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Iranian Non-Responding Contact Method German Cockroach Permethrin Resistance Strains Resulting from Field Pressure Pyrethroid Spraying

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Abstract: The present study investigated the probable responsible reasons for non-response the *B. germanica* to permethrin only in World Health Organization glass jar method after insecticide spraying control failure with pyrethroid insecticide groups in Islamic Republic of Iran. Eleven German cockroach strains were collected from field populations of nine infested kitchen student dormitories and two infested hospitals after insecticide spraying control failure with pyrethroid insecticide groups in Iran. The current study, in World Health Organization glass jar and knock down methods conducted on newly emerged adult males. All feral strains, with various levels of resistance to pyrethroids, in World Health Organization glass jar method at 400 min (6 h) time exposures, was not observed mortality, while the susceptible strain was observed 100% mortality in 25 min time exposures. Susceptible strain at LT_{50} after assessing on mortality data from the replicates by probit analysis in World Health Organization glass jar method was 15.3 min. In this study, all feral strains in World Health Organization glass jar method after 400 min (6 h) time exposures, was not observed mortality, that showed these strains very high-level resistance to permethrin. In the knock down method, the resistance ratios were 3.6 to 26.1-folds compare with the susceptible reference strain. In a comparison, among this study and previous studies resistance ratios of 8.6 to 17.7-folds for permethrin in topical application, indicated that German cockroach have had under pressure spraying. German cockroach have had in vicinity to pyrethroid insecticides especially permethrin in these locations in long period for non-responding to permethrin insecticide only in World Health Organization glass jar method, and the field evident confirm this subject.

Key words: German cockroach, pyrethroid, insecticide resistance

INTRODUCTION

The German cockroach, *Blattella germanica* L. is an important household insect pest worldwide. In addition to its economic importance, the German cockroach is a significant health hazard and acts as a mechanical vector and reservoir for pathogenic agents (Pridgeon *et al.*, 2002). It is a major source of indoor allergens and responsible for an increased incidence of asthma (Roberts, 1996) and have added emphasis to the need for the control of this insect pest.

Extensive use of insecticides has led to the development of resistance in German cockroach to a wide range of insecticides including organochlorines, organophosphates, carbamates and pyrethroids (Cochran, 1997). Pyrethroid (PYRs), a family of synthetic insecticides, are an important class of pesticides because they rapidly paralyze insects and less persistent in the environment. The effectiveness and low mammalian

toxicity of PYRs insecticides relative to other classes of pesticides have resulted in these compounds being widely used for German cockroach control. Despite frequent use of insecticides, control failures in some field populations have been reported resulting from the development of resistance (Scott *et al.*, 1990; Atkinson *et al.*, 1991; Valles and Yu, 1996; Dong *et al.*, 1998; Valles, 1999; Valles *et al.*, 2000; Wei *et al.*, 2001).

Diverse methods have been used to estimate insecticide resistance in the German cockroach. Ladonni (2001) considered the insecticide resistance status of the German cockroach against permethrin with three different bioassays, KT_{50} , LD_{50} and LT_{50} methods. The results indicate that in all three bioassays, cockroaches showed resistance ratios of 6.7-, 12.8- and 7.5-fold for KT_{50} , LD_{50} and LT_{50} , respectively compared with those from a susceptible strain. Comparison among three methods all field strains exhibited no significant resistance level. During studying of insecticide resistance in German

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cockroach was observed a rare condition that this pest non-response to permethrin only in World Health Organization glass jar method and which was surprised, but the other methods, topical application and knock down, for detecting insecticide resistance is completely normal as previous studies (Ladonni, 2000, 2001).

The present study investigated the probable responsible reasons for non-response the *B. germanica* to permethrin only in World Health Organization glass jar method after insecticide spraying control failure with pyrethroid insecticide groups in Islamic Republic of Iran.

MATERIALS AND METHODS

Insects: The study was conducted on twelve field collected *B. germanica* strains in 2003. The susceptible strain SS has been held at the Cockroach Insectary of the Department of Medical Entomology and Vector Control, School of Public Health and Institute of Public Health Researches, Tehran University of Medical Sciences without exposure to insecticide for >30 years (Ladonni, 2001). The D₁-D₈, M and H₁-H₂ strains were collected from field populations of nine infested kitchen student dormitories and two infested hospitals after insecticide spraying control failure in Tehran during 2001 and 2003, respectively (Nasirian *et al.*, 2006a, b). Individual cockroaches used for experiments were adult male <1 month old in all cases.

Cockroaches collecting and rearing: Cockroaches were collected in the last hours of the night with a piece of a radiology film (10×10 cm) for the flexibility nature and transferred to an apparatus by hand catch. The apparatus manufactured from two parts (Fig. 1 a), the upper inside surface of the upper part (5 cm) was lightly greased with petroleum jelly to prevent cockroaches from escaping. After collecting cockroaches transferred to insectary and at the insectary the lower part separated from the upper part and cockroaches transferred to glass rearing jars to prevent cockroaches from greasing (Fig. 1a) (Nasirian *et al.*, 2006a, b).

All cockroaches were maintained and colonized at 27±2°C, 60±10% RH and a photoperiod of 12: 12 (L: D) h in the insectary at the School of Public Health and Institute of Public Health Researches, Tehran University of Medical Sciences. Each strain was kept in separate labeled glass rearing jars of the same size (4 L) (Fig. 1b). Cockroaches were provided with rodent diet, a cotton plugged water vial and a cardboard as a harborage.

Chemicals and reagents: Technical grade insecticide: permethrin (93.6%), cis: trans 60: 40 (Zeneca, Haslemere, UK), CO₂ as an anesthetic, and acetone as a solvent were used.



Fig. 1: (a) Two parts separated of cockroach collecting apparatus view (b) view of the cockroach insectary

Bioassay: Tarsal-contact method test using World Health Organization glass jar and knock down methods were used to evaluate permethrin resistance in eleven well-collected strains of German cockroach (Cochran, 1987, 1997). Technical grade permethrin, 93.6% (technical grade) cis: trans 60: 40 (Zeneca, Haslemere) was prepared in acetone and 2.5 mL was pipetted into a 0.4-litre glass jar. The jars were rolled horizontally over a flat surface until all of the acetone had evaporated, so that the insecticide was deposited evenly over the inner surface of the jar.

In a series of contact experiments, a concentration of 7 mg m⁻² permethrin (technical grade) at a 25 min exposure time was found to be a discriminating dose for adult males to continue the all experiments. Adult male cockroaches (1-3 week old) were treated with 5-6 time exposures and each time exposure was replicated 3-6 times (10 cockroaches for each replicate). Control groups received acetone alone. A 5-6 time exposure giving >0 and <100% mortality at 24 h after insecticide time exposures was used for each experiment. Permethrin exposures males were placed in plastic Petri dishes (150 by 25 mm), 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 when prodded, they were considered dead.

In a series of knock down experiments, a concentration of 20 mg m⁻² permethrin (technical grade) was found to be a discriminating dose for adults to continue the all experiments and the time of knock down cockroaches were recorded and continued until the last cockroaches, which were knockdown.

Statistical analysis: Mortality data from the replicates were pooled and the time exposure mortality and knockdown cockroaches were assessed by probit analysis (Finney, 1972), with a SPSS package on an IBM computer.

RESULTS AND DISCUSSION

Eleven German cockroach strains were collected from field populations of nine infested kitchen student dormitories and two infested hospitals after a control failure with pyrethroid group insecticide spraying in Iran. The current study, in World Health Organization glass jar and knock down methods conducted on newly emerged adult males. All feral strains in World Health Organization glass jar method at 400 min (6 h) time exposures, was not observed mortality (Table 1, Fig. 2) while the susceptible strain was observed 100% mortality in 25 min time exposures (Table 2, Fig. 2). Susceptible strain at LT₅₀ after assessing on mortality data from the replicates by probit analysis in World Health Organization glass jar method was 15.3 min. In the knockdown method, the resistance ratios were 3.6 to 26.1-folds compare with the susceptible reference strain (Table 3).

Nasirian *et al.* (2006a, b) in studies conducted on these strains reported resistance ratios of 8.6 to 17.7-folds for permethrin in topical application (Nasirian *et al.*, 2006a, b). Ladonni (2000, 2001) reported that observed no significant resistance level among diverse methods detecting insecticide resistance, topical application, knock down and World Health Organization glass jar method (Ladonni, 2000, 2001). While, in this study, all feral strains only in World Health Organization glass jar method in a rare condition in the world, was non-responding to permethrin, that will showed these strains have had under pressure spraying to pyrethroid insecticides and very high-level resistance to permethrin (Fig. 1).

In studies by Nasirian *et al.* (2006c, d) investigated fipronil toxicity against these strains of German cockroach in topical application and contact methods. There are no significant differences between the two bioassay (topical application and contact methods) in the toxicity of the fipronil against these strains of German cockroach. The RRs of these strains to fipronil ranged from 1.5 to 2.6-fold (Nasirian *et al.*, 2006c, d). Since fipronil has not been

Table 1: Bioassay results from each field collected strain of adult male *B. germanica* at 7 mg m⁻² permethrin (technical grade) in World Health Organization glass jar method

| Time exposure (min) | Treated numbers (in 3-6 replicates) | Results after 24 h holding time | | Mortality (%) |
|---------------------|-------------------------------------|---------------------------------|------|---------------|
| | | Alive | Dead | |
| 100 | 30 | 30 | 0 | 0 |
| 150 | 50 | 50 | 0 | 0 |
| 200 | 60 | 60 | 0 | 0 |
| 250 | 40 | 40 | 0 | 0 |
| 300 | 50 | 50 | 0 | 0 |
| 350 | 60 | 60 | 0 | 0 |
| 400 | 30 | 30 | 0 | 0 |

Table 2: Bioassay results from adult male *B. germanica* susceptible strain at 7 mgm⁻² permethrin (technical grade) in World Health Organization glass jar method

| Time exposure (min) | Treated numbers (in 3 replicates) | Results after 24h holding time | | Mortality (%) |
|---------------------|-----------------------------------|--------------------------------|------|---------------|
| | | Alive | Dead | |
| 5 | 30 | 29 | 1 | 3.3 |
| 10 | 30 | 25 | 5 | 16.7 |
| 15 | 30 | 27 | 13 | 43.3 |
| 20 | 30 | 18 | 22 | 73.3 |
| 22.5 | 30 | 1 | 29 | 96.7 |
| 25 | 30 | 0 | 30 | 100.0 |

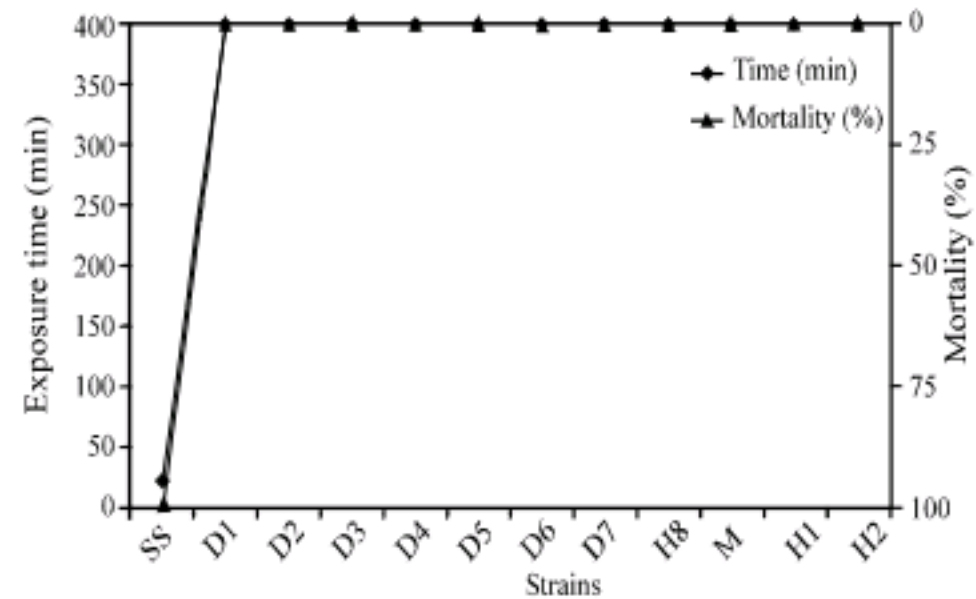


Fig. 2: Toxicity of permethrin to susceptible and field population strains by World Health Organization glass jar method

used previously in this area, so this relatively low resistance could be due to cross-resistance to dieldrin (Limoe *et al.*, 2007). These studies in comparison to present study also showed that German cockroach have had very high-level resistance to permethrin for non-responding to insecticide only in World Health Organization glass jar method in vicinity to long period pyrethroid insecticide spraying.

In conclusion, the German cockroach only in World Health Organization glass jar method was not observed mortality while the susceptible strain was observed 100% mortality, which probably due to extensive use of pyrethroid insecticides and the field evident confirms this

Table 3: Toxicity of permethrin to a susceptible and feral-reared German cockroach strains by knockdown method at 20 mg m⁻² permethrin (technical grade) in knock down method

| Strains | Permethrin in knock down method | | | | | |
|----------------|---------------------------------|---------------|-----------------------|-----------------------|-----------------------|------|
| | n | Slope Mean±SE | χ ² -value | KT ₅₀ (CI) | KT ₉₀ (CI) | RR |
| SS | 33 | 0.40±0.050 | 4.1 | 11.1 (10.2-11.6) | 014.4 (13.7-15.6) | – |
| D ₁ | 30 | 0.01±0.001 | 5.2 | 270.2 (221.3-361.5) | 496.2 (393.4-727.5) | 24.3 |
| D ₂ | 30 | 0.02±0.002 | 12.3 | 128.9 (111.1-144.9) | 195.9 (175.0-233.7) | 11.6 |
| D ₃ | 30 | 0.04±0.004 | 11.3 | 40.3 (29.6-50.2) | 076.6 (64.4-96.4) | 03.6 |
| D ₄ | 30 | 0.02±0.004 | 11.7 | 58.4 (18.9-231.3) | 110.3 (71.5-232.4) | 05.3 |
| D ₅ | 30 | 0.01±0.004 | 2.8 | 142.6 (104.4-283.5) | 256.4 (177.9-569.7) | 12.8 |
| D ₆ | 30 | 0.02±0.010 | 0.3 | 112.4 (89.3-502.3) | 167.0 (119.1-1024.7) | 10.1 |
| D ₇ | 36 | 0.02±0.050 | 2.0 | 121.8 (94.2-223.1) | 198.8 (144.1-416.9) | 11.0 |
| H ₈ | 30 | 0.01±0.002 | 3.5 | 127.8 (110.4-150.7) | 221.9 (190.5-274.1) | 11.5 |
| M | 30 | 0.01±0.002 | 1.2 | 290.2 (273.6-309.5) | 405.3 (372.7-461.3) | 26.1 |
| H ₁ | 30 | 0.02±0.003 | 7.0 | 75.1 (66.3-86.3) | 135.9 (117.9-165.7) | 06.8 |
| H ₂ | 33 | 0.01±0.002 | 19.0 | 93.7 (30.0-344.3) | 209.9 (131.5-1306.5) | 08.4 |

subject. It would be suggested that further studies on mechanism of pyrethroid insecticide resistance should be conducted.

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