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Synergists action of piperonyl butoxide and S,S,S-tributyl phosphorotrithioate on toxicity of carbamate insecticides against *Blattella germanica*

Alireza Sanei Dehkordi[1,2], Yaser Salim Abadi[3,4], Hasan Nasirian[5], Teymour Hazratian[6], Mohammad Amin Gorouhi[7], Saideh Yousefi[8], Azim Paksa[9]✉

[1]*Department of Medical Entomology and Vector Control, Faculty of Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

[2]*Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

[3]*Department of Health Services and Health Promotion, School of Health, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

[4]*Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

[5]*Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

[6]*Tabriz University of Medical Sciences, Tabriz, Iran*

[7]*Department of Medical Entomology and Vector Control, School of Health, Kerman University of Medical Sciences, Kerman, Iran*

[8]*Sirjan Faculty of Medical Sciences, Kerman University of Medical Sciences, Kerman, Iran*

[9]*Department of Parasitology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran*

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ABSTRACT

Objective: To determine the synergists action of piperonyl butoxide (PBO) and S,S,S-tributyl phosphorotrithioate (DEF) on toxicity of carbamate insecticides against *Blattella germanica* in Tehran city.

Methods: In the current study, German cockroach strains were collected from several hospitals and dormitories in Tehran. At the beginning, different concentrations of bendiocarb and carbaryl (insecticides belong to carbamate group) were determined by surface contact on a susceptible strain. Then, the level of susceptibility and type of resistance mechanisms in the collected strains from contaminated sites to the afore-mentioned insecticides were studied by using PBO and DEF synergists with different insecticide ratios to synergist (1:0, 1:1, 1:2, 1:3).

Results: The DEF synergist along with bendiocarb and carbaryl completely eliminated the resistance in all strains but PBO did not completely eliminate the resistance in the strains of Mofid, Alvand, Valiasr hospitals and Shariati dormitory. Generally, the impact of DEF was observed in the removing resistance more than PBO.

Conclusions: In most of these strains, resistance to bendiocarb and carbaryl is completely eliminated by DEF, showing a very high role of estraze enzymes in resistance to bendiocarb and carbaryl. But in most strains PBO does not remove the resistance because other mechanisms, such as reduced cuticle penetration and insensitivity to the acetylcholine esterase enzyme, may be involved.

1. Introduction

German cockroach, known as *Blattella germanica* (*B. germanica*), is a pest of residential houses, hospitals, hotels, and dormitories. This insect carries a variety of fungi, viruses, pathogens, and always affects human health. Therefore, the control of this species is very important [1–9]. According to the recommendations of different methods for control, the use of pesticides is the most important method against German cockroach. However, due to continuous, overexposure and prolonged use, German cockroach is gradually resistant to the used pesticides and the subsequent spraying would have not any impact due to the types of produced resistance [10–19]. In the world, several synergistic studies which survey the

First author: Alireza Sanei Dehkordi, Department of Medical Entomology and Vector Control, Faculty of Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
✉Corresponding author: Azim Pksa, Department of Parasitology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran.
Tel: +98 09141288848
E-mail: a.paksa@yahoo.com
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resistance level of different groups of insecticides belonging to phosphorus, carbamate, and pyrethroids on German cockroach has been done. Synergistic studies with piperonyl butoxide (PBO) indicate the role of monoxidase system and S,S,S-tributyl phosphorotrithioate (DEF) also indicates the role of esterase enzymes activity in resistance to these insecticides [16–23]. Carbamate insecticides are derivatives of carbamate acid. Bendiocarb has contact, digestive and somewhat systemic properties that affect different species of insects and storage pests. As one of the most commonly used insecticides in the carbamate group, carbaryl has an impact on a wide range of insects through contact and digestive tract [24]. Some of the synergists contribute to the enhancement and stability of insecticidal properties of carbamates [21,22]. Synergists are chemical products that do not have a pesticide effect but the pesticides properties enhance and improve the active ingredient in them. PBO synergist has a wide range that is used in insecticides containing active substances pyrethroids, ruthenones, organophosphates, and carbamates such as bendiocarb and carbaryl. Without PBO enzymes intervening in the metabolism of the insect, especially enzymes of the cytochrome group P_{450} can detoxify the active substances of insecticide before impact. Adding PBO to a pesticide reduces the active dose which is needed to produce the desired effect [21,22,25–29]. Having been widely recognized as an insecticide synergist, DEF is responsible for the hydrolysis metabolism inhibition and usually used in experiments related to metabolism of insecticides that have Astrid links like carbamates including bendiocarb and carbaryl [30–38]. Achieving success in the control of German cockroach requires a precise execution of a resistance management program for insecticides based on the pre-awareness of the occurrence resistance. On the other hand, without a comprehensive study on diagnosis and identification of different mechanisms of resistance that cause inactivate insecticides inside the body of German cockroaches, the resistance management of insecticides is not possible. In Iran, German cockroaches have been found resistant to diazinon, acyclic, bendiocarb, permethrin, cypermethrin, deltamethrin, lambdacyhalothrin, and propoxur insecticides [14,39–42]. Moreover the resistance mechanisms against permethrin and dichlorodiphenyl-trichloroethane have been determined by using dimethyl carbonate, PBO and DEF synergists [43–45]. In Iran, up to now there is no study about resistance mechanisms against carbamate group insecticides on German cockroaches. Therefore, the present study has been conducted in Tehran City in order to determine the resistance mechanisms in German cockroaches against bendiocarb and carbaryl insecticides with *in vivo* method by using PBO and DEF synergists. By doing this study and having an awareness of resistance mechanisms, it is possible to manage successfully the resistance phenomenon in this pest to carbamate insecticides.

2. Materials and methods

2.1. Strains

In this experimental study, a laboratory strain of *B. germanica* which belonged to the Insectarium of School of Health, Tehran University of Medical Sciences without contact with insecticide since 1989 was used as a susceptible strain. Over 8 different strains of *B. germanica* collected from 4 hospitals including Mofid, Alvand, Shariati, Vali-Asr; two clinics: Shahid Kalantari Building and Amir-Al Momenin and two student dormitories (Shariati and Kooy) of Tehran University of Medical Sciences were used as wild strains. After collecting wild strains from different mentioned sites, all samples were transferred to glass containers in the insectarium containing bread, starch, sugar and water for breeding under laboratory conditions: temperature (27 ± 2) °C, relative humidity (60 ± 10)% and photo period (12/12). Obtained results of wild strains were compared with those of the susceptible strain.

2.2. Used insecticides

In the current investigation, the purites of bendiocarb, carbaryl, PBO and DEF were 98%, 98%, 98% and 97%, respectively. In addition, the acetone was used as a solvent. For impregnating the inner surface of the test container with intended insecticide, at first dimensions of interior surface of the intended glass container was measured (radius = 4 cm and height = 8.5 cm) (188/4 cm^2), and then by using the contact method by Scharf *et al*, the specific insecticides diluted with acetone at a specific concentration were in a glass bottom. The solvent (acetone) was evaporated by rotating steadily the container and the uniform layer of poison remained in the inner wall of the container. Thus, discriminative concentration was determined in the scale (mg/m^2) [46].

2.3. Discriminative concentration determination

First, the desired logarithmic concentrations were prepared from the pure bendiocarb and carbaryl insecticides. Then, 1 mL of the logarithmic prepared concentration in the test glass was poured out and volume was adjusted to 2 mL with acetone. With a uniform rotation of the container, the solvent (acetone) was steamed and the uniform layer of poison remained in the inner wall of the container. In each logarithmic concentration, four replicates and one control (each replicate of 10 adults belongs to susceptible strain) were done. Among five concentrations of the insecticides, which were embedded in the interior of the glass container, the concentration that caused 99% mortality was considered as a discriminative concentration. Then, the susceptibility levels of the collected wild strains were determined by their discriminative concentrations. Among various concentrations of insecticides on the susceptible strains, discriminative concentrations of bendiocarb and carbaryl was 66.34 mg/m^2 and 390.11 mg/m^2 respectively after 30 min contact with the impregnated dishes.

2.4. Synergist bioassays

Synergist tests were conducted by *in vivo* and using PBO and DEF synergists with a contact method [47]. In this method, maximum of the sub lethal of synergists were used with the value of 1 to 1, 2 to 1 and 3 to 1 synergist ratio to synchronous insecticide [47]. These tests were performed in 4 replicates of each replicate with 10 adult male cockroaches. For each synergistic test, simultaneously, two controls were assigned to acetone and the other to the synergists. Finally data were analyzed with ANOVA test, using SPSS Version 13.0 program and $P < 0.05$ was considered as significant difference.

3. Results

In the current study the synergist effects of PBO and DEF against bendiocarb and carbaryl insecticides were tested with a contact method on the mature male of *B. germanica*. The results were shown in Tables 1–4. The results of synergistic tests showed that the resistance to the bendiocarb in the strains of Mofid Hospital (27.5%–30.0%), Alvand Hospital (22.5%–47.5%), Amir-Al Momenin Clinic (25.0%–52.5%), Shariati Hospital (30%–50%), Valiasr Hospital (57.5%–67.5%), Shahid Kalantari Clinic (0%–100%), Shariati dormitory (0.0%–42.5%) and Kooy dormitory (82.5%–100.0%) under impact of PBO which was reduced with the ratio of 1:1, 2:1 and 3:1 (Table 1). It could be seen that there was a significant difference between using synergist PBO and lack of PBO ($P < 0.05$). The resistance to the bendiocarb insecticide in strains of Mofid Hospital (47.5%–80.0%), Alvand Hospital (25%–55%), Amir-Al Momenin Clinic (45.0%–52.5%), Shariati Hospital (47.5%–50.0%), Valiasr Hospital (55.0%–67.5%), Shahid Kalantari Clinic (0%–100%), Shariati dormitory (15%–100%) and Kooy dormitory (85%–100%) was reduced under effect of DEF synergists with the ratio of 1:1, 2:1 and 3:1 (Table 2). It showed a significant difference between using DEF synergist and lack of DEF ($P < 0.05$). Moreover, the resistance to the carbaryl insecticide in strains of Mofid Hospital (0.0%–62.5%), Alvand Hospital (75%–100%), Amir-Al Momenin Clinic (20%–95%), Shariati Hospital (72.5%–85.0%), Valiasr Hospital (10%–85%), Shahid Kalantari Clinic (97.5%–100.0%),

Table 1

Mortality percent of bendiocarb with PBO against different strains of *B. germanica*.

Strain	Insecticide ratio to synergist			
	1:0	1:1	1:2	1:3
Lab (Susceptible)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Mofid Hospital	20.0 ± 5.0	47.5 ± 6.2	50.0 ± 5.0	50.0 ± 4.8
Alvand Hospital	45.0 ± 5.1	67.5 ± 5.7	75.0 ± 5.7	92.5 ± 5.9
Shariati Hospital	50.0 ± 4.2	80.0 ± 5.4	100.0 ± 0.0	100.0 ± 0.0
Valiasr Hospital	32.5 ± 5.3	90.0 ± 5.0	97.5 ± 1.2	100.0 ± 0.0
Amir-Al Momenin Clinic	47.5 ± 5.0	72.5 ± 5.7	75.0 ± 5.2	100.0 ± 0.0
Shahid Kalantari Clinic	0.0 ± 0.0	0.0 ± 0.0	90.0 ± 5.0	100.0 ± 0.0
Shariati dormitory	0.0 ± 0.0	0.0 ± 0.0	37.5 ± 11.2	42.5 ± 3.8
Kooy dormitory	0.0 ± 0.0	82.5 ± 3.7	97.5 ± 1.2	100.0 ± 0.0

Table 2

Mortality percent of bendiocarb with DEF against different strains of *B. germanica*.

Strain	Insecticide ratio to synergist			
	1:0	1:1	1:2	1:3
Lab (Susceptible)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Mofid Hospital	20.0 ± 5.0	67.5 ± 6.2	100.0 ± 0.0	100.0 ± 0.0
Alvand Hospital	45.0 ± 5.1	70.0 ± 10.0	100.0 ± 0.0	100.0 ± 0.0
Shariati Hospital	50.0 ± 4.1	97.5 ± 3.7	97.5 ± 1.2	100.0 ± 0.0
Valiasr Hospital	32.5 ± 5.3	87.5 ± 6.2	100 ± 0.0	100.0 ± 0.0
Amir-Al Momenin Clinic	47.5 ± 5.0	92.5 ± 3.7	97.5 ± 1.2	100.0 ± 0.0
Shahid Kalantari Clinic	0.0 ± 0.0	0.0 ± 0.0	80.0 ± 5.6	100.0 ± 0.0
Shariati dormitory	0.0 ± 0.0	15.0 ± 2.5	100.0 ± 0.0	100.0 ± 0.0
Kooy dormitory	0.0 ± 0.0	85.0 ± 5.2	100.0 ± 0.0	100.0 ± 0.0

Table 3

Mortality percent of carbaryl with PBO against different strains of *B. germanica*.

Strain	Insecticide ratio to synergist			
	1:0	1:1	1:2	1:3
Lab (Susceptible)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Mofid Hospital	0.0 ± 0.0	0.0 ± 0.0	55.0 ± 5.7	62.5 ± 5.3
Alvand Hospital	0.0 ± 0.0	75.0 ± 5.2	95.0 ± 5.2	100.0 ± 0.0
Shariati Hospital	15.0 ± 5.7	87.5 ± 1.2	100.0 ± 0.0	100.0 ± 0.0
Valiasr Hospital	0.0 ± 0.0	10.0 ± 5.0	80.0 ± 10.0	85.0 ± 5.2
Amir-Al Momenin Clinic	5.0 ± 1.2	25.0 ± 5.2	62.5 ± 3.7	100.0 ± 0.0
Shahid Kalantari Clinic	0.0 ± 0.0	97.5 ± 1.2	97.5 ± 1.2	100.0 ± 0.0
Shariati dormitory	0.0 ± 0.0	0.0 ± 0.0	72.5 ± 5.7	75.0 ± 5.7
Kooy dormitory	0.0 ± 0.0	17.5 ± 5.1	90.0 ± 6.5	100.0 ± 0.0

Table 4

Mortality percent of carbaryl with DEF against different strains of *B. germanica*.

Strain	Insecticide ratio to synergist			
	1:0	1:1	1:2	1:3
Lab (Susceptible)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Mofid Hospital	0.0 ± 0.0	80.00 ± 10.00	100.0 ± 0.0	100.0 ± 0.0
Alvand Hospital	0.0 ± 0.0	95.00 ± 5.20	100.0 ± 0.0	100.0 ± 0.0
Shariati Hospital	15.57 ± 5.70	95.00 ± 5.20	100.0 ± 0.0	100.0 ± 0.0
Valiasr Hospital	0.0 ± 0.0	80.00 ± 10.00	80.00 ± 10.00	100.0 ± 0.0
Amir-Al Momenin Clinic	5.00 ± 1.20	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Shahid Kalantari Clinic	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Shariati dormitory	0.0 ± 0.0	25.00 ± 5.20	95.00 ± 5.20	100.0 ± 0.0
Kooy dormitory	0.0 ± 0.0	25.00 ± 5.20	100.0 ± 0.0	100.0 ± 0.0

Shariati dormitory (0%–75%) and Kooy dormitory (17.5%–100.0%) was reduced under effect of PBO synergist with the ratio of 1:1, 2:1 and 3:1 (Table 3), demonstrating a significant difference between using PBO synergist and lack of PBO ($P < 0.05$). Also, results of synergist experiments showed that the resistance to the carbaryl in strains of Mofid Hospital (80%–100%), Alvand Hospital (95%–100%), Amir-Al Momenin Clinic (95%–100%), Shariati Hospital (80%–100%), Valiasr Hospital (80%–100%), Shahid Kalantari Clinic (100%), Shariati dormitory (25%–100%) and Kooy dormitory (25%–100%) under impact of DEF synergist was reduced with the ratio of 1:1, 2:1 and 3:1 (Table 4). A significant difference was observed between using DEF synergist and lack of DEF ($P < 0.05$).

4. Discussion

Results of DEF synergist tests on the bendiocarb and carbaryl manifested that the resistance level in all tested strains was completely eliminated which showed a very high role of esterase enzymes in resistance to these insecticides in each of these strains. But PBO synergist used in the strains of Mofid, Valiasr, Alvand hospitals and Shariati dormitory could not completely eliminated the resistance because other mechanisms may interfere with resistance to these insecticides such as reduced cuticle penetration and insensitivity to the acetylcholinesterase enzyme. Besides, the resistance to these insecticides could be due to excessive use of insecticides from the carbamate group for the control of German cockroaches. The conducted studies across the world about the susceptibility level and resistance mechanisms in German cockroach against the propoxur, bygone and bendiocarb has shown that by using PBO and DEF resistance against these insecticides has incompletely lost and has been inhibited by mono-oxygenase and esterase enzymes [16,17,20]. In this study, generally, the impact of DEF on breaking the resistance was observed more than PBO that indicates the role of hydrolytic enzymes as the main source of resistance and the cytochrome P450 mono-oxygenases enzyme as a contributing factor in resistance to bendiocarb and carbaryl in the mentioned strains. Synergists have an effect on the enzymatic systems of insects, which exacerbate the effect of insecticides and cause increased susceptibility to insecticides in insects. Synergistic tests can identify the enzyme systems that are involved in the resistance phenomenon [47]. Studies conducted in recent years indicate that esters and mono-oxygenases P450 have been involved in the resistance of *B. germanica* against insecticides belonging to carbamate group [20,36,48–52] and confirm the current study that is done for the first time in Iran. Controlling German cockroaches successfully requires a precise execution of a resistance management program against insecticides based on the prediction of resistance. Considering the results of this study and acquired knowledge of the resistance mechanisms, it is not recommended to use insecticides related to carbamate group for control of German cockroach. Instead, according to conducted studies, the novel insecticides should be used such as Esinosada or poisonous baits, whose mechanism of action is different from the insecticides of the carbamate group, including the poisonous baits of – imidacloprid and fipronil [44,53–57]. On the other hand, the management of resistance to insecticides is not possible except carrying out a comprehensive study on the diagnosis and identification of various mechanisms of resistance which make insecticides ineffective against the German cockroach. It is suggested that in the future further studies will be conducted to examine other mechanisms, such as decreasing cuticle penetration and insensitivity to the acetylcholinesterase enzyme in this field. In conclusion, the resistance against bendiocarb and carbaryl is completely eliminated by DEF in most of these strains, which indicates a key role of esterase enzymes in the development of resistance to carbaryl and bendiocarb while PBO does not remove the resistance completely which may be attributed to other mechanisms such as reduced cuticle penetration and insensitivity to the acetylcholine esterase enzyme.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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