

Elevated esterase detection in insecticide resistant German cockroaches (Dictyoptera: Blattellidae) using modified Pasteur-Georghiou's filter-paper method

Lee, C.Y., Yap, H.H. and Chong, N.L.

School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

Abstract. Five field populations of the German cockroach, *Blattella germanica* (L.) (HangTuah, Melia II, ChiliPadi I, GoldenSand and TsimShaShui) were collected from several hotels and restaurants in Malaysia. Resistance levels, as determined by World Health Organization (WHO) glass jar method were moderate for carbamate insecticides (5.4 - 13.3) and low for pyrethroids (1.5 - 3.6), although topical bioassays demonstrated a higher resistance level in all strains. Non-specific esterase activity in individual insects from these field populations was detected using modified Pasteur-Georghiou's filter paper method with α - and β -naphthyl acetate as model substrates. Intensity of dot-staining on filter paper was visually scored from 1 - 5 (low to heavy). Esterase activity was well-correlated with propoxur resistance levels. Results obtained from this study was confirmed with microassays using microplate reader.

The German cockroach, *Blattella germanica* (L.) is an important urban insect pest in many parts of the world. In Malaysia, they are abundant in hotels and food preparative outlets (Yap *et al.*, 1991; Lee *et al.*, 1993). Uncoordinated insecticide usage and over-reliance on a particular group of insecticides has led to the development of insecticide resistance in the German cockroach. In Malaysia, German cockroach resistance to insecticides was found in 1994 where moderate to high levels of resistance to carbamates (propoxur and bendiocarb) and low to moderate resistance levels to pyrethroids (permethrin, cypermethrin, deltamethrin and phenothrin) were detected in field collected strains from hotels and restaurants using topical dose-response bioassays (Lee 1995; Lee *et al.*, 1994; Lee *et al.*, 1995; Lee *et al.*, 1996a).

The resistance mechanism is an important aspect in insecticide resistance studies because it provide basic information that can be used to improve resistance management options. In the German cockroach, the involvement of reduced cuticular penetration, monooxygenase, esterase and glutathione S-transferase, altered acetylcholinesterase and *kdr*-type resistance as resistance mechanisms had been reported earlier (Cochran, 1995). Synergism and biochemical studies have implicated that four strains of

German cockroaches collected in Malaysia have the possible presence of multiple resistance mechanisms (monooxygenase, esterase, glutathione S-transferase and altered acetylcholinesterase) (Lee, 1995; Lee *et al.*, 1996b; Lee *et al.*, in review). In that study, the authors conducted enzyme microassays using kinetic microplate technique (Hemingway *et al.*, 1993a; 1993b). Detection of metabolic-based resistance with this technique is very accurate; however, it requires a microplate reader and is also relatively expensive to conduct.

To overcome the above mentioned shortcomings, especially for workers from developing countries where research funds are insufficient, the authors have modified the filter-paper method proposed by Pasteur & Georghiou (1981) for their studies on mosquito resistance, for detection of elevated esterase activity in the German cockroach. Insecticide bioassays using WHO glass jars were also carried out to generate baseline susceptibility levels to several insecticides. Results obtained from earlier studies using topical bioassays and microplate technique were compared with the current one.

MATERIALS AND METHODS

Collection sites

Five field populations were collected from two hotels (Melia 2 and GoldenSand) and three food outlets (HangTuah, ChiliPadi 1 and TsimShaShui) in Kuala Lumpur and Penang, Malaysia, using baited glass jar left overnight. The trapped cockroaches were brought back to the laboratory and reared for 2 – 3 generations to achieve enough numbers for experiments. A laboratory susceptible strain (ICI) was used for comparison.

Insecticides

Technical grade of four insecticides, representing the carbamate (propoxur and bendiocarb) and pyrethroid (permethrin and cypermethrin) groups were used in bioassays. Insecticides used were diluted in analytical grade acetone at the following concentrations: propoxur and bendiocarb: 0.1%; permethrin: 0.05% and cypermethrin: 0.01%.

Bioassay method

The World Health Organization glass jar method for testing cockroaches was used in this study (Busvine, 1971). Insecticide solution in acetone (2.5 ml) was pipetted into a 0.5 l glass jar (surface area = 340 cm²) with its upper inside lip smeared with petroleum jelly. It was then deposited evenly on the inner surface by rolling the jar on a flat surface until all the acetone had evaporated. Ten adult males were introduced into the jar and knockdown of the cockroaches was recorded at selected time intervals. Each assay was replicated 4 – 6x for all strains. Data were subjected to probit analysis using a computer program developed by Finney (1971). Resistance ratio (RR) was calculated by dividing the KT_{50} values for each strain with the corresponding knockdown time recorded for the ICI (susceptible) strain. Results obtained were compared with those reported by Lee *et al.* (1996a) using topical application.

Modified filter-paper method

Twenty five first instars were knocked down in a freezer for 5 mins prior to the experiment.

Upon knockdown, the cockroaches were transferred individually into the wells of an ice-cube container, each contained 100 µl of sodium phosphate buffer/Triton X-100 (0.5% Triton X-100 in 0.1 M sodium phosphate buffer, pH 7.2). The cockroaches were crushed individually using the bottom of a test tube. Two µl of each homogenate sample was then pipetted onto two pieces of Whatman No. 2 disk filter paper (surface area = 50 cm²), respectively. When all 25 samples have been pipetted, the first filter paper was incubated in freshly prepared α -naphthyl acetate working solution (0.2% α -naphthyl acetate in 0.1 M sodium phosphate buffer, pH 7.2) for 1 min, followed by addition of 2 ml of staining solution (0.05 g Fast Garnet GBC in 2 ml distilled water) and further incubation for another 1 min. The same procedure was done with the second filter paper using β -naphthyl acetate as model substrate. Esterase activities were detected as a purplish-coloured dot for α - or a pink dot for β -naphthyl acetate. The filter papers were then air-dried for 2 – 3 hours and each kept in tightened plastic bag in a refrigerator for storage. Each experiment was replicated 4x with 25 cockroaches per experiment ($n = 100$ for each strain). Each dot was visually scored from 1 – 5 (low to heavy coloured) and the mean score for each strain was calculated. Results obtained were compared with those in Lee (1995) and Lee *et al.* (in review) using enzyme microassay techniques.

Correlation between propoxur resistance level and esterase activity

Propoxur resistance level was correlated with results from modified filter paper test and subjected to polynomial regression analysis, using a statistical analysis software (STATGRAPHICS Version 5.0).

RESULTS AND DISCUSSION

Low to moderate resistance (5.4 – 13.3x) to carbamate insecticides (propoxur and bendiocarb) were detected in all field collected strains of the German cockroach (Table 1). The highest resistance level to propoxur and bendiocarb

Table 1. Resistance profiles of five field populations of the German cockroach collected in Malaysia to some common insecticides, tested with WHO glass jar method.

Insecticide (% conc.)	Strain	n	RR ₅₀ (Glass jar method)	RR ₅₀ (topical) (from Lee <i>et al.</i> 1996a)
propoxur (0.1%)	HangTuah	50	10.7	39.7
	Melia2	50	11.5	53.1
	ChiliPadi 1	50	9.8	36.3
	GoldenSand	50	8.3	17.6
	TsimShaShui	50	5.4	7.5
bendiocarb (0.1%)	HangTuah	50	11.9	>62.8
	Melia 2	50	13.3	>61.4
	ChiliPadi 1	60	10.2	48.8
	GoldenSand	50	5.1	30.7
	TsimShaShui	50	9.0	11.3
permethrin (0.05%)	HangTuah	50	3.2	-
	Melia 2	40	2.2	14.2
	ChiliPadi 1	49	2.1	14.5
	GoldenSand	50	1.9	1.2
	TsimShaShui	40	2.7	7.4
cypermethrin (0.01%)	HangTuah	40	3.6	22.0
	Melia 2	50	2.2	13.1
	ChiliPadi 1	50	1.8	8.3
	GoldenSand	50	1.5	1.3
	TsimShaShui	50	1.5	5.9

was demonstrated by the Melia 2 strain, a strain collected from a hotel in Kuala Lumpur. On pyrethroid resistance, low resistance levels (<4x) were detected in all field strains. When compared with topical method (Lee *et al.*, 1996a), the glass jar method was less sensitive (Scott *et al.*, 1986; Milio *et al.*, 1987; Scharf *et al.*, 1995), although it may give a better reflection of the type of insecticidal exposure that cockroaches received in the field (Cochran, 1995). There is also a possibility of having behavioural resistance interacting with physiological resistance, as reported by Lee *et al.* (1996a) where they found that some strains of cockroaches remained relatively immobile

after contact with insecticides. This behavioural reduced insecticidal pickups by tarsal pads, thus resulting in slower knockdown and lower mortality. In the current study, the TSS strain was seen to demonstrate this behaviour, thus explaining why its susceptibility to bendiocarb is lower when compared with the GS strain, despite the latter having a higher resistance level to the topical insecticide treatment.

Esterase activities in the field strains were well-detected by the filter-paper method. The Melia 2 strain demonstrated the highest esterase activity among all field strains (Table 2). The results obtained also corresponded well with those reported in Lee (1995) and Lee

Table 2. Mean esterase activities in susceptible and five populations of the German cockroach collected from Kuala Lumpur and Penang, Malaysia.

Model Substrate	Strain	n	Mean esterase activity (0 – 5)	Mean esterase activity ¹ (nmol naphthol/min/mg protein)
α-naphthyl acetate				
	ICI	100	1.5	0.054
	HangTuah	100	3.8	0.227
	Melia 2	100	4.1	0.339
	ChiliPadi 1	100	3.3	0.225
	GoldenSand	100	2.5	-
	TsimShaShui	100	2.2	0.183
β-naphthyl acetate				
	ICI	100	1.8	0.154
	HangTuah	100	4.0	0.287
	Melia 2	100	4.5	0.360
	ChiliPadi 1	100	3.4	0.296
	GoldenSand	100	2.5	-
	TsimShaShui	100	2.3	0.203

¹ Data obtained from Lee (1995) and Lee *et al.* (in review).

et al. (in review) using microplate reader technique.

Esterase activity correlated well with propoxur resistance level following second order polynomial relationships. Using model substrate α-naphthyl acetate, the relationship generated was

$$y_1 = 1.561 - 0.024x + 0.021x^2, R^2 = 0.964$$

(where y_1 = intensity score and x = propoxur resistance level; $y_1 < 5$, $x > 1$).

When using model substrate β-naphthyl acetate, the following relationship was obtained:

$$y_2 = 2.028 - 0.192x + 0.035x^2, R^2 = 0.961$$

(where y_2 = intensity score and x = propoxur resistance level; $y_2 < 5$, $x > 1$).

In the study on the same cockroach strains, Lee (1995) and Lee *et al.* (in review) also recorded a similar trend of results when using microplate reader and topical application. In a temephos resistant strain of *Culex quinquefasciatus*

mosquitoes, Peiris & Hemingway (1990) showed that resistance to many organophosphates were correlated with esterase activity.

Homogenate aliquots from the same individuals were used in the assay against both model substrates. Therefore, comparison between hydrolytic activity on α- and β-naphthyl acetate was possible. In this study, it was seen that all strains showed greater hydrolytic activity against β-naphthyl acetate than α-naphthyl acetate (Table 2). By using the equations generated above, it can be shown that hydrolytic activity against β-naphthyl acetate is 1.77x greater than that towards α-naphthyl acetate.

The filter paper method had earlier been proposed by Pasteur & Georghiou (1981) for detecting elevated esterase in mosquitoes. In this current study, the working solutions were modified to suit the purpose of the study. First instars were used instead of the adult males, as they were usually available in big numbers. From our observation, there was no difference between esterase activity in these two stages in absolute units after protein content in the cock-

roach body was taken into consideration (Lee, C.Y., unpublished). Similar results were observed by Valles *et al.* (1994) where there was no significant difference in esterase and GST activity, and cytochrome P₄₅₀ content between last instars and adult males.

The modified Pasteur-Georghiou's filter paper test suggested is a cheap and simple, yet useful method for detecting elevated esterase in the German cockroach in the field. The prospect is good for developing a simple test kit containing all the required working solutions and a visual score card showing the progressive intensity of the dot colour for intensity measurement. It can be used practically by any technician without the need for biochemistry knowledge.

Acknowledgements. The authors are grateful to RST Lee (Pest Dynamics) for the collection of field cockroaches and SY Lim for his assistance in cockroach rearing.

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