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PERMETHRIN TOXICITY AND SYNERGISTIC EFFECT OF PIPERNOYL BUTOXIDE IN THE FIRST NYMPHAL STAGE OF *BLATELLA GERMANICA* (*ORTHOPTERA: BLATTELLIDAE*)

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Abstract

Tarsal-contact method test using glass petri-dish rather than the conventional glass jar was used to evaluate the permethrin toxicity and synergism effect of piperonal butoxide (PBO) on nymphae of five strains of *Blattella germanica* (L.). The test was carried out on 2-3 days old of first nymphal stage, at four replicates of 10 cockroaches. The susceptible strain showed LT_{50} and KT_{50} of 12.81 and 8.41 minutes respectively. The order of resistance for all strains were GIR>BOY1>BOY2>FAY>SUS. The wild strains showed resistance ratios varied over a range from 4.2 to 6.45 folds for mortality tests and 17 to 23.24 folds for knock-down tests.

Both method of tests showed similar pattern of susceptibility in the wild strains. Comparison of the resistance ratios for mortality and knock-down tests revealed that knock-down test has always showing higher resistance ratio than the mortality test. Toxicity of permethrin alone or with the synergist at different ratios on the susceptible and resistant strains showed that the PBO has modified the response of the wild and susceptible strains to permethrin. The result indicated that increasing the amounts of synergist form 1 to 3 ratio in presence of insecticide, effectively will increase the mortalities, compared with permethrin alone. The 1:3 ratio compietely eliminates the resistance in the wild strains, indicating that PBO could inhibit the activity of mixed-function oxidases as a major metabolic pathway in the resistant strains.

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Introduction

Insecticide resistance in the German cockroach, *Blattella germanica* (L), is a substantial problem that contributes to control problems in many areas (2,7,10,11) German cockroaches have developed resistance to a wide range of insecticides, including organophosphate, organochlorine and pyrethroids insecticides (3,8,9,19).

Although efforts at monitoring for resistance in field-collected strains at the adult stage of German cockroaches have been successful (2), very little information is available on the development of resistance in this species, particularly in the nymphal stage.

This study was under taken to measure the resistance spectrum of permethrin in nymphal stage of four strains of German cockroaches and to gain preliminary information on the mechanism of resistance in these strains with the use of synergist (piperonal butoxide).

Materials and methods

Five strains of German cockroaches were used in this study: 1) SUS a susceptible strain obtained from our insectary at the School of Public Health, Tehran University of Medical Sciences, which has been reared continuously since 1975 without any insecticide exposure. 2) GIR a wild strain, collected from a girlish dormitory of Tehran University. 3) FAY strain, a wild strain collected from Fayyaz Bakhash hospital in Tehran. 4) BOY1 and BOY2 strains, collected from two separated boys dormitories of Tehran University. In order to avoid any heterogeneities in the collected strains, each strain was allowed to rear individually in the rearing jars, then the tests were carried out at the F1 generation. The following insecticide and synergist were used: 1) Permethrin, 93.6% A.I cis/trans: 60/40, Zeneca, 2)Cytochrome p-450 monoxygenase inhibitor = piperonyl butoxide (PBO), 100%, Zigma.

The test was carried out according to the method recommended by Cochran, 1997 with some modification. Insecticide test was conducted in glass petri-dish (9-10 cm of diameter) rather than glass jar. The inside of surfaces of two pieces of petri-dishes were coated with 2 ml of known quantities of acetone insecticide or synergist

solution (1 ml for each inside surfaces). The petri-dishes were rotated evenly in a hood until the acetone evaporated.

After many preliminary knock-down and mortality tests, 15 mg/m² of permethrin in technical grade at 25 minutes of exposure was recognized as a discriminating dose. The tests were carried out on 2-3 days old of first nymphal stage in 3-4 replicates of 10 for each strain. Time-mortality/knockdown responses were recorded at discriminating dose at initially 2-3 minutes interval for susceptible, and longer intervals for resistant strains. Responses were recorded until 90-100% knock-down or mortality occurred at the end of exposure time or after 24 hour holding period respectively. Synergist tests were conducted on both susceptible and resistant strains. The synergist concentrations were related to insecticide concentration by a definite ratio of 1:1, 1:2 and 1:3 according to the method recommended (1). The data from the replicates were pooled and analyzed by probit analysis using a SPSS package on an IBM computer (6). Resistance ratios were calculated as LT_{50}/and or KT_{50} of wild strains divided by LT_{50}/and or KT_{50} of susceptible strain.

Results and discussion

The results of mortality and knock-down tests with permethrin on the five strains are presented in tables 1 and 2. The SUS-strain showed an initial LT_{50} of 12.81 minutes. The wild strains showed resistance ratios varied over a range from 4.2 to 6.45 folds. The order of resistance in the strains compared with the susceptible strain were: GIR>BOY1>BOY2>FAY> SUS with the resistance ratios of 6.44>5.41>5>4.2 folds respectively (see table 1).

Similarly for the knock-down tests, the resistance ratios was calculated based on the KT_{50} of susceptible strain to the KT_{50} of the wild strains. the SUS strain showed a KT_{50} of 8.41 minutes. Therefore the order of resistance for the strains were: BOY1>GIR>SUS and the resistance ratios varied over a range from 17 to 23.24 folds. Both testing methods showed similar pattern of susceptibility in all strains tested.

Comparison of the resistance ratios for mortality and knock-down test revealed that knock-down test always showing higher resistance ratio than the mortality tests. This probably could be a good indication for rapid detection of resistance in a any field population. Toxicity of permrthrin alone or with the synergist at different ratios and synergist alone (PBO) on the susceptible and GIR and BOY1 strains are shown in table 3. PBO modified the response of the wild and susceptible strains to permethrin. The result indicated that increasing the amounts of synergist form 1 to 3 ratio in the presence of insecticide, effectively will increase the mortalities, compared with permethrin alone. The 1:3 ratio completely eliminated the resistance in the GIR and the BOY1 strains, indicating that PBO could inhibit the activity of mixed-function oxidases as a major metabolic pathway in the resistant strains. The effect of three synergists on different strains of German cockroaches, resistant to bendiocarb and pyrethrin was studied (1).

The result support the idea that resistance may be attributed to an enhanced microsomal mixed-function oxidases system which is effectively inhibited by either synergist. Comparison of our results with earlier study showing some similarities in the pattern of resistance. In contract, it was found that resistance to pyrethrins (9.5 fold) in the Keny strain was unaffected by the synergists PBO or S,S,S-tributylphosphorotrithioate (DEF)(10). It was suggested that metabolism is not involved in that case of resistance and *kdr-like* mechanism is involved in the resistance.

Table. 1- The probit regression line parameters of susceptibility tests of different strains of German cockroach

Strain	a[1]	Slope±S.E	LT50 (95% C.L.)	LT_{90} (95% C.L.)	X^2 (d.f.)	RR*
SUS	-4.5214	4.081±0.871	12.81 (10.20-14.77)	26.41 (21.74-40.01)	1.65(2)	-
GIR	-3.4771	1.814±0.308	82.58 (219.03-138.05)	420.14 (219.03-1483.17)	4.181 (5)	6.45
BOY1	-3.4291	1.8625±0.295	69.35 (54.43-97.14)	338.21 (200.54-879.19)	10.732(5)	5.41
BOY2	-2.0689	1.1452±0.243	64.06 (41.95-143.49)	842.82 (288.36-10625)	1.53(5)	5
FAY	-3.3472	1.9346±0.315	53.72 (42.38-70.47)	246.97 (155.99-574.75)	14.608(4)	4.2

RR* = Resistance ratio a[1]= Y-intercept

Table. 3- Effect of synergist (PBO), permethrin, synergist plus permethrin on susceptibility of different strains of *Blattella germanica*

Strains	Exposure time (Minutes)	Insecticide/synergist				
		Synergist (PBO)	Permethrin	Permethrin + Synergist (1:1)	Permethrin + Synergist (1:2)	Permethrin + Synergist (1:3)
SUS	5	(40) 0	(40) 22.5	(40) 55	(40) 60	(40) 100
GIR	25	(40) 0	(40) 40	(40) 40	(40) 75	(40) 100
BOY1	60	(30) 0	(30) 16.6	(30) 30	(30) 70	(30) 100

The figures in brackets represent The number of cockroaches tested

Table. 2- The probit regression line parameters of knock down tests on different strains of German cockroach

Strains	a^1	Slope ± S.E	KT_{50} (95% C.L.)	KT_{∞} (95% C.L.)	X^2 (d.F.)	RR*
SUS	-1.4847	1.60 ± 0.261	8.41 (6.04-10.90)	52.95 (34.36-115.30)	17.32 (13)	-
GIR	-4.0914	1.8986 ± 0.485	142.88 (102.48-326.6)	676.13 (305.10-6009.48)	2.174 (11)	17
BOY1	-1.47	0.6416 ± 0.15	195.47 (97.39-837.9)	19433.69 (2736.57-3363401.59)	10.473 (6)	23.24

RR*= Resistance ratio a^1= Y intercept

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