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Dillapiole Mediated Esterase Inhibition in Insecticide Resistant *Spodoptera litura* (Fabricius)

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Synergism of insecticide toxicity by dillapiole was examined in the third instar laboratory-reared populations of *Spodoptera litura* collected from Delhi and Guntur. The Guntur population is 10.2, 7.0, 4.8 and 2.3 fold resistant to lambdacyhalothrin, cypermethrin, fenvalarate and profenophos, respectively. Synergistic studies with various insecticides showed that there was significant increase in mortality in both Delhi and Guntur populations. Dillapiole synergized cypermethrin and lambdacyhalothrin against both the Delhi and Guntur population whereas, profenophos synergism with dillapiole was evident only against Guntur population. The combination with fenvalarate was antagonistic to both the population. Esterase inhibition by dillapiole did not occur immediately after dosing, but exhibited maximum inhibition around 3-4 h after dosage in case of Delhi population (susceptible). In Delhi population of *S. litura* the inhibition of esterase by dillapiole was not instantaneous but reflected only after 2-3 h. On the contrary in Guntur population, the inhibition of esterase occurred over a longer period and this led to an increase in synergistic ratio value of all insecticides. Though there was a reduction in toxicity with less proportion of synergist, increase in the proportion of synergist in the mixture led to an increase in toxicity. The ability of dillapiole in suppression of esterase activity was relatively less compared to that of its dihydro - derivative in terms of the time for which the esterase activity was suppressed.

Key words: *Spodoptera litura*, dillapiole, esterase, synergist

The tobacco caterpillar *Spodoptera litura* (Fab.) is a serious polyphagous pest infesting more than 120 host plants all over India. Pyrethroid insecticides were introduced in India in 1980 for the control of this pest on cotton, since it developed resistance to benzene hexa chloride (BHC)[1], lindane and endosulfan[2]. This has also developed resistance to synthetic pyrethroids[3,4] and quinalphos and monocrotophos[5]. The resistance developed by *S. litura,* a major pest of economically important crops has been reported from many parts of India and world[6,7]. In recent years, its outbreaks have been more common in south Asia especially in India[8,9]. The third instar larvae of *S. litura* from Guntur (Andhra Pradesh) showed four to five fold resistance against cypermethrin and fenvalarate as compared to Delhi population. The Delhi population of *S. litura* was 25.3 times more susceptible than Guntur to cypermethrin and fenvalarate[10].

The increasingly wide spread occurrence of resistance to synthetic pyrethroids is a serious threat to control and manage *S. litura*. There has been mounting interest in the use of synergist to reduce this resistance by combined application of insecticide with synergists[11]. One of the ways to enhance the toxicity of the insecticide is the addition of non-toxic synergistic compounds with insecticide[12]. Mixture of insecticides and synergists was applied on crop pests to minimize the cost of pesticides. A highly effective, expensive insecticide might be used at a diluted rate with a less expensive chemical to give satisfactory control of a target insect and minimize the contamination of agro ecosystem. In these multifarious uses, the synergists are likely to become an indispensable part of the pest control strategies in future.

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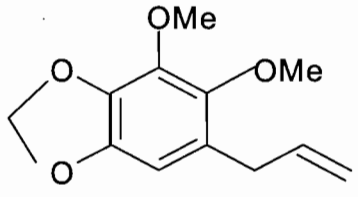
MATERIALS AND METHODS

Insects: The egg masses of *S. litura* were collected from the cauliflower fields of the Indian Agricultural Research Institute, New Delhi and Peddavadalapudi, Guntur district of Andhra Pradesh. Thereafter, rearing was done under laboratory conditions on tender castor leaves under controlled conditions at 27 ± 1 ^{0} C and 60 ± 5 % RH.

Chemicals: The insecticides used were technical grade material of cypermethrin 93.0 % and fenvalarate 92.5% from

Rallis India Limited, Mumbai, lambdacyhalothrin 93.2 % and profenophos 89.0 % from Syngenta India Limited, Mumbai. Acetone was used for the preparation of stock solution.

Synergist: Dillapiole



Dillapiole (5-allyl 6, 7-dimethoxy- 1,3–benzodioxole), is a plant based insecticide synergist. It is obtained from *Anethum sowa* Roxb (Indian dill) seeds following extraction of seeds with n- hexane. After evaporation of the solvent dil oil was subjected to fractional distillation to obtain heavier fraction rich in dillapiole (95.0%).

Insecticide bioassay: The toxicity of four different insecticides *viz.*, cypermethrin, fenvalarate, lambdacy-halothrin and profenofos, formulated either alone or in combination with dillapiole were determined against the third instar larvae of *S. litura* (Delhi and Guntur populations). Technical grade materials of insecticides and dillapiole were used for the preparation of stock solution (20.0 %). Further the stock solution of insecticides (20 %) were diluted in such a way as to maintain the level of solvent (acetone) and emulsifier (triton-x100) at 5.0 and 0.5 per cent respectively in the final concentrations. One percent stock solution of dillapiole was prepared from the 20.0 % stock solution. A mixed formulation of each of the insecticide with dillapiole in four ratios *viz.*, 1:1, 1:2, 1:5 and 1:10 was prepared by mixing the two stock solutions of equal concentrations depending upon the ratio to be maintained. Further, serial dilutions were done. Castor leaf discs of approximately 6 cm diameter were dipped in the required concentrations of insecticides or their mixed formulations with dillapiole for twenty seconds and then dried. The treated leaf discs were then transferred to clean jars (15x 10 cm). In each jar 15 larvae were released. The jars were kept at 27 ± 1°C and the larval mortality was recorded 24 h after the treatment. There were three replications for each concentration and untreated control. The LC_{50} values were calculated by probit analysis[13]. The data were subjected to regression analysis. They were also categorized as synergistic and antagonistic with respect to each combination of a particular insecticide and synergist as per Sarup[14].

Dillapiole treatment and extraction of esterase: Third instar larvae of *S. litura*, weighing about 25–30 mg were sorted out from the rearing jar and kept separately for preconditioning at ambient temperature. The treatment operation involved the application of 1µl of dillapiole (30 mM) in acetone, applied by a micropipette to the dorsal thorax. Samples of insects were obtained for esterase assay at one-hour intervals (treatment group) and two-hour intervals (control group) after treatment with dillapiole. Following the treatment, larvae were kept at a constant temperature of 27 ± 1 °C with adequate food for varying periods of time up to 24 h. Thirty insects in each treatment were mass homogenized in 2 ml homogenization buffer (100 mM phosphate buffer, pH 7.0 containing 1mM each of EDTA, PTU, PMSF and 20 % glycerol), and the homogenates were subjected to centrifugation at 10,000 rpm for 20 min. The volume of the supernatant obtained from centrifugation was made up to 2 ml using phosphate buffer (100 mM, pH 7.0). 100 µl of aliquot was taken from the supernatant in a 1.5 ml microcentrifuge tube and the volume made up to one ml. This solution was used to assay the esterase activity and named as enzyme assay solution.

Determination of esterase activity: Esterase activity was determined according to the method described by Kranthi[15]. Fifty microlitres of enzyme assay solution was taken in a 10 ml test tube and the volume made up to one ml with 950 µl phosphate buffer (40 mM, pH 6.8) and then five ml of substrate solution (1 ml of 30 mM α- naphthyl acetate in 99 ml of phosphate buffer, 40 mM, pH 6.8) was added to each test tube. One ml of 40 mM phosphate buffer with 5 ml of substrate solution without the enzyme assay solution was kept as control. The whole set was maintained in dark for 20 min at 30° C with occasional shaking. After incubation, one ml of staining solution (2 parts of 1 % Fast blue BB solution in 5 parts of 5 % SDS) was added to each tube including control and the tubes were kept in dark for 20 min at room temperature. 1 – Naphthol was produced as a product during the esterase action on the substrate (α-napthyl acetate). This 1-napthol was coupled with fast blue BB salt (Sigma, USA). A strong blue color was produced, which was measured at its absorbance maxima of 590 nm, on a double beam spectrophotometer (Perkin Elmer ë 3B). For the calibration of the 1- naphthol produced, the procedure of [16] as detailed by Kranthi (2005) was followed. Enzyme inhibition was expressed as the mean percentage of activity remaining (with respect to an un-inhibited control) for dillapiole. Three individual assays of esterase activity were made for each time interval.

RESULTS AND DISCUSSION

Toxicity bioassay: Synergistic studies with various insecticides showed that there was significant increase in mortality in both Delhi and Guntur populations (Figure 1a and Figure 1b, Table 1). Dillapiole synergized cypermethrin and lambdacyhalothrin against both the Delhi and Guntur population whereas, profenophos synergism with dillapiole was evident only against Guntur population. The combination with fenvalarate was antagonistic to both the population. Methylene dioxy phenyl (mdp) compounds are known to synergise the insecticides, which are chiefly metabolized by mixed function oxidases. Dillapiole and dihydrodillapiole exhibited synergism and antagonism with DDT and malathion, respectively, against *Cylas formicarius*[17]. These compounds are indigenous substitutes for safrole for the production of methylene dioxy phenyl synergists, which also showed good synergism with carbaryl against red flour beetle *Tribolium castaneum*[18].

Esterase assay: Esterase inhibition by dillapiole did not occur immediately after dosing, but exhibited maximum inhibition around 3-4 h after dosage in case of Delhi population (susceptible) (Figure 2a and Table 2). The activity of esterase subsequently recovered at 7 h post treatment. However, the recovered esterase was not stable over time; it again declined around 10–11h post treatment, thereafter the activity restored at 12 h post treatment. Thus, the esterase activity in Delhi population appears to be bi-phasic, whereas, the reduction in the esterase activity of Guntur population was instantaneous initially and sustained for about 9 h post treatment. There was a gradual decrease in

Table 1. Synergistic Ratio of different insecticides with dillapiole against Delhi and Guntur *S. litura*

Insecticide	LC_{50}					Synergistic Ratio (SR)			
	Without synergist	With synergist				$SR=LC_{50}$ without synergist/ LC_{50} with synergist			
Delhi		**1**	**2**	**5**	**10**	**1**	**2**	**5**	**10**
Cypermethrin	0.017	0.01	0.009	0.008	0.005	1.627	1.919	2.012	3.3
Fenvalarate	0.031	0.027	0.028	0.039	0.032	1.169	1.103	0.791	0.978
lambdacyhalothrin	0.021	0.015	0.01	0.01	0.008	1.432	2.01	2.111	2.787
Profenophos	0.064	0.055	0.066	0.077	0.069	1.148	0.961	0.825	0.922
Guntur									
Cypermethrin	0.12	0.13	0.11	0.072	0.07	0.87	1.05	1.62	1.68
Fenvalarate	0.143	0.164	0.151	0.132	0.123	0.88	0.95	1.08	1.17
Lambdacyhalothrin	0.19	0.145	0.14	0.07	0.061	1.31	1.83	2.41	3.14
Profenophos	0.149	0.107	0.093	0.074	0.069	1.39	1.61	2.0	2.17

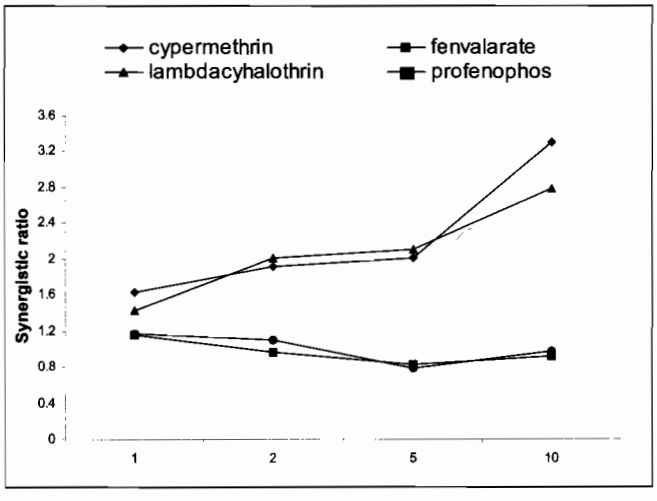


Figure 1a. Synergistic ratio of dillapiole with Delhi *S. litura*

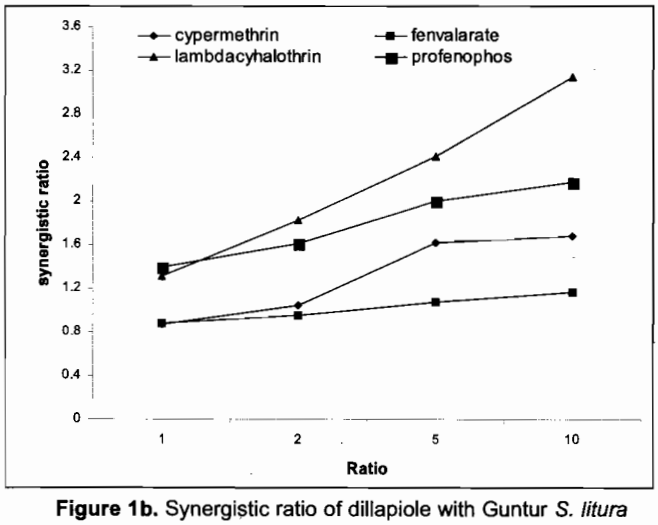


Figure 1b. Synergistic ratio of dillapiole with Guntur *S. litura*

Table 2. Esterase activity of Delhi and Guntur *S. litura* after the application of Dillapiole at different hour interval

	OD value 1- naphthol (μ moles/mg/min) Hours																		
Hours	0	1	2	3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24
Delhi	0.938	0.822	0.845	0.879	0.696	0.68	0.659	0.873	0.791	0.811	0.666	0.691	0.803	0.801	0.824	0.815	0.832	0.923	0.953
Guntur	0.947	0.801	0.655	0.564	0.492	0.464	0.646	0.569	0.652	0.759	0.785	0.726	0.708	0.722	0.802	0.815	0.643	0.688	0.639

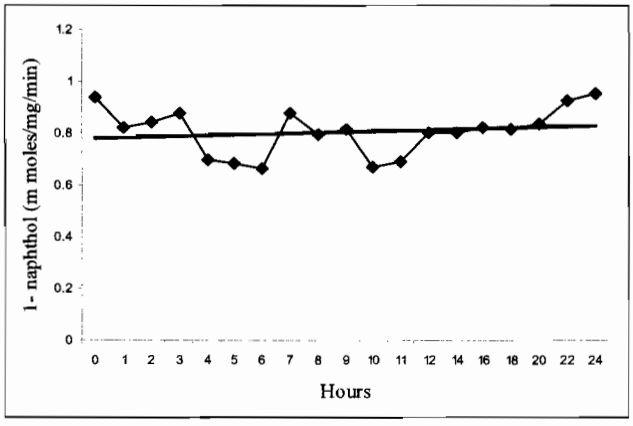


Figure 2a. Delhi S. litura treated with Dillapiole

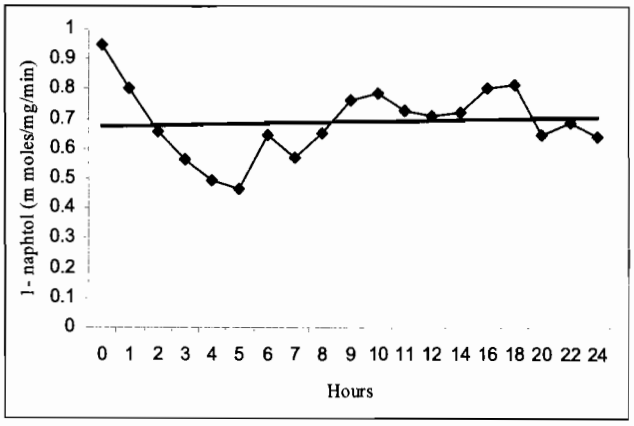


Figure 2b. Guntur S. litura treated with Dillapiole

the activity of esterase from 0.946 to 0.464 μmoles/mg/min and it equilibrated at 10 h post treatment. In Delhi population of *S. litura* the inhibition of esterase by dillapiole was not instantaneous but reflected only after 2-3 h. On the contrary in Guntur population (Figure 2b and Table 2) the inhibition of esterase occurred over a longer period, this inhibition lead to an increase in synergistic ratio value of all insecticides. Though there was a reduction in toxicity with less proportion of synergist, increase in the proportion of synergist in the mixture led to increase in toxicity. The ability of dillapiole in suppression of esterase activity was relatively less compared to that of its dihydro - derivative in terms of the time for which the esterase activity was suppressed.

The inhibition of esterase activity following topical application of dillapiole resulted in the reduction in the level of esterase in both Delhi and Guntur population. The increased esterase activity in the resistant population from Guntur and its suppression by application of synergists suggested the involvement of esterases in hydrolysis of pyrethroid insecticides. Young *et al*[19] presented evidence to show the over production of esterase isozymes which metabolized and sequestered pyrethroid insecticides in resistant *H. armigera* larvae and that the classical synergist PBO inhibited the same. Further, it was shown that PBO suppressed esterase activity 3-4 h post-treatment and that this activity was regained gradually and reached to a normal level at 24 h. Almost similar results were obtained against whitefly, *Bemicia tabaci* (Aleyrodidae), by the same group of workers.

The activity of esterase was very high in un-treated control of both the populations. The inhibition of esterase activity by piperonyl butoxide was very high in Guntur population, when compared to Delhi population. The hydrolytic activity of pyrethroid was found to be 3-6.5 times higher in resistant strain of *S. littoralis* compared to its susceptible strain[20]. In a resistant strain of *S. frugiperda* with at least 100 times higher LC_{50} values as that of the susceptible strain, Delmore *et al.*[21] showed that besides delayed penetration, ester hydrolysis was a predominant mechanism responsible for resistance. In the larvae of the mosquito *Culex quniquifasciatus*, ester hydrolysis was found to be a predominant mechanism of resistance but not in *Aedes aegypti*[22].

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