±1.75 (282.9-2517.9)
50	20.6	28.9				
100	36.0	41.2				
200	57.3	62.5				
400	93.8	86.7				

*Values are 24 and 48h LC₅₀ respectively; ** -Values are 24 and 48h LC₉₀ 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

and the proportion of *S. xanthocarpum* was increased and three different binary combinations were prepared. These pairs were WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3) and tests were carried out at concentrations of 5,10, 25, 50, 100 and 200 mg/l for *An. stephensi* (Table-3). A dose response curve was established to determine the 24 and 48h LC₅₀ and LC₉₀ 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 were prepared. These pairs were SX:WS (1:1), SX:WS (1:2) and SX:WS (1:3) and tests were carried out at concentrations of 10, 25, 50, 100 and 200 mg/l for *An. stephensi* (Table-4).

The 24h LC₅₀ values as determined for three binary mixtures viz. WS:SX (1:1), WS:SX (1:2) and WS:SX (1:3) were 32.7, 30.6 & 22.2 mg/l and the 48h LC₅₀ were 22.9, 16.3 & 12.9 mg/l respectively for this mosquito vector species. The 24h and 48h LC₉₀ values were also determined which were 149.4, 152.6, & 136.8 mg/l and 109.8, 110.0, & 64.0 mg/l respectively for this mosquito species (Table 3). From the results it is clear that *An. stephensi* was very much susceptible to the binary mixture WS:SX (1:3) (24h LC₅₀,

22.2 mg/l) as compared to the binary pairs WS:SX (1:2) (24h LC₅₀, 30.6 mg/l) and WS:SX (1:1) (24h LC₅₀, 32.7 mg/l) or in other words the efficacy goes up with the increase in the proportion of *S. xanthocarpum* extract.

Table 2. Efficacy of methanol extracts of *W. somnifera* (Red fruits) on larvae of different mosquito vectors

Mosquito Species/ Conc. (mg ⁻¹)	(%) Corrected Mortality		Regression Equation	Chi-Sq. (df)	24 hr LC ₅₀ & LC ₉₀ (Fiducial limits) mg ⁻¹	48 hr LC ₅₀ & LC ₉₀ (Fiducial limits) mg ⁻¹
	24h	48h				
<i>An. stephensi</i> Control 25 50 100 200 400	-	-	Y= 1.87x +1.19 ^a	0.15 ^a (3)	108.4 ± 1.16* (80.7-45.5)	66.3±1.13* (52.2-84.2)
	11.2	12.8				
	28.3	45.0	Y= 2.40x+0.63 ^b	0.87 ^b (3)	523.1±1.42** (263.1-1040.1)	226.4±1.29** (137.7-372.3)
	45.5	66.4				
	71.8	84.8				
	84.4	95.5				
<i>Ae. aegypti</i> Control 25 50 100 200 400	-	-	Y= 1.78x+1.22	0.35 (3)	135.1±1.17 (98.9 - 184.5)	79.0±1.17 (57.8 - 107.9)
	8.3	20.8				
	25.0	37.5	Y=1.68x+1.81	0.66 (3)	710.7±1.46 (339.2-1489.2)	455.0±1.15 (202.3 - 1023.3)
	41.2	51.5				
	61.2	75.5				
	79.2	93.8				
<i>Cx. quinque-fasciatus</i> Control 50 100 200 400 500	-	-	Y=1.22x+1.28	1.36 (3)	1092.1±1.54 (470.0-2537.2)	456.0±1.23 (306.5 - 679.5)
	3.3	3.4				
	14.1	17.7	Y=1.61x+0.72	2.46 (3)	2159.4±2.78 (1640.5-9012.0)	1850.0±1.68 (1031.8-4872.9)
	19.8	35.0				
	27.0	43.4				
	34.1	49.6				

*Values are 24 and 48h LC₅₀ respectively; ** -Values are 24 and 48h LC₉₀ 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

The 24h LC₅₀ 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 & 63.8 mg/l and the 48h LC₅₀ were 22.9, 29.1 & 50.2 mg/l respectively for this mosquito vector species i.e. *An. stephensi*. The 24h and 48h LC₉₀ values were also determined which were 149.4, 316.8, & 471.7 mg/l and 109.8, 203.2, & 361.9 mg/l respectively for this mosquito species (Table 4). From the results it is clear that *An. stephensi* was very much susceptible to the binary mixture SX:WS (1:1) (24h LC₅₀, 32.7 mg/l) as compared to the binary pairs SX:WS (1:2) (24h LC₅₀, 47.5 mg/l) and SX:WS (1:3) (24h LC₅₀, 63.8 mg/l) 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 concentration 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 5 & Fig. 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 6 & Fig. 2). Experiments have also been done on different fractions viz. 1/2, 1/3, 1/4th of the LC_{50} values of SX and WS and it was observed that synergistic effect was observed even up to 1/4th of the LC_{50} of these two plant extracts (Table 7 & Fig.3)

Table 3. Efficacy of combined methanol extracts of *W. somnifera* (WS) and *S. xanthocarpum* (SX) on larvae of *An. stephensi*

Mosquito Species/ Conc. (mg^{-1})	(%) Corrected Mortality		Regression Equation	Chi-Sq. (df)	24 hr LC_{50} & LC_{90} (Fiducial limits) mg^{-1}	48 hr LC_{50} & LC_{90} (Fiducial limits) mg^{-1}
	24h	48h				
(WS:SX) 1:1 Control 10 25 50 100 200	-	-	Y=1.94x + 2.06 ^a	0.22 ^a (3)	32.7±1.16* (24.5-43.6)	22.9±1.17* (16.8-31.4)
	17.1	28.2				
	40.4	46.5	Y=1.88x + 2.44 ^b	0.98 ^b (3)	149.4±1.40** (77.6-287.8)	109.8±1.43** (54.2-222.6)
	61.9	74.2				
	83.5	92.8				
	95.9	97.9				
(WS:SX) 1:2 Control 5 10 25 50 100	-	-	Y =1.48x + 2.80	2.26 (3)	30.6 ± 1.20 (21.3 - 43.8)	16.3 ± 1.20 (11.5 - 23.1)
	8.0	19.0				
	30.3	42.4	Y = 1.54x + 3.13	0.64 (3)	152.6 ± 1.56 (93.4 - 530.3)	110.0 ± 1.54 (47.2 - 256.4)
	45.5	61.6				
	66.0	74.0				
	72.4	90.5				
(WS:SX) 1:3 Control 5 10 25 50 100	-	-	Y= 1.62x + 2.81	1.34 (3)	22.2 ± 1.18 (16.1 -30.8)	12.9± 1.17 (9.4 - 17.6)
	12.2	21.4				
	30.9	44.3	Y = 1.84x + 2.96	0.29 (3)	136.8 ± 1.45 (66.0 - 283.4)	64.0 ± 1.43 (31.9 - 128.4)
	60.6	71.7				
	70.7	85.9				
	79.6	91.8				

*Values are 24 and 48h LC_{50} respectively; ** -Values are 24 and 48h 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

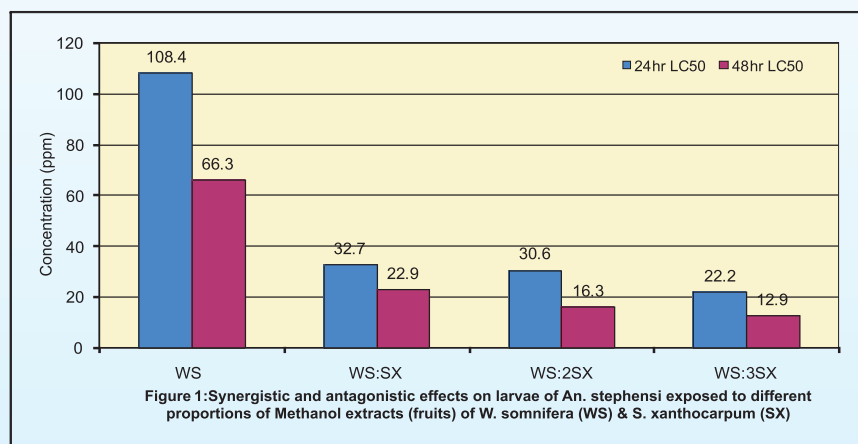


Table 4. Efficacy of combined methanol extracts of *S. xanthocarpum* (SX) and *W. somnifera* (WS) on larvae of *An. stephensi*

Mosquito Species/ Conc. (mg ⁻¹)	(% Corrected Mortality		Regression Equation	Chi- Sq. (df)	24 hr LC ₅₀ & LC ₉₀ (Fiducial limits) mg ⁻¹	48 hr LC ₅₀ & LC ₉₀ (Fiducial limits) mg ⁻¹
	24h	48h				
(SX:WS) 1:1 Control 10 25 50 100 200	-	-	Y=1.94x + 2.06 ^a	0.22 ^a (3)	32.7±1.16* (24.5-43.6)	22.9±1.17** (16.8-31.4)
	17.1	28.2				
	40.4	46.5	Y=1.88x +2.44 ^a	0.98 ^b (3)	149.4±1.40* (77.6-287.8)	109.8±1.43** (54.2-222.6)
	61.9	74.2				
	83.5	92.8				
	95.9	97.9				
(SX:WS) 1:2 Control 10 25 50 100 200	-	-	Y= 1.55x + 2.40	0.57 (3)	47.5± 1.20 (33.4 - 67.6)	29.1±1.20 (20.3 - 41.5)
	16.0	28.0	Y =1.51x + 2.76	1.48 (3)	316.8±1.59 (128.1 - 783.57)	203.2±1.63 (77.9 - 529.8)
	30.0	41.0				
	49.5	61.6				
	73.5	87.8				
	82.8	92.9				
(SX:WS) 1:3 Control 10 25 50 100 200	-	-	Y = 1.47x + 2.34	0.25 (3)	63.8 ± 1.21 (44.2 - 92.1)	50.2 ± 1.21 (34.5 - 73.1)
	12.0	16.8	Y =1.49x + 2.46	1.73 (3)	471.7 ± 1.65 (175.8 - 1258.4)	361.9 ± 1.74 (122.6 - 1068.1)
	27.6	32.3				
	40.8	43.0				
	60.6	72.3				
	80.4	90.2				

*Values are 24 and 48h LC₅₀ respectively; ** -Values are 24 and 48h LC₉₀ 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

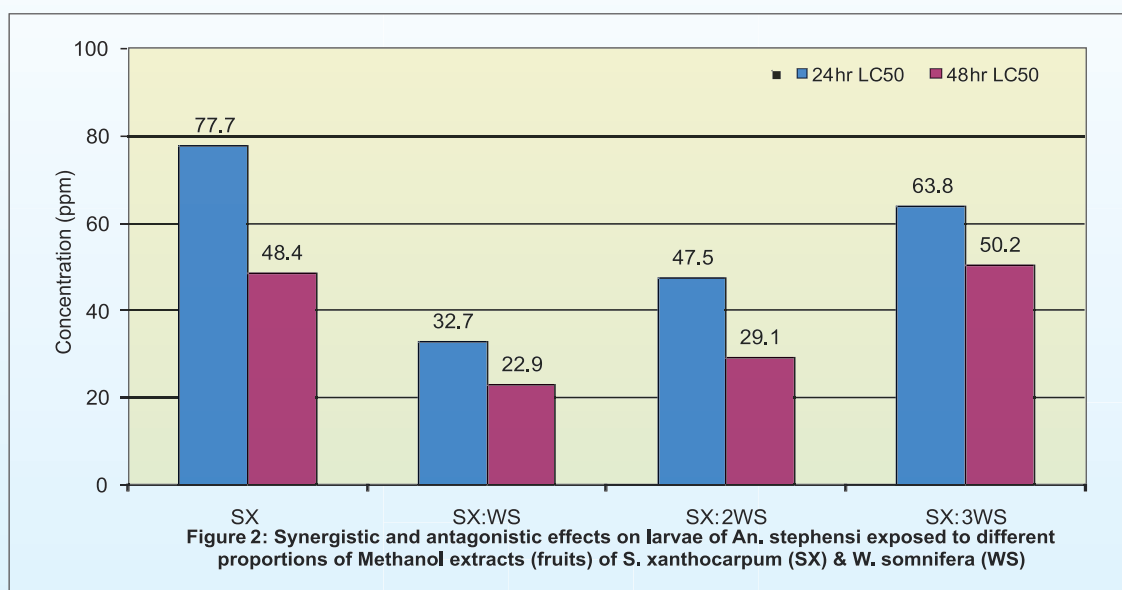


Table 5. Synergistic and antagonistic effects on larvae of *An. stephensi* when exposed to different proportions of methanol extracts (Fruits) of *W. somnifera* (WS) and *S. xanthocarpum* (SX)

Treatment Ratios	Exp. time	Regression Equation	Chi Sq. (χ^2)	LC ₅₀ ± SE Fiducial limits (mg ⁻¹)	CTC	SF	Type of Response	LC ₉₀ ± SE Fiducial limits (mg ⁻¹)	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	Syn	149.4 ± 1.40 (77.6-287.8)	350.1	3.50	Syn
	48	Y = 1.88x + 2.44	0.98	22.9 ± 1.17 (16.8-31.4)	289.5	2.90	Syn	109.8 ± 1.43 (54.2-222.6)	206.2	2.06	Syn
WS:SX (1:2)	24	Y = 1.48x + 2.80	2.26	30.6 ± 1.20 (21.3 - 43.8)	354.2	3.54	Syn	152.6 ± 1.56 (93.4 - 530.3)	342.8	3.43	Syn
	48	Y = 1.54x + 3.13	0.64	16.3 ± 1.20 (11.5-23.1)	406.7	4.07	Syn	110.0 ± 1.54 (47.2 - 256.4)	205.8	2.06	Syn
WS:SX (1:3)	24	Y = 1.62x + 2.81	1.34	22.2 ± 1.18 (16.1 -30.8)	488.3	4.88	Syn	136.8 ± 1.45 (66.0 - 283.4)	382.4	3.82	Syn
	48	Y = 1.84x + 2.96	0.29	12.9 ± 1.17 (9.4 - 17.6)	514.0	5.14	Syn	64.0 ± 1.43 (31.9 - 128.4)	353.8	3.54	Syn

SF - Synergistic Factor; CTC - Co-Toxicity Coefficient
All values of LC₅₀ and LC₉₀ are in mg⁻¹

Table 6. Synergistic and antagonistic effects on larvae of *An. stephensi* when exposed to different proportions of methanol extracts (Fruits) of *S. xanthocarpum* (SX) and *W. somnifera* (WS)

Treatment Ratios	Exp. time	Regression Equation	Chi Sq. (χ^2)	LC ₅₀ ± SE Fiducial limits (mg ⁻¹)	CTC	SF	Type of Response	LC ₉₀ ± SE Fiducial limits (mg ⁻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 LC₅₀ and LC₉₀ are in mg⁻¹

Table 7. Efficacy of methanol extracts of fruit of *S. xanthocarpum* and *W. somnifera* tested at different fractions of LC₅₀ each against *An. stephensi*

Treatment Combinations	No. Exp.	No. Dead after		% Experimental 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

