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Synergism of insecticides by enzyme inhibitors in the resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract: The effect of enzyme inhibitors piperonyl butoxide (PBO) and tribufos (DEF) was studied in combination with insecticides profenofos, methomyl, thiodicarb, cypermethrin, λ-cyhalothrin, bifenthrin, indoxacarb, and spinosad in the resistant Pakistani populations of *Spodoptera litura* using a leaf-dip bioassay. Both the inhibitors synergised carbamates methomyl and thiodicarb but showed no synergistic effect on an organophosphate profenofos. These inhibitors produced a synergism with cypermethrin but had no synergism with bifenthrin. PBO and DEF enhanced the toxicity of λ-cyhalothrin and indoxacarb in one population but not in the other. Spinosad was synergised by DEF but not by PBO. The potent synergism of carbamates, pyrethroids, indoxacarb and spinosad by PBO and DEF in the present study indicates that detoxification by cytochrome P450 monooxygenases and esterases is at least partially involved in imparting resistance to these insecticides in *S. litura*. However, a limited synergism of insecticides shown by both the synergists implies that other mechanisms such as target site insensitivity and reduced cuticular penetration may be more important mechanisms of resistance in the Pakistani populations of *S. litura*.

Key words: *Spodoptera litura*; insecticides; resistance; synergism; piperonyl butoxide; DEF

1 INTRODUCTION

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae), known as armyworm in Pakistan, has emerged as a serious pest of many economic crops such as cotton, tobacco, vegetables, edible legumes, and oilseeds in the past 10 years. These high-value crops have been subjected to frequent applications of pesticides to control *S. litura* and other pests. This led to the development of a broad-spectrum insecticide resistance in this pest in Pakistan (Ahmad *et al.*, 2007, 2008; Saleem *et al.*, 2008), India (Armes *et al.*, 1997; Kranthi *et al.*, 2001, 2002), and China (Zhou and Huang, 2002). The recently-introduced genetically-engineered crops expressing *Bacillus thuringiensis* (*Bt*) toxin Cry1Ac are effective in controlling many lepidopterous pests (Shelton *et al.*, 2002) but not *Spodoptera* species. *Spodoptera* pests are even gaining more importance on *Bt* crops having Cry1Ac and increasingly becoming resistant to insecticides applied for their control.

The most common mechanism of *S. litura* resistance to insecticides has been documented to be due to enhanced metabolism mediated through detoxification by cytochrome P450 monooxygenases and general esterases (Armes *et al.*, 1997; Zhou and Huang, 2002; Huang and Han, 2007; Ahmad, 2009). To find out the role of metabolic detoxification in the putatively resistant Pakistani populations of *S. litura*, synergists *viz.* PBO (piperonyl butoxide) as a monooxygenase inhibitor (Hodgson, 1999) and DEF (S, S, S-tributyl phosphorotrithioate, tribufos) as an esterase inhibitor (Jang *et al.*, 1992) were added to the selected insecticides in the present bioassays.

2 MATERIALS AND METHODS

2.1 Insects

Fifth or sixth instar larvae of *S. litura* were mostly collected within 50 km radius from various locations of Multan in the southern Punjab, Pakistan during 1998 - 2004. Each collection of about 400 larvae was made by walking through a 2-hectare block of a particular host crop in a zigzag manner to randomize collections. Larvae were fed in the laboratory on a semi-synthetic diet, which consisted of chickpea flour (300 g), ascorbic acid (4.7 g), methyl-4-hydroxybenzoate (3 g), sorbic acid (1.5 g), streptomycin (1.5 g), corn oil (12 mL),

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vitamin mixture (10 mL), yeast (48 g), and agar (17 g). Yeast and agar were dissolved in 800 mL of boiling water and added to other constituents premixed in 500 mL of water. Adults were fed on a solution containing sugar (50 g), vitamin mixture (10 mL), methyl-4-hydroxybenzoate (1 g), and distilled water (500 mL).

2.2 Insecticides and synergists

Commercial formulations of insecticides used in bioassays were: Curacron [profenofos, 500 g/L EC (emulsifiable concentrate); Syngenta, Basle, Switzerland], Lannate [methomyl, 400 g/kg SP(water soluble powder); DuPont Agricultural Products, Wilmington, DE, USA], Larvin [thiodicarb, 800 g/kg DF (dry flowable); Bayer CropScience, Leverkusen, Germany], Arrivo (cypermethrin, 100 g/L EC; FMC, Philadelphia, PA, USA), Karate (λ-cyhalothrin, 25 g/L EC; Syngenta), Talstar (bifenthrin, 100 g/L EC; FMC), Steward [indoxacarb, 150 g/L SC (suspension concentrate); Dupont], and Tracer (spinosad, 240 g/L SC; Dow AgroSciences, Indianapolis, IN, USA). The synergists PBO (91.3% EC) and DEF (70.5% EC) were obtained from Bayer.

2.3 Bioassays

Synergism bioassays were performed on seven populations of *S. litura*, which were resistant to organophosphates, carbamates and pyrethroids (Ahmad *et al.*, 2007). Newly moulted 2nd instar larvae from F_1 laboratory cultures were exposed to different insecticides using the leaf-dip method recommended by the Insecticide Resistance Action Committee (IRAC) (http://www.irac-online.org/resources/methods.asp) (Anonymous, 1990). Each time, the whole batch of insects was divided into four sets and the experiment conducted with and without synergists in parallel. Serial dilutions were prepared as mg/L of the active ingredient of the test compounds using distilled water or synergist solutions, as required, with the concentration based on the percentage of active ingredient of the formulated insecticides. Synergist solutions were made in distilled water as 20 mg/L for PBO as well as DEF. This was the maximum concentration of synergists that could be used without any deleterious effects on the 2nd instar larvae of *S. litura*. Five-centimeter diameter cotton (*Gossypium hirsutum*) leaf discs were cut and dipped into the test solutions for 10 seconds with gentle agitation, then allowed to dry on paper towel on both sides. Five larvae were released onto each leaf disc placed in a 5-cm-diameter Petri dish with adaxial side up. Eight replicates of five larvae were used for each concentration and 5 – 9 serial concentrations were used for each test insecticide. The same number of leaf discs per treatment was dipped into distilled water or synergist solution as an untreated check. Moistened filter papers were placed beneath leaf discs to avoid desiccation of leaves in Petri dishes. Before and after treatment, larvae were maintained at a constant temperature of 25 ± 2℃ with a photoperiod of 14 h.

2.4 Statistical analysis

Larval mortalities were scored 48 h after treatment. Larvae were considered dead if they failed to make a coordinated movement when prodded with a probe. Data were corrected for control mortality using Abbott's formula (Abbott, 1925) and analysed by probit analysis (Finney, 1971) using POLO-PLUS programme (LeOra Software, 2003). Lethal concentrations (LC_{50} and LC_{90}) were calculated and any two values compared were considered significantly different if their respective 95% confidence limits (CLs) did not overlap. To assess the degree of synergism, synergistic ratios (SRs) were calculated by dividing the LC_{50} or LC_{90} of the insecticide by the LC_{50} or LC_{90} of the insecticide plus synergist(s). The 95% CLs for the SRs were computed according to Robertson and Preisler (1992).

3 RESULTS

3.1 Profenofos

Metabolic inhibitors PBO and DEF did not synergise profenofos in the three resistant populations of *S. litura* (Table 1). Profenofos is probably less compromised by the attack of metabolic enzymes. The other resistance mechanisms such as insensitive cholinesterase and reduced cuticular penetration may be responsible for the resistance to profenofos in the Pakistani populations of *S. litura*.

3.2 Carbamates

Three populations tested for methomyl and two populations tested for thiodicarb showed good synergism by the use of both PBO and DEF (Table 1), implying that enzymes cytochrome P450 monooxygenases, esterases and probably glutathione S-transferases play a significant role in the detoxification of methomyl and thiodicarb in the resistant populations of *S. litura*. The extent of synergism by PBO and DEF was similar for methomyl and thiodicarb. However, the application of PBO and DEF could partially reduce LC values of methomyl and thiodicarb in the present study, indicating that other mechanisms such as insensitive cholinesterase and decreased cuticular penetration may also be involved in imparting resistance to carbamates in *S. litura*.

Table 1 Synergism of selected insecticides by piperonyl butoxide (PBO) and tribufos (DEF) in field populations of *Spodoptera litura*

Population	Treatment	Number of tested larvae	Fit of probit line				LC_{50} (mg/L) (95% CL)	SR at LC_{50} (95% CL)	LC_{90} (mg/L) (95% CL)	SR at LC_{90} (95% CL)
			Slope ± *SE*	x^2	*df*	*P*				
Shujabad[3]	Profenofos	320	1.89 ± 0.17	3.83	6	0.70	104 (83.3 – 129)		494 (360 – 754)	
	Profenofos + PBO	280	2.10 ± 0.21	4.88	5	0.43	79.9 (65.0 – 98.7)	1.3 (0.95 – 1.8)	325 (239 – 494)	1.5 (0.90 – 1.5)
	Profenofos + DEF	280	2.60 ± 0.26	2.85	5	0.72	143 (119 – 172)	0.73 (0.55 – 0.96)	446 (347 – 627)	1.1 (0.70 – 1.77)
	Profenofos + PBO + DEF	240	2.57 ± 0.27	4.38	4	0.36	81.8 (61.2 – 109)	1.3 (1.0 – 1.7)	258 (180 – 469)	1.9 (1.2 – 3.1)
Bosan[4]	Profenofos	320	1.63 ± 0.15	4.25	6	0.64	51.6 (40.7 – 65.7)		314 (219 – 513)	
	Profenofos + PBO	240	2.94 ± 0.31	2.14	4	0.71	84.4 (70.9 – 100)	0.61 (0.46 – 0.84)	231 (183 – 319)	1.4 (0.83 – 2.3)
	Profenofos + DEF	320	1.87 ± 0.17	4.00	6	0.68	95.3 (76.6 – 119)	0.54 (0.39 – 0.75)	460 (336 – 702)	0.68 (0.39 – 1.2)
	Profenofos + PBO + DEF	320	1.91 ± 0.17	3.26	6	0.78	45.4 (36.5 – 56.4)	1.1 (0.82 – 1.6)	213 (156 – 320)	1.5 (0.85 – 2.6)
Khanewal[5]	Profenofos	280	1.91 ± 0.23	4.97	5	0.42	67.5 (49.6 – 88.0)		315 (225 – 513)	
	Profenofos + PBO	240	2.01 ± 0.27	3.17	4	0.53	55.8 (41.8 – 72.4)	1.2 (0.8 – 1.8)	243 (170 – 416)	1.3 (0.7 – 2.4)
	Profenofos + DEF	280	1.80 ± 0.25	2.78	5	0.73	101 (71.8 – 134)	0.7 (0.4 – 1.0)	517 (351 – 948)	0.6 (0.3 – 1.2)
	Profenofos + PBO + DEF	280	1.88 ± 0.24	4.68	5	0.46	38.2 (28.1 – 49.8)	1.8 (1.2 – 2.7)	183 (130 – 301)	1.7 (1.0 – 3.1)
Lar[1]	Methomyl	240	2.49 ± 0.26	2.57	4	0.63	398 (329 – 482)		1 304 (997 – 1 901)	
	Methomyl + PBO	280	2.22 ± 0.22	1.81	5	0.87	202 (165 – 247)	2.0 (1.5 – 2.5)	763 (577 – 1 113)	1.7 (1.1 – 2.6)
	Methomyl + DEF	280	2.07 ± 0.20	4.96	5	0.42	128 (104 – 158)	3.1 (2.3 – 4.1)	535 (397 – 807)	2.4 (1.5 – 3.9)
	Methomyl + PBO + DEF	280	2.41 ± 0.24	2.61	5	0.76	53.0 (43.7 – 64.2)	7.5 (5.7 – 9.8)	180 (139 – 257)	7.2 (4.6 – 11)
Muzafargarh[2]	Methomyl	280	2.23 ± 0.22	3.61	5	0.61	289 (237 – 354)		1 086 (817 – 1 600)	
	Methomyl + PBO	280	2.57 ± 0.26	1.81	5	0.87	133 (110 – 160)	2.2 (1.7 – 2.9)	418 (325 – 588)	2.6 (1.7 – 4.1)
	Methomyl + DEF	240	2.54 ± 0.27	4.90	4	0.30	104 (76.7 – 142)	2.8 (2.1 – 3.6)	331 (224 – 653)	3.3 (2.1 – 5.2)
	Methomyl + PBO + DEF	240	2.76 ± 0.29	3.18	4	0.53	104 (86.9 – 125)	2.8 (2.1 – 3.7)	303 (236 – 428)	3.6 (2.3 – 5.6)
Shujabad	Methomyl	240	2.63 ± 0.28	4.62	4	0.33	603 (448 – 803)		1 854 (1 296 – 3 377)	
	Methomyl + PBO	240	2.59 ± 0.28	4.47	4	0.35	395 (297 – 528)	1.5 (1.2 – 1.9)	1 234 (854 – 2 289)	1.5 (1.0 – 2.2)
	Methomyl + DEF	280	2.32 ± 0.23	3.56	5	0.61	310 (255 – 378)	1.9 (1.5 – 2.5)	1 108 (840 – 1 619)	1.7 (1.1 – 2.5)
	Methomyl + PBO + DEF	240	2.95 ± 0.31	1.92	4	0.75	339 (285 – 403)	1.8 (1.4 – 2.2)	921 (730 – 1 273)	2.0 (1.4 – 2.9)
Muzafargarh	Thiodicarb	280	2.15 ± 0.21	2.50	5	0.78	377 (308 – 466)		1 483 (1 095 – 2 255)	
	Thiodicarb + PBO	280	2.11 ± 0.21	4.38	5	0.50	215 (175 – 265)	1.7 (1.3 – 2.3)	870 (651 – 1 291)	1.7 (1.0 – 2.7)

续表 1 Table 1 continued

Population	Treatment	Number of tested larvae	Fit of probit line				LC_{50} (mg/L) (95% CL)	SR at LC_{50} (95% CL)	LC_{90} (mg/L) (95% CL)	SR at LC_{90} (95% CL)
			Slope ± *SE*	x^2	*df*	*P*				
	Thiodicarb + DEF	280	2.12 ± 0.21	3.15	5	0.68	168 (136 – 207)	2.2 (1.6 – 3.1)	676 (500 – 1 024)	2.2 (1.3 – 3.6)
	Thiodicarb + PBO + DEF	240	2.62 ± 0.28	3.76	4	0.44	190 (158 – 229)	2.0 (1.4 – 2.7)	587 (454 – 842)	2.5 (1.5 – 4.1)
Shujabad	Thiodicarb	280	2.09 ± 0.21	4.39	5	0.49	235 (191 – 289)		962 (717 – 1 437)	
	Thiodicarb + PBO	320	1.95 ± 0.18	3.31	6	0.80	170 (137 – 210)	1.4 (1.0 – 1.9)	768 (569 – 1 146)	1.2 (0.75 – 2.1)
	Thiodicarb + DEF	280	2.23 ± 0.22	3.89	5	0.57	140 (115 – 172)	1.7 (1.3 – 2.3)	528 (398 – 777)	1.8 (1.1 – 3.0)
	Thiodicarb + PBO + DEF	280	2.47 ± 0.24	2.28	5	0.81	117 (96.9 – 142)	2.0 (1.5 – 2.7)	386 (298 – 548)	2.5 (1.6 – 4.0)
Shujabad	Cypermethrin	240	2.53 ± 0.27	4.53	4	0.34	22.3 (16.6 – 29.9)		71.4 (49.2 – 133)	
	Cypermethrin + PBO	280	1.99 ± 0.20	3.01	5	0.70	11.1 (9.00 – 13.9)	2.0 (1.5 – 2.7)	48.9 (35.4 – 76.4)	1.5 (0.9 – 2.4)
	Cypermethrin + DEF	280	2.37 ± 0.23	2.87	5	0.72	9.54 (7.86 – 11.6)	2.3 (1.8 – 3.1)	33.2 (25.3 – 48.1)	2.2 (1.4 – 3.4)
	Cypermethrin + PBO + DEF	280	2.12 ± 0.21	4.36	5	0.50	7.00 (5.68 – 8.60)	3.2 (2.4 – 4.2)	28.1 (21.1 – 41.6)	2.5 (1.6 – 4.0)
Bosan	Cypermethrin	280	2.60 ± 0.22	4.49	5	0.48	114 (93.5 – 140)		423 (321 – 613)	
	Cypermethrin + PBO	280	2.46 ± 0.24	2.28	5	0.81	81.1 (67.1 – 98.3)	1.4 (1.1 – 1.8)	269 (206 – 386)	1.6 (1.0 – 2.4)
	Cypermethrin + DEF	320	1.99 ± 0.18	3.30	6	0.77	26.8 (21.7 – 33.2)	4.3 (3.2 – 5.8)	118 (87.3 – 177)	3.6 (2.2 – 5.8)
	Cypermethrin + PBO + DEF	320	1.94 ± 0.18	3.66	6	0.72	22.3 (18.0 – 27.6)	5.1 (3.8 – 6.9)	102 (75.1 – 152)	4.1 (2.5 – 6.7)
Muzafargarh	λ-cyhalothrin	280	2.43 ± 0.24	2.29	5	0.81	152 (126 – 184)		512 (392 – 736)	
	λ-cyhalothrin + PBO	320	1.91 ± 0.18	3.78	6	0.71	73.4 (59.0 – 91.2)	2.1 (1.6 – 2.8)	345 (254 – 517)	1.5 (0.9 – 2.4)
	λ-cyhalothrin + DEF	280	2.14 ± 0.21	3.99	5	0.55	65.1 (53.0 – 80.0)	2.3 (1.8 – 3.1)	259 (193 – 385)	2.0 (1.3 – 3.2)
	λ-cyhalothrin + PBO + DEF	280	2.33 ± 0.23	3.11	5	0.68	47.8 (39.2 – 58.1)	3.2 (2.4 – 4.2)	169 (130 – 243)	3.0 (1.9 – 4.7)
Shujabad	λ-cyhalothrin	240	2.36 ± 0.25	3.48	4	0.48	94.6 (77.7 – 116)		330 (249 – 493)	
	λ-cyhalothrin + PBO	320	1.83 ± 0.17	5.05	6	0.54	96.9 (77.6 – 121)	0.98 (0.39 – 2.44)	485 (351 – 747)	0.68 (0.22 – 2.1)
	λ-cyhalothrin + DEF	320	1.93 ± 0.18	3.32	6	0.77	75.9 (61.1 – 94.1)	1.2 (0.92 – 1.67)	349 (258 – 522)	0.95 (0.58 – 1.5)
	λ-cyhalothrin + PBO + DEF	280	2.14 ± 0.21	4.22	5	0.52	67.6 (55.1 – 83.1)	1.4 (1.1 – 1.8)	268 (200 – 400)	1.2 (0.76 – 2.0)
Shujabad	Bifenthrin	280	1.99 ± 0.20	2.26	5	0.81	11.4 (9.20 – 14.2)		50.1 (36.3 – 78.3)	
	Bifenthrin + PBO	240	2.31 ± 0.25	2.30	4	0.68	14.3 (11.7 – 17.6)	0.80 (0.59 – 1.1)	51.3 (38.2 – 78.1)	0.98 (0.58 – 1.7)
	Bifenthrin + DEF	240	2.19 ± 0.24	1.49	4	0.83	17.7 (14.4 – 22.0)	0.65 (0.48 – 0.89)	68.2 (49.3 – 110)	0.73 (0.43 – 1.3)
	Bifenthrin + PBO + DEF	240	2.52 ± 0.27	4.89	4	0.30	11.0 (8.04 – 14.9)	1.0 (0.79 – 1.4)	35.4 (24.1 – 68.6)	1.4 (0.88 – 2.3)

续表 2 Table 2 continued

Population	Treatment	Number of tested larvae	Fit of probit line				LC_{50} (mg/L) (95% CL)	SR at LC_{50} (95% CL)	LC_{90} (mg/L) (95% CL)	SR at LC_{90} (95% CL)
			Slope ± *SE*	x^2	*df*	*P*				
Bosan	Bifenthrin	280	1.96 ± 0.19	3.92	5	0.56	14.8(11.9 – 18.4)		66.6(48.9 – 102)	
	Bifenthrin + PBO	320	2.14 ± 0.20	2.27	6	0.89	10.9(8.93 – 13.4)	1.4(1.0 – 1.8)	43.5(32.9 – 63.2)	1.5(0.95 – 2.5)
	Bifenthrin + DEF	320	1.83 ± 0.17	4.28	6	0.64	12.2(9.73 – 15.2)	1.2(0.89 – 1.7)	60.9(44.1 – 93.7)	1.1(0.65 – 1.8)
	Bifenthrin + PBO + DEF	320	1.77 ± 0.16	4.58	6	0.60	4.93(3.92 – 6.19)	3.0(2.2 – 4.1)	26.0(18.8 – 40.2)	2.6(1.5 – 4.3)
Mailsi[6]	Indoxacarb	280	1.60 ± 0.18	1.11	5	0.95	484(359 – 638)		3 055 (2 069 – 5 352)	
	Indoxacarb + PBO	280	1.91 ± 0.19	2.36	5	0.80	395(315 – 492)	1.2(0.9 – 1.8)	1 850 (1 350 – 2 852)	1.7(0.9 – 3.0)
	Indoxacarb + DEF	280	1.89 ± 0.20	3.70	5	0.59	424(326 – 540)	1.1(0.8 – 1.7)	2 026 (1 463 – 3 188)	1.5(0.8 – 2.9)
	Indoxacarb + PBO + DEF	200	2.70 ± 0.36	1.66	3	0.65	376(298 – 461)	1.3(0.9 – 1.9)	1 121 (864 – 1 647)	2.7(1.5 – 4.9)
Multan[7]	Indoxacarb	240	2.61 ± 0.28	1.85	4	0.76	320(266 – 386)		990(769 – 1 409)	
	Indoxacarb + PBO	200	3.42 ± 0.61	4.29	3	0.23	196(96.6 – 291)	1.6(1.3 – 2.1)	465(309 – 2 169)	2.1(1.4 – 3.3)
	Indoxacarb + DEF	240	2.38 ± 0.26	2.05	4	0.73	143(117 – 174)	2.2(1.7 – 2.9)	492(375 – 721)	2.0(1.3 – 3.1)
	Indoxacarb + PBO + DEF	280	2.35 ± 0.29	3.99	5	0.55	130(100 – 162)	2.5(1.8 – 3.3)	455(345 – 677)	2.2(1.4 – 3.4)
Multan	Spinosad	280	1.92 ± 0.19	2.98	5	0.70	36.7(29.4 – 45.8)		170(123 – 266)	
	Spinosad + PBO	320	1.76 ± 0.17	3.50	6	0.74	37.6(28.9 – 48.1)	1.0(0.7 – 1.4)	200(144 – 313)	0.9(0.5 – 1.5)
	Spinosad + DEF	320	1.93 ± 0.22	1.87	6	0.93	18.9(14.1 – 24.3)	1.9(1.4 – 2.8)	86.9(63.8 – 133)	2.0(1.2 – 3.3)
	Spinosad + PBO + DEF	360	1.55 ± 0.15	3.10	7	0.88	12.9(9.72 – 16.8)	2.8(2.0 – 4.1)	86.2(60.4 – 138)	2.0(1.1 – 3.5)

1: Berseem, April 1998; 2: Cotton, November 1998; 3: Cauliflower, January 1999; 4: Cabbage, August 1999; 5: Potato, March 2003; 6: Cotton, October 2003; 7: Cotton, October 2004.

3.3 Pyrethroids

Both PBO and DEF had a synergism with cypermethrin in both the populations of *S. litura* tested in the present studies (Table 1). In the case of λ-cyhalothrin, these inhibitors produced synergism in the Muzafargarh population but showed no synergism in the Shujabad population of *S. litura*. For bifenthrin, both PBO and DEF exhibited no synergism in both the populations tested, indicating the existence of non-metabolic resistance to bifenthrin in *S. litura*. The LC values of cypermethrin and λ-cyhalothrin indicate that there was still an appreciable resistance in *S. litura* that could not be overcome by the use of both the synergists. The resistance to bifenthrin as well as the remaining resistance to cypermethrin and λ-cyhalothrin may be due to mechanisms of decreased nerve sensitivity and/or reduced cuticular penetration.

3.4 New chemicals

PBO and DEF produced no synergism with indoxacarb in the Mailsi population but a significant synergism in the Multan population of *S. litura* (Table 1), showing an enzymatic metabolism of indoxacarb by cytochrome P450 monooxygenases and esterases in the Multan population only. In the case of spinosad, PBO exhibited no synergism but DEF synergised it in the Multan population. No synergism of indoxacarb in one population and a two-fold synergism in another either by PBO or DEF or PBO + DEF, and a two-fold synergism of spinosad by DEF (Table 1) could not fully eliminate the resistance to these new chemicals in the field populations of *S. litura*. This suggests that other mechanisms of resistance such as target-site insensitivity and/or reduced cuticular penetration may also be responsible for conferring resistance to indoxacarb and spinosad in the Pakistani *S. litura*.

4 DISCUSSION

PBO is a well-known inhibitor of cytochrome P450 monooxygenases; but recently, it has been shown to inhibit esterases as well (Gunning *et al.*, 1998; Young *et al.*, 2005, 2006; Kang *et al.*, 2006). On the other hand, DEF is an established esterase inhibitor, but it also acts as a substrate for cytochrome P450 monooxygenases (Sanchez-Arroyo *et al.*, 2001). However, for the purpose of present study, PBO and DEF were considered as inhibitors of cytochrome P450 monooxygenases and esterases respectively.

PBO and DEF showed no synergism with profenofos but exhibited a good synergism with methomyl and thiodicarb in the current studies. These inhibitors also produced no significant increase in toxicity of profenofos to tobacco budworm *Heliothis virescens* (F.) (Kanga and Plapp, 1994). On the other hand, both PBO and DEF had a synergism with methomyl in beet armyworm *Spodoptera exigua* (Hübner) from China (Wang *et al.*, 2002) and cotton bollworm *Helicoverpa armigera* (Hübner) from Australia (Gunning *et al.*, 1992), and therefore the resistance in these pests was attributable to cytochrome P450 monooxygenase and esterase detoxification of methomyl. PBO demonstrated a high synergism with carbaryl in *S. litura* in India (Radhika *et al.*, 2005). Contrarily, PBO and DEF did not decrease the level of carbaryl resistance in *H. armigera* from Thailand (Ahmad and McCaffery, 1991) and of methomyl resistance in the two-spotted mite *Tetranychus urticae* Koch from Greece (Tsagkarakou *et al.*, 2002).

There was a good synergism by both PBO and DEF in the case of cypermethrin in two populations and λ-cyhalothrin in one out of two populations, but no synergism of these inhibitors in the case of bifenthrin in two populations of *S. litura* tested in the present studies. Both the inhibitors were highly synergistic with cypermethrin in resistant strains of *S. exigua* (Wang *et al.*, 2002), fall armyworm *Spodoptera frugiperda* (Smith JE), corn earworm *Helicoverpa zea* (Boddie), black cutworm *Agrotis ipsilon* (Hufnagel) (Usmani and Knowles, 2001), and obliquebanded leafroller *Choristoneura rosaceana* (Harris) (Ahmad and Hollingworth, 2004). PBO and TPP (triphenyl phosphate), an esterase inhibitor, also demonstrated a high synergism with deltamethrin in resistant strains of *S. litura* from China (Huang and Han, 2007). PBO displayed a good synergistic effect with cypermethrin in *S. litura* (Armes *et al.*, 1997), *H. armigera* (Gunning *et al.*, 1991; Kranthi *et al.*, 2001; Yang *et al.*, 2005), *H. virescens* (McCaffery *et al.*, 1991; Martin *et al.*, 1997), cotton aphid *Aphis gossypii* Glover (Jhansi and Subbaratnum, 2004), oriental fruit fly *Bactrocera dorsalis* (Hendel) (Hsu *et al.*, 2004), and parasitoids *Diaeretiella rapae* (McIntosh) (Wu and Jiang, 2003) and *Apanteles plutellae* Kurdj (Wu and Jiang, 2004). The toxicity of cypermethrin was increased by DEF in cotton leafworm *Spodoptera littoralis* (Boisd.) (El-Sayed *et al.*, 1982) and *H. armigera* (Kranthi *et al.*, 1997).

Although not significant statistically, the synergistic effect of DEF on cypermethrin was more than PBO in the present studies. Conversely, PBO but not DEF strongly synergised *cis*-cypermethrin, *trans*-cypermethrin and fenvalerate in a resistant Thai strain of *H. armigera* (Ahmad and McCaffery, 1991). A very high synergism of cypermethrin, λ-cyhalothrin, deltamethrin and fenvalerate by PBO was found in the Pakistani and Chinese populations of *H. armigera* compared with a low synergism of these pyrethroids by DEF in the Chinese populations (Yang *et al.*, 2004, 2005). Biochemical studies confirmed an enhanced rate of pyrethroid detoxification by cytochrome P450 monooxygenase activity as a major metabolic mechanism in resistant *H. armigera* (Martin *et al.*, 2002; Yang *et al.*, 2004, 2005; Chen *et al.*, 2005) and *H. virescens* (Little *et al.*, 1989).

Metabolic inhibitors PBO and DEF enhanced the toxicity of λ-cyhalothrin in honey bee *Apis mellifera* L. (Johnson *et al.*, 2006) and German cockroach *Blattella germanica* (L.) (Valles, 1998). PBO and TPP also synergised λ-cyhalothrin in *T. urticae* and Banks grass mite *Oligonychus pratensis* (Banks) (Yang *et al.*, 2001). PBO eliminated most of the very high resistance to λ-cyhalothrin in a Chinese strain of *H. armigera* (Yang *et al.*, 2005). Contrary to the present studies, cytochrome P450 monooxygenases and esterases were involved in the detoxification of bifenthrin and thus imparting resistance to bifenthrin in mites *T. urticae* and *O. pratensis* (Yang *et al.*, 2001; van Leeuwen *et al.*, 2005). Esterase inhibitor DEF was able to strongly enhance the toxicity of bifenthrin in a resistant strain of *T. urticae* (van Leeuwen and Tirry, 2007).

Compared with other pyrethroids, bifenthrin and λ-cyhalothrin, which have the same acid moiety, seem to resist enzymatic attack as there was no synergism by PBO and DEF for bifenthrin in both the populations and for λ-cyhalothrin in one out of two populations of *S. litura* tested in the present study. This explains why some highly cypermethrin-resistant populations of *H. armigera* had a low resistance to bifenthrin and λ-cyhalothrin (Ahmad *et al.*, 1997; Yang *et al.*, 2005). Also, the Pakistani populations of *S. litura* that had moderate to high resistance to cyfluthrin were having very low resistance to bifenthrin (Ahmad *et al.*, 2007). The same phenomenon was found in pyrethroid-resistant populations of sweetpotato whitefly

Bemisia tabaci (Gennadius) (Byrne *et al.*, 1994) and cotton jassid *Amrasca devastans* (Distant) (Ahmad *et al.*, 1999).

PBO and DEF were synergistic with indoxacarb in one of the two populations of *S. litura* in the present studies. These enzyme inhibitors synergised indoxacarb in a highly resistant strain of *C. rosaceana*; however, the synergism by PBO was much higher than DEF, showing that PBO-suppressible metabolism mediated by cytochrome P450 monooxygenases was a critical mechanism of resistance to indoxacarb in *C. rosaceana* (Ahmad and Hollingworth, 2004). PBO also exhibited a high synergism with indoxacarb in the resistant strains of diamondback moth *Plutella xylostella* (L.) (Sayyed and Wright, 2006) and house fly *Musca domestica* (L.) (Shono *et al.*, 2004).

DEF, but not PBO, exhibited a synergism with spinosad in a field population of *S. litura* (Table 1), which is not understood. This DEF synergism is unlikely due to the inhibition of esterases, which would hydrolyze spinosad. Spinosad does contain an internal ester (lactone) in its macrolide structure that is exceedingly stable, sterically hindered system, which is not available for attack by esterases. Nevertheless, PBO and DEF were both synergistic with spinosad in the resistant *S. exigua* in China (Wang *et al.*, 2006). There was also a high synergism of spinosad by PBO in *B. tabaci* from China (Kang *et al.*, 2006). On the contrary, PBO and DEF did not synergise spinosad in a spinosad-selected strain of *M. domestica* (Shono and Scott, 2003).

A synergism of both carbamates and pyrethroids by PBO and DEF in the same populations of *S. litura* tested in the current studies suggests that these classes of insecticides are cross-resistant due to a common mechanism of metabolic detoxification by cytochrome P-450 monooxygenases and esterases, and this cross-resistance probably extends to new chemicals such as indoxacarb and spinosad. A low level of synergism shown by these enzyme inhibitors demonstrates that other mechanisms of resistance such as target-site insensitivity and decreased cuticular penetration may be of major importance for the observed resistance of *S. litura* to tested insecticides. This implies that the use of synergists will not combat insecticide resistance of *S. litura* under field conditions. The cross-resistance across diverse chemicals and existence of multiple resistance mechanisms in this pest thus make implementation of an insecticide resistance management strategy a difficult task. In this scenario, the valuable new compounds should be applied judiciously and their useful life can be prolonged by limiting their application to one or two sprays per season on a single crop.

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斜纹夜蛾抗性种群中酶抑制剂对杀虫剂的增效作用

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摘要: 采用浸液生测法研究了斜纹夜蛾 *Spodoptera litura* 巴基斯坦抗性种群中酶抑制剂[胡椒基丁醚(PBO)和脱叶膦(DEF)]对丙溴磷、灭多威、硫双灭多威、氯氰菊酯、氯氟氰菊酯、联苯菊酯、茚虫威和多杀菌素等杀虫剂的增效作用。结果表明:PPO 和 DEF 对氨基甲酸酯杀虫剂灭多威和硫双灭多威均具有增效作用,但对有机磷杀虫剂丙溴磷不具有增效作用。两种抑制剂对氯氰菊酯均产生增效作用,但对联苯菊酯没有增效作用。PPO 和 DEF 增加了氯氟氰菊酯对 Multan 种群的毒性,但没有增加其对 Mailsi 种群的毒性。DEF 对多杀菌素具有增效作用,但 PBO 对其没有增效作用。PBO 和 DEF 对氨基甲酸酯杀虫剂、拟除虫菊酯杀虫剂、茚虫威和多杀菌素具有明显的增效作用,这说明细胞色素 P450 单加氧酶和酯酶的解毒作用至少部分参与了斜纹夜蛾对这些杀虫剂的抗性过程。不过,两种增效剂对杀虫剂增效作用范围有限,暗示对于斜纹夜蛾巴基斯坦种群而言,其他的机制(如靶位点不敏感、表皮穿透作用降低)可能是更重要的抗性机制。

关键词: 斜纹夜蛾; 杀虫剂; 抗性; 增效作用; 胡椒基丁醚(PBO); 脱叶膦(DEF)

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