COMBINATION LARVICIDAL ACTION OF *SOLANUM XANTHOCARPUM* EXTRACT AND CERTAIN SYNTHETIC INSECTICIDES AGAINST FILARIAL VECTOR, *CULEX QUINQUEFASCIATUS* (SAY)

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Abstract. The combination activities of temephos, fenthion and petroleum ether extract of *Solanum xanthocarpum* were observed for their larvicidal activities against *Culex quinquefasciatus*. The combination of temephos and *S. xanthocarpum* was studied at ratios of 1:1, 1:2, and 1:4. Similar ratios were also used for the combination of fenthion and *S. xanthocarpum*. The temephos/plant extract combination acted antagonistically. The combination of fenthion and plant extract acted synergistically against the target organisms at a ratio of 1:1, which showed the best results of: LC_{50} 0.0144 and 0.0056 ppm and LC_{90} 0.0958 and 0.0209 ppm at 24 and 48 hours, respectively. The present study will be helpful in developing a commercial formulation for effective vector management.

Key words: fenthion, larvicide, *Solanum xanthocarpum*, synergism, temephos, *C. quinquefasciatus*

INTRODUCTION

Mosquitoes are responsible for the transmission of more diseases than any other group of arthropods and play an important role as etiologic agents of malaria, filariasis, dengue, yellow fever, Japanese encephalitis and other viral diseases (James, 1992). They are not only the vector for the transmission of these diseases, but are also an important irritating agent to man by causing allergic responses that include local skin reactions as well as systemic reactions, such as angioedema and urticaria (Peng *et al*, 1999).

The management of larvae through the use of larvicides is an ideal method for controlling mosquitoes by reducing mosquito breeding (Gluber, 1989). Since “adulticides” may only reduce the adult population temporarily, most mosquito control programs target the larval stage in their breeding sites with larvicides (El Hag *et al*, 1999, 2001). It is easier to control delicate mosquito larvae that have not yet left their aquatic habitat than to control adult mosquitoes. This method reduces the overall application of pesticides needed to control the mosquito population (Dharmagadda *et al*, 2005).

Synthetic pesticides are more effective

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and faster acting than botanicals or biopesticides, but indiscriminate application of chemical pesticides often leads to pest resistance to these insecticides, resulting in rebound of the vector population and its disease potential. Synergistic activity between synthetic pesticides and botanicals is a powerful and effective tool for the development of an efficient, more eco-friendly and less hazardous insect pest control strategy (Bernard and Philogne, 1993). The application of synergists has been preferred as a strategy to enhance the eco-friendliness and cost effectiveness of an insecticide by reducing the quantity needed to kill the target population and lengthen the residual activity. The role of synergists in resistance management is an accepted alternative for resistance management. The importance of proper selection of plant extracts as synergists in mixed formulations with different synthetic insecticides is being increasingly recognized in mosquito management. The mixture may provide less toxicity, prevent the development of resistance, have economic benefits and could be more effective than individual components of the mixture. Synthetic phytopesticide combinations are rarely evaluated as mosquito larvicides. In the present investigation, the joint action of *Solanum xanthocarpum* and the synthetic pesticides, fenthion and temephos were evaluated against the larvae of the filarial vector, *Culex quinquefasciatus*.

MATERIALS AND METHODS

The roots of *S. xanthocarpum* were collected from areas adjacent to Dayalbagh Educational Institute, Agra, washed and dried in the shade. Dried roots were chopped in small pieces of about 1 cm size using a falcon-stem cutter and subjected to extraction with petroleum ether in a Soxhlet apparatus for 72 hours (Saxena *et al*, 1994). After removing the respective solvent from the plant extract in a vacuum rotary evaporator, 6.38 grams of viscous paste were obtained per kilogram of dry plant material. Ten grams of the residues obtained was dissolved in 100 ml of ethanol to get stock solution of 100,000 ppm. Six test concentrations were prepared by further diluting the stock in ethanol at dilutions ranging from 7,500 to 20,000 ppm.

Fenthion and temephos (50% EC) were obtained from the district malaria office at Agra and diluted in dechlorinated tap water to obtain a concentration of 20 ppm stock each for both fenthion and temephos. Different test concentrations ranging from 0.0015 to 0.05 ppm were prepared by diluting these stock solutions.

Keeping the fenthion and temephos as standard solutions, the stock was mixed with the stock of the phytoextract in ratios of 1:1, 1:2 and 1:4. Test concentrations for each mixed formulation ratio were prepared by further diluting the combination mixture in water.

For bioassays of each combination, 1.0 ml of each of these test combinations was added to 249.0 ml of dechlorinated tap water, in 500 ml beakers, to obtain combinations of 0.001 to 0.150 ppm. Twenty, 3rd instar *Cx. quinquefasciatus* larvae obtained from lab culture were exposed to these working test combinations. Experiments were conducted in triplicate; controls for each series used 1.0 ml of ethanol and 249.0 ml of dechlorinated tap water were also conducted in parallel for each series according to standard WHO (1975) procedures at 27±1ºC and 85±5% relative humidity. Mortality observations were carried out at 24 and 48 hours post-treatment. To calculate the LC_{50} and LC_{90}, we used Probit Analysis (Finney, 1971).

The co-toxicity coefficient (Sarup *et al*, 1980) and synergistic factor (Kalayanasundaram and Das, 1985) for mixed formulation were 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 SF value > 1 indicates synergism and an SF value < 1 indicates antagonism.

RESULTS

In our study, fenthion gave an LC_{50} of 0.0189 ppm at 24 hours and 0.0095 ppm at 48 hours after exposure. It gave an LC_{90} value of 0.1504 at 24 hours and 0.0932 ppm at 48 hours after exposure. These data were used to determine the synergistic factor (Table 1).

The larvicidal activity of *S. xanthocarpum* and fenthion is shown in Table 1. At a ratio of 1:1 the LC_{50} was 0.0144 ppm and the LC_{90} was 0.0958 ppm at 24 hours and the LC_{50} was 0.0056 ppm and the LC_{90} was 0.0209 ppm at 48 hours after treatment. The ratio of 1:1 gave co-toxicity coefficients of 131.2500 and 156.9937 and synergistic factors of 1.3125 and 1.5699 for LC_{50} and LC_{90}, respectively, at 24 hours; which shows synergistic activity of the combination. The co-toxicity coefficients were 169.6429 and 445.9330 and the synergistic factors were 1.6964 and 4.4593 for LC_{50} and LC_{90}, respectively, at 48 hours after exposure; which shows synergistic activity.

At a ratio of 1:2 the LC_{50} results were 0.0177 and 0.0073 ppm and the LC_{90} results were 0.0949 and 0.0603 ppm at 24 and 48 hours after exposure, respectively. The ratio of 1:2 gave co-toxicity coefficients of 106.7797 and 158.4826 and synergistic factors of 1.0678 and 1.5848 for LC_{50} and LC_{90}, respectively, at 24 hours after, which shows synergism. The co-toxicity coefficients were 130.1370 and 154.5605 and the synergistic factors were 1.3014 and 1.5456 for LC_{50} and LC_{90}, respectively, at 48 hours, indicating synergistic activity of the combination.

At a ratio of 1:4 the LC_{50} was 0.0190 ppm and the LC_{90} was 0.1143 ppm at 24 hours after exposure and the LC_{50} was 0.0074 ppm and the LC_{90} was 0.0542 ppm at 48 hours after exposure. At a ratio of 1:4, the co-toxicity coefficients were 99.4737 and 131.5836 and the synergistic factors were 0.9947 and 1.3158 for LC_{50} and LC_{90}, respectively, at 24 hours. The co-toxicity coefficients and synergistic factors for LC_{50} showed antagonistic activity and the LC_{90} showed synergistic activity at 24 hours. The co-toxicity coefficients were 128.3784 and 171.9557 and the synergistic factors were 1.2838 and 1.7196 for LC_{50} and LC_{90}, respectively, at 48 hours; both showed synergistic activity. All the values remained well within 95% confidence limits.

The results of evaluating the larvicidal efficacy of *S. xanthocarpum* with temephos jointly against *Cx. quinquefasciatus* are seen in Table 2. The table shows that at a ratio of 1:1, the LC_{50} value was 0.0064 ppm and the LC_{90} value was 0.0331 ppm at 24 hours after exposure and the LC_{50} was 0.0053 ppm and the LC_{90} was 0.0302 ppm at 48 hours after exposure. *S. xanthocarpum* and temephos at a ratio of 1:1 possesses co-toxicity coefficients for an LC_{50} and LC_{90} of 64.0625 and 49.5468, respectively, at 24 hours, and synergistic factors for an LC_{50}

Table 1
Joint action of binary mixtures of fenthion and petroleum ether root extract of *Solanum xanthocarpum* against *Culex quinquefasciatus* larvae.

Treatment	Ratio	Exposure period (Hours)	Regression equation	Chi-square	LC_{50} ± SE (Fiducial limits) (ppm)	SF	CTC	Type of action	LC_{90} ± SE (Fiducial limits) (ppm)	SF	CTC	Type of action
Fenthion	-	24	Y = 6.03+1.42X	0.97	0.0189±0.0038 (0.0363-0.0115)	-	-	-	0.1504±0.0706 (0.2889-0.0120)	-	-	-
		48	Y = 6.32+1.29X	5.94	0.0095±0.0024 (0.0143-0.0048)	-	-	-	0.0932±0.0421 (0.1756-0.0107)	-	-	-
Fenthion+*S. xanthocarpum*												
	1:1	24	Y = 6.31+1.56X	2.51	0.0144±0.0027 (0.0196-0.0091)	1.3125	131.2500	Synergistic	0.0958±0.0377 (0.1697-0.0218)	1.5699	156.9937	Synergistic
		48	Y = 7.81+2.25X	3.19	0.0056±0.0009 (0.0074-0.0038)	1.6964	169.6429	Synergistic	0.0209±0.0043 (0.0294-0.0124)	4.4593	445.9330	Synergistic
	1:2	24	Y = 6.32+1.76X	2.84	0.0177±0.0030 (0.0237-0.0118)	1.0678	106.7797	Synergistic	0.0949±0.0337 (0.1609-0.0290)	1.5848	158.4826	Synergistic
		48	Y = 6.59+1.39X	4.77	0.0073±0.0016 (0.0105-0.0040)	1.3014	130.1370	Synergistic	0.0603±0.0231 (0.1055-0.151)	1.5456	154.5605	Synergistic
	1:4	24	Y = 6.19+1.64X	15.66	0.0190±0.0035 (0.0258-0.0122)	0.9947	99.4737	Antagonistic	0.1143±0.0463 (0.2051-0.0235)	1.3158	131.5836	Synergistic
		48	Y = 6.67+1.48X	7.98	0.0074±0.0016 (0.0105-0.0043)	1.2838	128.3784	Synergistic	0.0542±0.0189 (0.0913-0.0171)	1.7196	171.9557	Synergistic

CTC, Co-toxicity coefficient; SF, Synergistic factor

Table 2
Joint action of binary mixtures of temephos and petroleum ether root extract of *Solanum xanthocarpum* against *Culex quinquefasciatus* larvae.

Treatment	Ratio	Exposure period (Hours)	Regression equation	Chi-square	LC_{50} ± SE (Fiducial limits) (ppm)	SF	CTC	Type of action	LC_{90} ± SE (Fiducial limits) (ppm)	SF	CTC	Type of action
Temephos[a]	-	24	Y = 7.96+2.13X	0.7293	0.0041±0.0008 (0.0057-0.0026)	-	-	-	0.0164±0.0033 (0.0229-0.0099)	-	-	-
		48	Y = 8.25+2.11X	0.5442	0.0029±0.0007 (0.0042-0.0015)	-	-	-	0.0116±0.0022 (0.0160-0.0072)	-	-	-
Temephos+*S. xanthocarpum*												
	1:1	24	Y = 7.14+1.80X	0.8264	0.0064±0.0025 (0.0114-0.0014)	0.6406	64.0625	Antagonistic	0.0331±0.0076 (0.0479-0.0183)	0.4955	49.5468	Antagonistic
		48	Y = 7.17+1.70X	0.9101	0.0053±0.0025 (0.0102-0.0004)	0.50	50.00	Antagonistic	0.0302±0.0072 (0.0444-0.0161)	0.3841	38.4106	Antagonistic
	1:2	24	Y = 7.09+2.99X	10.2635	0.0199±0.0027 (0.0254-0.0146)	0.2060	20.6030	Antagonistic	0.0563±0.0085 (0.0702-0.0369)	0.2913	29.1297	Antagonistic
		48	Y = 7.25+2.58X	2.7660	0.0134±0.0024 (0.0182-0.0087)	0.2164	21.6418	Antagonistic	0.0422±0.0075 (0.0569-0.0276)	0.2749	27.4882	Antagonistic
	1:4	24	Y = 6.37+2.12X	2.0344	0.0224±0.0039 (0.0301-0.0148)	0.1830	18.3036	Antagonistic	0.0905±0.0189 (0.1278-0.0533)	0.1812	18.1216	Antagonistic
		48	Y = 6.48+1.92X	2.0174	0.0169±0.0036 (0.0239-0.0098)	0.1716	17.1598	Antagonistic	0.0785±0.0176 (0.1129-0.0440)	0.1478	14.7771	Antagonistic

CTC, Co-toxicity coefficient; SF, Synergistic factor
[a]The data reported by Mohan *et al* (2008).

and LC_{90} of 0.6406 and 0.4955 at 24 hours after exposure. The ratio of 1:1 gave co-toxicity coefficients for an LC_{50} and LC_{90} of 50.00 and 38.4106, respectively, at 48 hours, and synergistic factors for for LC_{50} and LC_{90} of 0.50 and 0.3841, respectively, at 48 hours after exposure. The values for the co-toxicity coefficients and synergistic factors after both 24 and 48 hours show antagonistic activity of the combination.

The ratio 1:2 gave an LC_{50} value of 0.0199 ppm and an LC_{90} value of 0.0563 ppm 24 hours after treatment and an LC_{50} of 0.0134 ppm and an LC_{90} of 0.0422 ppm 48 hours after treatment. The ratio of 1:2 gave co-toxicity coefficients of 20.6030 and 29.1297 for LC_{50} and LC_{90}, respectively, and synergistic factors of 0.2060 and 0.2913 for LC_{50} and LC_{90}, respectively, after 24 hours. At 48 hours at a ratio of 1:2, the co-toxicity coefficients were 21.6418 and 27.4882 for LC_{50} and LC_{90}, respectively, and the synergistic factors were 0.2164 and 0.2749 for LC_{50} and LC_{90}; the results show antagonism at both 24 and 48 hours after exposure.

The ratio of 1:4 gave an LC_{50} of 0.0224 ppm and an LC_{90} of 0.0905 at 24 hours and an LC_{50} of 0.0169 ppm and an LC_{90} of 0.0785 ppm at 48 hours after exposure. The ratio of 1:4 gave co-toxicity coefficients of 18.3036 and 18.1216 and synergistic factors of 0.1830 and 0.1812 for LC_{50} and LC_{90}, respectively, at 24 hours and co-toxicity coefficients of 17.1598 and 14.7771 and synergistic factors of 0.1716 and 0.1478 for LC_{50} and LC_{90}, respectively, at 48 hours. The values of the co-toxicity coefficients and synergistic factors indicate antagonism at both exposure periods.

DISCUSSION

The strategy of combining different vector control agents has proven to be advantageous in various pest management programs (Caraballo, 2000; Seyoum *et al*, 2002). Synergistic formulations may be more bioactive than individual pesticides against different pests. A lot more work has been done on the synergistic activity of synthetic-synthetic pesticides than plant-plant and plant-synthetic pesticide combinations against various insect pests.

The individual bioefficacy of petroleum ether root extract of *S. xanthocarpum* and temephos was studied and noted their LC_{50} values 41.28 and 38.48 ppm; 0.0041 and 0.0029 ppm and LC_{90} 111.16 and 80.83 ppm; 0.0164 and 0.0116 ppm after 24 and 48 hours of exposure, respectively (Mohan *et al*, 2006, 2008).

The combined effect of temephos and *S. xanthocarpum* possessed antagonistic activity in all observed ratios (1:1, 1:2 and 1:4). The synergistic factors reduced with increasing ratios at 24 hours: 64.0625 at 1:1, 20.6030 at 1:2 and 18.3036 at 1:4 for LC_{50}, and 49.5468 at 1:1, 29.1297 at 1:2 and 18.1216 at 1:4 for LC_{90}. The plant extract enhanced the larvicidal activity of fenthion, when it was mixed at all combinations. Synergism was also seen with temephos and extract: 131.2500 at 1:1, 106.7797 at 1:2 and 99.4737 at 1:4 at LC_{50}, and 156.9937 at 1:1, 158.4826 at 1:2 and 131.5836 at 1:4 at LC_{90}, 48 hours after treatment. The ratio of 1:4 exhibited antagonistic activity at 24 hours with LC_{50} values and acted synergistically at 48 hours. In the case of LC_{90}, synergistic activity was observed at both 24 and 48 hours. Increasing the plant extract decreased the efficacy of the combination. Similar observations were noted by George and Vincent (2005). It was also seen that synergistic activity was directly proportional to exposure period. This finding is supported by the observations of George and Vincent (2005) and Mohan *et al* (2006, 2007).

Combinations of synthetic pesticides are generally more effective and more economical with higher bioefficacy against target organisms but may be more environmentally. Nonsynthetic-synthetic combinations are probably preferable in insect pest management.

Thangam and Kathiresan (1991) reported this synergistic activity may be due to plant extract inhibiting some factors, such as detoxifying enzymes, in mosquito larvae, which can act against synthetic pesticides as observed in the larvae of *Aedes aegypti*. Our findings concerning synergism are supported by those of Thangam and Kathiresan (1991), who evaluated the synergistic properties of *Rhizophora apiculata, Caulerpa scalpelliformis* and *Dictyota dichotoma* alone and combined with DDT. Similarly, Kalayanadundaram and Das (1985) reported the larvicidal efficacy of some plant extracts in combination with phenthoate and fenthion against *Anopheles stephensi* and synergism was observed significantly during their studies on fenthion and *Vinca rosea, Leucus aspara, Pedalium murax, Clerodendron inerme, Turnera ulmifolia,* and *Parthenium hysterophorus* extract, with synergistic factors of 1.40, 1.31, 1.61, 1.48, 1.38, and 2.23, respectively.

Corbel *et al* (2003) observed synergistic activity between permethrin and propoxur giving a LC_{50} of 0.26 mg/liter and a synergistic factor of 1.54 against *Cx. quinquefasciatus* larvae. Similarly, Gaaboub *et al* (1981) studied the joint actions of malathion with benthiocard, drepamon, oxadiazon, propanil and trifluralin which gave LC_{50} values of 0.064, 0.048, 0.041, 0.040 and 0.050 ppm, respectively, and synergistic values of 1.0625, 1.42, 1.65, 1.70 and 2.4, respectively, demonstrating synergistic activity against *Culex pipiens*. The synergism between DEET (N, N-diethyltoluamide) and propoxur against *Ae. aegypti* was reported by Pennetier *et al* (2005). The synergism between synthetic pesticides is due to overwhelming the detoxification defence mechanisms of mosquitoes (Hemingway and Ranson, 2000) and reinforcing their joint effect though different biochemical mode(s) of action but could reflect variable tolerance (polymorphism) of mosquitoes (Corbel *et al*, 2003).

The toxicity of the binary mixtures of *Annona squamosa* and *Pongamia glabra* at ratios of 3:1, 1:1 and 1:3 against *Cx. quinquefasciatus* was evaluated by George and Vincent (2005), the LC_{50} values were 0.828, 0.288, and 1.120 ppm and the synergistic factors were 5.3, 15.1 and 3.9, respectively, demonstrating synergistic action at all tested ratios. The ratio of 1:1 appeared to be more effective than the ratios of 3:1 and 1:3 (George and Vincent, 2005). Mohan *et al* (2006) evaluated the joint action of cypermethrin and *S. xanthocarpum* petroleum ether root extract in ratios of 1:1, 1:2 and 1:4, against *Cx. quinquefasciatus* larvae giving synergistic factors of 1.09, 0.98 and 0.67, respectively; the ratio of 1:1 revealed synergism and the other ratios showed antagonism. With *An. stephensi*, all the ratios of 1:1, 1:2 and 1:4 act synergistically giving synergistic factors of 6.83, 6.47 and 4.99; the ratio of 1:1 acted more synergistically than the other ratios (Mohan *et at*, 2007). The combination of pesticide and synergist in an equal ratio worked synergistically to improve the efficacy of the pesticide more effectively than the other ratios. The present findings are similar to the findings of George and Vincent (2005) and Mohan *et al* (2006, 2007). It can be concluded that the combinations reported here at a ratio of 1:1 are an effective culicine mosquito larvicide.

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