

Original Article**Detection of Bendiocarb and Carbaryl Resistance Mechanisms among German Cockroach *Blattella germanica* (Blattaria: Blattellidae) Collected from Tabriz Hospitals, East Azerbaijan Province, Iran in 2013**

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Abstract

Background: Insecticide resistance is one of the serious problems for German cockroach control program. This study was conducted to determine the bendiocarb and Carbaryl resistance mechanisms in German cockroaches using the piperonyl butoxide (PBO).

Methods: Bioassay tests were conducted with 4 to 6 different concentrations of both insecticides with four replicates of 10 susceptible strain cockroaches per concentration to determine of discriminative concentration. After determining discriminative concentration, the result was compared to wild strain. The levels of susceptibility and resistance ratio (RR) and synergism ratio (SR) were calculated for each five wild strains. Moreover resistance mechanisms in wild strains were determined using PBO synergist in vivo.

Results: Hospital strains showed different levels of resistance to bendiocarb and carbaryl compared to susceptible strain. The bendiocarb and carbaryl resistance ratios ranged from 2.11 to 7.97 and 1.67 to 2 at LD₅₀ levels, respectively. The synergist PBO significantly enhanced the toxicity of bendiocarb and carbaryl to all strains with different degrees of synergist ratio, 1.31, 1.39, 3.61, 1.78, 1.62 and 2.1 fold for bendiocarb, 1.19, 1.18, 1.12, 1.29, 1.45 and 1.11- fold for carbaryl, suggesting monooxygenase involvement in bendiocarb and carbaryl resistance.

Conclusion: The synergetic effect of PBO had the highest effect on bendiocarb and resistance level was significantly reduced, which indicates the important role of monooxidase enzyme in creating resistance to Bendiocarb. Piperonyl butoxide did not have a significant synergistic effect on carbaryl and did not significantly break the resistance.

Keywords: German cockroach, Resistance mechanism, Carbaryl, Bendiocarb

Introduction

German cockroach is one of the most important pests in terms of hygiene with a global

spread. This insect is allergenic and lives in residential places, restaurants, hotels, hospi-

tals, food warehouses, and can transmit many pathogens such as bacteria, viruses, fungi, protozoa and parasite eggs to human through mechanical methods or their digestive tract. Hence, it is necessary to control this insect (Nasirian 2007, Behmanesh et al. 2010, Fakoozriba et al. 2010, Nejati et al. 2012).

Development of resistance to insecticides in German cockroach due to excessive consumption and long-term insecticide spraying is one of the serious problems for controlling this pest (Fahiminia et al. 2010, Nasirian 2010).

Many synergistic studies have been conducted in the world to study resistance mechanism of German cockroach to phosphorous, carbamate and pyrethroid insecticides (Payne et al. 1984, Lee et al. 1996, Shinji Kasai et al. 1998, El-Merhibi et al. 2004, Chai and Lee 2010).

Synergistic studies with piperonyl butoxide (PBO) indicate the role of monoxidase system in creating resistance to these insecticides (Hemingway et al. 1993, Valles et al. 1994, Hodgson and Levi 1998, Tozzi 1998).

Increasing resistance of German cockroach to organochlorine, organophosphate, carbamate, and pyrethroid insecticides has been reported (Cochran 1990, Ahad and Hollingworth 2004). The resistance mechanisms of German cockroach to phosphorous, carbamate and pyrethroid insecticides have been determined using PBO and S, S, S-tributyl phosphorotriothioate (DEF) synergists (Metcalf 1976, Rust 1987, Scott et al. 1990, Cochran 1990, Dong and Scott 1992, Lee et al. 1999).

In Iran, German cockroach is resistant to Pyicom, diazinon, actylic, permethrin, cypermethrin, deltamethrin, lambda-cyhalothrin and propoxur (Ladonni 1993, 1997). Resistance mechanisms to permethrin, DDT, chlorpyrifos, malathion have been determined using PBO, DEF and DMC synergists (Ladonni and Sadegheyani 1998, Paksa et al. 2011, Limoe et al. 2011).

Synergists are chemicals which lack pes-

ticide effect but increase pesticide properties of their active ingredient. Piperonyl butoxide prevents activity of P-450 cytochrom. If resistance is due to increase of metabolism using MFO, one can overcome resistance using PBO, which is MFO inhibitor. Synergists are widely used in insecticides containing active ingredient of pyrethrin, pyrethroids and carbamates. Without PBO, enzymes interfering in mechanism of the insect's body particularly enzymes of P-450 cytochrom group can detoxify active ingredient of insecticide before affecting. Adding PBO to an insecticide reduces the dose of active ingredient required for production of the desired effect and increases the toxicity of insecticide (Hodgson and Levi 1998).

Carbamate insecticides act in this way: when a nerve is stimulated, concentration of sodium and potassium ions changes inside and outside the nerve, which leads to neurotransmission. Neurotransmission from one nerve to another requires neurotransmitters such as acetylcholine, which acts in central nervous system (CNS) and in neuromuscular junction. Carbamate insecticides inhibit acetyl cholinesterase and in the absence of this enzyme, acetylcholine accumulates in synapses and neural transmission continues in synapses or neuromuscular junctions, which disrupts harmony and finally results in insect death (Talebi-Jahromi 2006).

In Iran, no study has been conducted on resistance mechanisms of German cockroach to bendiocarb and carbaryl. Therefore, the present study was conducted to investigate and determine resistance mechanisms of German cockroach to these poisons in vivo in Tabriz City using PBO synergist. Conducting this study and knowing resistance mechanisms in this pest, we can manage defeating this pest, and the phenomenon of resistance in this pest compared with insecticide of carbamate group particularly bendiocarb and carbaryl.

Materials and Methods

In this experimental study, five wild strains of German cockroach were trapped or manually collected from Behbood, Sina, Shafa, Taleghani and Noor Hospitals in Tabriz during summer 2013. Then, they were taken to laboratory and housed along with sensitive strains in special glass containers containing bread, starch, sugar and water under laboratory conditions at 27 ± 2 °C and humidity of $60\pm 10\%$ and lighting period of 12 h.

Chemicals used in this study included formulation of technical insecticides such as bendiocarb 97% ai, (technical grade), carbaryl 98% ai, (technical grade), (Cyanamid Agro, India), piperonyl butoxide synergist (PBO) 94% ai, (Zeneca, Haslemere, UK), a monooxygenase inhibitor for synergism study. CO₂ was used as anesthetic and acetone as solvent. Internal surface of the test containers was measured in order to calculate the amount of insecticide needed (Schraft et al. 1995).

Standard dilutions of insecticide with acetone were poured at the bottom of the glass container. The solvent was evaporated through constant rotation of the container, so a uniform layer of exterminator remained on the internal wall of the container. In this way, the desired concentration was used based on mg/m². After specifying the concentrations for sensitive strains, the level and mechanisms of resistance for wild strains were determined using piperonyl butoxide *in vivo*. Synergistic tests were conducted *in vivo* using PBO synergist concurrently with bendiocarb and carbaryl using Cochran contact method (Cochran 1990). These tests were performed in 4 replicates each with 10 male adult cockroaches. For each synergistic test, two controls were assigned to acetone and synergist. The data were analyzed using Probit software (Finny 1972). For this purpose, LD₅₀ was calculated with confidence level of 95%. To calculate resistance ratio

(RR), LD₅₀ of resistant strain was divided by LD₅₀ of sensitive strain and LD₅₀ of insecticide was divided by LD₅₀ (insecticide+synergist) to calculate synergistic rate (SR).

Results

Resistance to Bendiocarb

All five strains of German cockroach collected from hospitals showed different degrees of resistance to bendiocarb with confidence level of 95% based on resistance ratio (RR) compared with the sensitive strain (Table 1). Lethal dose 50 of bendiocarb was calculated between 38.44 and 306.21 mg/m² for sensitive strain and five collected strains with confidence level of 95% (Table 1). Resistance ratio of bendiocarb was obtained between 2.11 and 7.97 for five collected strains. The highest resistance ratio for bendiocarb was found 7.97 in strain of Behbood Hospital (Table 1).

Resistance to Carbaryl

Different levels of resistance to carbaryl were observed in all strains of German cockroach collected from hospitals (Table 2). LD₅₀ of carbaryl was calculated between 280.87 and 560.81 for sensitive strain and five collected strains with confidence level of 95% (Table 2). RR of carbaryl was obtained between 1.67 and 2 for five collected strains. The highest resistance ratio (RR) for carbaryl was found 2 in strain of Behbood Hospital (Table 2).

Synergistic effect of piperonyl butoxide on Bendiocarb

As for the synergistic effect of PBO on Bendiocarb, LD₅₀ for sensitive strain and five collected strains was obtained between 25.76 and 220.90 and the highest resistance ratio (RR) for bendiocarb was found to be 7.51 in strain of Behbood Hospital (Table 3).

By studying the synergistic effect, synergism ratio of bendiocarb was calculated 1.31, 1.39, 3.61, 1.78, 1.62 and 2.12 fold in sensitive strain and strains from Behbood, Sina, Shafa, Taleghani and Noor Hospitals, respectively and the highest synergism ratio of carbaryl (SR) was calculated 3.61 in Sina strain (Table 3).

Synergistic effect of piperonyl butoxide on carbaryl

Lethal dose 50 of carbaryl+PBO for all col-

lected strains was obtained between 235.77 and 480.64 and the highest (RR) for carbaryl was found to be 2.04 in Noor Hospital. As for synergistic effect, synergism ratio (SR) of carbaryl was calculated 1.19, 1.18, 1.12, 1.29, 1.45 and 1.11 fold in the sensitive strain and strains from Behbood, Sina, Shafa, Taleghani and Noor Hospitals, respectively and the highest synergism ratio (SR) of carbaryl (SR) was calculated 1.45 in Taleghani strain (Table 4).

Table 1. Lethal dose values for bendiocarb on susceptible and five hospital-collected strains of German cockroach

Strain	N	Y-intercept	Slope(SE)	X ² (df)	LD ₅₀ , 95% C.L	RR
Susceptible	200	-9.17	5.78±0.778	5.89 (3)	38.44	1
Behbood-H	200	-8.79	3.54±0.501	2.94 (2)	306.21	7.97
Sina-H	200	-3.93	1.1±0.265	5.71 (3)	92.96	2.42
Shafa-H	200	-4.92	2.58±0.305	3.03 (3)	80.92	2.11
Talaghani-H	200	-4.86	2.54±0.301	4.9 (3)	81.32	2.12
Nour-H	200	-6.10	2.72±0.434	4.37 (2)	175.61	4.57

RR: Resistance Ratio
H: Hospital
N: Number

Table 2. Lethal dose values for Carbaryl on susceptible and five hospital-collected strains of German cockroach

Strain	N	Y-intercept	Slope(SE)	X ² (df)	LD ₅₀ , 95% C.L	RR
Susceptible	200	-11.33	4.63±2.015	20.06 (3)	280.87	1
Behbood-H	200	-45.84	16.68±2.780	4.05 (2)	560.81	2
Sina-H	200	-69.88	25.86±11.740	18.28 (2)	504.06	1.8
Shafa-H	200	-41.90	15.68±2.592	2.10 (2)	469.1	1.67
Talaghani-H	200	-48.0365	17.71±2.145	1.680 (3)	514.96	1.83
Nour-H	200	-93.09	34.14±10.015	9.45 (2)	533.47	1.9

RR: Resistance Ratio
H: Hospital
N: Number

Table 3. Toxicity bendiocarb with and without piperonyl butoxide to five bendiocarb resistant strains of the German cockroach compared to the susceptible strain

Strain	Insecticide alone			Insecticide + PBO						
	N	LD ₅₀ , 95% C.L	RR	N	Y-intercept	Slope(SE)	X ² (df)	LD ₅₀ , 95% C.L	SR	RR
Susceptible	200	38.44	1	200	-8.37	5.70±0.753	2.50 (2)	29.42	1.31	1
Behbood-H	200	306.21	7.97	200	-9.33	3.98±0.990	7.57 (2)	220.90	1.39	7.51
Sina-H	200	92.96	2.42	200	-4.13	2.92±0.444	2.61 (2)	25.76	3.61	0.88
Shafa-H	200	80.92	2.11	200	-8.69	5.24±0.708	1.36 (2)	45.46	1.78	1.55
Talaghani-H	200	81.32	2.12	200	-6.62	3.89±0.493	5.08 (2)	50.11	1.62	2.81
Nour-H	200	175.61	4.57	200	-9.69	5.05±1.917	13.68(2)	82.65	2.12	2.12

H: Hospital

N: Number

Resistance Ratio: LD₅₀ of resistant strain/ LD₅₀ of susceptible strain

Synergist Ratio: LD₅₀ of insecticide alone/ LD₅₀ of insecticide + PBO

Table 4. Toxicity Carbaryl with and without piperonyl butoxide to five Carbaryl resistant strains of the German cockroach compared to the susceptible strain (SUS)

Strain	Insecticide alone			Insecticide + PBO						
	N	LD ₅₀ , 95% C.L	RR	N	Y-intercept	Slope(SE)	X ² (df)	LD ₅₀ , 95% C.L	SR	RR
Susceptible	200	280.87	1	200	-12.42	5.23±2.404	32.03 (3)	235.77	1.19	1
Behbood-H	200	560.81	2	200	-65.17	24.36±3.526	3.78 (2)	472.89	1.18	2.01
Sina-H	200	504.06	1.8	200	-46.90	17.69±4.519	6.34 (2)	448.22	1.12	1.9
Shafa-H	200	469.1	1.67	200	-70.68	27.61±7.532	7.41 (2)	362.98	1.29	1.53
Talaghani-H	200	514.96	1.83	200	-49.51	19.42±2.535	2.15 (2)	354.42	1.45	1.5
Nour-H	200	533.47	1.9	200	-67.09	25.02±7.164	9.34 (2)	480.64	1.11	2.04

H: Hospital

N: Number

Resistance Ratio: LD₅₀ of resistant strain/ LD₅₀ of susceptible strain

Synergist Ratio: LD₅₀ of insecticide alone/ LD₅₀ of insecticide+PBO

Discussion

The results of PBO synergistic tests on bendiocarb insecticide showed that resistance level was reduced in all tested strains. Synergism rate (SR) for bendiocarb on susceptible, Behbood, Sina, Shafa, Taleghani and Noor Hospitals of strain was 1.31, 1.39, 3.61, 1.78, 1.62, 2.12 fold, respectively.

Based on the obtained results in strain of Sina Hospital and Noor Hospital, the synergistic effect of PBO on bendiocarb insecticide showed the highest effect and resistance level was significantly reduced, which indicates the important role of monoxidase enzyme (MFO) in creating resistance to bendiocarb exterminator. In strain of Behbood, Taleghani and Shafa Hospitals, the synergistic effect of PBO was found to be low to medium.

These results indicated that monoxidase was not among the main factors of resistance but it was a cofactor of resistance compared with Bendiocarb. PBO did not have a significant synergistic effect on carbaryl insecticide and did not significantly break the resistance.

This result shows that monoxidase enzyme does not have a main role in creating resistance to carbaryl in this strain. A major part of resistance was created probably due to mechanisms such as reduced Cuticle penetration, esterase and other factors created due to fighting with German cockroach. Synergism rate (SR) for carbaryl no susceptible, Behbood, Sina, Shafa, Taleghani and Noor Hospitals of strain were 1.19, 1.18, 1.12, 1.29, 1.45, 1.11 fold respectively. In strains of Shafa and Taleghani hospitals, the synergistic effect of PBO on carbaryl, these two are different. These results are consistent with our previous studies on resistance mechanisms among German cockroach strains in vivo in Tehran (Limoe et al. 2006). Besides, the results of the present study are in line with those of another study on resistance mechanisms in some populations of the

German cockroaches from kashan (Paksa et al. 2014).

According to Reiersen, 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. Thus, the low resistance ratios of the three strains to chlorpyrifos 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, bendiocarb and specially chlorpyrifos may still provide adequate control of these strains (Reiersen et al. 1998). The findings show involvement of monooxygenase in cyfluthrin resistance.

In 1987, first Rust recognized resistance to diazinon and bendiocarb insecticides in different strains of German cockroach (Rust 1987).

A study was conducted by Hemingway in 1993 to investigate resistance of German cockroach to chlorpyrifos and propoxur compared with sensitive strain. Thirteen out of 14 strains showed resistance to chlorpyrifos and 12 strains showed resistance to propoxur. Synergistic studies with PBO also showed role of monoxidase system in creating resistance to these insecticides (Hemingway et al. 1993). In this study, an increase in activity of esterase was observed in strains resistant to a broad spectrum of organophosphates.

Lee et al. collected 12 strains of German cockroach from Malaysia in 1996 to provide direct evidence for mechanisms involved in resistance of German cockroach to propoxur, chlorpyrifos, cypermethrin and permethrin and performed synergistic tests after performing sensitivity tests and specifying their resistance ratio. In these studies, PBO and DEF synergists reduced resistance to propoxur, which

indicated involvement of monooxygenase resistance (Lee et al. 1996). However, these synergists did not affect resistance to cypermethrin and permethrin.

Scott et al. conducted a study in 1990 to determine sensitivity of 4 strains of German cockroach and the effect of synergists on increasing toxicity of pyrethrin, propoxur, malathion, deltamethrin, chlorpyrifos and bendiocarb insecticides using topical application method and showed that bendiocarb insecticides along with PBO and DEF synergists were applied in Kenly strain (resistant to bendiocarb and permethrin) and Rutgers strain (resistant to permethrin) and as a result of this test, resistance was reduced indicating the presence of hydrolytic and oxidase metabolism in creating resistance in the above strains (Scott et al. 1990).

Lee et al. collected 23 strains of German cockroach in 1999 and calculated their sensitivity to propoxur, baygon, bendiocarb and chlorpyrifos. Resistance in pesticides of carbamate group was incompletely removed by PBO and DEF synergists and probably, resistance was controlled by monooxygenase and esterase. Resistance to phosphorous insecticides is neutralized by DEF (Lee et al. 1999).

Generally, PBO reduces resistance level of but it does not remove resistance completely in the present study like the studies conducted in the world, which indicated enzymes of cytochrom P540 and monooxygenase as the factor helping create resistance to bendiocarb and carbaryl in the above strains. Monooxygenase P540 interferes in resistance of *B. germanica* to carbamate insecticides and confirms the present study which is conducted for the first time in Iran (Rust 1987, Scott et al. 1990, Hemingway et al. 1993, Lee et al. 1996, Lee et al. 1999).

To succeed in controlling German cockroach in management of resistance to exterminators, it seems vital to recognize resistance in the early stages and the mechanism of pest

resistance using bioassay tests. Such tests measure the sensitivity of insects. Therefore, knowing of resistance in order to control pests desirably is based on measurement of pest resistance before its occurring or development in conventional insecticides or new compounds.

Considering the obtained results of this study and receiving information about the resistance mechanisms, it is recommended not to use bendiocarb and carbaryl exterminators or to use them periodically and in places which have not been sprayed with them, or use new insecticides such as spinosad or poisonous baits with different mechanism of action from those of carbamate group such as poisonous baits of imidacloprid and fipronil (Nasirian 2007, Nasirian et al. 2011). Furthermore, it will not be possible to manage resistance to insecticides unless resistance mechanisms neutralizing the effect of insecticide inside body of German cockroach are studied comprehensively. Studies that are more comprehensive are recommended in this field to investigate other mechanisms such as reduced penetration of cuticle and insensitivity to acetylcholinesterase.

Conclusion

All five collected strains showed different levels of resistance compared with sensitive strain. As a result, RR of all strains was significant compared with sensitive strain. Piperonyl butoxide significantly increased toxicity of bendiocarb and carbaryl almost in all strains at different degrees, but resistance was not completely removed by PBO. These results, reduction of resistance, show involvement of monooxidase enzyme in creating resistance to these insecticides. Considering that resistance was not removed by PBO synergist, other mechanisms such as reduced Cuticle penetration and insensitivity to acetylcholinesterase may be involved.

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