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Determination of Mechanism of Insecticide Resistance Through Synergist, *s's's'*- tributyl phosphorotrithioate (DEF) in *Spodoptera litura* (Fab.) in Cotton

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ABSTRACT

In the present study the synergist, *s's's'*- tributyl phosphorotrithioate (DEF) was tested with conventional insecticides to know their synergistic effect on resistant Guntur strain of *Spodoptera litura* during *kharif* 2007-08 and 2008-09. Results revealed with synergistic factors of 2.85 and 2.90 at LD_{50} and 3.32 and 3.13 at LD_{90}, during *kharif* 2007-08 and 2008-09, respectively with chlorpyriphos. The corresponding synergistic values for quinalphos were 2.86 and 2.95 at LD_{50} and 3.42 and 3.34 at LD_{90} level. The synergistic factors for endosulfan were 1.19 and 1.43 at LD_{50} and 1.19 and 2.87 at LD_{90} level and the corresponding synergistic factors were 2.65 and 3.66 at LD_{50} and 2.47 and 3.74 at LD_{90} level for cypermethrin while these values were 2.18 and 3.27 at LD_{50} and 2.14 and 2.90 at LD_{90} level for methomyl. The level of resistance to chlorpyriphos, quinalphos, endosulfan, cypermethrin and methomyl were brought down significantly with DEF.

Key words : Insecticide resistance, *Spodoptera litura*, *s's's'*- tributyl phosphorotrithioate.

The 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 (Kranthi, 2005).

Mechanisms of insecticide resistance can be identified based on differential mortalities by combining various categories of synergists with insecticides (Prabhakar *et al*., 1988). The synergists act as useful indicators of metabolic mechanisms of resistance such as DEF for esterases (Casida, 1970). The synergist, *s's's'*- tributyl phosphorotrithioate (DEF) was tested with chlorpyriphos, quinalphos, endosulfan, cypermethrin and methomyl to know their synergistic effect.

MATERIAL AND METHODS

Experiments were carried out in the Department of Entomology, Agricultural College, Bapatla, Guntur district, Andhra Pradesh during two seasons *viz.*, *kharif,* 2007-08 and *kharif*, 2008-09. The third instar larvae weighing 30 mg ± 0.011 S.E. of Guntur strain of *S.litura* was selected as the test insect in this study because it showed higher degree of resistance to the insecticides compared to Prakasam strain. Chlorpyriphos, quinalphos, endosulfan, cypermethrin and methomyl were the test insecticides and synergist used in the study was *s's's'*- tributyl phosphorotrithioate (DEF) for esterase activity.

Bioassay was done by topical application method (FAO, 1971). The respective concentrations of the test insecticides and DEF were prepared separately in 1:10 ratio *i.e.,* the concentration of synergist was ten times more than that of the insecticide. The insecticide and synergistic mixture was prepared and from that two microlitres was applied to the third instar *S.litura* larvae by topical application method.

Mortality of the larvae was recorded at 24, 48 and 72 hours after treatment (HAT). The experiments were repeated so as to get mortality in the range of 5 – 90 per cent and the data were subjected to probit analysis (Finney, 1971) using MLP 3.08 software (Ross, 1987) and the respective $LD_{50,}$ LD_{90} and other parameters were calculated. The log dose probit (ldp) lines were drawn by plotting log dose (x) on x-axis and probits of

Table1. Toxicity of *S'S'S'*-tributyl phosphorotrithioate (DEF) with test insecticides to the resistant larvae of *S. litura* during *kharif,*2007 - 08.

S.No	Hours after treatment	LD_{50} µg / larva (95% FL)	LD_{90} µg / larva (95% FL)	Hetero geneity (^{2})	Slope ± S.E(b)	Regression equationY = a+bx
Chlorpyriphos + DEF						
1	24	0.1412 (0.0932 – 0.2202)	1.2982 (0.6906 - 3.5668)	4.27	1.33 ± 0.18	Y = 6.13 + 1.33x
2	48	0.1392 (0.0840 – 0.2100)	1.3216 (0.7980 – 3.7680)	1.06	1.27 ± 0.20	Y = 6.09 + 1.27x
3	72	0.1068 (0.0906 – 0.1920)	0.5825 (0.3820 – 0.7835)	2.71	1.74 ± 0.27	Y = 6.69 + 1.74x
Quinalphos + DEF						
1	24	0.2414 (0.1680 – 0.3660)	2.9340 (0.9920 - 7.1640)	1.98	1.42 ± 0.25	Y = 5.88 + 1.42x
2	48	0.1836 (0.1296 – 0.2992)	1.3946 (0.6644 – 6.1970)	3.20	1.47 ± 0.28	Y = 6.08 + 1.47x
3	72	0.1258 (0.0764 – 0.1860)	0.6486 (0.3200 – 0.8640)	4.11	1.80 ± 0.26	Y = 6.62 + 1.80x
Endosulfan + DEF						
1	24	2.5820 (1.8986 – 4.4480)	6.9717 (3.2400-22.6380)	4.91	2.99 ± 0.28	Y = 3.76 + 2.99x
2	48	2.2000 (0.0100 – 9.9700)	4.8320 (2.4600-10.2603)	6.24	1.35 ± 0.31	Y = 4.53 + 1.35x
3	72	1.7145 (1.3484 – 2.6873)	2.7703 (1.0240 – 5.6700)	5.49	6.15 ± 0.43	Y = 3.56 + 6.15x
Cypermethrin + DEF						
1	24	0.3876 (0.2700 – 0.6600)	2.8878 (1.3540 -14.3580)	3.03	1.47 ± 0.28	Y = 5.60 + 1.47x
2	48	0.2470 (0.0980 – 0.2720)	1.7500 (1.2440 – 9.7820)	4.76	1.09 ± 0.19	Y = 5.66 + 1.09x
3	72	0.1771 (0.1142 – 0.3200)	1.2836 (0.6324 – 4.1200)	4.20	1.49 ± 0.19	Y = 6.12 + 1.49x
Methomyl + DEF						
1	24	0.6020 (0.3620 – 1.3860)	7.8130 (2.7380–67.8660)	2.02	1.15 ± 0.21	Y = 5.25 + 1.15x
2	48	0.4702 (0.3074 – 0.7902)	5.3427 (2.3954–25.1646)	6.38	1.21 ± 0.21	Y = 5.40 + 1.21x
3	72	0.2410 (0.1540 – 0.4245)	2.0100 (1.5200 – 5.6320)	5.05	1.39 ± 0.19	Y = 5.86 + 1.39x

respective doses on y-axis (Finney, 1971). The Synergistic factor (SF) was calculated by dividing the LD_{50} and LD_{90} value of the individual test insecticide with the corresponding LD_{50} and LD_{90} value of the test insecticide + synergist mixture at 72 HAT.

$$\text{Synergistic ratio} = \frac{LD_{50} \text{ of the insecticide alone}}{LD_{50} \text{ of the (insecticide + synergists)}}$$

If the synergistic ratio is <1 – Antagonistic effect
>1 – Synergistic effect
=1 – Additive effect

RESULTS AND DISCUSSION

Chlorpyriphos + DEF

During *kharif* 2007-08, the LD_{50} and LD_{90} values of chlorpyriphos in combination with DEF to the third instar larvae of Guntur strain of *S. litura* were 0.1412 and 1.2982; 0.1392 and 1.3216; 0.1068 and 0.5825 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.3040 and 1.9340 µg / larva for chlorpyriphos alone at 72 HAT while the corresponding values in combination with DEF were 0.1068 and 0.5825 µg / larva, respectively at 72 HAT. (Table 1) The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 2.85 and 3.32, respectively during *kharif* 2007-08 (Table 3).

During *kharif* 2008-09, the LD_{50} and LD_{90} values of chlorpyriphos were 0.1268 and 1.3758; 0.1164 and 1.2260; 0.0903 and 0.3838 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.2620 and 1.2000 µg / larva for chlorpyriphos alone at 72 HAT while the corresponding values in combination with DEF were 0.0903 and 0.3838 µg / larva, respectively at 72 HAT (Table 2). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 2.90 and 3.13, respectively during *kharif* 2008-09 (Table 3).

Quinalphos + DEF

During *kharif* 2007-08, the LD_{50} and LD_{90} values of quinalphos in combination with DEF to the third instar larvae of Guntur strain of *S. litura* were 0.2414 and 2.9340; 0.1836 and 1.3946; 0.1258 and 0.6486 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.3600 and 2.2160 µg / larva for quinalphos alone at 72 HAT while the corresponding values in combination with DEF were 0.1258 and 0.6486 µg / larva, respectively at 72 HAT (Table 1). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 2.86 and 3.42, respectively during *kharif* 2007-08 (Table 3).

During *kharif* 2008-09, the LD_{50} and LD_{90} values of quinalphos were 0.2569 and 2.9333; 0.1446 and 1.0798; 0.1186 and 0.5113 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.3500 and 1.7100 µg / larva for quinalphos alone at 72 HAT while the corresponding values in combination with DEF were 0.1186 and 0.5113 µg / larva, respectively at 72 HAT (Table 2).The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 2.95 and 3.34, respectively during *kharif* 2008-09 (Table 3).

Endosulfan + DEF

During *kharif* 2007-08, the LD_{50} and LD_{90} values of endosulfan in combination with DEF to the third instar larvae of Guntur strain of *S. litura* were 2.5820 and 6.9717; 2.2000 and 4.8320; 1.7145 and 2.7703 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 2.0425 and 3.3052 µg / larva for endosulfan alone at 72 HAT while the corresponding values in combination with DEF were 1.7145 and 2.7703 µg / larva, respectively at 72 HAT (Table 1). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 1.19 and 1.19, respectively during *kharif* 2007-08 (Table 3).

During *kharif* 2008-09, the LD_{50} and LD_{90} values of endosulfan were 0.9620 and 2.5820; 0.7176 and 1.8864; 0.6985 and 1.1452 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 1.7072 and 3.2882 µg / larva for endosulfan alone at 72 HAT while the corresponding values in combination with DEF were 0.6985 and 1.1452 µg / larva, respectively at 72 HAT (Table 2). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 1.43 and 2.87, respectively during *kharif* 2008-09 (Table 3).

Cypermethrin + DEF

During *kharif* 2007-08, the LD_{50} and LD_{90} values of cypermethrin in combination with DEF to the third instar larvae of Guntur strain of *S. litura*

Table 2. Toxicity of *S'S'S'*- tributyl phosphorotrithioate (DEF) with test insecticides to the resistant larvae of *S. litura* during *kharif,* 2008 – 09.

S.No	Hours after treatment	LD_{50} µg / larva (95% FL)	LD_{90} µg / larva (95% FL)	Hetero geneity (^{2})	Slope ± S.E(b)	Regression equationY = a+bx
Chlorpyriphos + DEF						
1	24	0.1268 (0.0800 – 0.1920)	1.3758 (0.7140 – 4.5380)	0.75	1.24 ± 0.21	Y = 6.11 + 1.24x
2	48	0.1164 (0.0780 – 0.1820)	1.2260 (0.5640 – 6.9420)	1.23	1.25 ± 0.26	Y = 6.17 + 1.25x
3	72	0.0903 (0.0504 – 0.1421)	0.3838 (0.2060 – 0.5244)	1.18	2.04± 0.27	Y = 7.13 + 2.04x
Quinalphos + DEF						
1	24	0.2569 (0.1794 – 0.3942)	2.9333 (1.0318 – 7.7636)	5.34	1.43 ± 0.26	Y = 5.84 + 1.43x
2	48	0.1446 (0.0980 – 0.2060)	1.0798 (0.6260 – 2.8560)	4.34	1.47 ± 0.24	Y = 6.23 + 1.47x
3	72	0.1186 (0.0682 – 0.3240)	0.5113 (0.2100 – 0.9240)	4.29	2.02 ± 0.23	Y = 6.87 + 2.02x
Endosulfan + DEF						
1	24	0.9620 (0.8008 – 1.1846)	2.5820 (1.8986 – 4.4480)	2.52	2.99 ± 0.47	Y = 5.05 + 2.99x
2	48	0.7176 (0.5952 – 0.8624)	1.8864 (1.4546 – 2.8582)	7.62	3.05 ± 0.43	Y = 5.44 + 3.05x
3	72	0.6985 (0.3420 – 0.9320)	1.1452 (0.0920 – 5.6826)	4.12	5.97 ± 0.47	Y = 5.93 + 5.97x
Cypermethrin + DEF						
1	24	0.5179 (0.3004 – 1.0930)	13.1811 (4.0936- 174.004)	9.26	0.91 ± 0.18	Y = 5.26 + 0.91x
2	48	0.1976 (0.1300 – 0.2940)	1.7380 (0.9940 – 4.2300)	5.05	1.36 ± 0.19	Y = 5.96 + 1.36x
3	72	0.1145 (0.0908 – 0.2560)	0.6569 (0.4248 – 0.7243)	1.93	1.69 ± 0.27	Y = 6.59 + 1.69x
Methomyl + DEF						
1	24	0.3386 (0.2440 – 0.4500)	2.5088 (1.0900 – 2.8280)	4.12	1.97 ± 0.27	Y = 5.93 + 1.97x
2	48	0.1872 (0.1240 – 0.3500)	2.1286 (0.8340-19.6400)	0.72	1.21 ± 0.26	Y = 5.88 + 1.21x
3	72	0.1570 (0.1204 – 0.2842)	1.2360 (0.0909 – 1.5000)	2.83	1.43 ± 0.27	Y = 6.15 + 1.43x

were 0.3876 and 2.8878; 0.2470 and 1.7500; 0.1771 and 1.2836 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.4700 and 3.1680 µg / larva for cypermethrin alone at 72 HAT while the corresponding values in combination with DEF were 0.1771 and 1.2836 µg / larva, respectively at 72 HAT (Table 1).The synergistic factor due to TPP at LD_{50} and LD_{90} levels were 2.65 and 2.47, respectively during *kharif* 2007-08 (Table 3).

During *kharif* 2008-09, the LD_{50} and LD_{90} values of cypermethrin were 0.5179 and 13.1811; 0.1976 and 1.7380; 0.1145 and 0.6569 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.4200 and 2.4540 µg / larva for cypermethrin alone at 72 HAT while the corresponding values in combination with DEF were 0.1145 and 0.6569 µg / larva, respectively at 72 HAT (Table 2). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 3.66 and 3.74, respectively during *kharif* 2008-09 (Table 3).

Methomyl + DEF

During *kharif* 2007-08, the LD_{50} and LD_{90} values of methomyl in combination with DEF to the third instar larvae of Guntur strain of *S. litura* were 0.6020 and 7.8130; 0.4702 and 5.3427; 0.2410 and 2.0100 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.5247 and 4.2922 µg / larva for methomyl alone at 72 HAT while the corresponding values in combination with DEF were 0.2410 and 2.0100 µg / larva, respectively at 72 HAT (Table 1). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 2.18 and 2.14, respectively during *kharif* 2007-08(Table 3).

During *kharif* 2008-09, the LD_{50} and LD_{90} values of methomyl were 0.3386 and 2.5088; 0.1872 and 2.1286; 0.1570 and 1.2360 µg / larva at 24, 48 and 72 HAT, respectively. The Guntur strain of *S. litura* has recorded the LD_{50} and LD_{90} values of 0.5140 and 3.5895 µg / larva for methomyl alone at 72 HAT while the corresponding values in combination with DEF were 0.1570 and 1.2360 µg / larva, respectively at 72 HAT (Table 2). The synergistic factor due to DEF at LD_{50} and LD_{90} levels was 3.27 and 2.90, respectively during *kharif* 2008-09(Table 3).

From the above results it is evident that synergism with DEF was observed more in quinalphos followed by chlorpyriphos, cypermethrin, methomyl and endosulfan revealing that the levels of resistance to quinalphos followed by chlorpyriphos, cypermethrin, methomyl and endosulfan could be brought down successfully with the synergist DEF.

The present study is in accordance with Riskallah (1984) who reported higher degree of synergism with DEF when mixed with chlorpyriphos in resistant strain of *S. littoralis*. Ahmad and Mc Caffery (1991) and Yang *et al*. (2005) reported

Table 3. Synergism in *S. litura* to the test insecticides during *kharif,* 2007 – 08 and *kharif*, 2008 - 09.

Insecticide	LD_{50}	LD_{90}	Synergistic ratio (*kharif*, 2007 – 08)		LD_{50}	LD_{90}	Synergistic ratio (*kharif*, 2008 – 09)	
			LD_{50}	LD_{90}			LD_{50}	LD_{90}
Chlorpyriphos	**0.3040**	**1.9340**	—	—	**0.2620**	**1.2000**	—	—
Chlorpyriphos + DEF	0.1068	0.5825	2.85	3.32	0.0903	0.3838	2.90	3.13
Quinalphos	**0.3600**	**2.2160**	—	—	**0.3500**	**1.7100**	—	—
Quinalphos +DEF	0.1258	0.6486	2.86	3.42	0.1186	0.5113	2.95	3.34
Endosulfan	**2.0425**	**3.3052**	—	—	**1.7072**	**3.2882**	—	—
Endosulfan + DEF	1.7145	2.7703			0.6985	1.1452	1.43	2.87
Cypermethrin	**0.4700**	**3.1680**	—	—	**0.4200**	**2.4540**	—	—
Cypermethrin + DEF	0.1771	1.2836	2.65	2.47	0.1145	0.6569	3.66	3.74
Methomyl	**0.5247**	**4.2922**	—	—	**0.5140**	**3.5895**	—	—
Methomyl + DEF	0.2410	2.0100	2.18	2.14	0.1570	1.2360	3.27	2.90

synergistic effect of DEF with cypermethrin in *H.armigera*. From the present investigations it is evident that synergism of chlorpyriphos, quinalphos and endosulfan was more with DEF which clearly indicated that DEF could effectively reduce the esterase activity in the detoxification of chlorpyriphos, quinalphos and endosulfan whereas the synergism of cypermethrin and methomyl was more with DEF which clearly indicated that DEF could effectively reduce the esterase activity in detoxification of cypermethrin and methomyl.

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