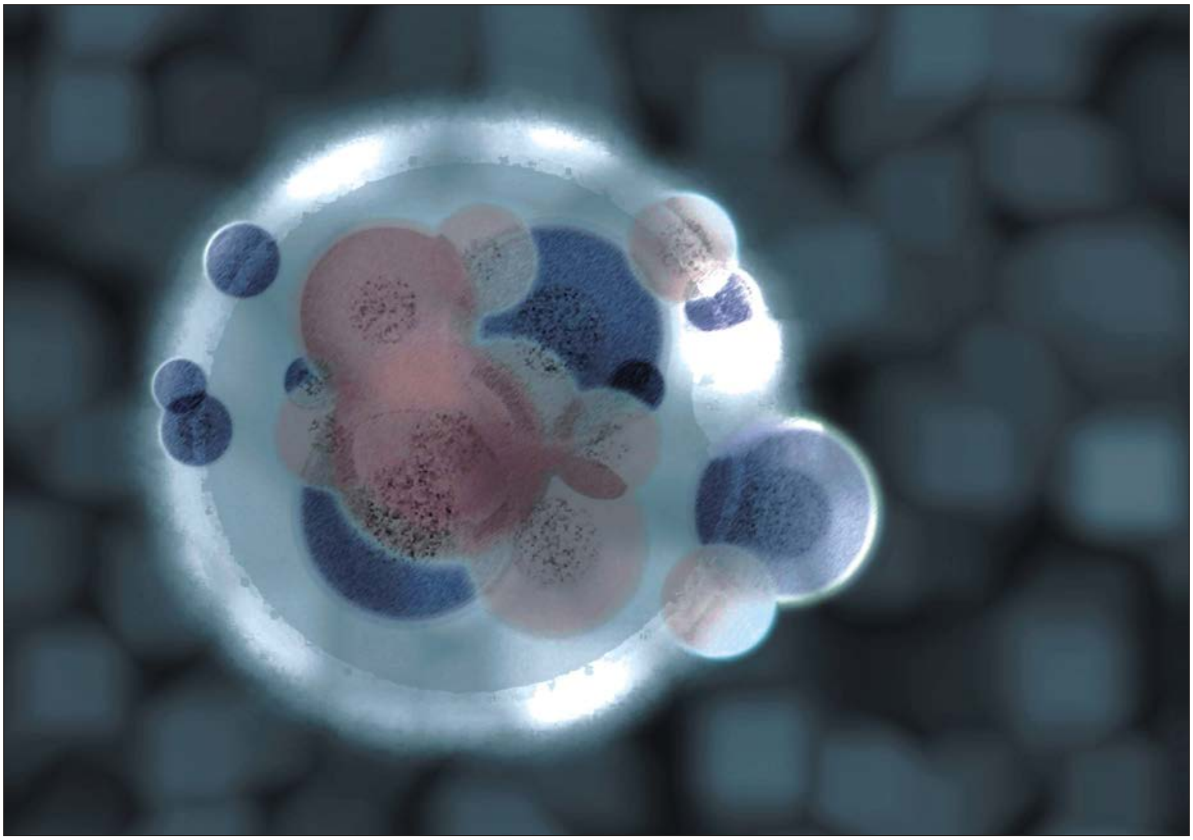

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Detection of Pyrethroid Resistance and Cross Resistance to DDT in Seven Field-collected Strains of the German Cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

[1]M. Limoee, [1]H. Ladonni, [2]A.A. Enayati, [1]H. Vatandoost and [1]M. Aboulhasani
[1]Department of Medical Entomology, School of Public Health,
Tehran University of Medical Sciences, Iran
[2]School of Public Health and Environmental Health Research Center,
Mazandaran University of Medical Sciences, Sari, Iran

Abstract: The insecticide resistance status in seven field collected strains of the German cockroach, *Blattella germanica* (L.) against three pyrethroids: permethrin, cypermethrin and cyfluthrin and cross resistance between these pyrethroids and organochlorine DDT were detected by glass jar test method. For detection of pyrethroid resistance in adult males of field collected strains, the glass jar knockdown test was used and the susceptibility level of each field strain was compared with that of a standard susceptible strain based on Resistance Ratio (RR) calculated by dividing the KT 50 of field strain by the KT 50 of standard susceptible strain. Tests were replicated three to six times in groups of ten cockroaches. For detection of DDT resistance in adult males of the field collected strains, the glass jar mortality test was used to compare the susceptibility level of each field strain with that of susceptible strain based on the mortality rate obtained from exposing them to a single discriminating dose. Tests were replicated three or four times in groups of ten cockroaches. The results of this study indicated that all the field-collected strains of German cockroach were resistant to three pyrethroids: permethrin, cypermethrin and cyfluthrin, i.e., the Resistance Ratios (RRs) of different strains ranged from 5.26 to 23.7 fold for permethrin, 2.9 to 20.7 fold for cypermethrin and 2.4 to 11.42 fold for cyfluthrin, respectively. The order of resistance level to three pyrethroid insecticides was permethrin>cypermethrin>cyfluthrin. Among these seven fields collected strains, five showed high resistance to organochlorine DDT indicating the possible cross resistance between three pyrethroid insecticides used in this study and the organochlorine DDT. Present results demonstrated the differential responses among field collected strains of German cockroach to pyrethroid and DDT insecticides. The information achieved on cross resistance between these three pyrethroid insecticides used in this study and organochlorine DDT could provide the preliminary information for a mechanistic study on possible mechanisms of insecticide resistance in pyrethroid resistant strains.

Key words: German cockroach, pyrethroid, DDT, resistance, cross resistance

INTRODUCTION

The German cockroach, *Blattella germanica* (L.), is a world-wide pest that is very difficult to control. Extensive usage of insecticides may develop resistance to a variety of insecticides. Cochran (1995) reported the resistance to different classes of insecticides such as organochlorines, organophosphates, carbamates and pyrethroids in field strains of German cockroach (Cochran, 1995). Lee *et al*. (1996) reported different levels of resistance to carbamates, organophosphates and pyrethroids in field collected strains of German cockroach in Malaysia.

The effectiveness and low mammalian toxicity of pyrethroid insecticides have resulted in this class of chemical compounds being extensively used for German cockroach control. Nevertheless, frequent use of pyrethroid insecticides has resulted in the development of resistance, causing control failures in some field populations (Valles and Yu, 1996; Dong *et al*., 1998).

Scharf *et al*. (1997) reported 80 fold resistance at LD 50 level to pyrethroid insecticide cypermethrin in a field collected strain of German cockroach from Indiana (Scharf *et al*., 1997). Ladonni (1997) reported different levels of resistance to permethrin, lambdacyhalothrin and sumithrin in field strains of German cockroach and suggested that the observed resistance to sumithrin was likely related to uses of other pyrethroids and hence developing cross-resistance to sumithrin.

Corresponding Author: M. Limoee, Department of Medical Entomology, School of Public Health, Tehran University of Medical Sciences, Iran

The Munsyana strain of German cockroach collected from Indiana, was found to have high level resistance to fenvalerate, displaying 825 fold resistance by topical application compared to a susceptible laboratory strain (Danxia *et al.*, 1998).

Cypermethrin resistance level was investigated in a strain (Aves) of German cockroach collected from Florida residence. Topical bioassay results indicated the Aves strain was 93-fold resistant to cypermethrin compared with a susceptible strain (Valles *et al.*, 2000).

A German cockroach strain, Apyr-R, was collected from Opelika, Alabama after control failures with pyrethroid insecticides. Levels of resistance to permethrin and deltamethrin in Apyr-R strain were 97 and 480 fold, respectively, compared with a susceptible strain, ACY (Wei *et al.*, 2001).

Pyrethroid insecticides have been used extensively to control the German cockroach in Iran and as a result, resistance to this class of insecticides appears to have become prevalent among its populations (Ladonni, 1993, 1997; Ladonni and Sadegheyani, 1998).

Although attempts were made to compare different test methods for detecting resistance of German cockroach strains in Iran (Ladonni, 2000; 2001), very little is known about the relationship between DDT and pyrethroid resistance in field collected strains of German cockroach. Therefore, using three commonly used pyrethroid insecticides, this study was designed to detect pyrethroid resistance and cross resistance to DDT in field-collected strains of the German cockroach.

Although synergistic and biochemical studies are necessary to provide direct evidence for detecting the mechanisms involved in resistance (Valles *et al.*, 1996; Valles, 1998; Danxia *et al.*, 1998; Valles *et al.*, 2000; Wei *et al.*, 2001), the investigation of cross resistance between pyrethroid insecticides and organochlorine DDT provides the preliminary information on possible involvement of glutathione s-transferase enzyme and/or target site insensitivity in pyrethroid resistant strains.

MATERIALS AND METHODS

Cockroach strains: Eight German cockroach strains were examined in this study:SUS is the standard susceptible strain maintained since 1975 in the insectary at School of Public Health. without exposure to insecticide; five strains. M. T, AZAR4. A22. ABAN21 were collected in 2003 from different student dormitories of Tehran University and a strain, BOOSTAN7 collected in 2002 from a student dormitory of Tarbeyat Modarres University and a strain, SAMAN collected in 2002 from residential area in Tehran. These student dormitories and residential area were sprayed with insecticides including pyrethroids, organophosphates and carbamates among which permethrin produced disappointing results (Ladonni, 2001). All cockroaches were maintained in an insectary at 27(2)°C, 60(10%) RH, with a photoperiod of 12:12 h (L:D). Each strain was kept in separate labeled glass rearing jar of the same size. Cockroaches were provided cat food and water. Tests were coducted on adult males of F2-F4 generations.

Chemicals used were permethrin, 93.6% (technical grade) cis:trans 60:40 (Zeneca, Haslemere, UK), Cypermethrin, 97.5% (technical grade), cyfluthrin, 97.5% (technical grade), DDT 98% (technical grade), (Dr. Ehrenstorfer GmbH), CO_2 as an anesthetic and acetone as a solvent.

Bioassays: Susceptibility of adult males of each field strain was compared with that of adult males of Susceptible Strain (SUS) for detection of permethrin, cypermethrin and cyfluthrin resistance. The glass jar test method used to obtain the time to knockdown of pyrethroid insecticide on German cockroach known as the KT method (Cochran, 1996) involves continuous exposure of cockroaches within glass jar (500 mL) coated with a known quantity of insecticide. The surfaces were prepared by placing 2 mL of the appropriate acetone insecticide solution into each jar. Jars were rotated evenly in a hood until the acetone was evaporated. Tests were replicated three to six times in groups of ten cockroaches.

In a series of knockdown experiments. a concentration of 15 mg m^{-2} permethrin and cypermethrin (technical grade) and 3 mg m^{-2} cyfluthrin (technical grade) at a 25 min exposure time was found to be a discriminating dose for adult males to separate resistant from susceptible individuals. Knockdown was recorded at discriminating dose at 5 min intervals.

Susceptibility of adult males of each strain to DDT was determined based on a discriminating dose of 600 mg m^{-2} DDT (technical grade). The adult males were exposed to treated glass jars for 30 min. Tests were replicated three or four times in groups of ten cockroaches per strain. Mortality rates were recorded at the end of exposure time and after a 24 h holding period.

Statistical analysis: Knockdown data from the replicates were pooled and subjected to probit analysis (Finy, 1972) using a statistical package (Probit, 1979) on a personal computer. Resistance ratios were calculated by dividing the KT 50 of the resistant strain by the KT 50 of the susceptible strain.

RESULTS AND DISCUSSION

Detection of permethrin resistance: The knockdown test method was chosen for detecting resistance as Ladonni and Sadegheyani (1998) found by comparison of the resistance ratios for mortality and knockdown test that knockdown test always showing higher resistance ratio than the mortality tests. Therefore, this probably could be a good indication for rapid detection of resistance in any field population. Results of Table 1 indicated that SAMAN strain had the least slope of regression line [1.108849(0.13568)] indicating the highest heterogeneity among the field strains. Comparison among Resistance Ratios (Rrs) with susceptible strain indicated that all the seven field collected strains had significant RR based on overlap of 95% confidence limits of the KT 50 values. Resistance ratios ranged from 5.26 (BOOSTAN7 strain) to 23.7 (ABAN21 strain). These results are consistent with previous studies on permethrin resistance in field strains in which resistance ratios varied over a range of 17 to 23.24 for knockdown tests using tarsal-contact method (Ladonni and Sadegheyani, 1998).

The order of resistance levels for seven field collected strains was as follows: ABAN21>A22>AZAR4> T>SAMAN>M>BOOSTAN7. Comparison among Rrs of all the field collected strains indicated that two strains (M and BOOSTAN7) had low level of resistance. three strains (T, AZAR4 and SAMAN) had moderate level of resistance and two strains (A22 and ABAN21) had high level of resistance to permethrin (Table 2).

Detection of cypermethrin resistance: Comparison among resistance ratios with susceptible strain indicated that all the seven field collected strains had significant RR based on overlap of 95% confidence limits of the KT50 values. Resistance ratios ranged from 2.9 (BOOSTAN7 strain) to 20.7 (ABAN21 strain). The order of resistance level for seven field collected strains was ABAN21>M>A22>T>SAMAN>AZAR4>BOOSTAN7 (Table 3). The most resistant strain to cypermethrin (ABAN21) in this study had significant difference in KT50 value based on overlap of 95% CL with other strains. M strain, the second resistant strain to cypermethrin, had also significant difference in KT50 value based on overlap of 95% CL with other strains. On the other hand, the differences in KT50 values of A22. T, SAMAN and AZAR4 strains based on overlap of 95% CL were not significant. The BOOSTAN7 strain with the lowest RR had significant difference in KT50 value based on overlap of 95% CL with other strains.

Finally comparison among RRs of all the field strains showed that BOOSTAN7 strain had low level of

Table 1: Probit regression line parameters for knock down results of dose-response tests on eight strains of *Blattella germanica*, KT 50 exposure time (min) giving 50% Knock down from tarsal contact with permethrin 15 mg m^{-2}, CL 95% confidence Limits of the mean value; RR, resistance ratio, compared to SUS at KT 50 level

Strain	Y-intercept	Slope(SE)	χ^2 (df)	KT 50 (95% CL)	RR
SUS*	0.632729	4.57601 (0.634194)	4.173 (3)	9.003(7.584-10.332)	1
M	0.354945	2.650417 (0.37405)	1.265 (2)	56.569(46.272-69.2)	6.28
T	1.421019	1.717873 (0.279594)	3.849 (3)	121.166(96.16-152.886)	13.5
AZAR4	-0.048337	2.336105 (0.352631)	0.116 (3)	144.879(122.73-174.27)	16.1
A22	-0.307071	2.312035 (0.396214)	0.955 (3)	197.429(166.19-252.81)	21.9
ABAN21	-0.349542	2.29735 (0.411204)	0.293 (3)	213.094(178.51-279.45)	23.7
BOOSTAN7	-0.028302	3.001379 (0.562339)	7.517 (3)	47.351(39.694-56.603)	5.26
SAMAN	2.747704	1.108849 (0.13568)	4.537 (8)	107.449(86.594-135.116)	11.9

*Reference standard susceptible strain, SUS

Table 2: The significance of differences of Resistance Ratios (RRs), compared to SUS# at KT 50 level from tarsal contact with permethrin 15 mg m^{-2} [AI] between different strains based on overlap of 95% CL of the KT50 values

STRAIN	M	T	AZAR4	A22	ABAN2	BOOST	SAMA
SUS#	6.28(S)*	13.5(S)	16.I(S)	21.9(S)	23.7(S)	5.26(S)	11.9(S)
M							
T	7.22(S)						
AZAR4	9.82(S)	2.6(NS)**					
A22	15.62(S)	8.4(S)	5.8(NS)				
ABAN21	17.42(S)	10.2(S)	7.6(S)	1.8(NS)			
BOOSTAN7	1.02(NS)	8.24(S)	10.84(S)	16.64(S)	18.44(S)		
SAMAN	5.62(S)	1.6(NS)	4.2(NS)	10(S)	11.8(S)	6.64(S)	

#Reference Standard Susceptible, SUS *Significant **Nonsignificant

Table 3: Probit regression line parameters for knock down results of dose- response tests on eight strains of *Blattella germanica*, KT50 exposure time(min) giving 50% Knock down from tarsal contact with cypermethrin 15 mg m^{-2}, CL 95% confidence Limits of the mean value; RR, resistance ratio, compared to SUS at KT50 level

Strain	Y-intercept	Slope(SE)	χ^2(df)	KT50(95% CL)	RR
SUS*	1.375104	3.703463	1.138	9.523	1
		(0.527148)	(2)	(8.134-10.893)	
M	1.490367	1.738267	0.683	104.48	10.97
		(0.333208)	(4)	(80.62-162.83)	
T	0.61043	2.537239	0.475	53.71	5.64
		(0.350565)	(3)	(44.797-63.53)	
AZAR4	-0.737502	3.375134	3.323	50.111	5.26
		(0.442348)	(4)	(44.03-56.521)	
A22	-0.882871	3.250238	0.973	64.563	5.78
		(0.497631)	(3)	(56.243-74.58)	
ABAN21	-3.648167	3.783756	1.163	193.021	20.27
		(0.771781)	(3)	(165.988-258.196)	
BOOSTAN7	1.896918	2.153211	6.964	27.615	2.9
		(0.3162)	(3)	(21.919-34.035)	
SAMAN	-1.198513	3.614263	2.553	51.882	5.45
		(0.549225)	(4)	(46.128-60.684)	

Reference Standard Susceptible Strain, SUS

Table 4: Probit regression line parameters for knock down results of dose- response tests on eight strains of *Blattella germanica*, KT 50 exposure time (min) giving 50% Knock down from tarsal contact with cyfluthrin 15 mg m^{-2}, CL 95% confidence limits of the mean value; RR, resistance ratio, compared to SUS at KT50 level

Strain	Y-intercept	Slope(SE)	χ^2(df)	KT 50 (95% CL)	RR
SUS*	0.65238	4.736266	3.545	8.278	1
		(0.712095)	(2)	(6.97-9.516)	
M	1.944641	1.878913	9.403	42.28	5.11
		(0.246363)	(5)	(33.31-51.69)	
T	-0.124974	3.24668	2.39	37.89	4.58
		(0.54311)	(2)	(32.435-44.741)	
AZAR4	2.414464	1.601821	2.942	41.127	4.97
		(0.236818)	(5)	(32.353-51.898)	
A22	-0.664833	3.266934	1.517	54.199	6.55
		(0.645629)	(2)	(46.501-68.423)	
ABAN21	-2.40063	3.745926	0.268	94.547	11.42
		(0.767013)	(3)	(82.927-119.85)	
BOOSTAN7	2.634036	1.822328	1.211	19.876	2.4
		(0.270696)	(3)	(14.731-25.336)	
SAMAN	1.930901	2.229744	8.047	23.792	2.87
		(0.279397)	(4)	(18.735-28.872)	

Reference Standard Susceptible Strain. SUS

Table 5: Mortality test data from 30 min exposure to a single dose 600 mg [AI] m^{-2} of DDT on adult males of eight German cockroach strains after 24 h holding time

Strain	Alive	Dead	Total	%Mortality
SUS	0	30	30	100
M	30	10	40	25
T	40	0	40	0
AZAR4	12	28	40	70
A22	40	0	40	0
ABAN21	40	0	40	0
BOOSTAN7	40	0	40	0
SAMAN	40	0	40	0

resistance, four strains (A22,T,SAMAN and AZAR4) had moderate level of resistance, M strain had relatively high level of resistance and ABAN21 strain had high level of resistance to cypermethrin.

Detection of cyfluthrin resistance: Comparison among resistance ratios with susceptible strain indicated that all the seven field collected strains had significant RR based on overlap of 95% CL of the KT50 values. Resistance ratios ranged from 2.4 (BOOSTAN7 strain) to 11.42 (ABAN21 strain) (Table 4). The order of resistance level for seven field collected strains was: ABAN21>A22>M>AZAR4>T>SAMAN>BOOSTAN7.

ABAN21 strain with highest RR had significant difference in KT50 value based on overlap of 95% CL with other strains. SAMAN and BOOSTAN7 strains with the lowest RRs, 2.87 and 2.4, respectively had no significant difference in KT50 value based on overlap of 95% CL with each other but had significant difference in KT50 values based on overlap of 95% CL with other strains.

Finally comparison among RRs of all the field collected strains indicated that two strains (BOOSTAN7 and SAMAN) had very low level of resistance, four strains (M, T, AZAR4 and A22) had low to moderate level of resistance and ABAN21 strain had relatively high level of resistance to cyfluthrin.

Comparison among results from knockdown assays of three pyrethroid insecticides on adult males of eight

German cockroach strains suggested that the order of resistance level to three pyrethroid insecticides was permethrin>cypermethrin>cyfluthrin. It can be suggested that the highest resistance level to permethrin, compared with the resistance levels to cypermethrin and cyfluthrin is due to a long history of residual application of permethrin for more than 20 years (Ladonni, 1997). Data presented here indicated the lowest resistance level to cyfluthrin compared to the other pyrethroid insecticides used in this study, probably because cyfluthrin has been introduced in Iran for house hold pest control since 1997, however, this insecticide has not been used extensively (Ladonni, 1997). The relatively high level of resistance to cypermethrin demonstrated in this study, may be due to extensive usage of this insecticide for German cockroach control in Iran. Zhai and Robinson (1991) reported high level of resistance to cypermethrin and control failure of cypermethrin against German cockroach after about 4 years of use.

Detection of DDT resistance: As it has been mentioned previously, M strain has a low level of resistance to permethrin, relatively high level of resistance to cypermethrin and moderate level of resistance to cyfluthrin, Table 5 shows that, this strain is relatively resistant to DDT. Although AZAR4 strain has moderate level of resistance to permethrin, cypermethrin and cyfluthrin, it has relatively low level of resistance to DDT. As it has been mentioned before, the strains, T, A22, ABAN21, BOOSTAN7 and SAMAN have different levels of resistance (from low to high) to permethrin, cypermethrin and cyfluthrin. Table 5 shows that these strains are also resistant to DDT. These results were similar to those of Lee *et al*. (1996) where five out of twelve field collected strains tested were resistant to DDT and some pyrethroid insecticides (Lee *et al*., 1996). Cross resistance often occurs between insecticides which are chemically related or which share a common mode of action. Present results demonstrate the differential responses among field collected strains of German cockroach to pyrethroid and DDT insecticides. Although the relationship between DDT and pyrethroid resistance was studied in a DDT resistant (kdr) strain of *B. germanica* in some areas (Scott and Matsumura, 1981, 1983; Scott *et al*., 1986), very little is known about the relationship between DDT and pyrethroid resistance in field collected strains of German cockroach in Iran.

Scott and Matsumura (1981, 1983) reported a DDT resistant strains of *B. germanica*, that was cross-resistant to all pyrethroids thus providing evidence for the first time of a kdr-type mechanism in *B. germanica* (Scott and Matsumura, 1981; 1983).

It can be suggested that there is a possibly cross resistance between pyrethroid insecticides used in this study and organochlorine insecticide DDT. The information achieved on cross resistance between three pyrethroid insecticides used in this study and DDT could provide the preliminary information for a mechanistic study on possible involvement of glutathione s- transferase enzyme and/or target site insensitivity in pyrethroid resistant strains.

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