Research Article

Synergism of Insecticides by Enzyme Inhibitors in Different Populations of *Cnaphalocrocis medinalis* in Tamil Nadu

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Abstract

The effect of different enzyme inhibitors like piperonyl butoxide (PBO), triphenyl phosphate (TPP) and diethyl maleate (DEM) were studied in combination with insecticides cartap hydrochloride, profenophos and chlorpyriphos in resistant populations of *Cnaphalocrocis medinalis* (Guenee) using seedling dipping bioassay method. Among the synthetic synergist PBO exhibited moderate level of synergism with cartap hydrochloride and there was no synergistic action with chlorpyriphos and profenophos. Similarly DEM exhibited moderate to high level of synergism with cartap hydrochloride and there was very low to low level of synergism with profenophos and chlorpyriphos. TPP was found very effective in suppressing the rice leaffolder resistance to cartap hydrochloride and it is moderately effective in suppressing resistance in case of chlorpyriphos and was low to moderate level with profenophos.

Keywords: *Cnaphalocrocis medinalis*, insecticides resistance, Suppression of resistance (SR), PBO, DEM, TPP

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Introduction

Rice leaf folder *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) is the most widely distributed foliage feeder found in rice. The large scale cultivation of high yielding varieties, excessive usage of nitrogenous fertilizers and continuous use of insecticides that has created resistance against *C. medinalis* population [1]. Many Asian countries suffered from outbreaks of serious infestations of rice leaf-folder including India, Japan, Malaysia, Vietnam, Korea and China [2]. The most common mechanism of resistance to insecticides has been documented to be due to enhanced metabolism mediated through detoxification by cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), mixed function oxidases (MFO) and esterases [3]. Synergist that interfere with the detoxification of insecticides in insects were of practical importance in achieving more efficient control of insects, increasing the spectrum of activity of an insecticide and also in restoring the activity of an insecticide against resistance strains of insects [4]. Synergism would, however, still be found in susceptible insect strains since the detoxification enzymes inhibited by the synergists are still present in baseline amounts. Synergists can be used as a chemical counter-measure for insecticide resistance.

Since the synergists act primarily by inhibiting a detoxification mechanism, they also help in identifying the metabolic pathway involved in the resistance.To find out the role of metabolic detoxification in the resistant populations of *C. medinalis*, synergists *viz*., PBO (piperonyl butoxide), DEM (Diethyl Maleate) and TPP (Triphenyl phosphate) were added to the some selected insecticides like cartap hydrochloride, chlorpyriphos and profenophos in this present bioassays. Piperonyl butoxide (PBO), Triphenyl phosphate (TPP) and Diethyl maleate (DEM) generally used as standard synergists. PBO is an inhibitor of both monooxygenases and esterases [5]. PBO has also been shown to inhibit AChE [6]. The effects of PBO therefore seem to be multiple, which could explain the high efficacy of PBO. TPP is an esterase inhibitor and DEM is often used as a standard for glutathione-S-transferase conferred resistance [7].

Materials and Methods

Insects

During the rice growing seasons, the larvae of *C. medinalis* were collected from various locations like Trichy, Bhavanisagar, Aduthurai and Coimbatore of Tamil Nadu and were cultured to the next generation (F1) in the laboratory on TN1 rice seedlings at Tamil Nadu Agricultural University (TNAU), Coimbatore.

Insecticides and synergists

In this bioassay studies, the commercial formulation of insecticides were: Profenophos 50 EC, Chlorpyriphos 20 EC and Cartap hydrochloride 50 SP. The synthetic synergists [Piperonyl butoxide (PBO); 94% pure, $C_{19}H_{30}O_5$ (338.44), Methylene dioxyphenol ring], [Diethyl maleate (DEM); 97% pure, $C_8H_{12}O_4$ (172.18), Ester] and [Triphenyl phosphate (TPP); 99% pure, $(C_6H_5O)_3PO$ (326.28), Ester (triester)] were obtained from Bayer.

Bioassay method

Synergism bioassays were performed on Aduthurai, Trichy, Bhavanisagar and Coimbatore populations of *C. medinalis.* The F_1 generation of 3rd instar larvae were exposed to some insecticides like cartap hydrochloride, chlorpyriphos, and profenophos by seedling dip bioassay method. Successive dilutions were prepared to get required concentration for the test compounds using distilled water and synergist solutions were prepared in distilled water as 50 ppm of PBO, DEM and TPP. Water only was used as control. Three weeks old rice seedlings (25cm ht.) were dipped in insecticidal plus synergist suspension for 30 seconds by rotating the pot upside down and dipping the leaves and stem into the solution and then seedlings were endorsed to pet dry. Filter papers were spread at the base of petri dish (6 cm dia.) and then allowed to hydrate by adding 1 mL of distilled water. The 5 cm sections of the leaves were cut and then layered onto the filter paper in petri dish. Approximately 30 leaf sections were used in each petri dish and ten third-instar larvae were shifted onto each Petri dish with a small paint brush. The Petri dishes were stored at temperature of 26°C, and 70% RH.

Statistical Analysis

Assessment of larval mortalities was done after 24 and 48 hours. Larvae were considered as dead unless their coordinated movements. There was a conversion of mortality values to percentages and adjusted for control mortalities using Abbott's formula [8]. The corrected mortality data was fed to probit analysis software for developing regression equations for dosage mortality responses and to determine the LC_{50} values. The resistance percentage (RP) was calculated by using the formula,

$$\text{Per cent resistance (RP)} = 1 - \frac{\text{No. of dead insects}}{\text{No. of insects tested}} \times 100$$

The standard error was worked out as,

$$\sqrt{\frac{p\,(100 - p)}{n - 1}}$$

Where, p = per cent insect surviving in discriminative dose, n = total no. of insects tested.

The suppression of resistance (SR) was worked out by the following formula,

$$SR = 1 - \frac{\text{(Survival in insecticide) - (Survival in insecticide + synergist)}}{\text{(Survival in insecticide)}} \times 100$$

Results and Discussion

Synergists with known metabolic functions were used in identifying the mechanisms involved in the resistance of rice leaffolder to insecticides. In the present investigations, synthetic synergists such as PBO, DEM and TPP were tested each along with cartap hydrochloride, chlorpyriphos and profenophos. PBO exhibited moderate level of synergism with cartap hydrochloride by reducing the resistance level from 22.5 to 50.00 per cent, suppression of resistance was 21.825, 20.833, 28.260 and 34.445 in Aduthurai, Bhavanisagar, Trichy and Coimbatore respectively, and there was no any synergistic action with chlorpyriphos and profenophos (**Table 1-3, Figures 1-3**).

The non-toxicity of PBO was also reported against *Lasioderma sericorne* Fab., *Spodoptera eridiana* (Cramer) and DBM, respectively [9]. PBO showed no synergistic effect on profenophos and spinosad on the resistant population of *S.litura* [10].The antagonic interaction of PBO with the organophosphate insecticides like azinphos-methyl and chlorpyrifos to the navel orangeworm *Amyelois transitella* laboratory strain signifying that they are possibly bioactivated by P450s and PBO not influenced the toxicity of the anthranilic diamide chlorantraniliprole [11]. Since PBO is cytochrome P450 monooxygenase inhibitor and there was less synergist effect found in case of PBO, so the present study indicates that detoxification by cytochrome P450 monooxygenases enzyme may least partially involved in imparting resistance to the testes insecticides.

Table 1 Efficacy of PBO, DEM, and TPP in the suppression of *C. medinalis* resistance to cartap hydrochloride

Locations	Cartap hydrochloride	PBO Cartap hydrochloride + PBO		DEM Cartap hydrochloride + DEM		TPP Cartap hydrochloride + TPP	
	RP ± SE	RP ± SE	SR (%)	RP ± SE	SR (%)	RP ± SE	SR (%)
Bhavanisagar	65.00±4.330 (53.728)[a]	50.00±5.773 (45.001)[a]	20.833	40.00±8.164 (39.232)[a]	39.583	45.00±9.575 (42.131)[a]	31.250
Aduthurai	56.25±4.800 (48.590)[a]	42.50±2.50 (40.541)[ab]	21.825	42.25±7.50 (40.541)[a]	25.476	35.00±2.886 (36.272)[a]	37.380
Coimbatore	40.00±3.535 (39.231)[b]	30.00±5.773 (33.210)[bc]	34.445	25.00±6.291 (30.000)[ab]	35.834	17.50±4.787 (24.729)[b]	52.500
Trichy	41.50±1.299 (40.106)[b]	22.50±6.291 (28.317)[c]	28.260	17.50±2.886 (24.729)[b]	57.880	15.00±2.887 (22.787)[b]	64.130

Figures in the parentheses are arcsin transformed values.
RP - Resistance Percentage; SE – Standard error; SR- Suppression of resistance
Means followed by common letter in a column are not significantly different at five percent level by DMRT.

Table 2 Efficacy of PBO, DEM, and TPP in the suppression of *C. medinalis* resistance to chlorpyriphos

Locations	Chlorpyriphos	PBO Chlorpyriphos + PBO		DEM Chlorpyriphos + DEM		TPP Chlorpyriphos + TPP	
	RP ± SE	RP ± SE	SR (%)	RP ± SE	SR (%)	RP ± SE	SR (%)
Bhavanisagar	73.33±5.443 (58.906)[a]	68.66±4.667 (55.956)[a]	5.833	53.34±6.66 (46.916)[a]	25.351	43.34±8.819 (41.172)[a]	40.271
Aduthurai	63.33±7.200 (52.731)[ab]	61.34±4.082 (51.554)[a]	3.777	54.66±6.110 (47.673)[a]	12.344	48.00±5.333 (43.853)[a]	27.773
Coimbatore	53.33±5.443 (46.909)[b]	48.67±4.666 (44.237)[b]	7.771	40.66±5.773 (39.616)[b]	23.880	35.00±3.334 (36.272)[b]	30.554
Trichy	51.34±3.810 (45.767)[b]	46.00±3.055 (42.705)[b]	9.919	43.33±6.359 (41.166)[b]	17.273	36.67±5.773 (37.268)[b]	31.515

Figures in the parentheses are arcsin transformed values.
RP - Resistance Percentage; SE – Standard error; SR- Suppression of resistance
Means followed by common letter in a column are not significantly different at five percent level by DMRT.

Table 3 Efficacy of PBO, DEM, and TPP in the suppression of *C. medinalis* resistance to profenophos

Locations	Profenophos	PBO Profenophos + PBO		DEM Profenophos + DEM		TPP Profenophos + TPP	
	RP ± SE	RP ± SE	SR (%)	RP ± SE	SR (%)	RP ± SE	SR (%)
Bhavanisagar	61.33±1.088 (51.548)[a]	56.67±4.409 (48.834)[a]	7.638	51.67±5.925 (45.958)[a]	15.833	50.00±5.773 (45.000)[a]	18.402
Aduthurai	50.00±2.828 (45.000)[ab]	43.34±5.773 (41.173)[ab]	11.645	48.33±3.334 (44.042)[a]	4.978	43.34±2.886 (41.172)[a]	12.554
Coimbatore	46.67±5.443 (43.090)[b]	40.00±3.334 (39.232)[b]	11.111	43.34±8.819 (41.173)[b]	8.334	38.34±4.409 (38.258)[ab]	13.888
Trichy	43.34±7.200 (41.172)[b]	36.67±8.164 (37.268)[b]	13.888	41.60±5.773 (40.164)[b]	5.773	26.67±6.667 (31.094)[b]	38.887

Figures in the parentheses are arcsin transformed values.
RP - Resistance Percentage; SE – Standard error; SR- Suppression of resistance
Means followed by common letter in a column are not significantly different at five percent level by DMRT.

However, a limited synergism of insecticides shown by PBO implies that other mechanisms such as target site insensitivity and reduced cuticular penetration may be more important mechanisms of resistance of *C. medinalis*. In this study, the efficacy of DEM exhibited moderate to high level of synergism with cartap hydrochloride by reducing the resistance level from 17.50 to 42.25 per cent, the SR was 25.476 to 57.880 per cent. There was very low to low level of synergism with profenophos and chlorpyriphos showing SR ranging from 4.978 to 15.833 and 12.344 to 25.351 per cent respectively. The susceptibility of *chilo suppressalis* to chlorantraniliprole was observed by addition of PBO, S,S,S-tributyl phosphorotrithioate (DEF) and DEM. PBO was found synergistic effect whereas others like DEM and DEF had no synergist effect [12].

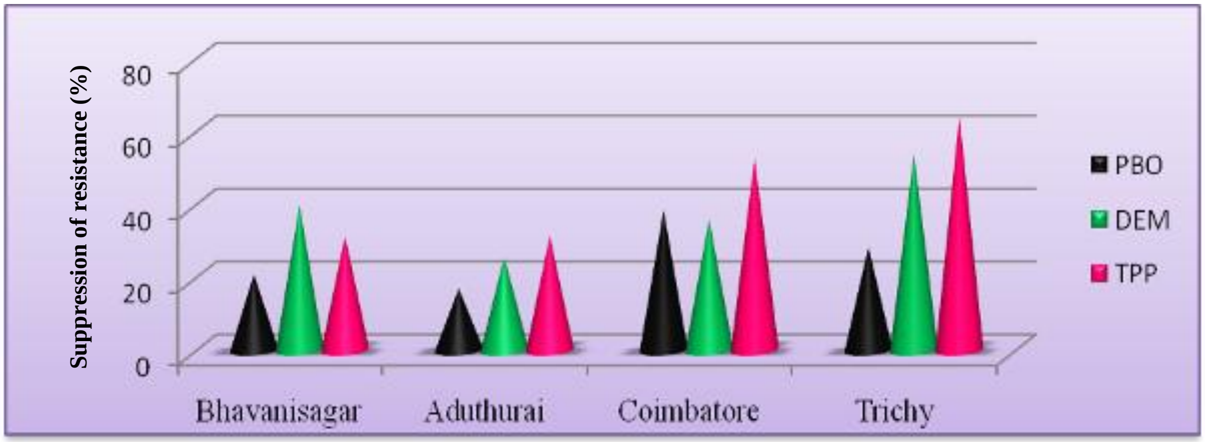

Figure 1 Efficacy of synthetic synergists in the suppression of *C. medinalis* resistance to cartap hydrochloride

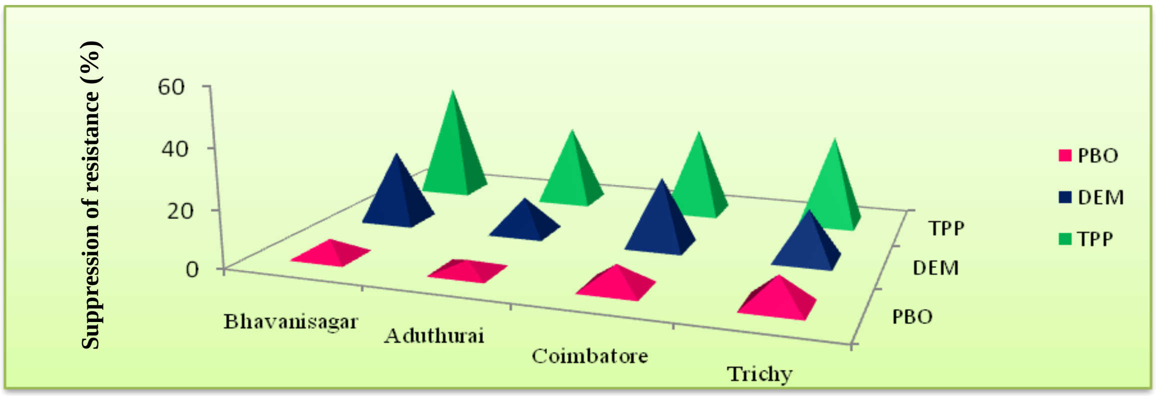

Figure 2 Efficacy of synthetic synergists in the suppression of *C. medinalis* resistance to chlorpyriphos

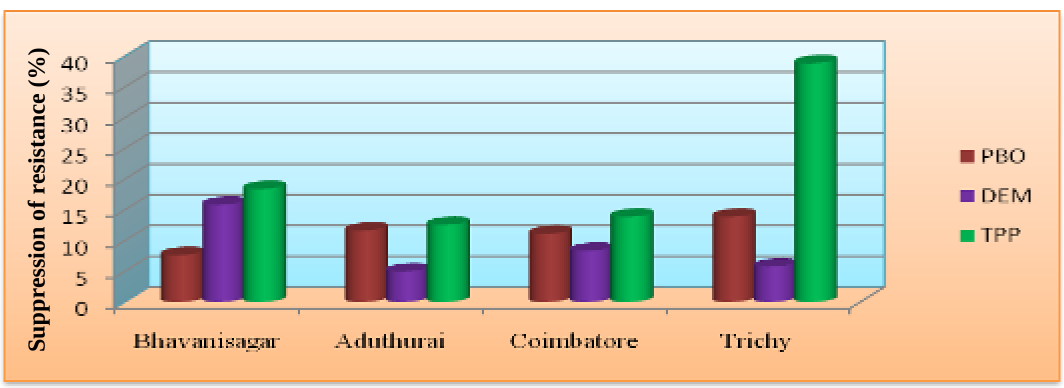

Figure 3 Efficacy of synthetic synergists in the suppression of *C. medinalis* resistance to profenophos

The level of resistance decreased from 65 to 23.33 per cent and SR ranged from 14.88 to 64.11 per cent when DEM was mixed with quinalphos [13]. DEM exhibited no synergistic activity with fenvalerate and monocrotophos. DEM was also not influenced the toxicity of the anthranilidic amide chlorantraniliprole [11]. Some scientist employed GST inhibitor to find out the mechanism of insecticide resistance involved in case of Musca domestica, H. armigera and Platynotaidaeu salis (Walker) [14]. The finding of the present study indicated the involvement of GST in the buildup of resistance to cartap hydrochloride. The synergist effect revealed that the synergistic ratios of PBO, TPP and DEF with fipronil in susceptible and resistant strains of C. suppressalis were 7.55, 1.93, and 2.91 fold respectively, and DEM exhibited no obvious synergistic action [15]. The synergism experiment was done to indicate the role of detoxicating enzymes by using the synergist like TPP, PBO and DEM in C. suppressalis resistant population with triazophos and the results revealed that the suppression ratio of TPP (SR, 1.92) and PBO (SR 1.63), While DEM (SR 0.83) [16].

In this study the synergism of TPP with chlorpyriphos by reducing the resistance level SR ranges from 27.77 to

40.27 per cent and there was also low to moderate level of SR with profenophos ranging from 12.554 to 38.887 per cent, while there was appreciable suppression of resistance to cartap hydrochloride, SR was ranging from 31.250 to 64.130 per cent. The resistance to monocrotophos was suppressed by TPP showing highest SR of 50.00 per cent and decrease in level of resistance from 62.30 to 31.15 per cent. TPP exhibited no synergistic activity with fenvalerate and quinalphos [13]. The non-toxicity of TPP and DEM was reported against *Helicoverpa armigera* and TPP, which is an esterase-inhibiting synergist for characterizing the mechanism of resistance [17]. Among the all synthetic synergist TPP was found effective against rice leaffolder *C. medinalis*. As TPP is showing more effective results as compare to DEM, PBO and TPP is esterase inhibitors, so overall results suggest that *C. medinalis* detoxify insecticides used in management through enhanced esterases activity, and resistance may begin to develop by this route.

Conclusion

As the toxicity of several insecticides is limited by detoxifying esterases and other enzyme systems in resistant insects hence the enzyme inhibitors may enhance the potency of insecticides in these insects. The present study result implies that the use of synergists will not combat the development of insecticide resistance of *C. medinalis*. The cross resistance across diverse insecticides and existence of multiple mechanism of resistance makes insecticide resistance management implementation strategy difficult. In this current scenario, some new valuable compounds can be applied judiciously and their utility could be prolonged by limiting their application rate.

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