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SYNERGISM OF INSECTICIDAL ACTION BY SOME BENZO 1,3-DIOXOLE COMPOUNDS IN *SPODOPTERA LITURA* (FABRICIUS)

K. Shankarganesh, S. Walia*, S. Dhingra and J.K.S Bhandari

Division of Entomology,
Indian Agricultural Research Institute, New Delhi-110012

ABSTRACT

Toxicity of different insecticides with synergists was evaluated against 4 and 7 day old larvae of *Spodoptera litura* (Fabricius). Based on the LC_{50} obtained, the 4 day-old larvae was of 8.0, 2.1, 4.4 and 11.2 - times susceptible than that of 7 day- old larvae to cypermethrin, fenvalerate, lambdacyhalothrin and profenophos, respectively. The joint action ratios for different insecticides synergist mixture indicates that piperonyl butoxide and dihydrodillapiole increased the toxicity of all the pyrethroids to the 4 day- old larvae, whereas dillapiole synergised only cypermethrin. However, an additive action was obtained when vanillin oxime ether was used in mixed formulations with each of the insecticides *viz.,* cypermethrin, fenvalerate, and lambdacyhalothrin. Synergist bioassays with the organophosphorous insecticide; profenophos resulted in additive action only with each of the synergists. The combination of piperonyl butoxide with each of the four insecticides *viz.,* cypermethrin, fenvalerate, lambdacyhalothrin and profenophos, indicated synergistic effect against 7 day- old larvae. Also, plant based insecticide synergists i.e. dillapiole and the more stable reduced derivative dihydrodillapiole exhibited synergistic action when mixed with cypermethrin, lambdacyhalothrin and profenophos. Yet only an additive action was obtained when combined with fenvalerate. On the other hand, vanillin oxime ether synergised only cypermethrin and lambdacyhalothrin.

Key words: Insecticide, resistance, *Spodoptera litura*, synergist, relative susceptibility.

The tobacco caterpillar *Spodoptera litura* (F.) is a serious polyphagous pest infesting more than 120 host plants all over India. The main thrust of Indian farming community has so far been unilaterally on synthetic insecticides, especially on synthetic pyrethroids and organophosphorus compounds to combat menace caused by this pest. Pyrethroid insecticides were introduced in to India in 1980 for the control of this pest on cotton, which has become resistant to benzene hexa chloride (BHC) (Srivastava and Joshi, 1965), lindane and endosulfan (Ramakrishnan *et al.*, 1984), synthetic pyrethroids (Murugesan and Dhingra, 1995) and quinalphos and monocrotophos (Armes *et al.,* 1997 and Kumar and Regupathy, 2001). During the last 30 years, it has become a major pest on cotton, groundnut and mungbean (Armes *et al.,* 1997).

The increasingly widespread occurrence of resistance to insecticides is a serious threat to the control and management of many important insect pests. There has been mounting interest in the use of synergists to reduce some of these resistance incidents by combined application (Wilkins *et al.,* 1995). The use of piperonyl butoxide, sesame oil, triphenyl phosphate, diethyl maleate and other compounds to suppress resistance to pyrethroids and organophosphates has been well documented in India and abroad (Sridevi and Dhingra 1996; Young *et al.,* 2005 and 2006; Huang *et al.,* 2006). Experimentally a number of compounds containing methylene dioxyphenyl and a polyalkoxy side chain as synergophoric groups and oxime ethers have shown synergism with a range of insecticides against different insects (Walia *et al.,* 1985).

This article reports the synergism of three commonly used synthetic pyrethroids cypermethrin. fenvalerate, lambdacyhalothrin and one of the organophosphate, profenophos with the plant based insecticide synergist dillapiole, a natural constituent of *Anethum sowa,* dihydrodillapiole and synthetic compound vanillin oxime ether along with the standard synergist piperonyl butoxide in 4 and 7 day- old larvae of *S. litura* by leaf dip method.

MATERIALS AND METHODS

The egg masses of *S. litura* were collected from the cauliflower fields of I ARI, New Delhi during 2006-07 and rearing of larvae was done on tender castor leaves under controlled conditions at 27 ± 1 ° C and

* Division of Agricultural Chemicals, IARI, New Delhi-110012

60 ± 5 % RH. The insecticides used were technical grade material of cypermethrin and fenvalerate 92.5% (Rallis India Limited, Mumbai), lambdacyhalothrin 93.2% and profenophos 89.0% (Syngenta India Limited, Mumbai). Acetone was used for the preparation of stock solution.

Synergists

The technical product containing 90.0 per cent of the active ingredient synergists *viz*., piperonyl butoxide (PBO), dillapiole, dihydrodillapiole was obtained from Division of Agricultural Chemicals, IARI, New Delhi. For the preparation of vanillin oxime ether, the procedure of Chowdhury (1993) was followed.

Insecticide bioassay

The toxicities of four different insecticides *viz*., cypermethrin, fenvalerate, lambdacyhalothrin and profenofos, formulated either alone or in combination with different synergists were determined against the second and third instar larvae of *S. litura.* The stock solutions (20 %) were diluted in such a way as to maintain the level of solvent (acetone) and emulsifier (triton-xl00) at 5.0 and 0.5 per cent respectively in the final concentrations. The mixture of an insecticide with a particular synergist in each of the four ratios viz, 1:1, 1:2, 1:5 and 1:10 were prepared by mixing the two stock solutions of equal concentrations depending upon the ratio to be maintained. Further dilutions of the mixture of aforesaid stock solution of a particular proportion were done in the same way, as in the case of the formulation of an insecticide used alone, to obtain the final emulsions. Castor leaf discs of approximately 6 cm diameter were dipped in the required concentrations of insecticides or their mixed formulations with dihydrodillapiole for twenty seconds and then dried. The treated leaf discs were then transferred to clean jars (15x10 cm). In each jar 15 larvae were released. The jars were kept at 27 ± 1°C and the larval mortality was recorded 24h after the treatment. There were three replications for each treatment. The LC_{50} values were calculated by probit analysis (Finney, 1971). The data were subjected to regression analysis. The relationship between probit of mortality and concentration of insecticide and non toxic synergist was expressed by the regression equation (Finney, 1971).

$y = a + b_1 x + b_2 x_1$ where,

$$x_1 = \frac{z_1}{(c + z_1)}$$

In this equation, y is the probit of mortality; x is log of the concentration of toxicant; z_1 is the concentration of the non-toxic synergist; a, b_1, b_2 and c being the constants. Sarup *et al.* (1980) categorized the synergistic and antagonistic with respect to each combination of a particular insecticide and synergist based on Joint Action Ratio (JAR). According to them the JAR of the mixture with weighting coefficient was calculated as follows:

$$\text{Joint Action Ratio} = \text{anti log} = \frac{b_2}{b_1}$$

The usual yardstick value of joint action ratio is ≥ 1.05 indicating synergism, between 0.95 and 1.05 the additive action and those between 0 and 0.95 the antagonism.

RESULTS AND DISCUSSION

Assessment of the relative susceptibility of 4 and 7 day-old larvae of *S. litura* by taking the LC_{50} value of third instar larvae as unity revealed that 4 day- old larvae was 8.0, 2.1, 4.4 and 11.2 - times susceptible than that of 7 day- old larvae to cypermethrin, fenvalerate, lambdacyhalothrin and profenophos respectively (Table 1).

Table 1. Susceptibility of 4 and 7 day- old larvae of Delhi population of *S. litura* to different insecticides

Insecticides	LC_{50} %		Relative susceptibility
	4 day-old	7 day-old	
Cypermethrin	0.0014	0.0112	8.0
Fenvalerate	0.0150	0.0314	2.1
Lambdacy-halothrin	0.0025	0.0110	4.4
Profenophos	0.0059	0.0662	11.2

The increased tolerance of older larvae to insecticides may be due to age dependent changes in mixed function oxidase enzyme systems of advancing instars. Similar results were reported by Balasubramanian and Balasubramanian (1984).

According to them, the third instar larvae of *S. litura* were about 4-times less susceptible than the second instar larvae to permethrin and fenvalerate; about 2-times to deltamethrin and 1.4-times to cypermethrin and fenvalerate. On the contrary, Mayuravalli *et al.,* (1985) found that deltamethrin was 2-times more toxic to the first instar than fifth instar larva. Rao and Dhingra (1997) and Sudhakar and Dhingra (2002) also indicated that LC_{50} values increased gradually from the first to fourth instar of *S. litura.*

The joint action ratios of the four insecticides *viz.,* cypermethrin, fenvalerate, lambdacyhalothrin and profenophos formulated separately with each of the four synergists *viz..* piperonyl butoxide, dillapiole, dihydrodillapiole and vanillin oxime ether to 4 and 7 day-old larvae of *S.litura* are given in Tables (2-5).

Piperonyl butoxide and dihydrodillapiole increased the toxicity of all the pyrethroids to the 4 day- old larvae, whereas Dillapiole synergised only cypermethrin. However an additive action was obtained when vanillin oxime ether was used in mixed formulalions with each of the insecticides *viz.,* cypermethrin, fenvalrate, and lambdacyhalotrin. Synergist bioassays with the organophosphorous insecticide; profenophos resulted in additive action only with each of the synergists.

The combination of piperonyl butoxide with each of the four insecticides *viz.*, cypermethrin, fenvalrate, lambdacyhalothrin and profenophos, indicated synergistic effect against 7 day- old larvae. Also plant based insecticide synergists dillapiole and the more stable reduced derivative dihydrodillapiole exhibited synergistic action when mixed with cypermethrin,

Table 2. Effect of different synergists on the toxicity of cypermethrin and fenvalerate to 4 day- old larvae of *S.litura* by leaf dip method

Insecticide/ Synergist	Regression equation	R^2 (%)	Joint action ratio
a) Cypermethrin			
Piperonyl butoxide	$Y = -7.995 + 1.094X + 0.665 \frac{Z_1}{(-0.00000529 + Z_1)}$	82.9	61.84
Dillapiole	$Y = -9.110 + 1.188X + 0.182 \frac{Z_1}{(-0.00000529 + Z_1)}$	83.59	1.17
Dihydrodillapiole	$Y = -8.559 + 1.135X + 0.554 \frac{Z_1}{(-0.00000529 + Z_1)}$	76.86	1.63
Vanillin oxime ether	$Y = -7.694 + 1.084X + (-0.00865) \frac{Z_1}{(-0.00000470 + Z_1)}$	99.92	0.99
b) Fenvalrate			
Piperonyl butoxide	$Y = -5.603 + 0.819X + 0.403 \frac{Z_1}{(-0.00001071 + Z_1)}$	86.85	1.64
Dillapiole	$Y = -5.607 + 0.816X + 0.00255 \frac{Z_1}{(-0.00001041 + Z_1)}$	85.46	1.003
Dihydrodillapiole	$Y = -5.456 + 0.802X + 0.912 \frac{Z_1}{(-0.00001279 + Z_1)}$	88.61	1.12
Vanillin oxime ether	$Y = -4.917 + 0.758X + 0.00141 \frac{Z_1}{(-0.00002058 + Z_1)}$	86.97	1.00

X = log of the concentration of toxicant; Y = probit of mortality; Z_1 = concentration of the non-toxic synergist

Table 3. Effect of different synergists on the toxicity of lambdacyhalothrin and profenophos to 4 day- old larvae of *S.litura* by leaf dip method

Insecticide/ Synergist	Regression equation	R^2 (%)	Joint action ratio
Lambdacyhalothrin			
Piperonyl butoxide	$Y = -8.610 + 1.116 X + 0.1651 \dfrac{Z_1}{(-0.00000715 + Z_1)}$	79.60	1.16
Dillapiole	$Y = -7.917 + 1.051 X + 0.0039 \dfrac{Z_1}{(-0.00000285 + Z_1)}$	83.77	1.003
Dihydrodillapiole	$Y = -6.658 + 0.964 X + 0.1141 \dfrac{Z_1}{(-0.00000613 + Z_1)}$	79.58	1.10
Vanillin oxime ether	$Y = -7.389 + 1.009 X + (-0.01149) \dfrac{Z_1}{(-0.00000592 + Z_1)}$	87.39	0.98
Profenophos			
Piperonyl butoxide	$Y = -11.055 + 1.277 X + 0.000489 \dfrac{Z_1}{(-0.00001661 + Z_1)}$	84.16	1.004
Dillapiole	$Y = -10.547 + 1.221 X + (-0.01123) \dfrac{Z_1}{(-0.00001247 + Z_1)}$	95.26	0.99
Dihydrodillapiole	$Y = -9.323 + 1.148 X + 0.2020 \dfrac{Z_1}{(-0.00001661 + Z_1)}$	85.89	0.98
Vanillin oxime ether	$Y = -11.005 + 1.268 X + (-0.01444) \dfrac{Z_1}{(-0.00001400 + Z_1)}$	86.79	0.98

X = log of the concentration of toxicant; Y = probit of mortality; Z_1 = concentration of the non-toxic synergist

lambdacyhalothrin and profenophos. Yet only an additive action was obtained when combined with fenvalerate. On the other hand, vanillin oxime ether synergised only cypermethrin and lambdacyhalothrin. Thus, the results indicated that with the increase in the age of the larvae of *S. litura* the type of action changed from additive to synergistic especially with the organophosphorous compound *i.e.* profenophos in combination with piperonyl butoxide, dillapiole and dihydrodillapiole.

Synergistic effect of piperonyl butoxide with each of the three synthetic pyrethroids viz., cypermethrin, fenvalerate and lambdacyhalothrin was clearly evident against both the instars except the combination of piperonyl butoxide with profenophos which exhibited additive action against 4 day- old larvae but synergistic effect on the 7 day old- larvae of *S. litura*. In conformity with these results PBO exhibited synergistic action with cypermethrin against *H. armigera* (Phokela and Mehrotra, 1993), in range caterpillars (Hagler *et al.*, 1997) and seven and nine day-old larvae of *S. litura* (Rao and Dhingra, 1997). Earlier, Campanhola and Plapp (1989) also reported synergistic effect of PBO when combined with cypermethrin and tested against susceptible and resistant neonates of *H. virescens.*

Table 4. Effect of different synergists on the toxicity of cypermethrin to 7 day-old larvae of *S. litura* by leaf dip method

Insecticide/ Synergist	Regression equation	R^2 (%)	Joint action ratio
Cypermethrin	**Leaf dip method**		
Piperonyl butoxide	$Y = - 0.676 + 0.438 X + 0.0305 \frac{Z_1}{(-0.00013319+ Z_1)}$	86.79	1.07
Dillapiole	$Y = - 0.332 + 0.412 X + 0.0318 \frac{Z_1}{(-0.00009954+ Z_1)}$	82.64	1.08
Dihydrodillapiole	$Y = - 0.125 + 0.368 X + 0.01488 \frac{Z_1}{(-0.00003499+ Z_1)}$	79.58	1.05
Vanillin oxime ether	$Y = - 0.204 + 0.389 X + 0.01834 \frac{Z_1}{(-0.00002068+ Z_1)}$	83.37	1.05
Fenvalerate			
Piperonyl butoxide	$Y = - 5.909 + 0.817 X + 0.0966 \frac{Z_1}{(-0.00001583+ Z_1)}$	85.14	1.13
Dillapiole	$Y = - 8.999 + 0.894 X + -0.0167 \frac{Z_1}{(-0.00005100+ Z_1)}$	75.50	0.98
Dihydrodillapiole	$Y = - 5.914 + 0.812 X + (-0.00854) \frac{Z_1}{(-0.00002240+ Z_1)}$	79.58	0.99
Vanillin oxime ether	$Y = - 6.740 + 0.868 X + 0.02129 \frac{Z_1}{(-0.00002033+ Z_1}$	82.62	1.03

X = log of the concentration of toxicant; Y = probit of mortality; Z_1 = concentration of the non-toxic synergist

Martin *et al.,*(1997) also obtained significant synergism of cypermethrin toxicity by PBO in three of the four tobacco bud worm larval strains but PBO had no effect on profenophos susceptibility in adult tobacco bud worms collected from Missisipi and Texas in a contact bioassay. Synergism of methamidophos. fipronil, avermectin, fenvalerate and cypermethrin with PBO was even recorded against *Diaeretiella rapae* (Wu *et al.*, 2004). Synergistic activity of PBO is expected due to the presence of methylene dioxyphenyl and a polyalhoxy side chain as a synergophoric group (Walia *et al.,* 2004). PBO has already been reported as a synergist for different insecticides against a number of insect pests. The present study further confirms the synergistic action of this synergists with various synthetic pyrethroids.

The benzo 1,3 dioxole group is well known for insecticide synergism. The methoxy substituent consistently had synergism enhancing effect. They are most active with pyrethrum insecticides against *Tribolium castaneum* (Devakumar and Saxena, 1994). Methylene dioxy phenyl (mdp) compounds are known to synergise the insecticides, which are chiefly metabolized by mixed function oxidases. Dillapiole and dihydrodillapiole are indigenous substitutes for safrole for the production of mdp synergist. According to Dhingra and Sarup (1981), dillapiole and dihydrodillapiole exhibited additive action with pyrethrum, lindane, malathion, diazinon and dichlorvos when applied to the adults of *T. castaneum.* Additive effect of lindane and dichlorvos with dillapiole and

Table 5. Effect of different synergists on the toxicity of lambdacyhalothrin and profenophos to 7 day- old larvae of *S. litura* by leaf dip method.

Insecticide/ Synergist	Regression equation	R^2 (%)	Joint action ratio
Lambdacyhalothrin			
Piperonyl butoxide	$Y = -7.646 + 0.983\,X + 0.0695 \dfrac{Z_1}{(-0.00001776 + Z_1)}$	83.73	1.07
Dillapiole	$Y = -8.634 + 1.043\,X + 0.1049 \dfrac{Z_1}{(-0.00001103 + Z_1)}$	82.74	1.10
Dihydrodillapiole	$Y = -7.674 + 0.981\,X + 0.185 \dfrac{Z_1}{(-0.00001081 + Z_1)}$	82.23	1.21
Vanillin oxime ether	$Y = -8.621 + 1.054\,X + -0.047386 \dfrac{Z_1}{(-0.00001063 + Z_1)}$	84.11	1.05
Profenophos			
Piperonyl butoxide	$Y = -3.598 + 0.637\,X + 0.0464 \dfrac{Z_1}{(-0.00009400 + Z_1)}$	82.28	1.07
Dillapiole	$Y = -3.076 + 0.587\,X + 0.00116 \dfrac{Z_1}{(-0.00005826 + Z_1)}$	60.69	1.19
Dihydrodillapiole	$Y = -3.353 + 0.633\,X + 0.0848 \dfrac{Z_1}{(-0.00016237 + Z_1)}$	82.05	1.14
Vanillin oxime ether	$Y = -9.550 + 1.053\,X + (-0.00568) \dfrac{Z_1}{(-0.00021855 + Z_1)}$	87.48	0.99

X = log of the concentration of toxicant; y = probit of mortality; Z1 = concentration of the non-toxic synergist

dihydrodillapiole was also recorded in the naturally tolerant *Cylas formicarius* (Dhingra and Sarup, 1979). However, an apparent level of synergism was detected with carbaryl in red flour beetle, *Tribolium castaneum* (Walia *et al.,* 1985). The synergistic activity of oxime ethers containing a benzo-1, 3 -dioxole with various insecticides against *T. castaneum* was reported by Walia *et al.* (1985) and in *H. virescens* by Brown and Bryson (1996).

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