Original article

Insecticide resistance studies on German cockroach (*Blattella germanica*) strains to malathion, propoxur and lambdacyhalothrin

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Background: The German cockroach has been known as the most invading human dwelling cockroach species. For managing the insecticide resistance, it is periodically a substantial need to evaluate susceptibility level of the German cockroaches to consuming insecticides.
Objective: To evaluate resistance of the German cockroaches to malathion, propoxur and lambdacyhalothrin.
Methods: Three cockroach strains including susceptible reference, and two wild strains were provided, maintained and colonized. The wild strains were collected from two hospitals of Sanandaj in Iran. Surface contact method was used for bioassay using standard glass jar procedure. The adult male cockroaches were treated with 5 - 6 insecticide dose exposures at a 30-min period. The dose exposures of malathion were 6.25, 12.5, 25, 50 and 100, and 25, 50, 100, 200 and 400 mg/m^2 for susceptible and wild cockroach strains, respectively. The dose exposures of propoxur were 5, 10, 20, 40 and 80, and 20, 40, 80, 160 and 320 mg/m^2 for susceptible and wild cockroach strains, respectively. The dose exposures of lambdacyhalothrin were 0.075, 0.15, 0.3, 0.6 and 1.2, and 0.3, 0.6, 1.2, 2.4 and 4.8 mg/m^2 for susceptible and wild cockroach strains, respectively. Each dose exposures were replicated 3 times (10 cockroaches for each replicate). Control groups received acetone alone.
Results: The lethal doses$_{50}$ (LDs$_{50}$) of the susceptible strain were 30.01, 16.56 and 0.303 for malathion, propoxur and lambdacyhalothrin respectively. While the LDs$_{50}$ of the wild cockroach strains were 157.45, 74.59 and 0.506 for malathion, propoxur and lambdacyhalothrin, respectively. Compared to the susceptible strain, the wild German cockroach strains were more tolerant to malathion and propoxur. Compared to the susceptible strain the wild German cockroach strains were more sensitive to lambdacyhalothrin, confirmed also by a significant difference (P = 0.033). The results of probit analysis and regression lines of treated insecticides indicate that the German cockroach was resistant to malathion and propoxur while susceptible to lambdacyhalothrin. The resistance ratio$_{50}$ (RR$_{50}$) of the malathion and propoxur insecticides were about 5.0 folds.
Conclusion: The study supports that malathion and propoxur should not be used for control of the German cockroaches. An appropriate choice is to use lambdacyhalothrin due to negative cross-resistance effect which occurs here. The study also provides a baseline of resistance data towards a few insecticides in a few German cockroach populations in Iran.

Keywords: *Blattella germanica*, insecticide resistance, lambdacyhalothrin, malathion, propoxur.

Among cockroaches of various species, the German cockroach (*Blattella germanica*: Blattaria, Blattellidae) has been known as the most invading cockroach species of the human dwelling areas [1 – 9] with a variety of medical importance. [9 - 15] Recently, it has been observed that about 25 fungal and 61 bacterial species were recovered from the German cockroaches associated with high morbidity and mortality in immune-compromised patients in the hospital environments. [16 – 17] Therefore the control of this important human dwelling pest is necessary. The use of insecticides is still the most important tool

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Received: January 30, 2020
Revised: March 13, 2020
Accepted: April 8, 2020

for control of the German cockroaches.[18] In order to predict and manage the insecticides resistance phenomenon, it is periodically a substantial need to evaluate the sensitivity level of the German cockroach to consuming insecticides. Various studies have been done in this area [19 – 23] but there is a need to be performed in other region. In this regard, a study is designed to evaluate the sensitivity level of the German cockroaches to malathion, propoxur and lambdacyhalothrin insecticides. The aim of this study is to determine the susceptibility level of the wild German cockroach strains from Sanandaj of Iran to malathion, propoxur and lambdacyhalothrin insecticides.

Materials and methods

Sites of cockroach collection

Cockroach collecting was done in Sanandaj, the center of Kurdistan Province in 2016. Kurdistan Province is home to natural beauty. It has abundant tourist attractions in terms of cultural heritage, history and handicrafts. The area has a semi-arid Mediterranean climate and its coordinates are: 35° 20ˆN - 46° 50ˆE. To do the study, we provided 3 cockroach strains including a standard susceptible strain, and two wild German cockroach strains. The standard susceptible (SS) strain was provided by the School of Public Health, Tehran University of Medical Sciences (TUMS) which had been maintained since 1975 in the insectary, without exposure to any insecticide. Two wild German cockroach strains including a strain from Besat Hospital of Kurdistan University of Medical Sciences and a strain from a hospital of Iranian Social Security Organization of Kurdistan (Tamin-e Ejtemaei hospital) were collected.

Cockroaches collecting and rearing

Cockroaches were collected using Nasirian H, *et al*. procedure.[24] Each cockroach strain after collecting were delivered into a large glass jar, maintained and colonized at 24 ± 2ÚC, 40 ± 5% RH, and a photoperiod of 12:12 (L:D) h in the insectary at the Department of Medical Entomology, School of Medicine, Hamadan University of Medical Sciences. The cockroaches were provided with rodent diet, a cotton plugged water vial and a cardboard as a harborage.

Chemicals and reagents

Malathion (technical grade, 98.0%), propoxur (technical grade, 95.0%) and lambdacyhalothrin (technical grade, 95.0%) as insecticides, CO_2 as an anesthetic, and acetone as a solvent were used. Malathion was provided by Asia Agro Chemicals & Fertilizers (Khurja, Dist. Bulandshahr, India). Propoxur and lambdacyhalothrin were provided by Kalyani Industries Pavt. Ltd. (Maharashtra, India).

Surface contact method bioassay

Surface contact method was used for bioassay using a glass jar. The glass jar inner surface areas were 188.4 cm^2. Technical grade of the insecticides was diluted by acetone solvent. To dilute insecticide concentrations 1 ml volume solvent considered contains insecticide doses. Then 1 ml of diluted insecticide was pipetted into the glass jar. To deposit the insecticide evenly over the inner surface of the glass jars, the glass jars were rolled horizontally over a flat surface until all of the acetone had evaporated.

In a series of contact experiments, a concentration of technical grade at a 30-min exposure time was found to be a sufficient time for exposure of adult males to insecticide treated of the glass jar inner surfaces.[9, 25 - 28] Then adult male cockroaches were treated with 5 - 6 insecticide dose exposures at a 30-min time. After preliminary experiments, the dose exposures of insecticides were determined. The determined dose exposures of malathion insecticide were 6.25, 12.5, 25, 50 and 100, and 25, 50, 100, 200 and 400 mg/m^2 for susceptible and wild cockroach strains, respectively. Finally, the determined dose exposures of propoxur insecticide were also 5, 10, 20, 40 and 80, and 20, 40, 80, 160 and 320 mg/m^2 for susceptible and wild cockroach strains, respectively. Finally the determined dose exposures of lambdacyhalothrin insecticide were 0.075, 0.15, 0.3, 0.6 and 1.2, and 0.3, 0.6, 1.2, 2.4 and 4.8 mg/m^2 for susceptible and wild cockroach strains, respectively. Each dose exposures were replicated 3 times (10 cockroaches for each replicate). Control groups received acetone alone. All insecticide dose exposures were given > 0.0% and < 100.0% mortality at 24 h after insecticide dose exposures. Insecticide-exposed males were placed in plastic dishes, provided with food and water, and monitored for mortality for 24 h under the same temperature and photoperiod as the colony. If insects on their backs were unable to right themselves, they were considered dead. All experiments were replicated 3 times and 10 cockroaches for each replicate.

Statistical analysis

IBM SPSS Statistics Data Editor Version 23 was used to analyze statistical analysis of the data on a computer. Mortality data from the replicates were pooled and the dose exposure mortality was assessed by probit regression analysis. One-sample Kolmogorov-Smirnov test was used to check the normality of the data distribution. After checking, to make sure that the distribution of data was normal, ANOVA was used to compare treated male cockroaches to insecticides for any significant difference. Microsoft Office Excel (2010 version) was used to calculate equation and draw the mortality and regression lines of insecticide treated cockroaches.

Results

Cockroach insecticide susceptibility level

Compared to the susceptible strain the wild German cockroach strains were more tolerant to malathion and propoxur, while they were susceptible to lambdacyhalothrin. In other words, the wild German cockroach strains were resistant to malathion and propoxur. Compared to the susceptible strain, the malathion mortality lines of the wild German cockroach strains locate the right of the susceptible strain. Unlike malathion, the lambdacyhalothrin mortality line of the susceptible strain locate the right of the wild German cockroach strains. While the propoxur mortality lines of the wild and susceptible cockroach strains overlap (Figure 1). Statistical analysis showed a significant difference ($P = 0.036$) between treated insecticides against the German cockroach strains. There was also a significant differences between treated malathion and lambdacyhalothrin ($P = 0.033$) against the German cockroach strains followed by Post Hoc Tests.

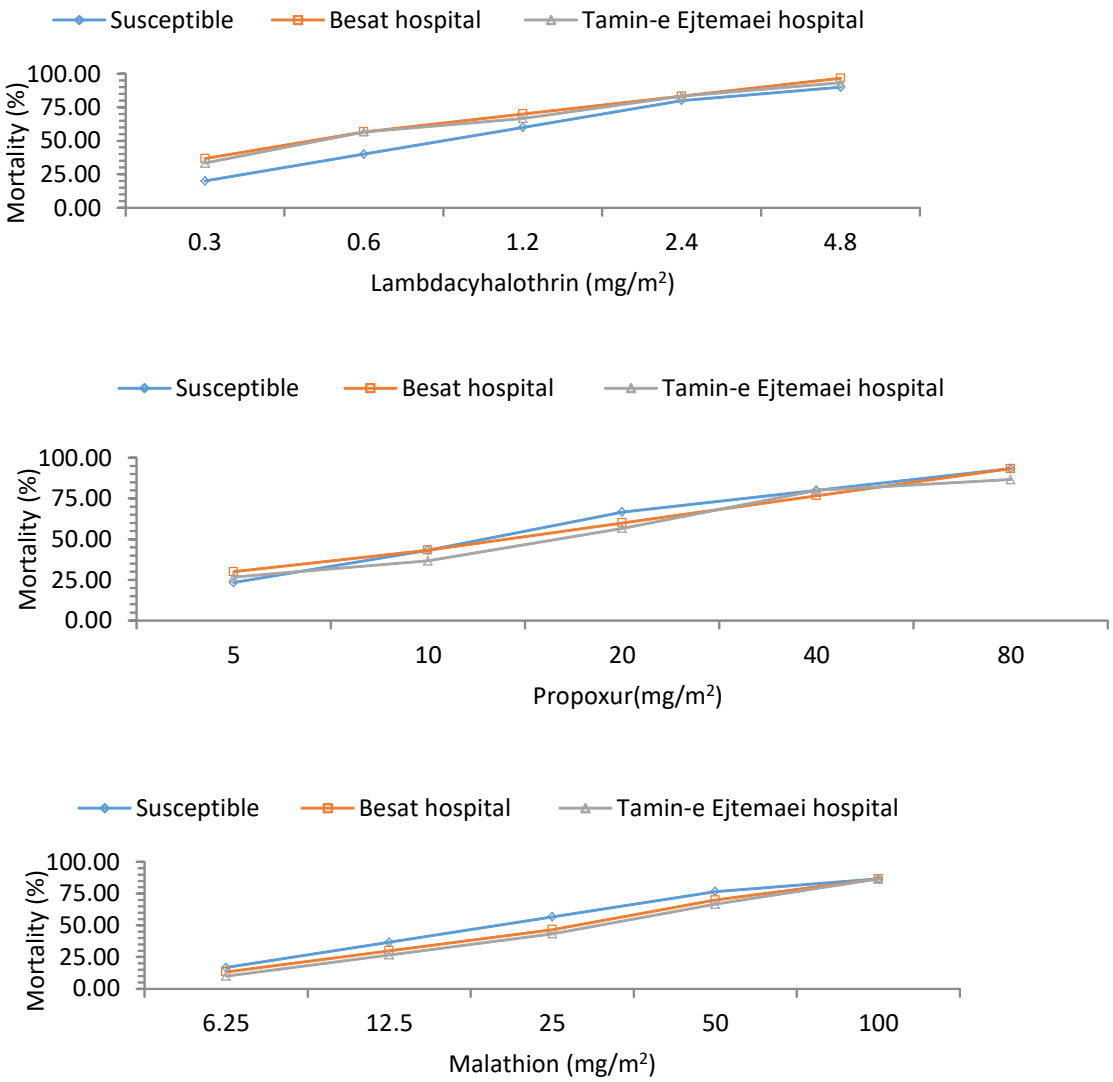

Figure 1. Mortality of the German cockroach strains after treated insecticides. There is a significant difference ($P = 0.036$) between treated insecticides against the German cockroach strains. There is also a significant difference between treated malathion and lambdacyhalothrin ($P = 0.033$) against the German cockroach strains followed by Post Hoc Tests.

Table 1. Probit regression analysis of insecticides against the German cockroach strains.

Malathion										
Strain	n[a]	Intercept ± SE	Slope ± SE	X^2	df	P	LD_{50}[b](LB-UB)[c]	RR_{50}	LD_{90}[b](LB-UB)[c]	RR_{90}
S	150	0.02 ± 0.004	-0.62 ± 0.17	7.4	3	0.06	30.01 (5.7 - 86.5)	0.0	92.57 (57.3 - 814.0)	0.0
BH	150	0.005 ± 0.001	-0.82 ± 0.17	4.7	3	0.19	150.42 (109.3 - 195.1)	5.0	384.24 (312.6 - 519.6)	4.2
TEH	150	0.006 ± 0.001	-0.95 ± 0.18	4.7	3	0.19	164.47 (125.6 - 209.2)	5.5	387.24 (317.8 - 514.5)	4.2
Propoxur										
S	150	0.03 ± 0.006	-0.48 ± 0.17	5.8	3	0.12	16.56 (3.3 - 38.3)	0.0	60.60 (38.7 - 269.2)	0.0
BH	150	0.007 ± 0.001	-0.45 ± 0.17	1.8	3	0.62	67.20 (24.9 - 100.2)	4.1	258.25 (204.3 - 369.9)	4.3
TEH	150	0.006 ± 0.001	-0.49 ± 0.16	5.1	3	0.16	81.98 (37.7 - 118.8)	5.0	297.15 (234.7 - 427.6)	4.9
Lambdacyhalothrin										
S	150	1.8 ± 0.33	-0.55 ± 0.17	6.6	3	0.08	0.303 (0.07 - 0.75)	0.0	1.006 (0.63 - 5.7)	0.0
BH	150	0.48 ± 0.105	-0.23 ± 0.0.17	2.3	3	0.51	0.477 (0.05 - 0.97)	1.6	3.15 (2.4 - 4.7)	3.1
TEH	150	0.42 ± 0.0.09	-0.22 ± 0.0.17	3.9	3	0.27	0.534 (0.17 - 1.1)	1.8	3.61 (2.8 - 5.4)	3.6

[a]Numbers of cockroach treated; [b]LD_{50}, LD_{90} values in mg/m^2 (LB, Lower bound and UB, Upper bound of 95% confidence interval); S, Susceptible; BH, Besat hospital and THE, Tamin-e Ejtemaei hospital strains.

Probit regression analysis

The slope of regression lines shows the heterogeneity and genetic variation of a strain population. The higher slope of a regression line indicates the less uniformity of a strain population. Further analysis also indicates genetic diversity of a strain population. Compared to the susceptible strain the wild German cockroach strains were heterogenous, based on the slope of the regression lines and X^2 of the cockroach population strains (Table 1).

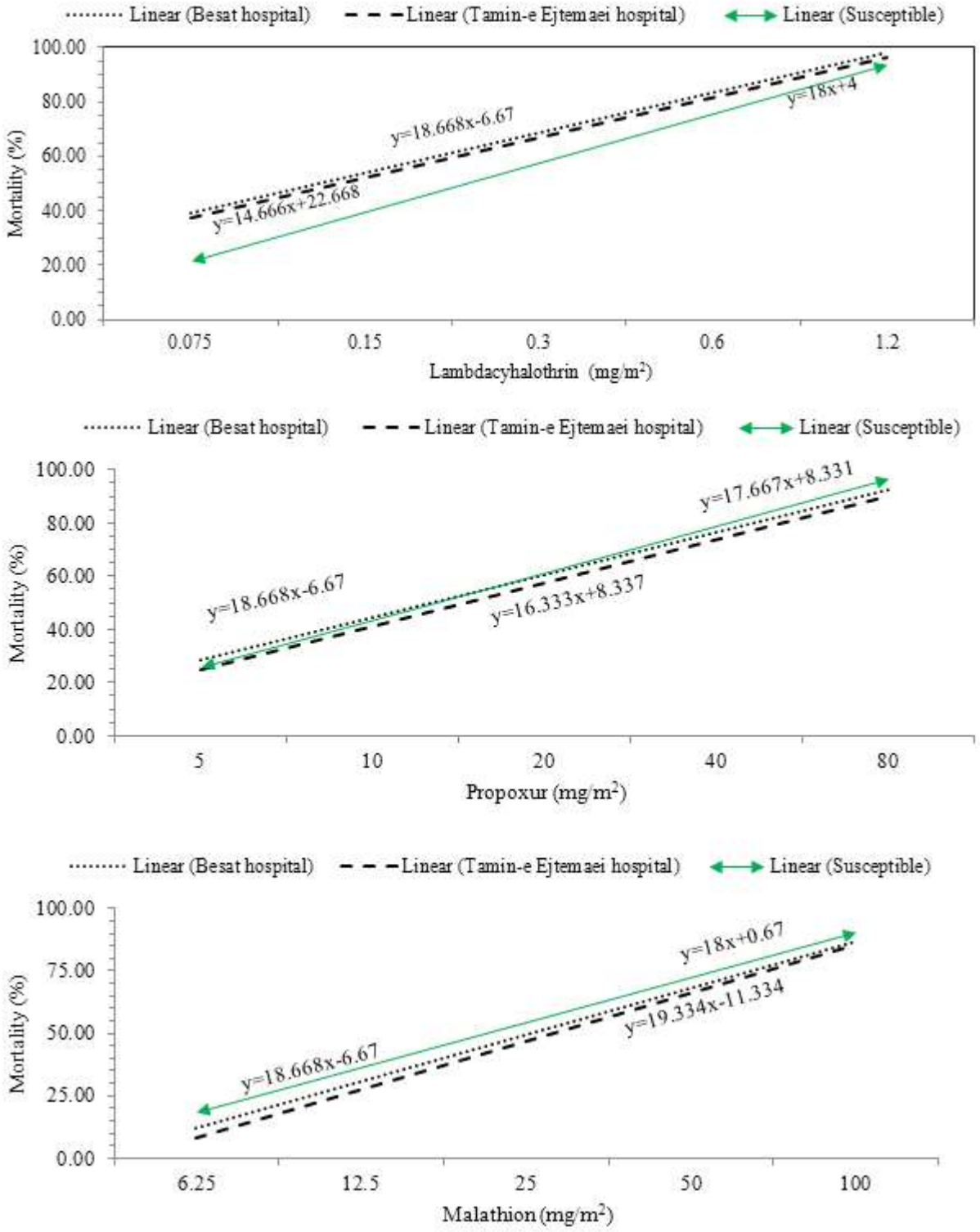

Figure 2. Regression lines of treated insecticides against the German cockroach strains.

The lethal doses$_{50}$ (LDs$_{50}$) of the susceptible strain were 30.01, 16.56 and 0.303 for malathion, propoxur and lambdacyhalothrin, respectively. While the LDs$_{50}$ of the wild cockroach strains were 157.45, 74.59 and 0.506 for malathion, propoxur and lambdacyhalothrin, respectively. The resistance ratio$_{50}$ (RR$_{50}$) of the malathion and propoxur were about 5.0 folds. While the RR$_{50}$ of the lambdacyhalothrin insecticides were about 1.7 folds. Compared to the susceptible strain, the wild cockroach strains were resistant to malathion and propoxur. Unlike malathion and propoxur, the wild cockroach strains were susceptible to the lambdacyhalothrin (Table 1). There was also a significant differences between malathion and lambdacyhalothrin insecticides (P = 0.033) against the German cockroach strains.

Compared to the susceptible strain, the regression lines of the wild cockroach strains treated with malathion locate the right of the susceptible strain. Unlike malathion, the lambdacyhalothrin regression line of the susceptible strain locate the right of the wild cockroach strains, while the propoxur regression lines of the wild and susceptible cockroach strains overlap (Figure 2). Based on the slope of regression lines of the German cockroach strains which were decreased (Table 1), and mortality of the German cockroach strains (Figure 2) and regression lines of treated insecticides (Figure 2), the population of the wild German cockroach strains were heterogenous.

Discussion

The present study was designed to evaluate the susceptibility level of the German cockroach strains to malathion, propoxur and lambdacyhalothrin insecticides for managing the insecticides resistance.

According to probit analysis and regression lines of the treated insecticides, the results of the study indicate that the German cockroach was resistant to malathion and propoxur while susceptible to lambdacyhalothrin. The RR$_{50}$ of the malathion and propoxur insecticides were about 5.0 folds and the malathion regression lines locate the right of the susceptible strain (Table 2). Further analysis showed a significant differences between treated insecticides against the German cockroach strains. There was a significant differences between treated malathion and lambdacyhalothrin insecticides against the German cockroach strains followed by Post Hoc Tests.

In accord with this study, it is well documented that the German cockroach is resistant to malathion [21, 29 - 33] and propoxur. [29 – 40] While unlike the current study, it is also well documented that the German cockroach is resistant to lambdacyhalothrin. [32, 33, 41, 43] Unlike malathion and propoxur insecticides, the wild cockroach strains were susceptible to lambdacyhalothrin (Table 2). Compared to the susceptible strain the lambdacyhalothrin mortality and regression lines of the wild cockroach strain locate the left (Figure 2). This means that the wild cockroach strains were more sensitive to lambdacyhalothrin, compared to the susceptible strain. This fact is also confirmed by observing a significant differences between treated lambdacyhalothrin against the German cockroach strains compared to the other insecticides. It may be due to negative cross-resistance effect which occurs when a novel allele confers resistance to one toxic chemical or environmental factor and hyper-susceptibility to another.[43] That is to say, a trade-off occurs in which the benefit of resistance to one factor arising at the cost of greater susceptibility to a second factor. [44 - 46] Sequential deployment of negative cross-resistance insecticides is useful for insect control in few situations. [43]

Resistance level of the German cockroaches to the carbamates (propoxur), organophosphates (chlorpyrifos), pyrethroids (beta-cyfluthrin, deltamethrin), and phenyl pyrazole (fipronil) has increased to higher levels even in field populations. However, moderate level of resistance is found in German cockroaches against neonicotinoids (imidacloprid) and the oxadiazines like indoxacarb. [47, 48] Currently, the German cockroaches are being emerged resistant to a wide range of insecticides including organophosphates, carbamates, pyrethroids, phenyl pyrazoles, spinosad, neonicotinoids and oxadiazines. [7, 23 – 25, 28 – 30, 48]

The study supports that it had better not use the malathion and propoxur insecticides for control of the German cockroaches in these circumstances to prevent insecticide resistance or developing management. An appropriate choice is to use baits, particularly gel baits. [8, 26, 27, 48] The other appropriate choice is to use lambdacyhalothrin due to negative cross-resistance effect which occurs here.

Conclusion

Like results of the study, it is well documented that the German cockroach is resistant to malathion and propoxur, while due to development of negative cross-resistance to lambdacyhalothrin which occurs

here, the German cockroach is susceptible to lambdacyhalothrin. The study supports that it had better not use the malathion and propoxur insecticides for control of the German cockroaches in these circumstances for preventing, developing and managing of the insecticide resistance. An appropriate choice is to use baits particularly gel baits. The other appropriate choice is to use lambdacyhalothrin due to negative cross-resistance effect. This study provides a baseline of resistance data towards insecticides in German cockroach populations in Iran. The RR_{50} of the malathion and propoxur insecticides were about 5.0 folds. It is periodically a substantial need to evaluate sensitivity level of German cockroaches to consuming insecticides for predicting, preventing and managing of the insecticide resistance.

Acknowledgements

This study is the second author's M.Sc. thesis in Medical Entomology and Vector Control from the Department of Medical Entomology, School of Medicine, financially supported by Research Deputy of Hamadan University of Medical Sciences (Grant No: 9412187345).

Conflict of interest

We declare, hereby, that we have no conflict of interest.

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