Characterization of pyrethroid and carbamate resistance in a Malaysian field collected strain of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

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Abstract. A field strain of the German cockroach (Melia), collected from the kitchen of a hotel in Kuala Lumpur, Malaysia demonstrated moderate resistance to bendiocarb and low resistance to propoxur and deltamethrin when tested with modified W.H.O. glass jar method. Low resistance was also detected when the strain was tested with DDT. Resistance level to bendiocarb and deltamethrin was partially suppressed with piperonyl butoxide (PBO) and *S,S,S*-triphosphorotrithioate (DEF), suggesting the possible involvement of monooxygenase and elevated esterase in the resistance. There is also an indication of the involvement of *kdr*-type resistance in this strain due to inability of PBO and DEF to suppress pyrethroid resistance. Biochemical assays using model substrate a- and b-naphthyl acetate with modified microplate technique demonstrated elevated esterase activity in the ML strain. A high elevated esterase gene frequency was estimated in this strain using Hardy-Weinberg equilibrium equation.

INTRODUCTION

Control failures due to insecticide resistance in the German cockroach is a common problem to the pest control industry nowadays (Lee 1997). Since 1952 when the first case of German cockroach resistance to chlordane was detected, many other incidents have since been documented (Webb 1961; MacDonald & Cochran 1968; Barson & McCheyne 1979; Cochran 1987; 1989; Horwood *et al.* 1991, Hemingway et al. 1993a, 1993b, Lee 1997, 1998, Lee *et al.* 1996, Lee *et al.* 1997a, Lee *et al.* 1997b). Information on the mechanism of insecticide resistance have been scarce when compared to those reported on their baseline susceptibility to insecticides. These information are extremely important as they can assist in resistance management programme. In this study, we characterized pyrethroid and carbamate resistance in a field collected strain from Kuala Lumpur, Malaysia. Synergism and biochemical assays were also executed to determine possible underlying resistance mechanisms in the strain studied.

MATERIALS AND METHODS

Strain

The field collected strain used in this study (ML) was collected from the main kitchen of a luxury four-star hotel (name of hotel withheld) in Kuala Lumpur, Malaysia in January 1997. This hotel was treated with propoxur formulation from 1978 – 1993, and lambda-cyhalothrin- and pirimiphos-methyl-based formulations since 1993. Cockroaches were trapped by placing 0.4 liter glass jars and baited with a piece of beer-soaked white bread for 48 hours.

Upon collection, the cockroaches were brought back to the laboratory and reared for 2-3 generations under condition of 28 ± 2°C, 75 ± 5% R.H. and 12 hours photoperiod, to achieve enough numbers for the experiments. Food and water were provided *ad libitum*.

Chemicals

Technical grade insecticides diluted in analytical grade acetone (>99%) used in this study were propoxur (99.5% a.i.), bendiocarb (95% a.i.), deltamethrin (99.8% a.i.) and DDT (>98% a.i.). These insecticides were provided by their respective companies. All other chemicals used in this study were purchased from Fluka (Switzerland) and Sigma (USA).

Baseline insecticide susceptibility and synergism studies

Ten adult males were introduced into 0.45 liter glass jar coated earlier on its inner base surface with 20 µg/cm^2 of insecticide. The inner surface (except the base) of the jar was smeared with a thin layer of petroleum jelly to prevent the cockroach from escaping. Knock down of the cockroaches was recorded at selected time interval up to 2 hours (for all insecticides except DDT).

For synergism studies, cockroaches were topically treated with 100 µg PBO or 30 µg DEF on the first abdominal segment approximately two hours prior to insecticidal bioassays. All experiments were replicated three times.

Non-specific elevated esterase assays and gene frequency

Biochemical assays were modified to study elevated esterase activity. As ELISA microplate reader was not available, an eye score technique was used in the biochemical assay. Ten to 12 early nymphs were immobilized in –70°C for 10 minutes. They were then each mixed with 100 ml of sodium phosphate buffer (0.1 M, pH 7.2) containing 5% of NP-40, and were crushed using the bottom of glass test tube. Twenty five ml of individual homogenate was then transferred into microplate well which was pre-added with 100 ml of 0.03 M a-naphthyl acetate (diluted in analytical grade acetone). The mixture was allowed to incubate for 10 minutes before 50 ml of 0.5% Fast Blue B solution was added. The colour of the mixture was scored after 5 minutes. Experiment was repeated for model substrate b-naphthyl acetate at similar concentration. For each model substrate, a minimum of 100 individuals were used. The colour intensity was scored based on the following criteria: 0 – light colour, 1- moderate colour, 2 – dark colour, 3 – very dark colour. Elevated esterase gene frequency was calculated by assuming they are inherited mono-factorially. Based on the biochemical assay results of the susceptible strain [assuming the susceptible strain consists only of susceptible homozygotes (SS)], the frequency of S-allele in the resistant strain was estimated by calculating the percentage population which showed enzyme activity at and below score 1 shown in the susceptible strain. Frequency of R alleles was then calculated since 1 – S = R.

Data analyses

Data on insecticide bioassays and synergism studies were pooled and subjected to probit analysis (Robertson & Preisler 1992) using POLO-PC software (LeOra Software 1997). Resistance and synergism ratios at LT_{50} were calculated using RR-PC97 (CY Lee, unpublished programme), a simple software programme developed based on the procedures described in Robertson & Preisler (1992). For detecting difference between elevated esterase activity in susceptible and resistant strains, Kolmogorov-Smirnov two sample test ($p = 0.05$) was executed using Statgraphics® version 5.0 statistical package.

RESULTS AND DISCUSSION

ML strain showed moderate resistance to bendiocarb and low resistance to deltamethrin and propoxur (Table 1). Prior to collection, ML population had been subjected to Baygon® EC (active ingredient: propoxur) treatment for more than 15 years. Since 1993, ICON® (active ingredient: lambdacyhalothrin) and Actellic® (active ingredient: pirimiphos-methyl) had been used in rotation to replace Baygon® against this population. Under such circumstances, propoxur resistance which was detected earlier in this population when it was collected in 1993 (Lee *et al.* 1996), may have diminished. Zhai & Robinson (1996) had reported that pyrethroid resistance level in a field population declined more than 75x within three years in the absence of pyrethroid usage. This may explained why our current results showed low resistance to propoxur, due to its absence of usage for a period of time.

Resistance to bendiocarb were partially suppressed by PBO and DEF in ML strain, suggesting possible involvement of monooxygenase and esterase in the resistance (Table 1). Several earlier studies had reported that both monoxygenase and esterase are involved in bendiocarb resistance (Cochran 1987, Scott *et al.* 1990, Lee *et al.* 1996). However, in this study, propoxur resistance was not suppressed by both synergists (Table 1). As DEF is a general esterase inhibitor, this may indicate that specific esterase isozymes involved in the propoxur resistance was not suppressible by the synergist (Lee 1995). The involvement of elevated esterase was confirmed with biochemical assays where ML strain showed significant elevated esterase activity with resistance gene frequency estimated at 0.83 ± 0.05. Most individuals in the ML strains demonstrated greater esterase activity against both model substrates with heavier colour intensity, when compared to that of the ICI susceptible strain (Figure 1). Colour intensities and their scores of a fraction of ML individuals tested in the biochemical assays were shown in Figure 2 (against a-naphthyl acetate) and Figure 3 (against b-naphthyl acetate). Similar findings with organophosphate and carbamate resistant strains had been found earlier Siegfried & Scott (1992), Hemingway *et al.* (1993a) and Lee *et al.* (1997a).

Deltamethrin resistance in ML strain was not suppressed with PBO and DEF which possibly ruled out the involvement of monooxygenase and esterase in the resistance (Table 1). The ML strain also showed resistance to DDT, although it had not been exposed to that insecticide. As DDT and deltamethrin (pyrethroid) showed similar mode of insecticidal action, there is a possibility that both insecticides had been overcome by the same resistance mechanism, possibly *kdr*-type resistance. A similar finding was reported by Amichot *et al.* (1992) where a *kdr*-type resistant strain of *Drosophila melanogaster* demonstrated cross-resistance between DDT and deltamethrin. This resistance mechanisms had also been

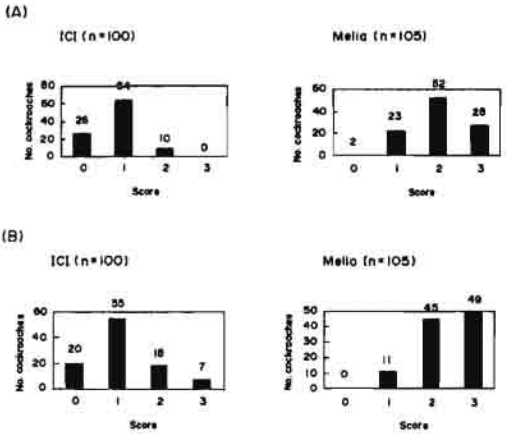
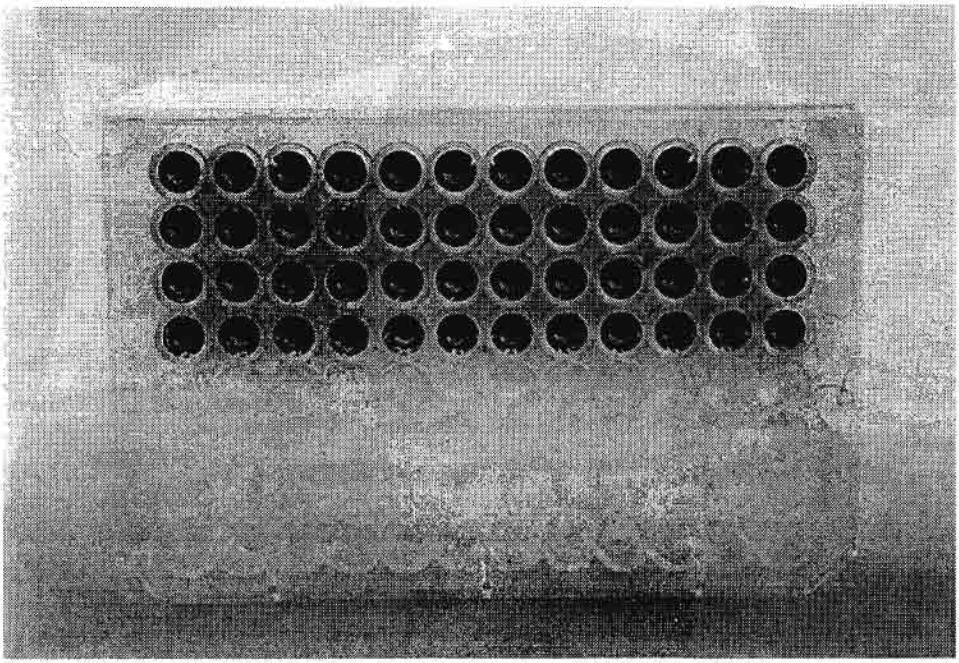


Figure 1. Esterase activity profiles in population of susceptible (ICI) and resisant strain (ML) of the German cockroach when using model substrate (A) α-naphthyl acetate, (B) β-naphthyl acetate.

Insecticide +synergist	Strain	n	LT_{50}(95% FL) (min)	LT_{95} (95% FL) (min)	Regression slope ± S.E.	χ^2 (DF)	SR_{50}[1] (95% FL)	RR_{50} (95% FL)
Propoxur	ICI	50	16.4 (15.9 – 16.9)	20.5 (19.7 – 21.8)	16.9 ± 1.8	0.80 (3)	–	–
(alone)	ML	30	21.4 (20.3 – 22.2)	27.5 (26.0 – 30.4)	15.2 ± 2.6	0.04 (5)	–	1.3 (1.2 – 1.4)
+ PBO	ICI	30	11.8 (11.2 – 12.7)	13.9 (13.0 – 16.2)	22.5 ± 3.0	3.64 (3)	1.4 (1.3 – 1.5)	–
	ML	30	16.6 (15.8 – 17.4)	24.1 (22.1 – 27.3)	10.1 ± 1.2	3.78 (5)	1.3 (1.2 – 1.4)	1.4 (1.3 – 1.5)
+ DEF	ICI	30	15.0 (13.5 – 16.6)	18.4 (16.6 – 27.1)	18.7 ± 2.8	2.09 (2)	1.1 (1.0 – 1.2)	–
	ML	30	18.0 (17.1 – 18.7)	23.1 (21.8 – 25.4)	15.1 ± 2.3	0.51 (3)	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)
Bendiocarb	ICI	50	20.0 (19.4 – 20.6)	28.2 (26.8 – 30.0)	11.1 ± 0.9	1.01 (7)	–	–
(alone)	ML	50	62.3 (58.7 – 65.9)	128.0 (109.6 – 166.2)	5.3 ± 0.4	45.8 (20)	–	3.1 (3.0 – 3.3)
+ PBO	ICI	30	18.2 (17.4 – 18.9)	23.3 (22.0 – 25.6)	15.3 ± 2.3	0.01 (3)	1.1 (1.0 - 1.2)	–
	ML	30	28.8 (27.7 – 29.9)	41.8 (39.1 – 46.2)	10.1 ± 1.1	0.87 (8)	2.2 (2.1 – 2.3)	1.6 (1.5 – 1.7)
+ DEF	ICI	30	13.2 (12.7 – 13.8)	17.1 (16.0 – 19.4)	15.9 ± 2.1	0.96 (3)	1.5 (1.4 – 1.6)	–
	ML	30	27.6 (26.3 – 28.8)	44.2 (40.9 – 49.3)	8.1 ± 0.8	1.83 (10)	2.3 (2.1 – 2.4)	2.1 (2.0 – 2.2)
Deltamethrin	ICI	50	5.5 (5.3 – 5.7)	8.7 (8.2 – 9.5)	8.3 ± 0.7	5.83 (8)	–	–
(alone)	ML	50	11.8 (11.0 – 12.4)	22.6 (21.1 – 24.9)	5.8 ± 0.5	5.92 (13)	–	2.1 (2.0 – 2.3)
+ PBO	ICI	30	5.4 (4.9 – 5.9)	9.5 (8.3 – 11.7)	7.6 ± 0.9	6.10 (5)	1.0 (0.9 – 1.1)	–
	ML	30	10.3 (9.8 – 10.9)	16.3 (14.8 – 18.8)	8.3 ± 0.9	1.48 (7)	1.1 (1.1 – 1.2)	1.9 (1.8 – 2.1)
+ DEF	ICI	30	6.4 (5.9 - 6.9)	12.2 (10.6 – 15.4)	5.9 ± 0.8	2.47 (5)		0.9 (0.8 – 1.0)
	ML	30	13.3 (12.7 – 14.0)	20.0 (17.9 – 24.4)	9.3 ± 1.5	1.97 (5)	0.9 (0.8 – 1.0)	2.1 (1.9 – 2.3)
DDT	ICI	30	1111.3 (972.8 – 1249.8)	1512.0 (1418.1 – 1742.1)	12.3 ± 2.8	1.42 (3)	–	–
	ML	30	*	*	–	–	–	–

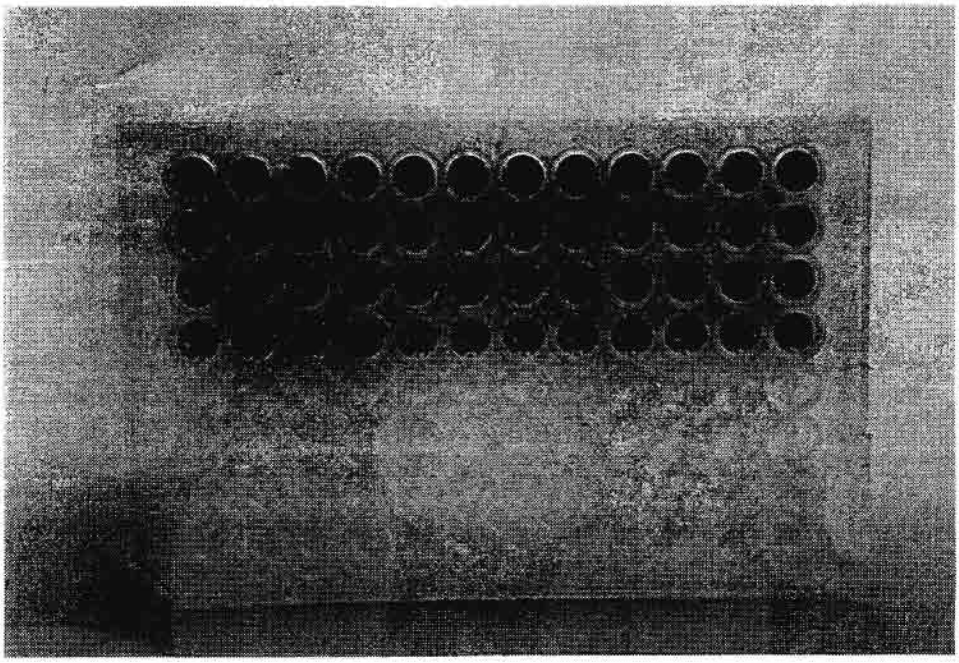
[1]SR = synergistic ratio.

* Only 7% mortality was observed up to 72 hours of DDT exposure.



	1	2	3	4	5	6	7	8	9	10	11	12
A	3	3	3	3	3	3	3	3	3	3	3	3
B	3	3	0	2	3	3	1	3	2	2	3	1
C	2	2	3	3	3	3	3	2	2	2	3	3
D	3	2	3	1	1	3	3	3	2	3	3	3

Figure 2. Colour intensities and their scores from esterase biochemical assay using α-naphthyl acetate as model substrate, in a fraction of ML population tested (n=48).



	1	2	3	4	5	6	7	8	9	10	11	12
A	(3)	(2)	(3)	(3)	(2)	(2)	(3)	(2)	(2)	(3)	(2)	(3)
B	(3)	(2)	(2)	(3)	(3)	(3)	(0)	(3)	(2)	(2)	(3)	(1)
C	(2)	(3)	(3)	(2)	(3)	(3)	(3)	(3)	(2)	(3)	(3)	(3)
D	(2)	(2)	(2)	(1)	(2)	(1)	(3)	(2)	(2)	(2)	(3)	(2)

Figure 3. Colour intensities and their scores from esterase biochemical assay using α-naphthyl acetate as model substrate, in a fraction of ML population tested (n=48).

found in many insect pests which conferred cross-resistance between DDT and other pyrethroids (Scott & Matsumura 1981, 1983, Chadwick et al. 1984, Atkinson *et al.* 1993, Lee *et al.* 1996).

The glass jar method used in this study had been described being less sensitive to detect insecticide resistance when compared to the topical application method (Milio *et al.* 1987) due to the fact that the actual amount of insecticide picked up by the cockroaches could not be determined (Cochran 1995). In addition, if the tested strains showed behavioural resistance to the insecticides, i.e. reduced activity upon coming in contact with the insecticide (Zhai & Robinson 1992, Lee *et al.* 1996), the results would no longer reflect the physiological resistance, but also its interaction with behavioural resistance as well. This will make interpretation of results more difficult to be apprehended.

In this study, non-specific esterase in both strains were seen to have a higher hydrolytic rate against b-naphthyl acetate than a-naphthyl acetate. Hemingway *et al.* (1993a) had reported earlier that five out of 15 strains that they screened for resistance mechanisms showed greater esterase activity against b- when compared to a-naphthyl acetate. Similar results had also been reported in organophosphate-resistant black flies (*Simulium dannosum*) (Hemingway *et al.* 1989). Four Malaysian resistant strains of the German cockroach also demonstrated similar activity against this model substrate (Lee *et al.* 1998).

The simple biochemical assay method for elevated esterase used in this study is a modification of that reported earlier by Hemingway *et al.* (1993a) and Lee *et al.* (1998). Instead of using spectrofotometric method to measure kinetic rate of the enzymes, enzyme activity is scored by visually determining the colour intensity of the product of the enzyme activity (i.e., a- and b-naphthol). Another simple method for determining esterase activity which uses similar principle had been reported by Pasteur & Georghiou (1981) and Lee *et al.* (1997b) which used filter-paper to replace microplate. Beside reducing the cost of assay, the filter paper method also allows permanent record which can be rechecked in the future.

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