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Effect of synergists Piperonyl butoxide, *S'S'S'* tribute phosphorotrithioate and Triphenyl phosphate on resistant Bapatla strain of *Tribolium castaneum* Herbst

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Abstract

A laboratory experiment was carried out in the Department of Entomology to know the toxic effect of synergists on commonly used insecticides against *Tribolium castaneum* Herbst. The synergists such as PBO, DEF and TPP showed synergistic factors of 89.58, 238.80 and 153.57 at LC_{50} and 3.17, 6.0 and 503 at $LC_{99.9}$ level respectively with malathion. The corresponding synergistic values for dichlorvos were 58.8, 35.33 and 15.14 at LC_{50} and 2.50, 2.13 and 0.32 at $LC_{99.9}$ level, while these values were 56.42, 25.48 and 21.03 at LC_{50} level and 1.80, 0.6 and 0.49 at $LC_{99.9}$ level for deltamethrin. The levels of resistance to malathion, dichlorvos and deltamethrin are brought down significantly with all the three synergists viz., PBO, DEF and TPP.

Keywords: *Tribolium castaneum*, synergists, malathion, dichlorvos, deltamethrin, PBO, DEF, TPP

Introduction

The red flour beetle, *Tribolium castaneum* Herbst. is an important primary pest of flour and other milled products and secondary pest of all stored grains. Both the adults and larvae feed on broken grains or mechanically damaged grains and milled products. General practice of protecting stored food grains is through insecticides and continuous usage of these protectants has resulted in health hazards and increased resistance to insecticides in storage pests.

Synergists increase the lethality of insecticides by inhibiting insecticide detoxifying enzymes. This enables synergists to be used as tools for elucidating resistance mechanisms, especially if they are specific inhibitors of a particular resistance conferring mechanism such as detoxification of enzymes and also play a significant role in enhancing toxicity on the resistant strain to a greater extent [1]. Mechanisms of insecticide resistance can be identified based on differential mortalities by combining various categories of synergists with insecticides [2]. The synergists act as useful indicators of metabolic mechanisms of resistance such as Piperonyl Butoxide (PBO) for Mixed Function Oxidases (MFO) systems [3], *S'S'S'* tribute phosphorotrithioate (DEF) and Triphenyl phosphate (TPP) for esterases [4].

Materials and Methods

The experiment was carried out in the Department of Entomology, Agricultural College, Bapatla, Guntur district, Andhra Pradesh during 2015-2016. Adults of *T. castaneum* of Bapatla strain were collected from the Central Warehousing Corporation, Bapatla were brought to the department laboratory in wheat flour containing yeast powder in 10:1 ratio at 32±2 ^{0}C and 75 percent relative humidity and it was selected as test insect because of it showed higher degree of resistance compared to the remaining test strains viz., Amalapuram, Eluru, Machilipatnam, Visakhapatnam, Srikakulam, Tirupathi and Nellore strains. Malathion, dichlorvos and deltamethrin were the test insecticides and synergists used in the study were PBO, DEF and TPP. The adult beetles of one week old were subjected to the bioassay following Jute Cloth Disc Impregnation method [5]. The respective concentrations of test insecticides and synergists were prepared separately in 1:10 ratio. The insecticide and synergistic mixture was prepared and from that two microlitres was applied to the *T. castaneum* by jute cloth disc impregnation method.

Mortality data were recorded at 24, 48 and 72 hours after the treatment (HAT). The experiment was repeated with a wide range of concentrations initially followed by a narrow range so as to get mortality in the range of 5 – 90% and the data were subjected probit analysis [6], using SPSS to calculate LC_{50}, $LC_{99.9}$ and other parameters were calculated. The log concentration probit (1cp) lines were drawn by plotting log concentrations on X-axis and probits on Y-axis and the response of test insect populations was studied at different concentrations of the test insecticides. The Synergistic Factor (SF) was calculated by dividing the LC_{50} and $LC_{99.9}$ value of the individual test insecticide with the corresponding LC_{50} and $LC_{99.9}$ value of the test insecticide + synergist mixture at 72 HAT.

$$Synergistic\ ratio = \frac{LC50\ of\ insecticide\ alone}{LC50\ of\ (Insecticide + Synergists)}$$

If the synergistic ratio is <1 - Antagonistic effect, >1 - Synergistic effect, = 1 – No effect

Results and Discussion

The Bapatla strain of *T. castaneum* has recorded the LC_{50} and $LC_{99.9}$ values of 0.2150 and 1.6000 percent for malathion alone at 72 HAT while those of malathion + PBO were 0.6899 and 0.5033 percent; malathion + DEF were 0.0009 and 0.2353 percent; malathion + TPP were 0.0097 and 2.7308 percent, respectively. The synergistic factors recorded at LC_{50} and $LC_{99.9}$ levels were 89.58 and 3.17 due to PBO, 238.80 and 6.70 due to DEF and 153.57 and 5.03 due to TPP, respectively at 72 HAT (Table 1).

From the above results, it is evident that synergism of malathion with DEF (SF = 238.8 and 6.7) and TPP (SF = 153.57 and 5.03) was more which clearly indicated that both DEF and TPP could effectively reduce the esterase activity in the detoxification of malathion. Similarly, the inhibition of carboxyl esterases activity in malathion degradation by TPP was observed in *P. interpunctella* [7]. Malathion with PBO also showed synergistic effect (SF = 89.58 and 3.17) confirming the oxidative detoxification by MFO. Similarly, the synergism of malathion with PBO (SF = 7.08) at $LC_{99.9}$ in *Cryptolestes ferrugineus* (Stephens) confirming the oxidative detoxification by MFO [8].

From the results of the present study, it is clear that the levels of resistance to malathion could be brought down successfully with all the three synergists *viz.*, PBO, DEF and TPP and the degree of synergism was in the decreasing order of DEF > TPP > PBO. The decreasing order of synergism with DEF, PBO and TPP revealed the role of esterases as well as mixed function oxidases in the detoxification of malathion.

The Bapatla strain of *T. castaneum* has recorded the LC_{50} and $LC_{99.9}$ values of 0.0530 and 0.4030 percent for dichlorvos alone at 72 HAT and that of the combination of dichlorvos + PBO were 0.0009 and 0.1579 percent; dichlorvos + DEF were 0.0015 and 0.1884 percent; dichlorvos + TPP were 0.0035 and 1.2288 percent, respectively. The synergistic factors recorded at LC_{50} and $LC_{99.9}$ levels were 58.80 and 2.50 due to PBO, 35.33 and 2.13 due to DEF and 15.14 and 0.32 due to TPP, respectively at 72 HAT (Table 1).

It is evident that synergism of dichlorvos with PBO (SF = 58.8 and 2.50) was more confirming the active role of MFO in detoxification of dichlorvos. Synergism of dichlorvos with PBO in *C. ferrugineus* (SF = 1.93) confirming the oxidative detoxification [8]. DEF also showed the synergistic factors of 35.33 and 2.13 revealing the inhibition of esterases activity in dichlorvos resistance. TPP also showed synergism but to a less extent (SF = 15.14 and 0.32) revealing the partial inhibition of carboxyl esterases activity in dichlorvos degradation. However, the degree of synergism was more with PBO, followed by DEF and TPP revealing the active role of mixed function oxidases than esterase activity. Among esterases, the activity of carboxyl esterases is less in the detoxification of dichlorvos.

The Bapatla strain of *T. castaneum* has recorded the LC_{50} and $LC_{99.9}$ values of 0.1580 and 0.7550 percent for deltamethrin alone at 72 HAT while those of deltamethrin + PBO were 0.0028 and 0.4097 percent; deltamethrin + DEF were 0.0062 and 1.1321 percent; deltamethrin + TPP were 0.0097 and 2.7308 percent, respectively. The synergistic factors recorded at LC_{50} and $LC_{99.9}$ levels were 56.42 and 1.80 due to PBO, 25.48 and 0.60 due to DEF and 21.03 and 0.49 due to TPP, respectively at 72 HAT (Table 1).

The synergism of deltamethrin with PBO (SF = 56.42 and 1.8) and DEF (SF = 25.48 and 0.6) was more revealing that both PBO and DEF could effectively reduce the MFO's activity and esterase activity respectively in detoxification of deltamethrin. Similarly the inhibition of MFO's activity and esterase activity in deltamethrin degradation by PBO and DEF observed in *T. castaneum* [9], whereas the role of inhibition of oxidative detoxification by MFO by PBO in *Sitophilus zeamais* (Motsch) was reported with synergistic factor of 5.2 [10] and in *T. castaneum* with synergistic ratio of 2.3 and 2.5 at 1:4 and 1:8 ratios of deltamethrin and PBO [11].

Table 1: Toxicity of commonly used insecticides with Synergists such as PBO, DEF and TPP against Bapatla strain of *T. castaneum*

Insecticidal treatment	LC_{50} % (95%FL)	$LC_{99.9}$ % (95%FL)	Slope b (±SE)	Heterogeneity χ2	Regression Equation Y=a+bx	Synergistic ratio	
						LC_{50}	$LC_{99.9}$
Malathion	0.2150(0.099-0.538)	1.6000(0.594-2.983)	3.00(±0.32)	52.52	Y=2.70+3.00x	-	-
Malathion+PBO	0.0024(0.0003-0.0007)	0.5033(0.1570-3.2706)	1.00(±0.12)	0.67	Y=2.50+1.00x	89.58	3.17
Malathion+DEF	0.0009(0.0006-0.0013)	0.2353(0.0798-1.327)	1.25(±0.11)	3.08	Y=4.00+1.25x	238.80	6.70
Malathion+TPP	0.0014(0.0010-0.0020)	0.3179(0.1058-1.8315)	1.07(±0.11)	0.91	Y= 3.14+1.07x	153.57	5.03
Dichlorvos	0.0530(0.041-0.065)	0.403(0.248-1.034)	3.00(±0.26)	8.41	Y=4.40+3.00x	-	-
Dichlorvos+PBO	0.0009(0.0006-0.0013)	0.1579(0.0590-0.7135)	1.25(±0.12)	2.95	Y=4.00+1.25x	58.80	2.50
Dichlorvos+DEF	0.0015(0.0011-0.0021)	0.1884(0.0739-0.7912)	1.50(±0.12)	3.52	Y=4.50+1.50x	35.33	2.13
Dichlorvos+TPP	0.0035(0.0025-0.0050)	1.2288(0.4995-4.2707)	1.00(±0.08)	3.14	Y=2.50+1.00x	15.14	0.32
Deltamethrin	0.158(0.143-0.174)	0.755(0.556-1.244)	3.00(±0.43)	0.94	Y=4.00+3.00x	-	-
Deltamethrin +PBO	0.0028(0.0020-0.0042)	0.4097(0.1392-2.2595)	1.25(±0.12)	6.32	Y=3.50+1.25x	56.42	1.80
Deltamethrin +DEF	0.0062(0.0041-0.0108)	1.1321(0.2955-10.9503)	1.25(±0.13)	5.15	Y=3.00+1.25x	25.48	0.60
Deltamethrin +TPP	0.0097(0.0066-0.0154)	2.7308(0.8459-15.9779)	1.00(±0.10)	6.17	Y=2.00+1.00x	21.03	0.49

Lethal concentrations and 95% fiducial limits (FL) were estimated using probit analysis (SPSS 16.0).
The Chi-square test revealed the homogeneity of the test population (*P*<0.05 %).

Conclusion

From the results of this research experiment, it is clear that the levels of resistance to deltamethrin could be brought down successfully with all the three synergists *viz.*, PBO, DEF and TPP. At LC_{50} and $LC_{99.9}$ level the role of esterases was more than the MFO system in the detoxification of deltamethrin. Among the esterases, carboxyl esterases activity is low due to less synergism with TPP (21.03 and 0.49). The degree of synergism of dichlorvos and deltamethrin was more with PBO followed by DEF and TPP revealing the active role of MFOs mixed function oxidases than esterase activity.

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