



EFFECT OF PRE-TREATMENT OF SYNERGISTS ON THE TOXICITY OF INSECTICIDES TO *SPODOPTERA LITURA* (FABRICIUS)*

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ABSTRACT

The pre treatment of different synergists before the application of insecticide proved synergistic over the period of time (up to 24hr). The toxicity of cypermethrin, when pre- treated with different synergist showed wide variation in mortality of *Spodoptera litura* larvae. The highest mortality of 83.33% was observed when piperonyl butoxide (PBO) was applied 8hr before the application of cypermethrin. PBO, dillapiole and dihydrodillapiole increased more than 50% fenvalerate induced mortality from 2-14, 6-14 and 4-8 hr, respectively at post-treatment. The highest mortality of 80% by lambda cyhalothrin was observed at 6 and 8 hr post-treatment of PBO and dihydrodillapiole. The toxicity of profenophos increased with pre-treatment of each of the synergists. The highest mortality of 70% and 76.67% was observed at 8 and 10 hr post-treatment of dillapiole and PBO respectively. The mortality obtained between 6 to 12 hr pretreatment of PBO, and dillapiole varied from 53.33 to 70% and 63.33 to 46.67%. There was a highly significant increase in the mortality due to cypermethrin, fenvalerate, lambda-cyhalothrin and profenophos when pre-treatment at 6 to 10 hrs with PBO was done. A delay of 8-10 hr prior to insecticide application was beneficial in case of dihydrodillapiole whereas a delay of 6-14 hr following pre-treatment of dillapiole increased the synergistic effects with various insecticides.

Key words: Insecticide resistance, *Spodoptera litura*, synergists, relative susceptibility

Spodoptera litura is a polyphagous pest of national importance. In recent years, its outbreaks have been more common due to development of insecticide resistance (Kranthi *et al.*, 2002; Kodandram and Dhingra, 2006) and subsequent failure in control measures. Most of the insecticides, especially pyrethroids and carbamates failed to provide adequate control. The development of multiple resistance remains far more challenging to the entomologists, which paved the way for search of new molecules or combination of chemicals for effective control. Insecticide synergism as possible resistance management proposition has been discussed globally because the synergists act primarily by inhibiting a specific pathway of metabolism (Casida, 1970; Georgiou and Mellon, 1983). Studies by Farnham (1986) using the house fly, *Musca domestica* suggested that a pre-treatment with PBO 2hr before insecticidal application was beneficial.

Huang and Han (2007) also observed increase in the toxicity against *S. litura* by pre-treatment with PBO and triphenyl phosphate (TPP) one hr before the application of deltamethrin. Keeping this in view, the present experiments was conducted to study the effect

of pre treatment of larvae with synergist on the toxicity of insecticides.

MATERIALS AND METHODS

The egg masses of *S. litura* were collected from the cauliflower fields of IARI, New Delhi during 2006-07 and reared on tender castor leaves under controlled conditions at $27\pm 1^{\circ}\text{C}$ and $60\pm 5\%\text{RH}$. The insecticides used were technical grade material of cypermethrin and fenvalerate 92.5% (Rallis India limited, Mumbai), lambda cyhalothrin 93.2% and profenophos 89.0% (Syngenta India Limited, Mumbai). Acetone was used for the preparation of stock solution. The technical product containing 90.0 per cent of the active ingredient synergists *viz.*, piperonyl butoxide (PBO), dillapiole and dihydrodillapiole were obtained from Division of Agricultural Chemicals, IARI, New Delhi. For the preparation of vanillin oxime ether, the procedure of Chowdhury *et al.*, (1998) was followed.

S. litura larvae were treated with 1 μl of particular synergist (30mM) in acetone, by micropipette on the dorsal thorax. Then, larvae were maintained at constant temperature of $27\pm 1^{\circ}\text{C}$ with adequate food for varying

periods of time up to 24hr prior to insecticide application. Larvae were then treated with 1µl of pre-determined dose of (LC₂₅) insecticide by micropipette on the dorsal thorax. Each treatment including control was replicated three times. Control was maintained separately applying 1µl of acetone. The post treatment operations involved transferring of glass jars containing treated *S. litura* to 27±1°C and 60 ± 5% RH conditions. Treated larvae were observed for mortality 24 hr after treatment. The larvae showing no motion on prodding with a needle and moribund larvae showing only slight movement were considered dead.

RESULTS AND DISCUSSION

The toxicity of cypermethrin, when pre-treated with different synergists showed that there is a wide variation in mortality after pre-treatment with synergists at varying periods of time (up to 24 hr) prior to insecticide application (Table 1). Mortality did not occur immediately after the application of cypermethrin. However, it increased from two-hour post-treatment of PBO, dillapiole, dihydrodillapiole and vanillin oxime ether. More than 50% mortality was observed from 4-10, 8-18, 10-16 and 10-12 hrs in case of pre-treatment with PBO, dillapiole, dihydrodillapiole and vanillin oxime ether, respectively. The highest mean per cent mortality of 83.33 was observed when PBO was applied 8hr before the application of cypermethrin. Similarly dillapiole, dihydrodillapiole and vanillin oxime ether resulted in highest mean per cent mortality of 60.0, 53.33; 63.33 at 14, 10 and 12 hr post treatment of cypermethrin. The toxicity of cypermethrin prolonged up to 20hr post treatment of PBO.

PBO, dillapiole and dihydrodillapiole increased more than 50 per cent fenvalerate (Table 2) induced mortality from 2-14, 6-14 and 4-8 hr, respectively at post-treatment. On the other hand, pretreatment of vanillin oxime ether at varying periods of time did not significantly increase the toxicity except at 6hr post-treatment (63.33% mortality). There was a gradual increase in the mortality caused by lambda cyhalothrin following pre-treatment with different synergists from 0 to 8hr post treatment, thereafter the mortality decreased (Table 3). The highest mortality of 80% was observed at 6 and 8 hr post-treatment of PBO and dihydrodillapiole, and 53.33% at 6hr post treatment of dillapiole and vanillin oxime ether. The mortality obtained after 14hr post-treatment of dillapiole, dihydrodillapiole and vanillin oxime ether was significantly low, but persisted up to 24 hrs post-treatment in case of PBO.

The toxicity of profenophos increased with pre-treatment of the synergists (Table 4). The mean per cent mortality obtained at 0 to 4hr and 18 to 24 hr was between 0 to 20. The highest mortality of 70% and 76.67% was observed at 8 and 10 hr post-treatment of dillapiole and PBO, respectively. The mortality obtained between 6 to 12 hr pretreatment of PBO and dillapiole varied from 53.33 to 70% and 63.33 to 46.67%. But there was only 50 and 56.67% mean mortality observed at 8 and 10hr prior to the application of vanillin oxime ether.

The present findings support the belief that the toxicity of an insecticide varied over the period of time following pretreatment of different synergists. There was a highly significant increase in the mortality due to cypermethrin, fenvalerate, lambda-cyhalothrin and profenophos when pre-treatment at 6 to 10 hrs with PBO was done. A delay of 8-10 hr prior to insecticide application was beneficial in case of dihydrodillapiole, whereas a delay of 6-14 hr following pre-treatment of dillapiole increased the synergistic effects.

These findings revealed that the success of various synergists could be enhanced merely by using an appropriate pre-treatment time. These results reflect the findings of Gunning *et al.* (1999) who found that treatment of PBO prior to pyrethroid application achieved higher levels of control of pyrethroid resistant *Helicoverpa armigera*. Young *et al.* (2006) also suggested 5 hr pre-treatment of PBO prior to pyrethroid application for the control of *H. armigera* and silver leaf whitefly, *Bemisia tabaci*.

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Table 1. Effect of pre treatment of synergists on the toxicity of cypermethrin against 7-day old larvae of *Spodoptera litura*

Hours	Cypermethrin				Mean
	PBO	DP	DDP	VOE	(Hours)
0	13.33 (17.72) ^c	0.00 (0.03) ^e	0.00 (0.03) ^c	0.00 (0.03) ^e	3.33 (4.45) ^b
2	43.33 (41.07) ^c	20.00 (26.57) ^c	43.33 (41.15) ^{abc}	0.00 (0.03) ^e	26.67 (27.20) ^e
4	56.67 (48.93) ^b	13.33 (17.72) ^d	33.33 (34.93) ^c	16.67 (23.86) ^d	30.00 (31.36) ^d
6	76.67 (61.22) ^a	43.33 (41.07) ^b	16.67 (19.93) ^d	23.33 (28.78) ^{cd}	40.00 (37.75) ^{ee}
8	83.33 (66.14) ^a	50.00 (45.08) ^{ab}	40.00 (39.15) ^{bc}	43.33 (41.15) ^b	54.17 (47.88) ^{ba}
10	73.33 (59.00) ^a	53.33 (46.92) ^{ab}	53.33 (46.92) ^a	56.67 (48.93) ^a	59.17 (50.44) ^a
12	40.00 (39.23) ^c	50.00 (45.00) ^{ab}	46.67 (43.08) ^{ab}	63.33 (53.07) ^a	50.00 (45.09) ^{bac}
14	46.67 (43.08) ^{bc}	60.00 (50.85) ^a	50.00 (45.00) ^{ab}	30.00 (33.00) ^c	46.67 (42.98) ^{bdc}
16	43.33 (41.07) ^c	56.67 (48.85) ^a	50.00 (45.08) ^{ab}	20.00 (26.57) ^{cd}	42.50 (40.39) ^{dc}
18	23.33 (28.78) ^d	50.00 (45.00) ^{ab}	33.33 (34.93) ^c	3.33 (6.16) ^e	27.50 (28.72) ^e
20	46.67 (43.08) ^{bc}	3.33 (6.16) ^e	13.33 (17.72) ^d	0.00 (0.03) ^e	15.83 (16.75) ^f
22	23.33 (28.78) ^d	20.00 (26.07) ^c	0.00 (0.03) ^e	0.00 (0.03) ^e	10.83 (13.73) ^{fg}
24	16.67 (23.86) ^{de}	13.33 (17.72) ^d	0.00 (0.03) ^e	0.00 (0.03) ^e	7.50g (10.41)
Control	0.00 (0.03) ^f	0.00 (0.03) ^e	0.00 (0.03) ^e	0.00 (0.03) ^e	
Mean (Treat)	45.13 (41.69) ^a	33.33 (32.08) ^b	29.23 (28.30) ^c	19.74 (20.13) ^d	
CD at 5% (Treat)	3.28				
CD at 5% (Hours)	5.91				
CD at 5% (Treat x Hours)	11.82				

Values in parentheses are arc sine transformed values; CD values are for the transformed values; Mean with at least one letter common are at par.

Table 2. Effect of pre-treatment of synergists on the toxicity of fenvalerate against 7-day old larvae of *Spodoptera litura*

Hours	Fenvalerate				Mean (Hours)
	PBO	DP	DDP	VOE	
0	23.33 (28.29) ^h	16.67 (23.86) ^e	0.00 (0.03) ^h	6.67 (8.87) ^f	11.67 (15.26) ⁱ
2	56.67 (48.85) ^{de}	36.67 (37.14) ^d	26.67 (31.00) ^{ef}	26.67 (31.00) ^{de}	36.67 (36.99) ^{ef}
4	46.67 (43.08) ^{ef}	40.00 (39.15) ^{cd}	53.33 (46.92) ^c	30.00 (33.00) ^{cde}	42.50 (40.54) ^{def}
6	76.67 (69.18) ^a	60.00 (50.85) ^{ab}	76.67 (61.22) ^b	63.33 (52.78) ^a	69.17 (58.51) ^a
8	73.33 (59.00) ^{bc}	50.00 (45.00) ^{bc}	86.67 (75.61) ^a	36.67 (37.22) ^{bcd}	61.67 (54.21) ^{ba}
10	73.33 (59.71) ^b	63.33 (53.15) ^a	43.33 (41.07) ^{cd}	43.33 (41.07) ^b	55.83 (48.75) ^{bc}
12	63.33 (52.86) ^{cd}	36.67 (37.14) ^d	33.33 (35.01) ^{de}	40.00 (38.86) ^{bcd}	43.33 (40.97) ^{de}
14	56.67 (48.85) ^{de}	60.00 (50.85) ^{ab}	46.67 (42.99) ^c	40.00 (39.15) ^{bc}	50.83 (45.46) ^{dc}
16	36.67 (37.14) ^{fg}	33.33 (35.22) ^d	26.67 (31.00) ^{ef}	26.67 (30.00) ^e	30.83 (33.34) ^{ef}
18	46.67 (43.08) ^{ef}	33.33 (34.93) ^d	23.33 (28.29) ^{efg}	3.33 (6.16) ^{fg}	26.67 (28.11) ^{hg}
20	26.67 (31.00) ^{gh}	20.00 (26.57) ^c	16.67 (23.86) ^{fg}	6.67 (8.87) ^f	17.50 (22.57) ^h
22	20.00 (26.07) ^h	6.67 (8.87) ^f	10.00 (11.09) ^h	0.00 (0.03) ^g	9.17 (11.51) ⁱ
24	23.33 (28.78) ^h	0.00 (0.03) ^g	16.67 (23.36) ^g	0.00 (0.03) ^g	10.00 (13.05) ⁱ
Control	0.00 (0.03) ⁱ	0.00 (0.03) ^g	0.00 (0.03) ⁱ	0.00 (0.03) ^g	
Mean (Treat)	47.95 (44.30) ^a	35.13 (34.06) ^b	35.38 (34.73) ^b	24.87 (25.16) ^c	
CD at 5% (Treat)	4.02				
CD at 5% (Hours)	7.25				
CD at 5% (Treat x Hours)	14.51				

Values in parentheses are arc sine transformed values; CD values are for the transformed values; Mean with at least one letter common are at par.

Table 3. Effect of pre-treatment of synergists on the toxicity of lambda cyhalothrin against 7-day old larvae of *Spodoptera litura*

Hours	Lambda cyhalothrin				Mean (Hours)
	PBO	DP	DDP	VOE	
0	16.67 (23.86) ^{def}	6.67 (8.87) ^d	13.33 (17.72) ^d	10.00 (11.09) ^d	11.67 (15.38) ^f
2	16.67 (19.93) ^{ef}	20.00 (21.94) ^c	46.67 (43.08) ^{bc}	33.33 (35.22) ^b	29.17 (30.04) ^{cd}
4	43.33 (41.07) ^c	23.33 (28.78) ^c	53.33 (46.92) ^b	43.33 (41.07) ^{ab}	40.83 (39.46) ^b
6	80.00 (63.43) ^a	53.33 (46.92) ^a	73.33 (59.00) ^a	53.33 (47.01) ^a	65.00 (54.09) ^a
8	63.33 (53.07) ^b	46.67 (42.99) ^{ab}	80.00 (63.43) ^a	33.33 (35.22) ^b	55.83 (48.68) ^a
10	50.00 (45.00) ^c	53.33 (46.92) ^a	50.00 (45.00) ^{bc}	20.00 (26.07) ^c	43.33 (40.75) ^b
12	40.00 (39.15) ^c	36.67 (36.93) ^b	40.00 (38.86) ^c	13.33 (17.22) ^d	32.50 (33.04) ^{cb}
14	26.67 (30.79) ^d	23.33 (28.78) ^c	6.67 (8.87) ^e	20.00 (26.57) ^c	19.17 (23.75) ^{ed}
16	16.67 (19.93) ^{ef}	6.67 (8.87) ^d	10.00 (15.01) ^{de}	6.67 (12.30) ^d	10.00 (14.03) ^{df}
18	16.67 (23.86) ^{def}	16.67 (23.86) ^c	6.67 (8.87) ^e	10.00 (15.01) ^d	12.50 (17.90) ^{ef}
20	16.67 (23.86) ^{def}	6.67 (8.87) ^d	6.67 (8.87) ^e	0.00 (0.03) ^e	7.50 (10.41) ^{df}
22	20.00 (26.57) ^{de}	0.00 (0.03) ^e	0.00 (0.03) ^f	0.00 (0.03) ^e	5.00 (6.66) ^e
24	20.00 (26.07) ^{de}	0.00 (0.03) ^e	0.00 (0.03) ^f	0.00 (0.03) ^e	5.00 (6.54) ^e
Control	0.00 (0.03) ^f	0.00 (0.03) ^e	0.00 0.03	0.00 0.03e	
Mean (Treat)	32.82 (33.58) ^a	22.56 (23.37) ^{bc}	18.72 (20.53) ^{bb}	18.72 (20.53) ^c	
CD at 5% (Treat)	4.34				
CD at 5% (Hours)	7.82				
CD at 5% (Treat x Hours)	15.66				

Values in parentheses are arc sine transformed values; CD values are for the transformed values; Mean with at least one letter common are at par.

Table 4. Effect of pre-treatment of synergists on the toxicity of profenophos against 7-day old larvae of *Spodoptera litura*

Hours	Profenophos				Mean (Hours)
	PBO	DP	DDP	VOE	
0	13.33 (17.22) ^f	6.67 (8.87) ^{de}	6.67 (8.87) ^d	0.00 (0.03) ^f	6.67 (8.75) ^d
2	26.67 (30.79) ^{de}	10.00 (15.01) ^{de}	3.33 (6.16) ^d	0.00 (0.03) ^f	10.00 (13.00) ^{cd}
4	23.33 (28.78) ^{de}	26.67 (31.00) ^c	23.33 (28.78) ^{de}	26.67 (31.00) ^d	25.00 (29.89) ^b
6	53.33 (46.92) ^b	63.33 (52.78) ^a	33.33 (35.01) ^{ab}	40.00 (38.86) ^{bc}	47.50 (43.39) ^a
8	73.33 (59.21) ^a	70.00 (57.29) ^a	43.33 (40.78) ^a	50.00 (45.00) ^{ab}	59.17 (50.57) ^a
10	76.67 (61.22) ^a	60.00 (50.85) ^a	33.33 (34.93) ^{ab}	56.67 (48.93) ^a	56.67 (48.98) ^a
12	70.00 (65.25) ^a	46.67 (43.08) ^b	26.67 (30.29) ^b	46.67 (42.99) ^b	47.50 (45.40) ^a
14	33.33 (35.22) ^{cd}	26.67 (31.00) ^c	13.33 (17.72) ^c	40.00 (38.86) ^{bc}	28.33 (30.70) ^b
16	43.33 (41.07) ^{bc}	20.00 (26.07) ^c	23.33 (28.08) ^b	30.00 (33.21) ^{cd}	29.17 (32.11) ^b
18	20.00 (26.57) ^e	13.33 (17.72) ^d	13.33 (17.72) ^c	6.67 (8.87) ^e	13.33 (17.72) ^c
20	23.33 (28.78) ^{de}	6.67 (8.87) ^c	0.00 (0.03) ^e	3.33 (6.16) ^{ef}	8.33 (10.96) ^{cd}
22	20.00 (26.57) ^e	0.00 (0.03) ^f	0.00 (0.03) ^e	6.67 (8.87) ^e	6.67 (8.87) ^d
24	16.67 (23.86) ^e	0.00 (0.03) ^f	0.00 (0.03) ^e	0.00 (0.03) ^f	4.17 (5.98) ^d
Control	0.00 (0.03) ^f	0.00 (0.03) ^f	0.00 (0.03) ^e	0.00 (0.03) ^f	
Mean (Treat)	37.95 (37.80) ^a	26.92 (26.35) ^b	16.92 (19.11) ^c	23.59 (23.29) ^{ab}	
CD at 5% (Treat)	4.36				
CD at 5% (Hours)	7.87				
CD at 5% (Treat x Hours)	15.75				

Values in parentheses are arc sine transformed values; CD values are for the transformed values; Mean with at least one letter common are at par.

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