Insecticide resistance and synergism of three field-collected strains of the German cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from hospitals in Kermanshah, Iran

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Received 31 August 2010; received in revised form 19 November 2010; accepted 29 November 2010

Abstract. The development of insecticide resistance in the German cockroach, *Blattella germanica* (L.) is a serious problem in controlling this medically important household pest. The insecticide resistance status in three hospital-collected strains of the German cockroach using four commonly used insecticides from different classes (permethrin, cypermethrin, bendiocarb and chlorpyrifos) was detected by topical bioassay method and preliminary information on possible involvement of monooxygenases in permethrin resistant strains employing synergist piperonyl butoxide (PBO) was obtained. For each insecticide, four to six concentrations resulting in >0% and <100% mortality were used. Three to six replicates of 10 cockroaches per concentration were conducted. For synergism studies, 100µg PBO per gram body weight of cockroach as the maximum sublethal dose was administered to the first abdominal segment 1 h before insecticide treatment. The differences between LD₅₀ (µg/g) values were considered statistically significant only when the 95% confidence intervals did not overlap. The resistance ratio and synergism ratio were calculated for each insecticide. All three hospital-collected strains of the German cockroach showed different levels of resistance to permethrin and cypermethrin based on resistance ratios compared with SUS strain. Permethrin and cypermethrin resistance ratios ranged from 11.61 to 17.64 and 11.45 to 26.45 at LD₅₀ levels, respectively. Low to moderate levels of bendiocarb resistance and low level of chlorpyrifos resistance were also observed in the hospital-collected strains under study. The synergist piperonyl butoxide (PBO) significantly enhanced the toxicity of permethrin to all strains with different degrees of synergist ratio, 2.45-, 1.87-, 2.51- and 2.38-fold, suggesting monooxygenase involvement in permethrin resistance.

INTRODUCTION

The German cockroach, *Blattella germanica* (L.) has been recognized as a serious health problem because of its potential to harbour and transmit human disease-causing pathogens as well as inducing asthma (Roberts, 1996; Pai et al., 2003, 2005).
reported different levels of resistance to carbamates, organophosphates and pyrethroids in field-collected strains of the German cockroach in Malaysia (Lee et al., 1996). Pai et al. (2005) determined the resistance of the German cockroach from hospitals and households to propoxur, chlorpyrifos and cypermethrin. Among the different classes of insecticides, pyrethroids have been extensively used for the German cockroach control because of their effectiveness and low mammalian toxicity. Nevertheless, frequent use of these compounds has resulted in the development of resistance. Scharf et al. (1997) reported 80-fold resistance at LD$_{50}$ level to cypermethrin in a field-collected strain of the German cockroach from Indiana. Wu et al. (1998) reported 825-fold resistance at LD$_{50}$ level to fenvalerate in the Munsyana strain of the German cockroach from Indiana. Valles et al. (2000) reported 93-fold resistance at LD$_{50}$ level to cypermethrin in a field strain of the German cockroach from Florida. Control failures in field populations of the German cockroach have been reported due to the development of permethrin resistance in Iran (Limoee et al., 2006), deltamethrin resistance in Singapore (Choo et al., 2000), and permethrin and deltamethrin resistance in Alabama (Wei et al., 2001). Pai et al. (2005) determined the resistance of the German cockroach from hospitals and households in Taiwan concluding that the resistance patterns of propoxur>chlorpyrifos>cypermethrin in hospital strains and propoxur>cypermethrin>chlorpyrifos in household strains, might be due to the frequency of application of the insecticides. Chai et al. (2010) studied the resistance status of 22 field-collected strains of the German cockroach from Singapore against six classes of insecticides using topical bioassay method. They found a range of resistance from 3-468-fold against pyrethroid insecticides and more than 20-fold resistance to OPs and carbamate compounds. Synergist bioassays revealed oxidases and esterases involvement in insecticide resistance in most of the strains leaving kdr and Rdl mutations as the probable underlying mechanism of insecticide resistance in the rest of the strains (Chai & Lee, 2010). In a study in Indonesia, relatively high levels of permethrin resistance were detected in field strains of the German cockroach which was attributable to oxidases. However, cypermethrin resistance was not suppressible by synergists, suggesting the involvement of a kdr-like mechanism (Ahmad et al., 2009).

Extensive use of insecticides from different classes such as organochlorine, organophosphates and pyrethroids resulted in development of resistance to different insecticides in various strains of the German cockroaches in Tehran, Iran (Ladonni, 1993, 1997; Ladonni & Sadegheyani, 1998; Limoee et al., 2006). Insecticide resistance was detected and its metabolic mechanisms were found in this pest in Tehran (Limoee et al., 2001), and attempts were made to compare different test methods for detecting resistance of the German cockroach strains (Ladonni, 2000, 2001). In a recent review article, the susceptibility of different strains of the German cockroach to different groups of insecticides were summarized (Nasirian, 2010). There is no information on insecticide resistance status in hospital strains of the German cockroach in Kermanshah, a western province of the country. Therefore, this study was undertaken to detect insecticide resistance status in three hospital-collected strains of the German cockroach using four commonly used insecticides from different classes (permethrin, cypermethrin, bendiocarb and chlorpyrifos), and to obtain preliminary information on possible involvement of monooxygenases in permethrin resistant strains employing synergist piperonyl butoxide (PBO).

MATERIALS AND METHODS

Cockroach strains

Four German cockroach strains were examined in this study: SUS is the standard susceptible strain maintained since 1975 in
the insectary at the School of Public Health, Tehran University of Medical Sciences without exposure to insecticide; three strains: T1, T2 and IH were collected in 2008 from different hospitals in Kermanshah.

Spraying with different insecticides including pyrethroids, organophosphates and carbamates failed to control the German cockroach in those hospitals (personal communication).

All cockroaches were maintained in an insectary at 27±2ºC, 60±10% RH, with a photoperiod of 12:12 h (L:D). Each strain was reared in the same size labeled glass jar. Cockroaches were provided with unlimited cat food and water. Tests were conducted on adult males of F2-F4 generations.

**Chemicals**

Chemical used were permethrin, 93.6% (technical grade) cis:trans 60:40, cypermethrin, 97.5% (technical grade), (Zeneca, Haslemere, UK), bendiocarb, 97% (technical grade), chlorpyrifos 97% (technical grade), (Cyanamid Agro, India) and synergist piperonyl butoxide (PBO) 93,6%, (Zeneca, Haslemere, UK), a monooxygenase inhibitor for synergism study. CO2 was used as anesthetic and acetone as solvent.

**Bioassays methods**

Bioassay tests were performed by topical application of 1µl of a known concentration of insecticide and synergist solution to the first abdominal segment of the insects, using a hand micro-applicator (Burkard, Scientific Ltd, UK) equipped with a 1.0 ml Hamilton glass syringe.

Adult male cockroaches were anesthetized with carbon dioxide for 20–30 seconds before insecticide treatment (Valles & Koehler, 1994). For each insecticide, four to six concentrations resulting in >0% and <100% mortality were used.

Three to six replicates of 10 cockroaches per concentration were conducted. For synergism study, 100µg PBO per gram body weight of cockroach, as the maximum sublethal dose, was placed on the first abdominal segment 1 h before insecticide treatment (Valles et al., 1997). Control groups received acetone or synergist alone. Treated cockroaches were kept for 24 h in Pyrex glass jars provided with food and water before scoring the mortality. Cockroaches were considered dead when they were unable to turn themselves to normal posture within one minute after being turned onto their dorsum.

**Data analysis**

Bioassay data were pooled and subjected to probit analysis (Finny, 1972) using a personal computer. The differences between LD50 values were considered statistically significant only when the 95% confidence intervals did not overlap. All LD50 values were converted from µg/cockroach to µg/g of cockroach body weight to avoid possible effect of weight differences on insecticide susceptibility. The resistance ratio (RR, LD50 of the resistant strain divided by LD50 of the susceptible strain) and synergism ratio (SR, LD50 of insecticide alone divided by LD50 of insecticide + synergist) were calculated for each insecticide.

**RESULTS**

**Pyrethroid resistance**

All three hospital-collected strains of the German cockroach showed different levels of resistance to permethrin and cypermethrin based on resistance ratios (RRs) compared with SUS strain (Table 1). Permethrin resistance ratios ranged from 11.61 to 17.64 at LD50 levels. Comparisons made between the 95% confidence intervals of the LD50 values of field strains with susceptible strain (SUS) indicated that all three strains had significantly different RR. The order of resistance levels for the field strains was as follows: IH>T2>T1 with the IH strain exhibiting the highest resistance ratio (RR= 17.64).

Different levels of resistance to cypermethrin were observed in all three hospital-collected strains of the German cockroach (Table 1) showing resistance
ratios ranging from 11.45 to 26.45 at LD_{50} levels. Examining the 95% confidence intervals of the LD_{50} values between the resistance ratios of the three field strains with susceptible strain (SUS) indicated that all had significantly different RR. The order of resistance levels for three hospital collected strains was as follows: T2>IH>T1 with the T2 strain showing the highest resistance ratio (RR= 26.45).

**Bendiocarb resistance**

Low to moderate levels of bendiocarb resistance were also observed in the hospital-collected strains under study (Table 2) with resistance ratios ranging from 2.93 to 4.91 at LD_{50} levels. The order of resistance levels was as follows: IH>T2>T1.

**Chlorpyrifos resistance**

Chlorpyrifos bioassay data presented in Table 2 shows that T2 and IH strains developed relatively significant resistance when compared with the susceptible strain (SUS) (RR: 2.0 < 2.18 ). There was no significant difference between LD_{50} values of T1 and susceptible (SUS) strain (Table 2).

### Table 1. Lethal dose values for permethrin and cypermethrin for insecticide susceptible and three hospital-collected strains of German cockroach

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Y- intercept</th>
<th>S lope (SE)</th>
<th>X^2 (df)</th>
<th>LD50 (95% CL) µg/g^a</th>
<th>RR^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUS^cPermethrin</td>
<td>201</td>
<td>0.99</td>
<td>3.89 (0.49)</td>
<td>3.621 (4)</td>
<td>10.70 (9.44-12.1)</td>
<td>1</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>169</td>
<td>3.11</td>
<td>3.3 (0.42)</td>
<td>3.57 (2)</td>
<td>3.74 (3.18-4.44)</td>
<td>1</td>
</tr>
<tr>
<td>T1Permethrin</td>
<td>169</td>
<td>-8.27</td>
<td>6.34 (0.86)</td>
<td>0.981 (2)</td>
<td>124.28 (114.31-135.71)</td>
<td>11.61^sig</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>145</td>
<td>0.78</td>
<td>2.58 (0.39)</td>
<td>3.1 (2)</td>
<td>42.84 (33.01-53.97)</td>
<td>11.45^sig</td>
</tr>
<tr>
<td>T2Permethrin</td>
<td>128</td>
<td>-3.59</td>
<td>4.03 (0.57)</td>
<td>5.283 (2)</td>
<td>161.38 (137.4-188.96)</td>
<td>15.1^sig</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>170</td>
<td>-0.47</td>
<td>2.74 (0.37)</td>
<td>0.665 (2)</td>
<td>98.91 (81.99-122.6)</td>
<td>26.45^sig</td>
</tr>
<tr>
<td>IHPermethrin</td>
<td>171</td>
<td>-6.73</td>
<td>5.15 (0.76)</td>
<td>2.637 (2)</td>
<td>188.77 (169.35-208.87)</td>
<td>17.64^sig</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>146</td>
<td>-0.73</td>
<td>3.14 (0.43)</td>
<td>3.086 (2)</td>
<td>66.62 (55.34-80.24)</td>
<td>17.81^sig</td>
</tr>
</tbody>
</table>

^a Micrograms of insecticide/g of cockroach body weight  
^b Resistance Ratio  
^c Susceptible strain  
.sig Significant

### Table 2. Lethal dose values for bendiocarb and chlorpyrifos for insecticide susceptible and three hospital-collected strains of German cockroach

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Y- intercept</th>
<th>S lope (SE)</th>
<th>X^2 (df)</th>
<th>LD50 (95% CL) µg/g^a</th>
<th>RR^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUSBendiocarb</td>
<td>143</td>
<td>0.8</td>
<td>2.96 (0.44)</td>
<td>0.199 (2)</td>
<td>26.4 (21.63-31.9)</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>183</td>
<td>1.31</td>
<td>4.91 (0.64)</td>
<td>6.96 (3)</td>
<td>5.66 (5.11-6.33)</td>
<td>1</td>
</tr>
<tr>
<td>T1Bendiocarb</td>
<td>164</td>
<td>0.95</td>
<td>2.14 (0.29)</td>
<td>4.714 (2)</td>
<td>77.44 (58.92-98.86)</td>
<td>2.93^sig</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>149</td>
<td>1.83</td>
<td>3.8 (0.66)</td>
<td>0.84 (2)</td>
<td>6.81 (5.73-7.84)</td>
<td>1.20^ns</td>
</tr>
<tr>
<td>T2Bendiocarb</td>
<td>189</td>
<td>0.8</td>
<td>2.02 (0.29)</td>
<td>5.531 (3)</td>
<td>118.62 (91.63-148.88)</td>
<td>4.50^sig</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>159</td>
<td>-1.01</td>
<td>5.7 (0.76)</td>
<td>1.02 (2)</td>
<td>11.31 (9.92-12.57)</td>
<td>2.00^sig</td>
</tr>
<tr>
<td>IHBendiocarb</td>
<td>201</td>
<td>1.65</td>
<td>1.58 (0.26)</td>
<td>0.397 (2)</td>
<td>129.62 (98.83-177.53)</td>
<td>4.91^sig</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>154</td>
<td>-0.19</td>
<td>4.76 (0.67)</td>
<td>2.76 (2)</td>
<td>12.32 (10.88-13.83)</td>
<td>2.18^sig</td>
</tr>
</tbody>
</table>

^a Micrograms of insecticide/g of cockroach body weight  
^b Resistance Ratio  
^c Susceptible strain  
.sig Significant  
^ns Nonsignificant
Synergism of PBO
Pretreatment of adult male German cockroaches of susceptible and three hospital-collected strains with synergist, PBO significantly enhanced the toxicity of permethrin to all strains with different degrees of SRs, 2.45-, 1.87-, 2.51- and 2.38-fold, respectively (Table 3). It can be inferred from the synergist bioassay data that Cytochrome P450 monoxygenases might be contributing to certain degree to the detoxification of permethrin.

DISCUSSION
According to Milio et al. (1987), Scott et al. (1990), Choo et al. (2000) and Ladonni (2001), the lethal dose (LD) method especially by topical application may be more appropriate for insecticide toxicological tests because the amount applied is precisely measured. Hence, this method was used throughout this study.

Different levels of permethrin and cypermethrin resistance were observed in field strains of the German cockroaches from hospitals of Kermanshah. These results are consistent with our previous studies on pyrethroid resistance in some populations of the German cockroaches from Tehran, Iran in which resistance ratios ranged from 6.28 to 23.7 for permethrin and 5.26 to 20.7 for cypermethrin (Limoee et al., 2006). Also the results of the present study are in accord with those of another study on permethrin resistance in some populations of the German cockroach, reporting RRrs ranging from 17 to 23.24 (Ladonni & Sadegheyani, 1998). High levels of pyrethroid resistance have been reported from Singapore and Indonesia (Chai & Lee 2010; Ahmad et al., 2009).

The following factors might be attributable to the development of pyrethroid resistance in the field strains of German cockroaches. Pyrethroid insecticides such as permethrin and cypermethrin have been used to control these cockroach populations in Tehran, Kermanshah and other provinces for the last two decades. They have recently been replaced with a mixture of carbamate and cypermethrin. Therefore, this continued use of pyrethroids has led to a relatively high selection pressure. Zhai & Robinson (1991) reported high level of resistance to cypermethrin and control failure of this pyrethroid insecticide against the German cockroach after about 4 years of use.

Milio et al. (1987) compared three methods for measuring the susceptibility of adult German cockroaches to formulated chlorpyrifos based on resistance ratios. The Lethal dose (LD) method showed the highest resistance ratio compared with the other test methods.

According to Reierson et al. (1998), 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.

On the other hand, Robinson & Zhai (1990, 1994) previously reported that when cypermethrin is replaced with chlorpyrifos against the German cockroach for three years, resistance levels to cypermethrin decreased. Therefore this negative cross-resistance may be of importance in the susceptibility of the strains tested in our study to chlorpyrifos.

The continued susceptibility of the hospital-collected strains of the German cockroach in this study to chlorpyrifos, could be due to the low frequency of application of this organophosphate insecticide, as Pai et al. (2005) had previously mentioned, and also the fact that permethrin has recently been replaced with chlorpyrifosin for controlling cockroaches.

The results of synergist study demonstrated that toxicity of permethrin to three hospital strains was slightly enhanced (1.87 – 2.38) by PBO, suggesting the possible involvement of monooxygenases in permethrin resistance. These findings were similar to those of our previous studies, Lee et al. (1996), Limoe et al. (1996, 2007) and Chai & Lee (2010) on PBO effect in decreasing permethrin resistance in different populations of the German cockroach. However, resistance levels to permethrin in T1 strain increased from 11.61 to 15.18 when pretreated with PBO. Also PBO caused a marginal increase in RR from 17.64 to 18.16 in IH strain. This effect has been reported frequently (Lee et al., 1996; Valles & Yu, 1996; Valles, 1998; Scott, 1999; Pridgeon et al., 2002; Limoe et al., 2007) and may be explained by the following reasons: (a) Cytochrome p450s exist in a number of different related forms and PBO may not inhibit all isozymes involved in the metabolism of the insecticide in question (Valles & Yu, 1996; Valles, 1998; Pridgeon et al., 2002), (b) The higher susceptibility of Cytochrome p450 isform (s) to the synergist in the SUS strain than T1 strain resulted in a greater effect of PBO on the SUS strain and (c) It is also reported that PBO enhances the absorption of insecticide molecules into the insect body (Rouch & Bruce, 1990). Further studies on the effect of synergist DEF on permethrin resistance and its cross resistance pattern to DDT are necessary for providing further in vivo evidence about the underlying resistance mechanisms to permethrin.

Acknowledgements. The authors would like to express their sincere gratitude to the authorities of the Deputy for Research and School of Public Health of Kermanshah University of Medical Sciences for providing the research grant and administrative assistance.

REFERENCES


