COMPARATIVE EFFICACY OF *SOLANUM XANTHOCARPUM* EXTRACTS ALONE AND IN COMBINATION WITH A SYNTHETIC PYRETHROID, CYPERMETHRIN, AGAINST MALARIA VECTOR, *ANOPHELES STEPHENSI*

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Abstract. With a goal of minimal application of environmentally hazardous chemical insecticides, the larvicidal activity of cypermethrin was studied alone and in combination with the root extract of *Solanum xanthocarpum* against anopheline larvae. Petroleum ether extract was observed to be the most toxic, with LC_{50} of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm at 24 and 48 hours after application, respectively, followed by carbon tetrachloride and methanol extracts. The values for cypermethrin were an LC_{50} of 0.0369 ppm after 24 hours and 0.0096 ppm after 48 hours and LC_{90} of 0.0142 and 0.0091 ppm after 24 and 48 hours, respectively. The ratios of cypermethrin and petroleum ether extracts tested were 1:1, 1:2 and 1:4. Of the various ratios tested, the cypermethrin and petroleum ether extract ratio of 1:1 was observed to be more efficient than the other combinations. From the individual efficacy of each constituent, synergism was noted. This is an ideal ecofriendly approach for the control of malaria vector, *Anopheles stephensi*.

INTRODUCTION

The adaptability of mosquitoes makes no single strategy for their control adequate. Synthetic insecticides are fast acting, highly active and cost effective, yet their continuous application has resulted in gradual deterioration of the environment (Tyagi, 2003). Moreover, they are toxic to non-target organisms. Botanical insecticides are now preferred as an ecofriendly alternative, but they are time consuming and require large amounts. To enhance their slower action and to reduce their requirement, an integrated scheme is required for efficient, ecofriendly, cost effective management of vectors covering the combined application of different aspects of vector control. Insecticide synergists have been recommended as powerful research tools for diagnosing resistance mechanisms, determining the confirmation of target sites and elucidating metabolic roots (Bernard and Philogne, 1993). The role of phyto products for synergistic activity along with synthetic pyrethroids is well known against different pests (Rao and Dhingra, 1997; Vastrad *et al,* 2002) and vectors (Thangam and Kathiresan, 1997). The present study is a further extension of the synergistic approach. It emphasizes the larvicidal activity of *Solanum xanthocarpum* extracts, a plant of significant insecticidal nature (Singh and Bansal, 2003; Mohan *et al,* 2005) in combination with cypermethrin, an efficient synthetic pyrethroid (Bansal and Singh, 2004; Mohan *et al,* 2004) against *Anopheles stephensi* larvae.

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MATERIALS AND METHODS

Larvicidal assay of phytoextracts

Roots of *Solanum xanthocarpum* were collected and dried in the shade after washing. Dried roots were chopped and extracted with petroleum ether, carbon tetrachloride and methanol in a Soxhlet Apparatus (Borosil, Mumbai, India) for 72 hours. Extracts were seperated from the solvents (petroleum ether, carbon tetrachloride and methanol) by Vacuum Rotary Evaporator (Biocraft Scientific Industries, Agra, India) to get a solidified crude residue. Residues obtained from each fraction were dissolved in alcohol independently to get stock solutions of 500 ppm for petroleum ether and carbon tetrachloride, and 10,000 ppm for methanol. Different test concentrations for larval exposure were prepared by further diluting these stocks. Twenty 3rd instar anopheline larvae were exposed to these test concentrations in 500 ml beakers containing 249 ml dechlorinated tap water and 1 ml of test concentration. Experiments were set up according to standard WHO (1975) procedures at 27 ± 1ºC at 85% relative humidity. Three solutions were developed from each extract along with a parallel control. Mortality observations were noted 24 and 48 hours post-treatment.

Cypermethrin bioassay

Cypermethrin (25% EC, RPG Life Sciences, Mumbai, India) purchased from the local market was diluted in dechlorinated tap water to obtain 20 ppm stock. Different test concentrations were prepared by diluting this stock and bioassays against larvae were conducted as for these phytoextracts. Mortality data were recorded 24 and 48 hours post-treatment.

Larval efficacy for phytoextract and cypermethrin combinations

For combination-based studies, 20 ppm cypermethrin and the most efficient phytoextract preparation were prepared. Keeping cypermethrin as a standard, its stock was mixed with the stock of the most potent extract in ratios of 1:1, 1:2 and 1:4. Test concentrations for each mixed formulation ratio were prepared by further diluting the combination in water. Larvicidal efficacy of each formulation was observed as above and mortality was noted 24 and 48 hours post-exposure.

Statistical analysis of mortality response

Mortality data obtained for the phytoextracts and cypermethrin bioassays and for the mixed formulations were analyzed by Probit Analysis (Finney, 1971) to obtain an regression equation, LC_{50} and LC_{90}, standard error and fiducial limits at 95% confidence limits. The co-toxicity coefficient (Sarup *et al*, 1980) and synergistic factor (Kalayanadundaram and Das, 1985) for the mixed formulation were also calculated after calculating the LC_{50} and LC_{90} for each combination.

$$\text{Co-toxicity coefficient} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with plant extract}} \times 100$$

$$\text{Synergistic factor (SF)} = \frac{\text{Toxicity of insecticide (alone)}}{\text{Toxicity of insecticide with plant extract}}$$

A value of SF >1 indicates synergism and an SF <1 indicates antagonism.

RESULTS

Bioassay of phytoextracts

Table 1 reveals the larvicidal efficacy of various extracts of *S. xanthocarpum*. Petroleum ether extract exhibited minimum LC_{50} of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm after 24 and 48 hours, respectively. Carbon tetrachloride extract showed LC_{50} of 22.57 and 15.81 ppm at 24 and 48 hours post-exposure and LC_{90} of 135.13 ppm at 24 hours and 130.55 ppm at 48 hours post-treatment. Methanol extract had LC_{50} of 161.55 ppm at 24 hours and 110.57 ppm at 48 hours

Table 1
Efficacy of different root extracts of *Solanum xanthocarpum* against larvae of *Anopheles stephensi*.

Solvent extract	Exposure (hrs)	Regression equation	χ^2	LC_{50} ± SE (Fiducial limits) ppm	Relative toxicity irrespective of time period	LC_{90} ± SE (Fiducial limits) ppm	Relative toxicity irrespective of time period
Carbon tetrachloride	24	1.87X+2.36	9.76	22.57±4.16 (13.22-29.52)	7.16	135.13±43.01 (50.82-219.44)	5.52
	48	1.40X+3.32	19.03	15.81±3.74 (8.48-23.14)	10.22	130.55±46.94 (38.54-222.55)	5.71
Methanol	24	1.93X-1.19	5.09	161.55±39.62 (83.89-239.21)	1.0	745.88±80.42 (588.26-903.50)	1.0
	48	1.68X-0.12	1.70	110.57±19.52 (72.31-148.83)	1.46	638.83±24.70 (590.42-687.24)	1.17
Petroleum ether	24	1.19X+3.64	0.18	1.41±0.42 (0.58-2.23)	114.57	16.94±4.93 (7.28-29.60)	44.03
	48	1.33X+3.71	3.18	0.93±0.21 (0.52-1.33)	173.71	8.48±1.28 (5.97-10.99)	87.96

and LC_{90} of 745.88 and 638.83 ppm at 24 and 48 hours. Cypermethrin exhibited LC_{50} of 0.0369 and 0.0096 ppm and LC_{90} of 0.1932 and 0.0559 ppm at 24 and 48 hours post-exposure, respectively. All the values were within a 95% confidence interval.

Combinatorial bioassay

The combined bioassay with cypermethrin and petroleum ether extract, the most effective extract, in different ratios are depicted in Table 2. The 1:1 ratio of cypermethrin and extract had an LC_{50} value of 0.0054 ppm and an LC_{90} value of 0.0142 ppm at 24 hours and an LC_{50} of 0.0036 ppm and an LC_{90} of 0.0091 ppm at 48 hours post- exposure. The ratio of 1:2 had LC_{50} and LC_{90} values of 0.0057 and 0.0174 ppm, respectively, at 24 hours and an LC_{50} of 0.0047 ppm and an LC_{90} of 0.0145 ppm at 48 hours post-treatment. At a ratio of 1:4, the LC_{50} and LC_{90} were 0.0074 ppm and 0.0111 ppm, respectively, at 24 hours and an LC_{50} of 0.0068 ppm and an LC_{90} of 0.0108 ppm at 48 hours post-exposure.

The co-toxicity coefficient for the 1:1 mixture were 683.3 and 266.67 at 24 and 48 hours, respectively for the LC_{50} and 1,360.56 and 614.29 at 24 and 48 hours for the LC_{90}. The synergistic factors for the 1:1 mixture were 6.83 and 2.67 at 24 and 48 hours for the LC_{50}, respcetively and 13.61 and 6.14 for the LC_{90}, respectively. With a ratio 1:2, the co-toxicity coefficients for the LC_{50} were 647.37 and 204.24 and the synergistic factors were 6.47 and 2.04 at 24 and 48 hours post-treatment, respectively. The co-toxicity coefficients for the LC_{90} were 1,110.35 and 385.52 and the synergistic factors were 11.10 and 3.86 at 24 and 48 hours post-treatment, respectively. At a ratio of 1:4, the co-toxicity coefficients for LC_{50} were 498.65 and 141.18 and the synergistic factors were 4.99 and 1.41 at 24 and 48 hours post-treatment, respectively. The co-toxicity coefficients for the LC_{90} were 1,740.54 and 517.59 and the synergistic factors were 17.41 and 5.18 at 24 and 48 hours, respectively.

DISCUSSION

The petroleum ether *S. xanthocarpum* extract exhibited maximum larvicidal activity against *An. stephensi* compared to the other

Table 2
Effect of *Solanum xanthocarpum* extract on the toxicity of cypermethrin against *Anopheles stephensi* larvae.

Cypermethrin/ Cypermethrin + *S. xanthocarpum*	Ratio	Exposure period (hours)	Regression equation	Chi-square	LC_{50} ± SE (Fiducial limits) ppm	Co-toxicity coefficient	Synergistic factor	Type of action	LC_{90} ± SE (Fiducial limits) ppm	Co-toxicity coefficient	Synergistic factor	Type of action
Cypermethrin	-	24	2.54+1.57X	1.05	0.0369±0.0067 (0.0215-0.0478)	-	-	-	0.1932±0.0873 (0.0219-0.3644)	-	-	-
		48	3.25+1.18X	0.15	0.0096±0.0018 (0.0060-0.0132)	-	-	-	0.0559±0.0165 (0.0236-0.0881)	-	-	-
Cypermethrin + *S. xanthocarpum*	1:1	24	3.07X+8.88	1.27	0.0054±0.0007 (0.0040-0.0069)	683.33	6.83	Synergistic	0.0142±0.0050 (0.0044-0.0240)	1,360.56	13.61	Synergistic
		48	3.20X+9.62	1.11	0.0036±0.0004 (0.0027-0.0045)	266.67	2.67	Synergistic	0.0091±0.0022 (0.0048-0.0133)	614.29	6.14	Synergistic
	1:2	24	2.66X+8.31	0.35	0.0057±0.0008 (0.0042-0.0073)	647.37	6.47	Synergistic	0.0174±0.0068 (0.0041-0.0307)	1,110.35	11.10	Synergistic
		48	2.65X+8.51	0.38	0.0047±0.0007 (0.0034-0.0062)	204.24	2.04	Synergistic	0.0145±0.0050 (0.0047-0.0243)	385.52	3.86	Synergistic
	1:4	24	7.25X+13.20	0.68	0.0074±0.0004 (0.0065-0.0083)	498.65	4.99	Synergistic	0.0111±0.0012 (0.0088-0.0134)	1,740.54	17.41	Synergistic
		48	6.34X+12.39	0.29	0.0068±0.0005 (0.0058-0.0078)	141.18	1.41	Synergistic	0.0108±0.0013 (0.0083-0.0134)	517.59	5.18	Synergistic

extracts. Our results regarding the larvicidal efficacy of this plant are supported by findings of Singh and Bansal (2003) who studied the larvicidal activity of aqueous fruit (LC_{50} = 0.058%) and root extract (LC_{50} =1.08%) of *S. xanthocarpum* against *An. stephensi*. Mohan *et al* (2005) reported the larvicidal activity of carbon tetrachloride fruit extracts of the same plant (LC_{50} 5.11 ppm) against the same vector species.

Studies regarding the combined application of cypermethrin and the extract reveal the synergistic action of the extract towards cypermethrin at all ratios. Of the different ratios studied, the 1:1 combination was the most effective against the larvae. On increasing the amount of the extract, the synergistic factor decreased signifying the synergistic activity of the combination decreases with increasing concentrations of plant extract. The synergistic activity may be due to the plant extract inhibiting some factors, such as detoxifying enzymes in mosquito larvae, which can act against synthetic chemicals, as reported in *Aedes aegypti* (Thangam and Kathiresan, 1991). These observations concerning synergism are supported by the findings of Thangam and Kathiresan

(1991), who studied the synergistic properties of *Rhizophora apiculata, Caulerpa scalpelliformis* and *Dictyota dichotoma* individually and with DDT. Likewise, the larvicidal activity of some plant extracts in combination with phenthoate and fenthion against *Anopheles stephensi* were studied by Kalayanadundaram and Das (1985). Significant synergism was observed between fenthion and *Vinca rosea*, *Leucus aspara, Pedalium murax, Clerodendron inerme, Turnera ulmifolia, Parthenium hysterophorus* extract. The synergistic factors were 1.40, 1.31, 1.61, 1.48, 1.38, 2.23, respectively. The petroleum ether root extract of *S. xanthocarpum* is a potential mosquito larvicide and its efficacy may be further increased with the use of cypermethrin due to synergism. This approach has dual benefits in minimizing the amount of both the ingredients and make the application more effective, economical and comparatively less hazardous to the environment; an ideal ecofriendly option for management of malaria vectors.

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