Iranian Biomedical Journal 11 (4): 251-258 (October 2007)

Biochemistry of Pyrethroid Resistance in German Cockroach (Dictyoptera, Blatellidae) from Hospitals of Sari, Iran

Ahmad Ali Enayati* and Farzad Motevalli Haghi

School of Public Health and Environmental Health Research Centre, Mazandaran University of Medical Sciences, Sari, Iran

Received 15 July 2006; revised 18 February 2007; accepted 11 March 2007

ABSTRACT

Background: The German cockroach is an important household insect pest mechanically involved in transmission of a variety of diseases to humans. Different classes of insecticides have extensively been used for its control leading to insecticide resistance development. Hence, for an optimal control of this pest, the status and underlying mechanisms of insecticide resistance should be studied in this group of insects. **Methods:** Adult German cockroaches were collected from Imam and Bouali Cina Hospitals **(**Sari, Iran**)** and subjected to bioassay using jar test method. The results were compared to those of a susceptible laboratory strain. Biochemical assays of esterases, monooxigenases and glutathione S-transferase (GST) levels were undertaken on German cockroaches from Imam and Bouali Cina Hospitals and the results were compared to a susceptible laboratory strain. **Results:** The LT_{50} values of the three strains were 20.24 ± 2.2, 19.87 ± 2.3 and 8.89 ± 0.26 for permethrin; 19.3 ± 3.05, 17.6 ± 0.68 and 8.8 ± 0.99 for deltamethrin; 19.64 ± 2.9, 18.66 ± 3.45 and 8.64 ± 0.62 min for cypermethrin, respectively. The mean α-esterase activity of Imam and Bouali Cina Hospitals and susceptible strains were 6.941 × 10-4, 6.940 × 10-4 and 8.01 × 10-5 nmol/min/mg protein; the mean β-esterase activity in those strains were 5.8 × 10-4, 4.25 × 10-4 and 7.28 × 10-5 nmol/min/mg protein; the mean content of p450 in the above-mentioned strains were 5.64 × 10-6, 1.89 × 10-6 and 1.2 × 10-6 nmol/mg protein; the mean GST activity were 6.66 × 10-2, 0.102 and 5.72 × 10-2 μmol/min/mg protein**,** respectively. **Conclusion:** The LT50 values and also the mean activity of all enzyme groups in field strains were significantly different from those of the susceptible strain, indicating a vigour tolerance to insecticides and pyrethroids in particular. Hence, insecticide resistance monitoring techniques should be put in place and also resistance management strategies and measures should be considered implementing in the area. *Iran. Biomed. J. 11 (4): 251-258, 2007*

Keywords: Pyrethroids, German cockroaches, Sari

INTRODUCTION

The German cockroach, *Blattella germanica L.*, is a very important urban pest in households, hospitals and residential areas alleged to be involved in mechanical transmission of a variety of diseases as well as causing insectophobia [1]. 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 [1-3]. There are voluminous amount of literature on the mechanisms of insecticide resistance in many insect species.

One of the most important underlying resistance mechanisms is qualitative and quantitative increase in the activity/level of enzymes responsible for insecticide resistance. Involvement of glutathione S-transferases (GST) [4-6], esterases [7, 8], mixed function oxidases (MFO) [9-11] and esterases [12-16] are evident in many insect groups including German cockroaches.

Insecticide resistance in German cockroaches was first detected decades ago in a strain in the USA against chlordane in 1952. Resistance to lindan in Poland in 1959, to lindan and dieldrin in Turkey in 1962 and to malathion, diazinon and phenthione in the 1970s in the USA were subsequently reported

*Corresponding Author; E-mail: tmaae@liverpool.ac.uk

[DOR: 20.1001.1.1028852.2007.11.4.3.5]

[DOI: -]

[4]. In recent years, pyrethroids have been used for pest control and as a result resistance to these insecticides occurred in German cockroaches from many parts of the world [17].

In a study of collected German cockroaches from several fields from Florida and California, USA, using piperonyl butoxide (PBO) and S,S,S- tributyl-phosphorotrithioate (DEF), the resistance levels reduced dramatically confirming the involvement of oxidases and esterases in pyrethroid resistance [18]. Studies on samples of German cockroaches collected from residential areas of Florida (USA) showed that oxidases and esterases are involved in resistance and metabolism of pyrethroids [17]. Electrophoretic studies on pyrethroid resistant strains of German cockroaches showed that esterases and oxidases are involved in resistance [15, 19].

The levels of resistance to permethrin and deltamethrin and possible mechanisms involved in resistance to these two pyrethroids were investigated in a strain collected from Alabama, using the synergists PBO and DEF. Resistance to permethrin and deltamethrin was partially suppressed by the synergists suggesting that P450 monooxigenases and hydrolases are involved in pyrethroid resistance [20].

Synergist studies using PBO and DEF on three strains of German cockroaches collected from Alabama revealed that, despite being from the same geographic origin, P450 monooxigenases and hydrolases are strongly involved in permethrin and deltamethrin resistance in one strain while playing a minor role in two other strains [21]. Undertaking biochemical assays, Lee *et al.* [22] characterized possible insecticide resistance mechanisms in four Malaysian field-collected strains of German cockroach. Elevated esterase activity was detected in all four strains, while elevated GST levels were present in only two strains studied. Classic bioassays and biochemical assays performed on German cockroaches collected from residential areas of Tehran (Iran) showed that oxidases, esterases and GSTs are involved in pyrethroid resistance [3, 23].

Attempts have been made to study the insecticide resistance in German cockroach strains from Iran, but these studies mainly involved dose-response bioassays with no indications of underlying resistance mechanisms [2, 3, 24, 25]. Information about the underlying resistance mechanisms enables us to undertake adequate vector control methods as well as proper resistance management strategies. Hence, biochemical studies are necessary to provide evidence about the mechanisms involved in resistance [17, 26, 27]. Therefore, in this study, along with classic bioassay on German cockroaches, biochemical studies were undertaken to determine general esterase and GST activities and mono-oxigenases cytochrome P450 contents in two field strains collected from University Hospitals (Sari, Iran).

These two strains of German cockroach were extensively under pyrethroid insecticides pressure in the last two decades. Permethrin (Copex 20% WP), a class I pyrethroid, has been intensively used until 1996 when it was replaced by cypermethrin and lambda cyhalothrin followed by cyfluthrin, all class II pyrethroids. Carbamates such as Ficam has also been occasionally used along with pyrethroids. Apparently, the pyrethroid use was more intensive in Imam Khomeini than in Bouali Cina Hospital (Habibi, personal communication).

MATERIALS AND METHODS

Cockroaches strains. Field strains of German cockroach were collected from Imam and Bouali Cina University Hospitals (Sari, Iran) and kept in insectary under standard conditions and used for bioassays and biochemical assays. A susceptible strain (SUS) maintained since 1975 in the insectary at School of Public Health, Tehran University without exposure to insecticide, was used for comparison.

Bioassays. Tarsal-contact method test using glass jar was used to evaluate the susceptibility status of the cockroaches to permethrin, deltamethrin and cypermethrin. These insecticides were used to cover both class I and II pyrethroids as they have been used for pest control in the studied Hospitals and also because they might be different in terms of resistance mechanisms. The tests were conducted on adult males of both susceptible and resistant strains at 3-6 replicates of 10 cockroaches. The inside surfaces of glass jars were coated with 2 ml of known quantities of insecticide solution in acetone. The glass jars were rotated evenly in a hood until the acetone evaporated. Exposure times were determined based on some preliminary experiments to find the minimum (T1) and maximum (Tn) exposure time (times that kill 5 and 95% of the samples, respectively). The rest of the exposure times were calculated using the following formulas:

$$K = (\log Tmax - \log Tmin)/(n - 1)$$

[DOR: 20.1001.1.1028852.2007.11.4.3.5]
[DOI: -]

Where K is a constant, Tmax is the maximum exposure time (Tn), Tmin **is** the minimum (T1) exposure time and n is the total number of exposure times. So the second exposure time is calculated as follows:

$$T2=\text{Antilog}\,(K+\log T1)$$

Mortality responses were scored 24 hour after the end of the exposure time.

Biochemical assays. Biochemical assays for general esterases, GSTs and monooxigenases were performed on adult cockroaches according to [28] with some modifications. Live adult males of German cockroaches (93 individuals per plate) were used for these assays. The thorax of each cockroach was separated and transferred into a microtitre plate well and homogenized in 200 µl of distilled water. All these steps were carried out at 4°C. The homogenates were spun at 1100 g in a Beckman Coulter centrifuge (Beckman, USA), at 4°C for 15 min and the resulting supernatant used as the enzyme source. For biochemical assays a microtitre plate reader (Bio-TEK, Instruments, Inc., USA) operated by a personal computer using KC Junior software (USA) was used to obtain endpoint or kinetic readings for general esterases, GSTs and monooxigenases assays according to Hemingway [28] with some modifications.

Monooxigenases assay. This assay measures the total amount of heme containing protein using a heme-peroxidase assay. As cytochrome P450s make up the bulk of the proteins in non-blood-fed insects, results can crudely be expressed as equivalent units of cytochrome P450 within the insect [29]. The reaction mixture in each well of the microtitre plate contained 20 µl of insect homogenate, 80 µl of 0. 625 M potassium phosphate buffer pH 7.2, 200 µl of 3, 3`, 5, 5` tetramethyl benzidine (TMBZ) solution (0.01 g TMBZ dissolved in 5 ml methanol plus 15 ml of 0.25 M sodium acetate buffer pH 5.0) and 25 µl of 3% hydrogen peroxide. The plates were incubated at room temperature for 2 hours and absorbance was measured at 450 nm as an endpoint in the plate reader. The values were compared with a standard curve of purified cytochrome C and were reported as equivalent units of cytochrome p450/mg protein corrected for the known content of cytochrome C and P450.

General esterases. General esterases activities with the substrates α- and β-naphthyl acetate were determined. Reaction mixtures contained 20 µl of insect homogenate in duplicate in adjacent microtitre plate wells (assigned α and β) and 200 µl of α- or β-naphthyl acetate solution (120 µl of 30 mM α- or β-naphthyl acetate dissolved in 12 ml 0.02 M phosphate buffer pH 7.2) respectively. The reaction mixtures were incubated at room temperature for 30 min before the addition of 50 µl of fast blue solution (0.023 g fast blue dissolved in 2.25 ml distilled water and 5.25 ml of 5% SDS 0.1 M sodium phosphate buffer pH 7) to each well. The plates were incubated at room temperature for 5 min and then absorbance was read at 570 nm as an endpoint value. The resulting optical densities (OD) were compared with standard curves of OD for known concentrations of the products α- and β-naphthol, respectively to convert the absorbances to product concentrations. The enzyme activities were reported as nmol of product formed/min/mg protein.

GST. GST activity was measured using a reaction mixture of 10 µl of the homogenate plus 200 µl of reduced glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) solution (10 mM reduced glutathione dissolved in 0.1 M phosphate buffer pH 6.5 and 3 mM CDNB originally dissolved in methanol). The increase in absorbance was measured at 340 nm for 5 min. The enzyme activity was reported as µM/min/mg protein using the extinction co-efficient corrected for the path length of the solution in the microtitre plate well.

RESULTS

Bioassays. The results of bioassays performed on adult German cockroaches using a concentration of 7 mg/m2 permethrin, deltamethrin and cypermethrin in jar tests were subjected to probit analysis and summarized in Table 1. The bioassays showed that there are statistically significant differences between the LT50 of Imam and Bouali Cina University Hospitals field strains with that of susceptible laboratory strain ($P<0.05$). Also the results showed that the order of LT50 of the strains is Imam > Bouali Cina > Laboratory susceptible strain.

Biochemical assays. Monooxigenase, general esterase and GST assays were carried out on adults of Imam and Bouali Cina Hospitals as well as susceptible strains of German cockroaches. All raw data were analysed using Microsoft Excel software and reported as the equivalent units of cytochrome

[DOR: 20.1001.1.1028852.2007.11.4.3.5]
[DOI: -]

Table 1. The results of probit analysis on the bioassay results performed on adults of Germen cockroaches from Imam, Bouali Cina Hospitals and Lab. Susceptible strains.

insecticide	strain	α	β	X2 (DF)	LT50	RR
Permethrin	Imam	-0.131	3.92	0.176 (2)	20.24 ± 2.200	2.20
	Bouali	-2.460	3.87	1.660 (2)	19.87 ± 2.300	2.23
	Lab.	0.891	4.32	1.500 (2)	8.89 ± 0.265	1.00
Deltamethrin	Imam	-2.290	3.89	0.360 (2)	19.30 ± 3.050	2.19
	Bouali	-0.568	4.47	0.340 (2)	17.60 ± 0.680	2.00
	Lab.	1.220	3.99	0.210 (2)	8.80 ± 0.990	1.00
Cypermethrin	Imam	0.920	4.58	0.136 (2)	19.64 ± 2.900	2.27
	Bouali	0.215	3.76	0.458 (2)	18.66 ± 3.450	2.15
	Lab.	1.008	4.26	0.104 (2)	8.64 ± 0.620	1.00

P450 for monooxygenases and activity rates for the latter two enzymes. The results of biochemical assays are summarized in Tables 2 and 3. The mean α-esterase activity of Imam and Bouali Cina Hospitals and susceptible strains were 6.941 × 10-4, 6.940 × 10-4 and 8.01 × 10-5 nmol/min/mg protein; the mean β-esterase activity in those strains were 5.8 × 10-4, 4.25 × 10-4 and 7.28 × 10-5 nmol/min/mg protein; the mean content of p450 in the above-mentioned strains were 5.64 × 10-6, 1.89 × 10-6 and 1.2 × 10-6 nmol/mg protein; the mean GST activity were 6.66 × 10-2, 0.102 and 5.72 × 10-2 μmol/min/mg protein, respectively. The enzyme activity or content ratio of Imam, Bouali Cina and lab strains are for α-esterase: 8.676 > 8.675 > 1, for β-esterase 8 > 5.9 > 1, for GST 1.75 > 1.6 > 1 and for P450: 4.6 > 1.58 > 1. Analysis of variance of the data showed that the differences between the enzymes activities in the field and lab strains were statistically significant ($\alpha<0.001$).

Table 2. Descriptive analysis of the results of biochemical assays performed on field and susceptible strains of German cockroaches from Sari, Iran.

	N	Mean	Std. Deviation	Std. Error	N	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALFA	1.00	93	6.940E-04	9.8651873E-07	1.622E-07	6.9364E-04	6.9429E-04	.000690	.000695
	2.00	93	8.011E-05	1.8172016E-05	2.570E-06	7.4947E-05	8.5276E-05	.000042	.000126
	3.00	93	6.941E-04	6.7456591E-07	1.472E-07	6.9378E-04	6.9440E-04	.000691	.000695
	Total	279	4.098E-04	3.0777932E-04	2.962E-05	3.5109E-04	4.6851E-04	.000042	.000695
BETA	1.00	93	4.255E-04	6.0977217E-04	1.002E-04	2.2224E-04	6.2885E-04	.000162	.003665
	2.00	93	7.286E-05	1.6836704E-05	2.381E-06	6.8078E-05	7.7648E-05	.000043	.000117
	3.00	93	5.801E-04	5.9893448E-04	1.307E-04	3.0743E-04	8.5270E-04	.000236	.002988
	Total	279	2.923E-04	4.8700346E-04	4.686E-05	1.9941E-04	3.8521E-04	.000043	.003665
GST	1.00	93	.10211804	.14163923	2.329E-02	5.4893E-02	.14934292	-.004496	.786506
	2.00	93	5.723E-02	2.1772439E-02	3.079E-03	5.1041E-02	6.3416E-02	.022267	.127905
	3.00	93	6.666E-02	3.9613421E-02	8.644E-03	4.8628E-02	8.4691E-02	.010625	.179163
	Total	279	7.444E-02	8.7608974E-02	8.430E-03	5.7729E-02	9.1153E-02	-.004496	.786506
MFO	1.00	93	1.892E-06	4.699387E-06	7.726E-07	3.2467E-07	3.4584E-06	-.000013	.0000161
	2.00	93	1.207E-06	9.287063E-07	1.755E-07	8.4682E-07	1.5671E-06	.0000005	.0000049
	3.00	93	5.648E-06	3.074432E-06	6.709E-07	4.2484E-06	7.0473E-06	.0000012	.0000132
	Total	279	2.586E-06	3.873487E-06	4.177E-07	1.7554E-06	3.4164E-06	-.000013	.0000161

GST, glutathione S-transferases; Alfa, α-esterases; Beta, β-esterases; P450, cytochrome P450; 1, Bouali Cina Hospital; 2, Lab susceptible strain; 3, Imam Hospital.

Table 3. One way ANOVA analysis of the results of biochemical assays performed on field and susceptible strains of German cockroaches from Sari, Iran.

Dependent Variable	(I) 1 = BouAli Cina 2 = sus 3 = Imam	(J) 1 = BouAli Cina 2 = sus 3 = Imam	(I-J) Mean Difference	Standard Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
ALFA	1.00	2.00	6.139E-04*	2.696E-06	.000	6.0744E-04	6.2026E-04
		3.00	-1.247E-07	3.396E-06	.999	-8.1991E-06	7.9496E-06
	2.00	1.00	-6.139E-04*	2.696E-06	.000	-6.2026E-04	-6.0744E-04
		3.00	-6.140E-04*	3.232E-06	.000	-6.2166E-04	-6.0629E-04
	3.00	1.00	1.247E-07	3.396E-06	.999	-7.9496E-06	8.1991E-06
		2.00	6.140E-04*	3.232E-06	.000	6.0629E-04	6.2166E-04
BETA	1.00	2.00	3.527E-04*	9.599E-05	.001	1.2447E-04	5.8090E-04
		3.00	-1.545E-04	1.209E-04	.411	-4.4205E-04	1.3300E-04
	2.00	1.00	-3.527E-04*	9.599E-05	.001	-5.8090E-04	-1.2447E-04
		3.00	-5.072E-04*	1.151E-04	.000	-7.8086E-04	-2.3355E-04
	3.00	1.00	1.545E-04	1.209E-04	.411	-1.3300E-04	4.4205E-04
		2.00	5.072E-04*	1.151E-04	.000	2.3355E-04	7.8086E-04
GST	1.00	2.00	4.489E-02*	1.865E-02	.047	5.4425E-04	8.9235E-02
		3.00	3.546E-02	2.350E-02	.291	-2.0411E-02	9.1329E-02
	2.00	1.00	-4.489E-02*	1.865E-02	.047	-8.9235E-02	-5.4425E-04
		3.00	-9.431E-03	2.237E-02	.907	-6.2606E-02	4.3744E-02
	3.00	1.00	-3.546E-02	2.350E-02	.291	-9.1329E-02	2.0411E-02
		2.00	9.431E-03	2.237E-02	.907	-4.3744E-02	6.2606E-02
MFO	1.00	2.00	6.846E-07	8.726E-07	.714	-1.3979E-06	2.7671E-06
		3.00	-3.756E-06*	9.518E-07	.000	-6.0278E-06	-1.4848E-06
	2.00	1.00	-6.846E-07	8.726E-07	.714	-2.7671E-06	1.3979E-06
		3.00	-4.441E-06*	1.006E-06	.000	-6.8410E-06	-2.0409E-06
	3.00	1.00	3.756E-06*	9.518E-07	.000	1.4848E-06	6.0278E-06
		2.00	4.441E-06*	1.006E-06	.000	2.0409E-06	6.8410E-06

GST, glutathione S-transferases; Alfa, α-esterases; Beta, β-esterases; P450, cytochrome P450; 1, BouAli Cina Hospital; 2, Lab susceptible strain; 3, Imam Hospital.

DISCUSSION

Monitoring insecticide resistance and its underlying mechanisms are crucially important in vector and pest control as well as resistance management. Classic bioassay is a powerful method for monitoring and measuring insecticide resistance; however, it lacks adequate sensitivity and does not give a clear picture of mechanisms involved in insecticide resistance.

In other words, when classic bioassay confirms insecticide resistance, it means that the insecticide resistance gene frequency is high enough to demonstrate insecticide resistance phenotype and also it is a little late to implement insecticide resistance management strategies for better resistance and pest control. However, when bioassay is undertaken along with more sensitive biochemical assays, more information and solid evidence come out about the insecticide resistance status and its possible mechanisms. In this study, possible mechanisms of insecticide resistance were investigated using bioassays based on WHO standards and biochemical assays according to Hemingway [28].

Advantages can be obtained from the results of biochemical assays in establishing a base line data for the activity or levels of enzymes responsible for insecticide resistance and also a better understanding of status of the insecticide resistance when the results compared to those of the susceptible strain.

Knowledge about the involvement and the order of importance of enzyme groups in conferring insecticide resistance is highly important in implementing resistance management strategies such as rotation, change, mixture, mosaic as well as taking advantage of integrated pest management measures.

German cockroaches have developed resistance to different groups of insecticides due to their widespread use [17, 26, 30]. This makes their control extremely difficult and a better

[DOR: 20.1001.1.1028852.2007.11.4.3.5]
[DOI: -]

understanding of the underlying mechanisms involved in insecticide resistance in these pests is required for successful pest control. Good and timely information about the resistance status of the vectors helps better pest as well as insecticide resistance management.

The results of bioassays performed on the field and the susceptible strains showed statistically significant differences. However, the resistance ratios of the field strains are more than 1.5 but less than 2.5 inferring a tolerance in the field populations of the German cockroaches to pyrethroids used. The results of studies performed on the same field strains ten years ago showed that the Germen cockroach populations were susceptible to pyrethroids tested (data not shown). This shows that over the last decade, extensive use of pyrethroids for pest control has changed the susceptibility status of the field collected Germen cockroach from Imam and Bouali Cina Hospitals. This implies that a trend of building up pyrethroid resistance has been started and developed over the years and sooner or latter may lead to full insecticide resistance.

Comparative analysis of the results of biochemical assays performed on the field and the susceptible strains revealed that esterases, monooxygenases and GSTs were at higher levels in the field strains. The increase in α- and β-esterases in field strains are about 6-9-times as much as in the susceptible strain. This reveals the outstanding importance of esterases in pyrethroid resistance in Germen cockroaches. Involvement of esterases in pyrethroid resistance was frequently shown in Germen cockroaches in the literature [17, 26, 30]. Oxidases are also involved in pyrethroid resistance in Germen cockroaches, not to the level of esterases though, with an enzyme activity ratio of 1.5-4.6-fold compared to the susceptible strain. Oxidases were shown to be also important in pyrethroid resistance in Germen cockroaches from other countries [17, 26, 30].

The order of importance and involvement of different enzymes in pyrethroid resistance in Germen cockroaches is α-esterase > β-esterase > oxidases > GSTs. Several studies on the involvement of different enzymes in insecticide resistance in different strains of Germen cockroaches from different parts of the world showed that esterases and oxidases are strongly involved in pyrethroid resistance [17, 26, 31, 32].

Similar reports about the role of these enzymes in insecticide resistance in other groups of insects are present in the literature [9-11].

There is a great deal of difference between the resistance ratio obtained by classic bioassay and enzyme activity/level ratio calculated from biochemical assays of the field and lab strains i.e. 2 to 2.5 when compared to more than 5. This clearly shows the higher sensitivity of the biochemical assays compared to the classic bioassays. However, it should be noted that not all of the elevated activities or quantity of enzymes could be interpreted as involved in insecticide resistance as there are more that 100 different genes for each of those enzymes not all of which important in resistance to a given insecticide.

The activity/level of different enzymes in Imam strain of German cockroach was more than that of the Bouali Cina strain. This is in accordance with the results of bioassays in which the resistance ratio was greater in Imam strain. This finding might be, in part, because of the relatively intensive use of pyrethroids in controlling the latter strain. Although none of the field collected strains showed insecticide resistance, the current status of the resistance when compared to the results of a study in 1996 on the same strains shows a trend of building up pyrethroid resistance in this pest. Based on the results of this study, although pyrethroids can still be theoretically used in controlling German cockroaches in the above mentioned Hospitals, it is wise to put the pesticide resistance management strategies in place to avoid or postpone further development of pyrethroid resistance.

The indications made by biochemical assays help the authorities and experts in implementing vector control and resistance management strategies in time and with higher chance of success. As esterases are the most prominent enzyme group in these two populations of the German cockroach followed by MFO system, and noting that almost all insecticide groups are more or less detoxified by esterases and MFO, the best control strategy would be non-chemical approaches such as environmental methods. However, if insecticides are to be used, efforts must be made to use strategies of insecticide resistance management such as rotation, mixture and mosaic. The frequency and intensity of pyrethroid use in those Hospitals is reduced in recent years and other control strategies especially environmental control approaches have been put in place (data not shown).

[DOR: 20.1001.1.1028852.2007.11.4.3.5]
[DOI: -]

ACKNOWLEDGEMENTS

This research was financially supported by the research deputy of Mazandaran University of Medical Sciences, Sari, Iran.

REFERENCES

1. Service, M.W. (2000) Medical Entomology for students. 2nd edition, Cambridge University Press, Cambridge, United Kingdom, p. 283.
2. Ladonni, H. (2000) Permethrin resistance ratios compared by two methods of testing nymphs of the German cockroach, *Blattella germanica*. *Med. Vet. Ent. 14 (2): 213-216*.
3. Limoee, M., Ladonni, H., Enayati, A., Vatandoost, H. and Aboulhassani, M. (2006) Detection of Pyrethroid Resistance and Cross-Resistance to DDT in seven field-collected strains of the German cockroach, *Blattella germanica (L.)* (Dictyoptera: Blattellidae). *J. Biol. Sci. 6: 382-387*.
4. Lee, C.Y., Yap, H.H., Chong, N.L., Lee, (1996) Insecticide resistance and synergism in field collected German cockroaches (Dictyoptera, Blatellidea) in peninsular Malaysia, *Bull. Entomol. Res., 86:675-682*.
5. Penilla, R.P., Rodrigues, A.D., Hemingway, J., Torres, J.L., Arredondo-Jimenez, J.I. and Rodriguez, M.H. (1998) Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. *Med. Vet. Ent. 12: 217-233*.
6. Ranson, H., Rossiter, L., Ortelli, F., Jensen, B., Wang, X., Roth, C.W., Collins, F.H. and Hemingway, J. (2001) Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. *Biochem. J. 359 (Pt 2): 295-304*.
7. Hemingway, J. (1985) Malathion carboxylesterase enzymes in *Anopheles arabiensis* from Sudan. *Pest. Biochem. Physiol. 23: 309-313*.
8. Vontas, J.G., Small, G.J. and Hemingway, J. (2000) Comparison of esterase gene amplification, gene expression and esterase activity in insecticide susceptible and resistant strains of the brown planthopper, Nilaparvata lugens (Stal). *Insect Mol. Biol. 9 (6): 655-660*.
9. Enayati, A.A., Vatandoost, H., Ladonni, H., Townson, H. and Hemingway, J. (2003) Molecular evidence for a kdr-like pyrethroid resistance mechanism in the malaria vector mosquito *Anopheles stephensi*. *Med. Vet. Ent. 17: 138-144*.
10. Enayati, A.A. and Hemingway, J. (2006) Pyrethroid insecticide resistance and treated bednets efficacy in malaria control. *Pest. Biochem. Physiol. 84 (2): 116-126*.
11. Kasai, S. and G., S.J. (2000) Overexpression of cytochrome P450 CYP6D1 is associated with monooxygenase-mediated pyrethroid resistance in house flies from Georgia. *Pest. Biochem. Physiol. 68: 34-41*.
12. Lee, C.Y., Hemingway, J., Yap, H.H. and Chong, N.L. (2000) Biochemical characterization of insecticide resistance in the German cockroach, *Blattella germanica*, from Malaysia. *Med. Vet. Ent. 14 (1): 11-18*.
13. Valles, S.M. and Strong, C.A. (2001) A microsomal esterase involved in cypermethrin resistance in the German cockroach, *Blattella germanica*. *Pest. Biochem. Physiol. 71 (1): 56-67*.
14. Wu, D., Scharf, M.E., Neal, J.J., Suiter, D.R. and Bennett, G.W. (1998) Mechanisms of fenvalerate resistance in the German cockroach, *Blattella germanica (L.). Pest. Biochem. Physiol. 61 (1): 53-62*.
15. Scharf, M.E., Hemingway, J., Small, G.J. and Bennett, G.W. (1997) Examination of esterases from insecticide resistant and susceptible strains of the German cockroach, *Blattella germanica (L.). Insect. Biochem. Mol. Biol. 27 (6): 489-497*.
16. Scharf, M.E., Neal, J.J. and Bennett, G.W. (1997) Changes of insecticide resistance levels and detoxication enzymes following insecticide selection in the German cockroach, *Blattella germanica (L.). Pest. Biochem. Physiol. 59 (2): 67-79*.
17. Valles, S.M., Dong, K. and Brenner, R. (2000) Mechanisms responsible for cypermethrin resistance in a strain of German cockroach, *Blattella germanica*. *Pest. Biochem. Physiol. 66: 195-205*.
18. Valles, S.M. (1998) Toxicological and biochemical studies with field p0opulations of the German cockroach, *Blattella germanica*. *Pest. Biochem. Physiol. 62 (3): 190-200*.
19. Scharf, M.E., Neal, J.J. and Bennett, G.W. (1997) Changes of insecticide resistance levels and detoxication enzymes following insecticide selection in the German cockroach, *Blattella germanica (L.). Pest. Biochem. Physiol. 59: 67-79*.
20. Wei, Y., Appel, A.G., Moar, W.J. and Liu, N. (2001) Pyrethroid resistance and cross-resistance in the German cockroach, *Blattella germanica (L). Pest. Manag. Sci. 57 (11): 1055-1059*.
21. Pridgeon, J.W., Appel, A.G., Moar, W.J. and Liu, N. (2002) Variability of resistance mechanisms in pyrethroid resistant German cockroaches (Dictyoptera: Blattellidae). *Pest. Biochem. Physiol. 73 (3): 149-156*.
22. Lee, C.Y., Hemingway, J., Yap, H.H. and Chong, N.L. (2000) Biochemical characterization of insecticide resistance in the German cockroach, *Blatella germanica*, from Malaysia. *Med. Vet. Ent. 14: 11-18*.
23. Limoee, M., Enayati, A., Ladonni, H., Vatandoost, H., Baseri, H. and Oshaghi, M.A. (2007) Various

mechanisms responsible for permethrin metabolic resistance in seven field-collected strains of the German cockroach, *Blattella germanica (L.)* (Dictyoptera: Blattellidae) from Iran. *Pest. Biochem. Physiol. 87: 138-146*.

24. Ladonni, H. (1993) Susceptibility of *Blattella germanica* to different insecticides in different hospitals in Tehran-Iran. *J. Entomol. Soc. Iran, 12: 53-60.*
25. Ladonni, H. (1997) Susceptibility of different field strains of *Blattella germanica* to four pyrethroids (Orthoptera: Blattellidae). *Iran. J. Publ. Health 26: 35-40.*
26. Valles, S.M. (1998) Toxicological and biochemical studies with field populations of the German cockroach, *Blattella germanica*. *Pest. Biochem. Physiol. 62: 190-200*.
27. Valles, S.M. and Yu, S.J. (1996) Detection and biochemical-characterization of insecticide resistance in the German-cockroach (Dictyoptera, Blattellidae). *J. Econ. Entomol. 89: 21-26*.
28. Hemingway, J. (1998) Field and laboratory manual for the mechanistic detection of insecticide resistance in insects. WHO-CTD/MAL/98.6, p. 35.
29. Brogdon, W.G., McAllister, J.C. and Vulule, J. (1997) Heme peroxidase activity measured in single mosquitoes identifies individuals expressing an elevated oxidase for insecticide resistance. *J. Am. Mosq. Control Assoc. 13 (3): 233-237*.
30. Scharf, M.E., Neal, J.J., Marcus, C.B. and Bennett, G.W. (1998) Cytochrome P450 purification and immunological detection in an insecticide resistant strain of German cockroach (*Blattella germanica, L.*). *Insect Biochem. Mol. Biol. 28 (1): 1-9*.
31. Anspaugh, D.D., Rose, R.L., Koehler, P.G., Hodgson, E. and Roe, R.M. (1994) Multiple mechanisms of pyrethroid resistance in the German cockroach, *Blattella germanica (L.)*. *Pest. Biochem. Physiol. 50: 138-140*.
32. Wu, D., Scharf, M.E., Neal, J.J., Suiter, D.R. and Bennett, G.W. (1998) Mechanisms of fenvalerate resistance in the German cockroach, *Blattella germanica (L.)*. *Pest. Biochem. Physiol. 61: 53-62*.

[DOR: 20.1001.1.1028852.2007.11.4.3.5]

[DOI: -]