Original Article

Toxicity of Pyrethroid and Organophosphorous Insecticides against Two Field Collected Strains of the German Cockroach *Blattella germanica* (Blattaria: Blattellidae) from Hospitals in Hamadan, Iran

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Abstract

Background: The German cockroach, *Blattella germanica* is a major hygienic pest and mechanical vector for pathogenic agents in hospitals and residential areas. The development of insecticide resistance is a serious problem in controlling of this pest. Toxicity of four commonly used insecticides (permethrin, cypermethrin, malathion and chlorpyrifos) against two hospital- collected strains of the German cockroach was investigated.

Methods: Topical bioassay methods were carried out for detecting insecticide susceptibility of adult male cockroaches. For each insecticide, four to six concentrations resulting in >0% and <100% mortality were used. Three to six replicates of 10 cockroaches per concentration were conducted. The differences between LD_{50} (µg/g) values were considered statistically significant only when the 95% confidence intervals did not overlap.

Results: Two hospital- collected strains of the German cockroach showed low to moderate levels of resistance to chlorpyrifos, permethrin, malathion and cypermethrin based on resistance ratios compared with susceptible strain.

Conclusion: The low level chlorpyrifos resistance suggesting this insecticide may still provide adequate control of these strains. While the obsereved moderate levels of resistance to cypermethrin could imply developing resistance to this compound.

Keywords: Insecticide resistance, Pyrethroid, Organophosphate, German cockroach

Introduction

The German cockroach, *Blattella germanica* (L) is a major hygienic pest in households, hospitals and residential areas. These insects have been recognized as mechanical vectors and reservoir for pathogenic agents (Pai et al. 2003, 2005, Kinfu and Erko 2008). In addition to mechanical transmission of pathogens, large indoor cockroach populations are also one of the causes inducing asthma (Roberts 1996, Miller and Koehler 2003, Kinfu and Erko 2008).

Insecticide resistance is now a serious problem, challenging the control of the German - cockroach. In addition, selection with some insecticides could confer resistance to the new insecticides through cross-resistance (Wei et al. 2006). The development of resistance to different classes of insecticides in field-collected strains of the German cockroach, *Blattella germanica* has been reported frequently (Cochran 1995, Lee et al. 1996, Pai et al. 2005).

Pai et al. (2005) determined the resistance of the German cockroach from hospitals and

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households to propoxur, chlorpyriphos and cypermethrin in Taiwan. They concluded that the resistance patterns of propoxur> chlorpyriphos> cypermethrin in hospital strains and propoxur> cypermethrin> chlorpyriphos in household strains, might be due to the frequency of application of the insecticides.

Chai and Lee (2010) determined the resistance of 22 field- collected strains of the German cockroach from various localities of Singapore to 6 classes of insecticides.

Among the different classes of insecticides, the effectiveness and low mammalian toxicity of pyrethroids have resulted in these compounds being extensively used for cockroach control. Nevertheless, due to frequent use of these compounds, control failure in some field populations have been reported resulting from the development of pyrethroid resistance (Walles and Yu, 1996, Dong 1998, Valles 1998, Valles et al. 2000, Wei et al. 2006).

The insecticides from different chemical groups including organophosphorous, carbamates and particularly pyrethroids have been used extensively to control the German cockroach in Iran and as a result, insecticide resistance appears to have become a prevalent among its populations (Ladonni 1993, 1997, Ladonni and Sadegheyani 1998, Limoee et al. 2006, 2011).

Earlier, we found pyrethroid resistance (including permethrin, cypermethrin and cyfluthrin) and cross resistance to DDT in seven field-collected strains from Tehran, Iran (Limoee et al. 2006). Synergistic studies and biochemical assays revealed that the metabolic mechanisms were involved in resistance to permethrin in those strains mentioned above (Limoee et al. 2007). Nasirian (2010) summarized the susceptibility of different strains of the German cockroach to different classes of insecticides in Iran (Nasirian 2010). In a recent article, resistance to organophosphorous carbamate and pyrethroid insecticides, were reported in three hospital- collected strains from Kermanshah, Iran. Permethrin resistance was suppressible by synergist PBO, suggesting the oxidases involvement in resistance (Limoee et al. 2011).

Although attempts were made to determine the insecticide resistance of the German cockroach populations from different provinces of Iran, such as Tehran and Kermanshah (Ladonni 1993, 1997, Ladonni and Sadegheyani 1998, Limoee et al. 2006, 2011, Nasirian 2006), there is no information on insecticide resistance status in hospital strains of the German cockroach in Hamadan, a western province of the country. Therefore, this study was undertaken to detect possible insecticide resistance in two hospital-collected strains of the German cockroach using four commonly used insecticides from different classes including permethrin, cypermethrin, malathion and chlorpyrifos.

Materials and Methods

Cockroach Strains

Three German cockroach strains were examined in this study: SUS is the standard susceptible strain maintained since 1975 in the insectary at the School of Public Health, Tehran University of Medical Sciences without exposure to insecticides; two strains EcH and FH were collected in 2010 from two hospitals in Hamadan.

Spraying with different insecticides including pyrethroids and organophosphates were not very effective in controlling of the German cockroach in those hospitals (personal communication).

All cockroaches were maintained in an insectary at 27±2 ºC, 60±10% RH, with a photoperiod of 12:12 h (L: D). Each strain was reared in the same size labeled glass jar. Cockroaches were provided with unlimited cat food and water. Tests were conducted on adult males of F_2–F_4 generations.

Chemicals

Chemicals used were permethrin, 93.7% (technical grade) cis:trans 60:40, cypermethrin, 97.5% (technical grade), (Zeneca, Haslemere,

UK), chlorpyripfos, 97% (technical grade) and malathion, 92% (technical grade), (Cyanamid Agro, India). CO_2 was used as anesthetic and acetone as solvent.

Bioassays methods

Bioassay tests were performed by topical application of 1µl of a known concentration of insecticide solution to the first abdominal segment of the insects, using a hand micro- applicator (Burkard, Scientific Ltd, UK) equipped with a 1.0 ml Hamilton glass syringe.

Adult male cockroaches were anesthetized with carbon dioxide for 20–30 seconds before insecticide treatment (Valles 1994). For each insecticide, four to six concentrations resulting in >0% and <100% mortality were used.

Three to six replications of 10 cockroaches per concentration were conducted. Control groups received acetone alone. Treated cockroaches were kept in Pyrex glass jars provided with food and water before scoring the mortality. Cockroaches were considered dead when they were unable to turn themselves to normal posture within one minute after being turned onto their dorsum.

Data analysis

Bioassay data were pooled and subjected to probit analysis (Finny 1972), using a personal computer. The differences between LD_{50} values were considered statistically significant only when the 95% confidence intervals did not overlap. All LD_{50} values were converted from µg/cockroach to µg/g of cockroach body weight to avoid possible effect of weight differences on insecticide susceptibility. The Resistance Ratios (RRs) were calculated by dividing the LD_{50} of the resistant strain by the LD_{50} of the susceptible strain.

Results

Toxicity of permethrin

Both hospital-collected strains of the German cockroach showed the similar levels of resistance to permethrin based on resistance ratios (RRs) compared with SUS strain (RR values for FH and ICH strains were 3.36 and 3.15, respectively), (Table 1).

Comparisons made between the 95% confidence intervals of the LD_{50} values of field strains with susceptible strain (SUS) indicated that both strains had significantly different RRs ($P< 0.05$) (Table 1).

Toxicity of cyprmethrin

Different levels of resistance to cypermethrin were observed in two hospital-collected strains of German cockroach based on RRs compared with susceptible strain (SUS) (Table 1) showing resistance ratios, 3.23 and 6.18 at LD_{50} levels, for ICH and FH strains, respectively.

Comparisons made between the 95% confidence intervals of the LD_{50} values of field strains with susceptible strain (SUS) indicated that both strains had significantly different RRs ($P< 0.05$) (Table 1).

Toxicity of malathion

Two hospital-collected strains of German cockroach showed the similar levels of resistance to malathion with significant RRs based on overlap of 95% confidence intervals of the LD_{50} values, compared with susceptible strain (SUS) ($P< 0.05$). Resistance ratio values for FH and ICH strains were 5.2 and 6.23, respectively (Table 2).

Toxicity of chlorpyriphos

Two hospital-collected strains of German cockroach had similar resistance levels to chlorpyriphos with significant RRs based on overlap of 95% confidence intervals of the LD_{50} values, compared with susceptible strain (SUS) ($P< 0.05$). Resistance ratio values for FH and ICH strains were 2.2 and 2.4, respectively (Table 2).

Table 1. Lethal dose values for permethrin and cypermethrin for insecticide susceptible and two hospital collected strains of German cockroach

Strain	n	Y- intercept	S lope(SE)	X^2 (df)	LD50 (95% CL) µg/g[a]	RR[b]
SUS[c] Permethrin	201	0.99	3.89 (0.49)	3.621 (4)	10.70 (9.44–12.1)	1
Cypermethrin	169	3.11	3.3 (0.42)	3.57 (2)	3.74 (3.18–4.44)	1
FH Permethrin	169	0.24	3.06 (0.37)	1.03 (3)	35.97 (29.56–43.79)	3.36[sig]
Cypermethrin	149	0.87	3.03 (0.40)	0.77 (3)	23.13 (18.96–28.46)	6.18[sig]
ICH Permethrin	149	0.81	2.74 (0.37)	1.30 (3)	33.70 (27.17–42.00)	3.15[sig]
Cypermethrin	190	2.83	2.01 (0.30)	2.63 (3)	12.07 (9.06–15.19)	3.23[sig]

[a] Micrograms of insecticide/g of cockroach body weight
[b]Resistance Ratio
[c]Susceptible strain
[sig]Significant

Table 2. Lethal dose values for malathion and chlorpyriphos for insecticide susceptible and two hospital collected strains of German cockroach

Strain	n	Y- intercept	S lope(SE)	X^2 (df)	LD50 (95% CL) µg/g[a]	RR[b]
SUS[c] malathion	157	1.39	2.71 (0.38)	1.39 (2)	21.47(17.48–26.11)	1
chlorpyriphos	183	1.31	4.91 (0.64)	6.96 (3)	5.66(5.11–6.33)	1
FH malathion	120	- 2.02	3.43 (0.52)	5.68 (2)	111.66(92.23–134.29)	5.20[sig]
chlorpyriphos	160	- 0.05	4.61 (0.61)	5.42 (2)	12.46(10.91–14.00)	2.20[sig]
ICH malathion	120	- 2.32	3.44 (0.56)	0.05 (2)	133.75(111.38–163.17)	6.23[sig]
chlorpyriphos	160	- 0.28	4.68 (0.66)	0.71 (2)	13.47(11.91–15.20)	2.40[sig]

[a]Micrograms of insecticide/g of cockroach body weight
[b]Resistance Ratio
[c]Susceptible strain
[sig]Significant

Discussion

In contrast to our previous study (Limoee et al. 2006, 2011), the present study showed the low to moderate resistance levels of collected strains to pyrethroid, organophosphorous and carbamate insecticides. Hence, it could be suggested that the resistance to those group of insecticides are developing.

Several researchers previously concluded that the lethal dose (LD) method especially by topical application might be appropriate for determining susceptibility levels of the German cockroach strains to different insecticides because the amount applied could be precisely measured (Milio 1987, Scott and Cochran 1990, Choo et al. 2000, Ladonni 2001). Thus, this method was used throughout this study.

According to Reierson et al. (1998) 10 fold resistance measured by topical application is the critical point above which operational control failures are likely to occur while, resistance ratio at 5x and below may still achieve a good control of the German cockroach population.

Although we observed the high level of resistance to permethrin and cypermethrin in different field collected strains of the German cockroach in our previous studies (Limoee et al. 2006, 2011), being consistent with another study on pyrethroid resistance in some populations of this insect (Ladonni and

Sadegheyani 1998), in present study, the low to moderate resistance levels of collected strains from hospitals of Hamadan to pyrethroid insecticides compared with the susceptible strain were detected. Our findings probably suggested that these strains had not been selected especially by permethrin for several generations. Nevertheless, cypermethrin resistance seemed to be higher (RR: 6.18) than resistance to permethrin.

Continued use of pyrethroids may lead to a relatively high selection pressure. As reported by Zhai and Robinson (1991), the use of cypermethrin against the German cockroach about 4 years resulted in high level of resistance and control failure (Zhai and Robinson 1991), while, the pyrethroid insecticides have been recently replaced with some carbamates or organophosphates such as bendiocarb and chlorpyriphos in controlling of German cockroach populations of hospitals in Hamadan, Iran (Personal communication).

We can conclude that according to Reirson et al. (1998), the resistance ratio of these two strains to chlorpyriphos compared with the susceptible strain probably reflect a relatively low levels of resistance to this compound because these strains have not been selected by chlorpyrifos for several generations. Hence chlorpyrifos may still provide adequate control of these strains. These results are consistent with our previous studies on chlorpyrifos resistance in some populations of the German cockroach from Kermanshah, Iran which resistance ratios ranged from 1.20 to 2.18 for chlorpyrifos (Limoee et al. 2011).

Lee et al. (1996), reported low levels of resistance to propoxure due to low frequency of insecticide applications (bi yearly). Similarly, the two strains in this study were subjected to a low frequency of malathion application and then replaced by chlorpyrifos (Personal communication). These strains exhibited moderate levels of resistance to malathion (>5x) probably because of low frequency of application and short term usage of malathion. Indeed, based on our findings in this study, it can be suggested that chlor-pyrifos could still be a potent toxic organo-phosphate in controlling of German cock-roach populations in the hospitals of Hamadan. On the other hand, a mixture of carbamate and cypermethrin has recently been used against hospital strains of German cockroach in Kermanshah (Limoee et al. 2011).

The majority of toxicological and biochemical studies suggest that insecticide resistance in the German cockroach can be mediated by a number of mechanisms, the most common being enhanced enzymatic metabolism (Walles and Yu 1996, Valles et al. 2000, Wei et al. 2001). The synergistic studies supported by biochemical assays implicated that p450 monooxygenases hydrolases were involved in permethrin resistance in some strains of this insect in Iran (Limoee et al. 2007).

Finally, we concluded that the low level chlorpyrifos resistance suggesting this insecticide may still provide adequate control of these strains. While the observed moderate levels of resistance to cypermethrin could imply developing resistance to this compound.

Thus, we propose further studies on changes insecticide resistance levels by using the different synergists following insecticide selection in these strains for providing *in vivo* evidences about the possible mechanisms of insecticide resistance.

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