

Determination of susceptibility levels of three different cockroach species including hospitals German cockroach, *Blattella germanica* L. (Blattodea: Blattellidae), to common insecticides, cypermethrin, propoxur and fenitrothion

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ABSTRACT

Objective: German cockroach is highly adapted to different environments like hospitals. This pest is an important carrier of pathogenic agents and allergic compounds. Hence, it is important to German cockroaches always be monitored and controlled. This study investigated the toxicity and resistance levels of technical-grade of cypermethrin, propoxur, and fenitrothion against adult males of four strains of the German cockroach.

Methods: Four German cockroaches' strains including laboratory-reared susceptible strain (S) and three hospital-collected strains (B, F, and Z) were tested in this study. Adult male cockroaches were treated topically with three technical grade insecticides on the first abdominal segment of the insects using a hand micro-applicator.

Results: Using topical application methods, fenitrothion showed the most toxicity to all four strains. The LD₅₀ values of fenitrothion in the susceptible strain (S) and the hospital-collected strains B, F, and Z were 1.89, 21.48, 25.73, and 31.55 µg/grBW, respectively. All hospital-collected strains showed different resistant levels to all insecticides. The field-collected Z strain was the most resistant strain to cypermethrin, propoxur, and fenitrothion. The resistance ratios of strain Z to cypermethrin, propoxur, and fenitrothion were 10.9, 10.47 and 16.67, respectively.

Conclusion: Based on the susceptibility and resistance ratios for each insecticide, we conclude that there are high rates of insecticide resistance in *Blattella germanica* from different hospitals of Sari County which can be caused by different insecticides treatment histories.

Keywords: Susceptibility, insecticide resistance, topical application, *Blattella germanica*, Iran

Introduction

Cockroaches as the oldest inhabitants of the planet have lived on earth for over 250 million years. This species has a worldwide distribution, especially in tropical and subtropical regions, and often lives in places such as hospitals, hotels, homes.^[1] To date, over 4,000 species of cockroaches have been reported from around the world. Among these, three species including German cockroach (*Blattella germanica* L.), American cockroach (*Periplaneta Americana* L.) and oriental cockroach (*Blatta orientalis* L.) mainly cause many health problems in urban areas.^[2] The particles from their feces, secretions and the exuviae is containing allergic compounds so

that, can cause asthma, dermatitis, itching and acute respiratory diseases.^[1,3-5] Furthermore, these species are naturally infected with about 40 different species of vertebrate pathogenic bacteria, such as *Citrobacter* and *Proteus*, viruses and fungi which are responsible for a wide range of infections. They are also capable of transmitting at least seven species of intestinal worms.^[1,6,7]

Among these important species, German cockroach is one of the most well-known and -common household pests.^[8] *B. germanica* is highly adapted to different environments and easily reproduces in human places such as homes, hospitals, kitchens, bakeries, and restaurants.^[9,10] This behavior of

B. germanica has caused that the German cockroach create a lot of economic damage to a wide range of the human products such as food sources and other human properties.^[2] But more importantly, *B. germanica* is an important carrier of bacteria and other pathogenic agents and due to its specific nutritional habits, fecal excretion, fast moving, and adaptability to living in residential environments, can mechanically transmit these pathogens to humans;^[10-12] which indicates the role of this species in the spread of various infections especially in nosocomial infections.^[13] Since this theme can endanger the health of people and society, as a result, it is important to German cockroaches always be monitored and controlled. In general, there are different ways to control cockroaches' populations such as prevention, non-chemical and chemical control.^[14,15] Among the methods aforementioned, chemical control is very common; usually by liquid, gel, solid, and foam formulations.^[4,10,16]

Most of the chemical insecticides used in the chemical control of German cockroaches are divided into three groups: pyrethroids, carbamates, and organophosphates.^[10,17] Unfortunately, the widespread and repeatedly using of these insecticides under cockroach control programs has caused that *B. germanica* be resistance to a wide range of chemical insecticides in many parts of the world including Iran.^[4,17] So that, among the "Top 20" insecticide-resistant insects species, German cockroach ranks is 17th.^[18] Moreover, occurrence of resistance, and consequently, repeated application of insecticides to the surfaces used may not only does not control the cockroach population but may also be harmful to humans health.^[4,19-21] Therefore, the success of the cockroach control programs may requires to careful implementation of management programs to identify different levels of cockroach resistance to insecticides. For example, the constant use of spray formulations of pyrethroids caused that German cockroach be resistance to this insecticide and causing the pyrethroids to be ineffective against German cockroach control programs.^[4]

In Iran, German cockroaches are found over a wide geographical range including in northern Iran; and due to the repeated use of insecticides pervasive resistance to different insecticides has been observed.^[17,22] More recently, resistance to cypermethrin,^[23] propoxur,^[24] and fenitrothion^[25] was detected in German cockroach populations collected from different parts of Iran. However, the studies on determination of resistance of German cockroach to insecticide in hospitals, where the patient and its residents are highly exposed to nosocomial infections,^[26-29] are very necessary. Because of the inefficiency of chemical control can lead to frequent use of pesticides and consequently not only does not control the German cockroaches, but also hospital patients and residents will be exposed to residuals of pesticides. Therefore, we planned a study to evaluate insecticides resistance in German cockroaches collected from three selected hospitals of Sati County; where three groups of insecticides including pyrethroids, carbamates, and organophosphates are frequently

used. In our study, resistance was characterized by topical application^[4] of cypermethrin, propoxur, and fenitrothion. The objective of this study was to compare different levels of cockroach resistance to these insecticides between susceptible and hospital populations. These findings can provide compelling support for successful implementation of German cockroach control programs especially in hospitals.

Materials and Methods

Cockroach populations

Four German cockroaches' strains were used in this study. Three strain of *B. germanica* were collected from different infested hospitals of Sari County (36.5659° N, 53.0586° E). The history of using of pyrethroids, carbamates, and organophosphates was also considered in selection of hospitals [Table 1]. One standard susceptible strain (control treatment) was also prepared from the medical entomology insectarium of Tehran University of Medical Sciences to determine resistance ratios (RR). This strain was maintained since 1975 in insectaria and has never exposed to the insecticides. Susceptible cockroaches were transferred to insectarium of entomology laboratory of Mazandaran University of Medical Sciences, Sari and were colonized in 25-L plastic container (40 × 40 × 40 cm) sealed with net cloth with fine mesh, under controlled conditions, temperature 27±2°C, relative humidity 70±5% and photoperiod of 12:12 (L: D) h.^[30] German cockroaches from infested hospitals were trapped and/or were manually hand caught using a broom. Traps were paper carton (40 × 40 × 40 cm) contained with bread + crushed newspaper, which was very good for attraction of cockroaches. Traps were placed at positions known to be infested with cockroaches, for example, kitchen, below the cooking range, dishwashers, sinks, bathroom, wardrobe, and patient rooms. Approximately 3 traps per place were set up in the last hours of the night until the next morning (from 18:00 PM to 8:00 AM). All live cockroaches were transferred to insectarium and were maintained in 25-L plastic container provided with water, bread and soya. Each strain was kept in separate labeled plastic container. The upper inside surface of the container (5 cm) was lightly smeared with edible paraffin to prevent cockroaches from escaping. Only healthy adult males of German cockroach were considered in all bioassays because of their weight and physiology are more uniform than females.^[31]

Body mass

Mean body masses of the each strain were measured by weighing 20 randomly selected male adult cockroaches of each hospital. Cockroaches were weighed on an analytical laboratory balance (HR-200, A&D Co. Japan) with a resolution of 0.0001 gr.

Chemicals

Technical grade of cypermethrin (>93% purity), propoxur (>97% purity), and fenitrothion (>95% purity) was a gift from Aryashimi Company (Iran). To solve the technical form of

insecticides, acetone solvent (>99.8% purity) was used. Dilutions ranged from 0 $\frac{\mu\text{gr}}{\text{gr}_{\text{B.W.}}}$ (acetone-only-control) to 250 $\frac{\mu\text{gr}}{\text{gr}_{\text{B.W.}}}$ (cypermethrin) or 800 $\frac{\mu\text{gr}}{\text{gr}_{\text{B.W.}}}$ (Propoxur) or 100 $\frac{\mu\text{gr}}{\text{gr}_{\text{B.W.}}}$ (fenitrothion), based on preliminary toxicity assays. Dilutions ranged of all technical insecticides are listed in Table 2.

Topical applications

Adult male cockroaches were briefly anesthetized with carbon dioxide (CO₂) (pressure, 20kPa) for 20–30 s before bioassay and separated into groups of 10^[4,32] in cylindrical plastic container (250 CC) sealed with net cloth with fine mesh. Each cockroach was treated topically with 2.0 ml of acetone containing predetermined doses [Table 2] of technical grade insecticides on the first abdominal segment of the insects using a hand micro-applicator (Burkard, Scientific Ltd, UK) equipped with a Luer 100 μl Hamilton glass syringe. After treatment, groups of 10 German cockroaches were placed in a plastic container with a piece of cotton soaked in water. Mortality was assessed 24 h post-application. Each dose of

insecticide and control was tested into three replications. 10 cockroaches considered in each replication. Only cockroach was considered dead that was left behind and unable to move.^[33] All topical applications tests were performed under aforementioned laboratory conditions, and each treatment was replicated 3 times on different days for a total of at least 30 males per concentration.^[34]

Statistical analysis

Mean masses of German cockroach strains were analyzed with one-way analysis of variance and Tukey's mean separation test in SAS Institute, Inc. (1985).^[34] The percentage mortality was corrected by Abbott's formula^[35] and then transformed to arcsine for analysis of variance (ANOVA, IBM SPSS V20.0). All corrected mortality means values were compared and separated on the basis of Fisher's least Significant Difference (LSD) value at $P=0.05$ probability level using of SAS Institute, Inc. (1985).^[36,37] Probit analyses were calculated using the Polo-Plus 2.0 program (LeOra Software, Berkeley, CA) to determine the LD₅₀ for the insecticides cypermethrin, propoxur, and fenitrothion for the susceptible and hospital collected cockroach populations. Lethal dose ratios (LCRs) test were also calculated with Polo Plus 2.0^[38] to compare the LD₅₀ values among insecticides and

Table 1: The information of German cockroaches' population sources

Hospital	Location	Established year	Reconstruction date	Number of beds	Number of sections	History of insecticide use during 2015–2019	Dosage (g/m ³)
Bu-Alicina	West of Sari	1973	2001	250	15	Pyrethroid Organophosphates Carbamates	7.80 2.85 0.1
Fatemeh-Zahra	Sari center	1941	1991	200	2	Pyrethroid Organophosphates Carbamates	8.35 1.31 0.21
Zare	East of Sari	1951	2008	200	2	Pyrethroid Organophosphates Carbamates	2.14 1.05 0

Table 2: Dilutions range of cypermethrin, propoxur and fenitrothion in different strains of *Blattella germanica*

Insecticides	Strain*	Dilutions range $\left(\frac{\text{gr}}{\text{gr}_{\text{B.W.}}^{**}}\right)$							
Cypermethrin	S	0.8	1.56	3.125	6.25	12.5	25	50	100
	B	3.125	6.25	12.5	25	50	100	200	-
	F	15.6	31.25	62.5	125	250	-	-	-
	Z	15.6	31.25	62.5	125	250	-	-	-
Propoxur	S	0.8	1.56	3.125	6.25	12.5	25	50	100
	B	25	50	100	200	400	600	800	-
	F	25	50	100	200	400	600	800	-
	Z	25	50	100	200	400	600	800	-
Fenitrothion	S	0.8	1.56	3.125	6.25	12.5	25	50	-
	B	12.5	25	50	75	100	-	-	-
	F	12.5	25	50	75	100	-	-	-
	Z	12.5	25	50	75	100	-	-	-

*Strain S is the susceptible laboratory strain; strains B, F, and Z are the field-collected strains in Bu-Alicina, Fatemeh-Zahra, and Zare hospitals, respectively. **BW is body weight of German cockroaches

strains.^[34] The Polo Plus was set at $P = 0.05$ probability level of choice to calculate the ratios of the LD_{50} and to establish whether ratios were significantly different or not at the 5% level. If the 95% confidence interval included 1, then the LD_{50} were considered not significantly different.^[38,39] The resistance to insecticides was quantified by calculating the Resistance Ratio^[40]

$$RR = \frac{LC_{50} \text{ of the hospital population}}{LC_{50} \text{ of the standard susceptible population}} \cdot I_f$$

RR < 1.5, the population is susceptible; if $1.5 \leq RR \leq 2.5$, the population is tolerant and tends to become resistant; if $RR > 2.5$, the population is resistance.^[41]

Results

Body mass

There was no significant difference in body mass among strains ($P = 0.53$, df [degree of freedom] = 3). Mean body mass calculated 0.0507, 0.0495, 0.0509, and 0.0518 gr for strains S, B, F, and Z, respectively [Table 3].

Topical application

There was no mortality in the control treatment during 24 h. The susceptible strain had an LD_{50} value at 8.32 (95% CI: 5.740–12.109) $\mu\text{g}/\text{grBW}$ for cypermethrin [Table 4]. The LD_{50} values of hospital collected strains for cypermethrin ranged from 63.205 (95% CI: 43.512–102.117) $\mu\text{g}/\text{grBW}$ for strain B to 76.501 (95% CI: 57.991–101.644) and 90.871 (95% CI: 70.376–118.952) $\mu\text{g}/\text{grBW}$ for strains F and Z, respectively [Table 4]. The lethal-dose ratio (LDRs) test showed LD_{50} values of hospital collected strains (B, F, and Z) were all significantly different than the susceptible strain (S) [Table 5]. The resistance ratios (RR) of the hospital-collected strains for cypermethrin calculated 7.6, 9.2, and 10.9 fold for strains B, F, and Z, respectively, and showed high-level resistance to permethrin [Table 4].

The LD_{50} value of the susceptible strain for propoxur was 14.678 $\mu\text{g}/\text{grBW}$, and ranged from 90.396 $\mu\text{g}/\text{grBW}$ for hospital-collected strains F to 122.793 and 153.653 $\mu\text{g}/\text{grBW}$ for hospital-collected strains B and Z, respectively [Table 4]. According to the lethal-dose ratio test, The LD_{50} values of all hospital -collected population were significantly greater than the susceptible strain [Table 5]. As is shown in Table 4, all hospital-collected strains had resistance ratios >6 and showed high-level resistance to propoxur [Table 4].

Table 3: Mean masses of German cockroach strains measured in grams

Strain	n	Mean \pm SD ^a
S	20	0.0507 \pm 0.0034a
B	20	0.0495 \pm 0.0060a
F	20	0.0509 \pm 0.0066a
Z	20	0.0518 \pm 0.0066a

^aMeans within a column followed by the same letter are not significantly different

The LD_{50} value of the susceptible strain for fenitrothion was 1.893 $\mu\text{g}/\text{grBW}$, and ranged from 21.485 $\mu\text{g}/\text{grBW}$ for hospital-collected strains B to 25.731 and 31.558 $\mu\text{g}/\text{grBW}$ for hospital-collected strains F and Z, respectively [Table 4]. According to the lethal-dose ratio test, The LD_{50} values of all hospital -collected population were significantly greater than the susceptible strain [Table 5]. All hospital-collected German cockroach strains showed resistance ratios >11 and showed high-level resistance to fenitrothion [Table 4].

Comparison of LD_{50} values of cypermethrin, propoxur, and fenitrothion in different hospitals base on the lethal-dose ratio test, showed that fenitrothion has significantly the highest amount of lethality and after that, the cypermethrin and propoxur are significantly in the second and third place, respectively [Table 6].

Determining the homogeneity/heterogeneity

For determining the homogeneity/heterogeneity response to cypermethrin, the slope of the log-dose probit relationship is shown in Figure 1. Among the field-collected strains, strain F and strain Z showed similar response in homogeneity, slightly higher than that of the strain B. This indicates that the populations are different in responding to cypermethrin doses and are heterogeneous [Figure 1 and Table 4]. For propoxur and fenitrothion dose–response regression lines showed that strains B, F, and Z showed similar response in homogeneity, slightly higher than that of the susceptible strain. This indicates that the field populations are not different in responding to cypermethrin doses and are homogenous [Figure 1 and Table 4]. Also based on the slopes of the dose–response regression lines all hospital populations for cypermethrin, propoxur and fenitrothion are far from the susceptible population and were significantly different from susceptible strain [Figure 1].

Discussion

Since using insecticides are still the most effective method of German cockroaches control, so repeating this method, especially with similar insecticides, always carries the risk of insecticides resistance.^[34] Therefore, study of insecticides resistance can certainly lead to a better understanding and implementation of German cockroach control programs.^[34] One of the most convenient ways to study the susceptibility of cockroaches to insecticides is using topical application method^[31,33] which was used in this study.

Fenitrothion had significantly the highest lethal effects to all four strains we tested in this study and can kill the German cockroach strains in lower values of LD_{50} . In confirmation of this result, Lukwa *et al.*^[42] showed application of fenitrothion caused drastic reduction in density of *B. germanica* in brick houses compared to other insecticides.^[42] Furthermore, Chang *et al.* (2009) observed high lethal effects of fenitrothion on

Table 4: Toxicity of cypermethrin, propoxur and fenitrothion topically applied to German cockroaches

Insecticide	Strain ^a	n	LD ₅₀ (95% CI; µg/grBW)	Slope (SE)	χ ²	df	RR ^b
Cypermethrin	S	160	8.32 (5.740–12.109)	1.468 (0.195)	5.704	6	-
	B	140	63.205 (43.512–102.117)	1.550 (0.247)	3.290	5	7.6
	F	100	76.501 (57.991–101.644)	2.608 (0.436)	2.457	3	9.2
	Z	100	90.871 (70.376–118.952)	2.944 (0.497)	2.001	3	10.9
Propoxur	S	160	14.678 (8.338–29.100)	1.357 (0.191)	8.041	6	-
	B	140	122.793 (85.783–168.894)	1.758 (0.260)	2.544	5	8.36
	F	140	90.396 (62.184–123.637)	1.850 (0.247)	1.762	5	6.16
	Z	140	153.653 (11.738–207.073)	1.932 (0.272)	4.111	5	10.47
Fenitrothion	S	140	1.893 (1.074–2.826)	1.469 (0.257)	1.318	5	-
	B	100	21.485 (14.406–28.117)	2.610 (0.500)	2.493	3	11.35
	F	100	25.731 (9.389–43.099)	2.813 (0.502)	4.520	3	13.6
	Z	100	31.558 (22863–40984)	2.467 (0.463)	2.837	3	16.67

^aStrain S is the susceptible laboratory strain; strains B, F, and Z are the field-collected strains in Bu-Alicina, Fatemeh-Zahra, and Zare hospitals, respectively. ^bRR: LD₅₀ of hospital-collected strains/LD₅₀ of susceptible strain

Table 5: Comparison of LD₅₀ values of Cypermethrin, Propoxur and Fenitrothion between hospital strains with susceptible strain of German cockroaches using LDRs (Lethal Dose Ratios) method (95% CI)

Comparison (LD ₅₀) of strains	Lethal Dose Ratios (95% CI) of insecticides		
	Cypermethrin	Propoxur	Fenitrothion
B-S	7.59 (4.37–13.17)*	8.36 (4.79–14.08)*	11.35 (6.53–19.71)*
F-S	9.18 (5.82–14.5)*	6.15 (3.64–10.39)*	13.59 (8.01–23.04)*
Z-S	10.91 (6.98–17.05)*	10.46 (6.32–17.32)*	16.67 (9.78–28.41)*
B-Z	0.69 (0.43–1.12)	0.79 (0.51–1.25)	0.68 (0.45–1.03)
B-F	0.82 (0.5–1.35)	1.35 (0.84–2.17)	0.83 (0.55–1.25)
Z-F	0.84 (0.58–1.21)	0.58 (0.37–0.92)*	0.81 (0.55–1.19)

LD₅₀ values for strains followed by * are significantly different than the LD₅₀ value of other strain according to the lethal-dose ratio test.^[38] Means compared lethal dose ratios (LDRs); if the 95% confidence interval includes 1, then the LD₅₀ is not significantly different. Strain S is the susceptible laboratory strain; strains B, F and Z are the field-collected strains in Bu-Alicina, Fatemeh-Zahra and Zare hospitals, respectively

Table 6: Comparison of LD₅₀ values of Cypermethrin, Propoxur, and Fenitrothion in different hospitals using LDRs (Lethal Dose Ratios) method (95% CI)

Comparison (LD ₅₀) of insecticides	Lethal Dose Ratios (95% CI) of insecticides			
	Bu-Alicina	Fatemeh-Zahra	Zare	Susceptible
Cypermethrin - Propoxur	0.515 (0.304–0.872)*	0.846 (0.551–1.301)	0.591 (0.399–0.877)*	1.763 (1.022–3.041)*
Cypermethrin - Fenitrothion	2.942 (1.759–4.921)*	2.973 (2.036–4.341)*	2.880 (1.982–4.184)*	0.227 (0.126–0.409)*
Propoxur - Fenitrothion	5.715 (3.633–8.991)*	3.513 (2.29–5.381)*	4.869 (3.235–7.330)*	0.129 (0.070–0.237)*

LD₅₀ values for strains followed by * are significantly different than the LD₅₀ value of other strain according to the lethal-dose ratio test.^[38] Means compared lethal dose ratios (LDRs); if the 95% confidence interval includes 1, then the LD₅₀ is not significantly different

German cockroaches which were collected from a Chinese restaurant with LD₅₀ of 1.24µg/insect.^[43] In this regard, it can be argued that fenitrothion is an organophosphorus insecticide that have generally high acute toxicity to human and other vertebrates and so are not often used in human places. Therefore, the selection pressure of fenitrothion on German cockroaches is low and consequently the highest lethal effects can be observed than other insecticides.

It should be noted that although fenitrothion had the highest lethal effects among the insecticides tested, the resistance ratios of the hospital-collected strains for fenitrothion were also higher than that of cypermethrin and propoxur. This

could be related to the overuse of fenitrothion due to more lethality compared to other insecticides in recent years and therefore increase the resistance ratio. This discussion is not far from the mind because Wu and Apple (2017) announced similar results; where the fipronil was more toxic than propoxur and imidacloprid tested on *B. germanica*, but the resistance ratios for fipronil was also higher than the other two insecticides. In justifying this, they pointed out that widespread use of fipronil, because of its high efficacy, can increase resistance ratio.^[34] Furthermore, our results showed that [Figure 1] the slope of the log-dose probit relationship of fenitrothion has a steeper slope than the other two insecticides which can be attributed to the fact that the population was

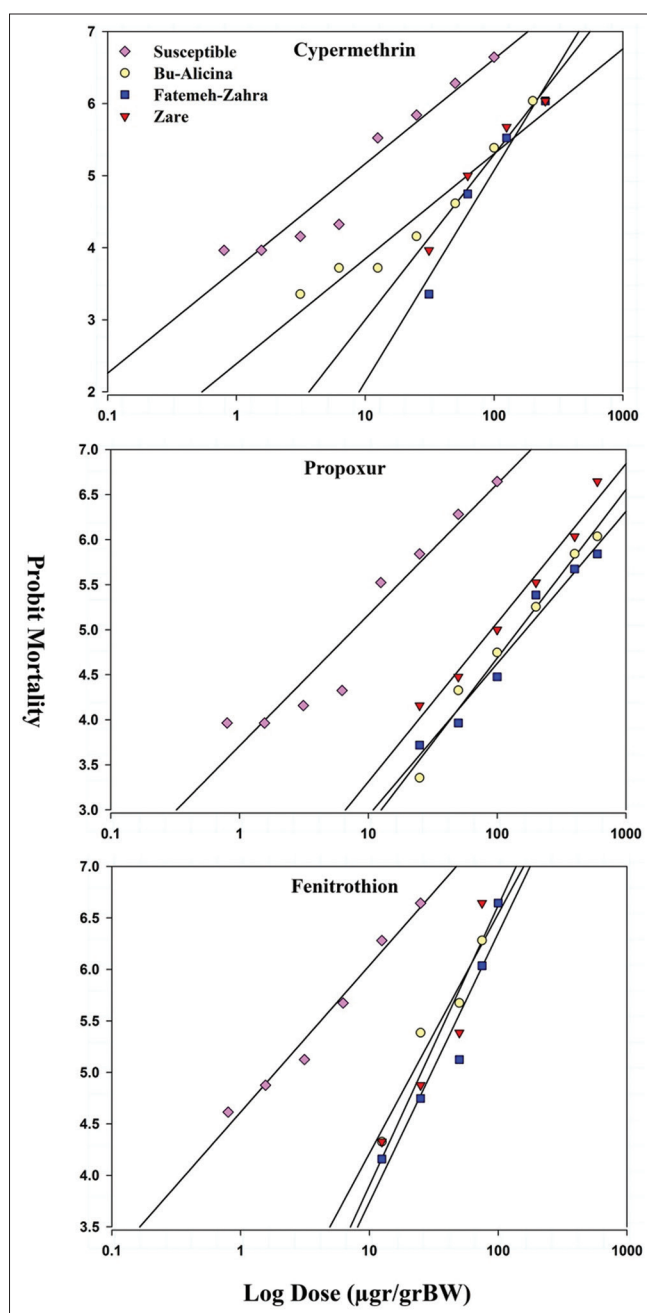


Figure 1: Comparison of regression lines from different hospital collected populations of German cockroaches bioassayed with cypermethrin, propoxur, and fenitrothion in Mazandaran Province, Northern Iran

under the selection pressure of the fenitrothion more than to cypermethrin and propoxur and consequently also showed more resistance ratio.

In our study, cypermethrin was the second most toxic insecticide to the all strains. Cypermethrin showed different ranges of resistance ratios that were consistent with other studies in Iran (ranged from 5.26 to 20.7 fold)^[23,44,45] and around the world.^[46,47] Z strain had RR >10 to cypermethrin and showed high resistance. The slope of the log-dose probit

relationship of cypermethrin in the F and Z strain varied from S strain and suggests that the majority of the hospitals strain had more heterogeneous individuals for resistance. Pyrethroids insecticides such as cypermethrin have been generally used in all parts of the world including Iran because of its effectiveness, low mammalian toxicity and rapid decomposition in the environment.^[33,48,49] This insecticide, especially in the past two decades, has been widely and frequently used in almost all provinces of Iran and consequently led to a relatively high selection pressure.^[33] A phenomenon that could simply lead to German cockroaches' resistance because most susceptible individuals are removed from the population and as a result, the remaining individuals progress to tolerance/resistance homogeneity.^[50] In this regard, it has been reported that continuous use of cypermethrin for almost four years causes resistance and as a result serve as a barrier in the control of German cockroaches.^[51] On the other hand, cross-resistance of *B. germanica* to pyrethroid insecticides seemed to have become prevalent among German cockroach populations and as a result, cause that this group of insecticides becomes useless against German cockroaches control programs.^[43]

In our results, propoxur had the least lethal effects to the all strains. Resistance to propoxur has been also determined with different high range of resistance ratio which is in accord with those of another study on propoxur resistance in populations of the German cockroach; reporting resistance ratios ranging from 2.86 to 31.14 in the hospitals and 6.93 to 62.50 in the households.^[47] In this regard repeated uses of the propoxur and/or same insecticide may lead to resistance in the cockroach population.^[47] The slope of the log-dose probit relationship of propoxur in all strain was similar with S strain and suggests that the majority of the hospitals strain had more homogeneous individuals for resistance and these German cockroach strains had similar response in homogeneity.^[34]

As shown in our studies, different levels of cypermethrin, propoxur and fenitrothion resistance were observed in all three hospitals strains of the German cockroaches. There are various theories about the phenomenon of German cockroaches' resistance to insecticides. In this regard the special life characteristics of German cockroach like the longevity and oviposition is one of the first reasons that can facilitate the development of resistance to many insecticides such as organophosphates, carbamates and pyrethroids.^[47,52] Another case is the occurrence of cross-resistance between different groups of insecticides which makes the German cockroach resistant to insecticides.^[33,53] Our results showed that the regression line of cypermethrin, propoxur and fenitrothion for B, F, and Z strains was almost steeper than S strain, which is an obvious reason that the populations has been affected by the selected pressure of insecticides and as a result showed a multiplier resistance to the susceptible population.^[50] Another reason for the emergence of cross resistance is considering the resistance ratios. Scott and

Wen (1997) reported that when resistance ratios was >4 it is possible that cross-resistance has occurred.^[54] Since our results indicated that all field strains of German cockroach showed values above five and/or 10-fold of resistance ratio to different insecticides; therefore, there is a high probability of occurrence of cross-resistance between the studied insecticides. The high values of resistance ratios in our study have another very important aspect. According to Reiersen *et al.* (1998), the resistance ratio at 5-fold and below, determined by topical application, can still achieve a good control of the German cockroach population while 10-fold resistance is the critical point because can lead to failure in German cockroach control programs.^[55] In view of these aforementioned points and with considering that all the studied strains showed resistance ratio close to 10 or higher, especially in Z strain, management in the control of German cockroaches in hospitals should be taken seriously.

Conclusion

The results of this study suggest that high resistance ratios of the three strains to cypermethrin, propoxur and fenitrothion compared with the susceptible strain most likely can be caused by life style of *B. germanica*, selection pressure or cross resistance; which are a serious threat to the successful control of German cockroaches in hospitals. Therefore, cautions should be exercised when using these insecticides in control against hospitals strains of *B. germanica*. Because this strain have been selected by cypermethrin, propoxur and fenitrothion for several generations and consequently may provide an inadequate control of *B. germanica* in aforementioned hospitals. In this regard rotational use of different groups of insecticides with a different mode of action can be suggested as a solution to the resistance problem of German cockroach.^[56] Furthermore, using mixtures of insecticides with insect growth regulators, inhibitors and/or synergists is another good solution.^[57,58]

Authors' Declaration Statements

Ethics approval and consent to participate

The study was approved by the ethics committee of the Mazandaran University of Medical Sciences under the code IR.MAZUMS.REC.1398.862.

Consent for publication

None.

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' Contributions

Author Contributions: A.E., S.F.M.H. designed the experiment; M.F.D., A.H., S.H.N., J.Y.C. collected data; analyzed the data and wrote the manuscript, A.E., M.F.D. read, corrected, and approved the manuscript. All authors have read and agreed to the published version of the manuscript.

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