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Insecticide toxicity on the adult German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

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ABSTRACT Thirteen insecticides (alpha-cypermethrin, beta-cyfluthrin, bendiocarb, bifenthrin, chlorpyrifos, cypermethrin, cyphenothrin, deltamethrin, etofenprox, fenitrothion, lambda-cyhalothrin, permethrin and propoxur) were tested topically against susceptible adult German cockroaches. Lambda-cyhalothrin was the most toxic compound against both sexes, while propoxur and bendiocarb were the least toxic to male and female, respectively. The Type II pyrethroids is the most toxic group of insecticide, followed by Type I pyrethroids, organophosphates and carbamates. Four factors (age, pre-treatment starvation, immobilizing techniques, and time of insecticidal application) affected adult male susceptibility to propoxur; however, susceptibility to deltamethrin was independent from the last two factors.

ABSTRAK Tiga belas insektisid (alpha-cypermethrin, beta-cyfluthrin, bendiocarb, bifenthrin, chlorpyrifos, cypermethrin, cyphenothrin, deltamethrin, etofenprox, fenitrothion, lambda-cyhalothrin, permethrin dan propoxur) diuji secara topikal terhadap lipas Jerman dewasa yang rentan. Lambda-cyhalothrin adalah bahan yang paling toksik kepada kedua jantina, manakala propoxur dan bendiocarb adalah paling kurang toksik kepada jantan dan betina, masing-masing. Piretroid jenis kedua adalah kumpulan insektisid yang paling toksik, diikuti pula oleh piretroid jenis pertama, organofosfat dan karbamat. Empat faktor (umur, kelaparan pra-rawatan, teknik immobilisi dan masa applikasi insektisid) didapati mempengaruhi kerentanan lipas jantan kepada propoxur; walau bagaimanapun, kerentanan kepada deltamethrin adalah bebas dari dua faktor yang terakhir.

(German cockroach, toxicity, age, immobilizing agents, starvation, application time)

INTRODUCTION

The German cockroach, *Blattella germanica* (L.), an important insect pest especially in the urban environment is present in domiciles, hotels and food establishments in many parts of the world. Current control effort on German cockroaches relies heavily on the use of neurotoxic insecticides. They are usually applied through the form of residual treatment and solid formulations (eg. bait) [1]. However, extensive usage of insecticides has led to the development of insecticide resistance in German cockroaches, especially in the U.S.A. [2-4].

The toxicity of insecticides against German cockroaches had been studied and reported. Abd-Elghafar *et al*. [5] compared the toxicity of ten insecticides on adult cockroaches (males, gravid and non-gravid females) and found that the non-gravid adult females were the most tolerant to all insecticides tested except to malathion (to which the males were most tolerant) among the three adult stages/sexes tested. Koehler *et al*. [6] who worked on both adult and nymphal stages in both susceptible and resistant strains, reported that late instar nymphs were the most tolerant among the stages tested and proposed that this factor should be considered when assessing insecticide susceptibility in German cockroaches.

The toxicity of insecticides may also be affected by physiological resistance, age, and reproductive status [7,8]. Other parameters including temperature [9], period of carbon dioxide immobilization [10], pre-treatment starvation [11], site of insecticide application [12] and time of insecticide application [13] have also been shown to affect insecticide susceptibility in German cockroaches.

This study was initiated to determine the toxicity of thirteen novel and conventional insecticides on adult male and virgin female German cockroaches. Prior to the evaluation of insecticide toxicity, it was observed that several factors (adult age, immobilizers, pre-treatment starvation and time of insecticide application) affected the toxicity results. Thus additional studies were also conducted to determine the effects of these factors on insecticide susceptibility in the German cockroach.

MATERIALS AND METHODS

Comparative toxicity of selected insecticides

Insects The susceptible strain of the German cockroach used in this study has been reared at the Vector Control Research Unit (VCRU), Universiti Sains Malaysia since 1980. It was obtained from the Institute for Medical Research, Kuala Lumpur, where it had been cultured from a wild strain collected in the 1970s. This strain is designated as the VCRU strain.

The cockroaches were reared in 3-liter glass jars or glass aquaria (30 x 25 x 25 cm) under the conditions: $26 \pm 2°C$; $60 \pm 5\%$ R.H. and 12 h photoperiod. The upper inside surface of the rearing container was smeared with petroleum jelly to prevent the cockroaches from escaping. Water and mouse pellet (Gold Coin 70P, supplied by F.E. Zuellig Pte. Ltd.) were provided *ad libitum*. For the purpose of this study, last instar nymphs were isolated from the colonies and placed in glass jars at approximately 200 cockroaches per jar. Each day, newly emerged adult cockroaches were segregated by sex to prevent mating. Adult cockroaches aged 3 to 10 days were used in most experiments except mentioned otherwise.

Insecticides Thirteen insecticides in the form of technical grade were used for toxicity testing: alpha-cypermethrin (96%), beta-cyfluthrin (98.7%), bifenthrin (94%), cypermethrin (94.5%), cyphenothrin (94.1%), deltamethrin (99.8%), etofenprox (96%), lambda-cyhalothrin (99.7%), permethrin (95.1%), chlorpyrifos (97.4%), fenitrothion (95%), propoxur (99.5%) and bendiocarb (95%). All insecticides were diluted in analytical grade acetone (>99%) for topical bioassay.

Topical bioassay The bioassay method used generally followed those of Scott *et al.* [12] and Abd-Elghafar *et al.* [5] with slight modification. Cockroaches were acclimatized in polyethylene cup (Swordman No. 14729, size A-1; 5 cm diam x 10 cm height) with food (mouse pellet) and water for 24 hours before insecticidal application. Ten adult male (or female) cockroaches were anaesthetized with CO_2 (pressure: 20 kpa) for not more than 20 secs prior to insecticide treatment. Upon immobilization, 1 µl of a predetermined dose of insecticide was applied to the first segment of the abdominal sternites, using a Burkard's PAX 100 electric-operated topical applicator (Burkard Scientific Ltd., Middlesex, U.K). A 1 µl hypodermic insulin glass syringe (Fortuna WG Co., Germany) fitted with a size 27 gauge needle was used to dispense the dose. The immobilized individual was held with a Martin's aluminium feather forcep to position the cockroach. In each experiment, 10-30 insects (depending on availability) were tested at each dose level. All experiments were done in the morning and replicated three to seven times. For the control, 1 µl of acetone was applied.

Upon treatment, the cockroaches were kept in clean polyethylene petri dishes (diam = 9 cm; height = 1.5 cm) with ten individuals per container, each provided with a piece of mouse pellet and a wet cotton bung. Mortality was scored at 24 hours post-treatment. The criterion for death was as follows: cockroaches lying on their back were touched on their abdomen with a forcep; those that were unable to back themselves to normal posture in two minutes were considered dead.

Effects of age, immobilizing technique, pre-treatment starvation and time of insecticide application on insecticide susceptibility in adult male German cockroaches

Insects The VCRU strain was used in this study. Only adult male cockroaches were tested because their weights and physiology are more uniform when compared with those of the adult female cockroaches [14]. The method of rearing cockroaches was similar to that described earlier. Newly emerged adult male cockroaches were isolated each day and kept until the required age for testing. For all experiments, cockroaches aged 3-14 days were used. Before insecticidal testing, the cockroaches were weighed in a group of 10 individuals with a minimum of five replicates and the mean masses per individual was generated.

Insecticides The effect of all the factors on susceptibility to propoxur and deltamethrin was studied, except for the case of pre-treatment starvation where susceptibility to only propoxur was studied. The procedure for the preparation of the required doses was essentially the same as that described earlier.

Effect of age Upon emergence into adult and after being segregated, the male cockroaches were kept until the following age for topical insecticidal tests: 1-2, 7-8, 15-16, 19-20, 29-30 and 59-60 days.

Effect of different immobilizing techniques Two immobilizing techniques were compared to determine their effect on insecticide susceptibility: (1) cockroaches were exposed for a period of 20 secs to CO_2 (pressure: 20 kpa), and (2) cockroaches confined in polyethylene cups were kept in the freezer compartment of a refrigerator (Sanyo SR 348F) at a temperature of −10 to −4°C for 80-90 secs.

Effect of pre-treatment starvation The insects were starved for 24 and 72 hours under the following nutritional conditions: food only (F), water only (W) and no food and water (no FW). Cockroaches receiving both food and water together served as control.

Effect of time of insecticide application Insecticidal testings were conducted at different times of a day. Prior to treatment, the insects were acclimatized with food and water at 12 h photoperiod for at least 96 hours. Treatment times were as follows: 0800, 1200, 1600, 2000 and 2400 hours. At scotophase period (2000 and 2400 hours), treatment was conducted under a 240-V table lamp with its fluorescent bulb wrapped up in a layer of red transparent plastic sheet (light intensity and wavelength not determined).

Topical bioassay The bioassay method used follows essentially that mentioned earlier. Upon treatment, the cockroaches were kept in clean petri dishes with food and water. Mortality was read at 24 hours post treatment. Each experiment consisted of 4-6 doses with 10 or 20 cockroaches each (depending on availability) and replicated 3-4 times.

Data analysis

Results from all replicates were tested with χ^2 test for uniformity [15] to ensure homogenity between replicates before being pooled, and subjected to probit analysis [16] using a computer programme developed by Daum [17]. A minimum of four dose-response points ranging from 10 to 90% mortality with at least 120 insects was used in the analysis for a minimum reliable estimation [18].

All LD_{50} values were converted from µg/insect to µg/g insect to avoid the possible effect of weight differences on toxicity. Test for statistical significance between LD_{50} values was the failure of their 95% fiducial limits to overlap.

RESULTS AND DISCUSSION

Comparative toxicity of selected insecticides

From the results obtained, the pyrethroids tested can be classified into two types based on their symptomology in the poisoned cockroaches. Type I consists of etofenprox and permethrin; these caused quick knockdown, hyperactivity and wriggling of abdomen in the affected insects. The other pyrethroids (beta-cyfluthrin, bifenthrin, cypermethrin, deltamethrin and lambda-cyhalothrin) were categorized as Type II pyrethroids; these effected hyporesponsiveness and leg paralysis in the poisoned insects. In this study, bifenthrin, although lacking the α-cyano moiety, caused type II symptomology in the cockroaches. This thus supports Scott's [19] suggestion that pyrethroids should be classified based on symptomology, electrophysiology or temperature-dependent characteristics, rather than on the absence or presence of the α-cyano moiety. In the current study, none of the pyrethroids demonstrated intermediate action as was found by Scott and Georghiou [20] for cyfluthrin in their study on house flies.

On toxicity studies of thirteen insecticides on adult males, lambda-cyhalothrin was the most toxic insecticide, followed by deltamethrin (Table 1). This was succeeded by both alpha-cypermethrin and beta-cyfluthrin which were equivalent in terms of toxicity. The next toxic compound in terms of ranking was bifenthrin, followed by cypermethrin (LD_{50} = 1.09 µg/g insect) and cyphenothrin (LD_{50} = 1.40 µg/g insect). In Type I pyrethroids, etofenprox was more toxic than permethrin. In the organophosphate group, chlorpyrifos had a higher insecticidal activity than fenitrothion. Both carbamates, i.e., bendicarb and propoxur, were the least toxic among the thirteen insecticides tested on adult males. Similar toxicity ranking was shown among the thirteen insecticides when tested on adult virgin females (Table 1), with the exception of the last two least toxic compounds in which propoxur (LD_{50} = 15.85 µg/g insect) was more toxic than bendiocarb (LD_{50} = 50.84 µg/g insect).

In this study, the adult males were at least twice more susceptible than the adult virgin females when comparison was made between LD_{50} values expressed in µg/insect. This may be due to female cockroaches having higher mass than the males due to differential lipid content and thus affecting insecticide toxicity [21,22]. However, when comparison was made using

Table 1. Comparative topical toxicity of selected insecticides against adult German cockroaches.

Group	Male			Female		
	n	LD_{50} (95% fiducial limit) (in µg/ g insect)	Slope±S.E	n	LD_{50} (95% fiducial limit) (in µg/ g insect)	Slope±SE
Type I pyrethroids						
permethrin	180	4.29 (3.98 - 4.61)	3.00 ± 0.17	180	9.27 (8.63 - 9.96)	2.69 ± 0.20
etofenprox	480	1.80 (1.66 - 1.95)	7.82 ± 0.64	120	5.40 (5.16 - 5.65)	5.33 ± 0.33
Type II pyrethroids						
deltamethrin	160	0.22 (0.21 - 0.23)	5.66 ± 0.47	270	0.24 (0.23 - 0.25)	7.10 ± 0.37
lambda-cyhalothrin	600	0.17 (0.16 - 0.19)	7.36 ± 0.45	120	0.19 (0.18 - 0.20)	6.94 ± 0.53
beta-cyfluthrin	450	0.32 (0.30 - 0.35)	7.42 ± 0.32	160	0.35 (0.33 - 0.36)	4.46 ± 0.30
alpha-cypermethrin	450	0.27 (0.25 - 0.30)	6.10 ± 0.39	120	0.29 (0.28 - 0.31)	6.40 ± 0.43
bifenthrin	450	0.64 (0.62 - 0.66)	5.47 ± 0.19	300	0.73 (0.70 - 0.76)	4.07 ± 0.25
cypermethrin	250	1.09 (1.06 - 1.11)	8.43 ± 0.38	120	1.29 (1.23 - 1.35)	5.86 ± 0.41
cyphenothrin	450	1.40 (1.35 - 1.45)	4.25 ± 0.16	450	1.72 (1.60 - 1.85)	6.24 ± 0.43
Organophosphates						
chlorpyrifos	160	5.21 (5.07 - 5.34)	8.62 ± 0.52	150	5.79 (5.53 - 6.05)	5.46 ± 0.42
fenitrothion	150	8.58 (8.27 - 8.90)	6.34 ± 0.40	120	13.35 (12.71 - 14.07)	5.06 ± 0.36
Carbamates						
propoxur	750	15.60 (15.01 - 16.27)	3.18 ± 0.12	360	15.85 (14.50 - 17.25)	3.50 ± 0.67
bendiocarb	750	12.83 (12.28 - 13.39)	2.38 ± 0.07	120	50.84 (48.14 - 53.59)	4.81 ± 0.33

LD_{50} values in µg/g insect (Table 1), both adult males and females showed overlap in their 95% fiducial limits in many cases especially when tested with Type II pyrethroids (alpha-cypermethrin, beta-cyfluthrin, deltamethrin and lambda-cyhalothrin). Koehler *et al.* [6] found that susceptible adult males were approximately two times more susceptible than the females when tested with bendiocarb, chlorpyrifos and cypermethrin and when comparison was made with µg/ insect. However, when the values were converted to µg/g insect, both males and females had similar LD_{50} values for both cypermethrin and chlorpyrifos. This observation is similar to that made in this study. This indicated that weight differences affect insecticide susceptibility.

In terms of group toxicity on both adult males and females, Type II pyethroids showed the highest insecticidal activity followed by Type I pyrethroids and organophosphates (Table 2). Carbamates was the least toxic group of insecticides tested. The group toxicity ranking for adult males found in this study corroborates well with that reported earlier by Abd-Elghafar *et al.* [5].

Effect of age on insecticide susceptibility

The mass of adult male German cockroaches showed

Table 2. Comparative toxicity of different groups of insecticide against adult male and female German cockroaches[1].

Group	n[2]	mean LD_{50} (95% fiducial limit) in µg/ g insect			
		Male		Female	
Type I pyrethroids	2	3.05 (2.82-3.28)	a	7.34 (6.90-7.81)	a
Type II pyrethroids	7	0.59 (0.56-0.61)	b	0.69 (0.65-0.73)	b
organophosphates	2	6.90 (6.67-7.12)	c	9.57 (9.12-10.06)	c
carbamates	2	14.22 (13.65-14.83)	d	33.35 (31.32-35.42)	d

[1]Based on LD_{50} values at 24 hours.
[2]Number of insecticides involved in the calculation of mean LD_{50} and 95% fiducial limit.
[3]Mean values followed by different letter within the same column are significantly different (based on non-overlap of 95% fiducial limit).

an increase with age over a period of 60 days (Table 3). The increase in weight is taken into consideration when expressing insecticide toxicity.

Table 3. Mean masses of individual adult male German cockroaches in relation to aging.

age (days)	mean ± S.E (mg/ insect)	95% confidence interval (mg)
1-2	48.36 ± 0.56	46.86 - 49.85
7-8	52.71 ± 0.56	51.22 - 54.20
15-16	53.56 ± 0.80	52.38 - 55.05
19-20	55.10 ± 0.87	53.61 - 56.59
29-30	55.74 ± 0.63	54.24 - 57.23
59-60	57.78 ± 1.16	56.21 - 59.36

In this study, the younger adult cockroaches were more tolerant to insecticides than the older ones except for cockroaches at 1-2 days post maturation. Adult males aged 7-8 days were most tolerant to propoxur when compared with those of other age groups (Table 4). For deltamethrin, although cockroaches aged 7-8 days had the highest susceptibility among all age groups tested, their susceptibility was not significantly different from those of age 15-16 days and 19-20 days as their 95% fiducial limit overlapped. Adult males aged 1-2 days were more susceptible when compared with those aged 7-8 days on being tested with propoxur and deltamethrin. Adult males at the age of 59-60 days were most susceptible to propoxur and deltamethrin among the six age groups tested.

In his study on the American cockroach, Murkerjea [23] also found that older adult cockroaches were more susceptible than younger ones. However, he did not study cockroaches aged 1-2 days post eclosion.

The susceptibility of the cockroaches was high at 1-2 days post-maturation; it then gradually decreased and reached a plateau until day 30. After that, the susceptibility increased again. This trend of susceptibility had also been observed earlier in adult male *Drosophila melanogaster;* when 0.01 µl of 25% DDT was applied topically, susceptibility was high in young flies. However, it rapidly declined with age, to minimum susceptibility at 5 days, thereafter increased again [24]. In the American cockroach, Turnquist and Brindley [25] found that both sexes demonstrated age-dependent changes in levels of cytochrome P_{450}. Cytochrome P_{450} level was very low in newly matured adults, but increased steadily and reached its peak at about 100 days old. Soon thereafter, it declined rapidly to the levels as found in the young insects earlier at 130-140 days old. In view of this, age-dependent enzyme activity may be the reason to our finding. Reports on age-dependent enzyme activity had been reported on glutathione S-transferase in *Aedes aegypti* mosquito [26] and malathion carboxylesterase in *Anopheles stephensi* mosquito [27].

In house fly, the susceptibility of the adult increased with age when tested against some carbamates [28,29]. This corresponded well with the *in vivo* inhibition of cholinesterase with carbamates used in the study and it

Table 4. Effect of age on susceptibility to propoxur and deltamethrin in adult male German cockroaches.

Insecticide	Age (d)	n	LD_{50} (95% fiducial limit)[1] (µg/ g insect)	Slope ± S.E	HF[2]
propoxur	1-2	400	10.96 (10.35-11.60)	2.19 ± 0.15	0.74
	7-8	400	14.89 (14.18-15.74)	3.04 ± 0.16	0.35
	15-16	400	10.94 (10.17-11.77)	4.15 ± 0.38	0.50
	19-20	400	10.81 (10.29-11.35)	5.35 ± 0.36	1.14
	29-30	400	9.89 (8.93-10.86)	3.71 ± 0.37	0.39
	59-60	150	6.87 (6.49-7.26)	4.40 ± 0.25	0.74
deltamethrin	1-2	400	0.14 (0.13-0.15)	5.20 ± 0.49	1.15
	7-8	400	0.21 (0.20-0.22)	5.94 ± 0.38	0.91
	15-16	400	0.20 (0.19-0.21)	8.19 ± 0.60	0.44
	19-20	400	0.20 (0.19-0.20)	7.91 ± 0.51	0.74
	29-30	400	0.18 (0.17-0.19)	6.31 ± 0.37	0.29
	59-60	150	0.11 (0.11-0.12)	5.96 ± 0.42	0.02

[1]A number of 4 replicates were conducted with 100 insects per replicate for each age group (except for age 59-60 days where only 3 replicates with 50 insects each. Mortalities were recorded at 24 hours post-treatment.
[2]HF = heterogenity factor = χ^2/ degree of freedom.

was suggested that the chemicals might vary in their rates of reaching the target site in different age groups because *in vitro* inhibition of cholinesterase did not vary with age [28].

Effect of immobilizing techniques on insecticide susceptibility

The effects of using CO_2 and chilling on susceptibility to propoxur and deltamethrin were compared in this study. Adult males immobilized with chilling method (LD_{50} = 9.79 µg/g insect) were more readily killed than those immobilized with CO_2 (LD_{50} = 15.62 µg/g insect) when tested with propoxur (Table 5). However, the susceptibility of adult males immobilized with both techniques did not differ significantly when tested with deltamethrin.

In other studies done earlier [30-32], chilling had been considered a superior immobilizing technique when compared to the use of CO_2 because it caused no adverse effect on insects. In this study, the two immobilizing techniques had different effects on the toxicity of different insecticides. It might be true that the chilling technique had affected the metabolic activity of the insects and caused an increase in susceptibility to propoxur. The chances of CO_2 causing the decrease in susceptibility to propoxur may not be possible, as it had been shown recently that as long as the exposure of CO_2 did not exceed 15 minutes, it will not increase chlorpyrifos toxicity in adult German cockroach [10]. In the current study, only a dose of CO_2 of 20 seconds was used, thus ruling out the possibility of CO_2 affecting propoxur toxicity.

Effect of pre-treatment starvation on insecticide susceptibility

The mass of adult male German cockroaches upon starvation for 24- and 72 hours are shown in Table 6. At 24 hours post-starvation, there appeared to be no significant difference in the cockroach mass at different nutritional regimes. However, as the period of starvation increased to 72 hours, cockroaches pre-starved without food and water had the lowest mass of all groups studied. Adult males which were provided only with water showed a higher mass when compared with those given only food.

At 24 hours after starvation, the LD_{50} values of adult males exposed to propoxur were 12.64 and 12.99 µg/g insect for those conditioned with food only and water only, respectively. This did not differ significantly from the control group (LD_{50} = 14.02 µg/g insect) (Table 6). On the other hand, cockroaches conditioned without food and water for 24 hours had significantly lower susceptibility (LD_{50} = 10.99 µg/g insect) when compared to that of the control.

However, as the period of starvation increased from 24 hours to 72 hours, differences in susceptibility of the cockroaches under all four nutritional regimes became clearer. Cockroaches conditioned without food and water were most susceptible (LD_{50} = 6.88 µg/g insect), followed by those conditioned with food only (LD_{50} = 8.30 µg/g insect). The probit regression slope of the group conditioned without food and water for 72 hours was steeper when compared with that of under other nutritional conditions, indicating the adverse effect of starvation on insecticide susceptibility. In addition, those conditioned with water were more tolerant when compared with those conditioned with food only.

The overall trend of susceptibility observed in this study was similar to that reported by Kramer *et al.* [11]. However, those acclimatised with food only, water only or starved had significantly higher susceptibility to propoxur than those found in the present study. This may be due to differential total body lipids, carbohy-

Table 5. Effect of immobilizing techniques on susceptibility to propoxur and deltamethrin in adult male German cockroaches.

Insecticide	Immobilizing technique	LD_{50} (95% fiducial limit)[1] (in µg/ g insect)	Slope ± S.E	HF[2]
propoxur	CO_2	15.62 (14.87 - 16.56)	3.07 ± 0.16	0.09
	chilling	9.79 (9.22 - 10.54)	3.31 ± 0.29	0.49
deltamethrin	CO_2	0.22 (0.21-0.23)	6.44 ± 0.56	1.05
	chilling	0.20 (0.19-0.21)	5.94 ± 0.43	0.35

[1]A number of 3 replicates were conducted with 50 insects each. (mean weight = 53.44 mg). Mortality was recorded at 24 hours post treatment.

[2]HF = heterogenity factor = χ^2/ degree of freedom.

Table 6. Effect of pretreatment starvation on susceptibility to propoxur in adult male German cockroaches.

Period of starvation	nutritional condition	mean masses (mg)	LD_{50} (95% F.L)[1] (in µg/ g insect)	Slope ± S.E	HF[2]
nil (control)	food & water	50.00 ± 0.70	14.02 (13.34-14.79)	3.07 ± 0.16	1.23
24 hours	food only	48.97 ± 0.50	12.64 (11.79-13.60)	3.14 ± 0.25	0.34
	water only	49.13 ± 0.89	12.99 (11.99-14.08)	2.68 ± 0.28	0.03
	no food & water	48.95 ± 0.71	10.99 (10.31-11.70)	3.54 ± 0.26	0.05
72 hours	food only	45.11 ± 0.40	8.30 (7.76-8.84)	3.62 ± 0.22	0.36
	water only	46.95 ± 0.84	11.32 (10.67-12.03)	3.87 ± 0.24	0.33
	no food & water	44.25 ± 0.95	6.88 (6.45-7.30)	4.39 ± 0.30	0.30

[1]Mortality was recorded at 24 hours post-treatment. A number of three replicates were conducted with 50 insects each.
[2]HF = heterogenity factor = χ^2/ degree of freedom.

Table 7. Effect of different time of application on insecticide susceptibility in adult male German cockroaches[1].

Insecticide	Time	n	LD_{50} [95% fiducial limit) (µg/ g insect)	Slope ± S.E.	HF[2]
propoxur	0800	400	13.23 (12.39 - 14.23)	3.73 ± 0.31	0.11
	1200	400	12.54 (11.80 - 13.37)	4.02 ± 0.32	0.24
	1600	400	11.04 (10.32 - 11.81)	3.59 ± 0.29	0.27
	2000	480	9.23 (8.68 - 9.77)	4.47 ± 0.32	0.29
	2400	400	10.68 (10.06 - 11.33)	4.14 ± 0.31	0.82
deltamethrin	0800	400	0.20 (0.19 - 0.21)	7.77 ± 0.59	<0.01
	1200	400	0.20 (0.19 - 0.21)	4.49 ± 0.39	0.79
	1600	400	0.22 (0.21 - 0.23)	7.88 ± 0.60	1.87
	2000	400	0.21 (0.20 - 0.22)	4.57 ± 0.52	0.67
	2400	400	0.21 (0.21 - 0.23)	4.48 ± 0.60	0.30

[1]Mortality was recorded at 24 hours post treatment. A number of five replicates were conducted with 80 cockroaches per replicate. Mean weight for adult males = 52.02 mg.
[2]HF = heterogenity factor = χ^2/ degree of freedom.

drates and uric acid contents in both strains (VCRU and Orlando-normal) as an increase in these factors has been shown to increase insecticide tolerance [11]. Different experimental conditions may also affect insecticide susceptibility.

In this study, adult males acclimatised with water only had a higher tolerance to propoxur when compared with those with food only. Earlier, Kramer *et al.* [33] reported that propoxur induced water loss in affected insects and attributed it to be caused by increased metabolism and loss of spiracle control in the insects. Propoxur is metabolized by hydroxylation which requires the input of a hydroxyl group, possibly coming from the body water [8]. With such a phenomenon, the impact of hydration would have been greater in those insects which have earlier been deprived of water rather than food.

It would be interesting to determine how nutritional factors affect the resistance level in insecticide-resistant German cockroaches due to the fact that these insects usually have a higher total body fat content than their susceptible counterparts (CY Lee, unpublished data). This subject warrants further investigation because one of the criteria for a successful German cockroach control programme is to minimize the food and water source of the cockroach population.

Effect of time of insecticide application on insecticide susceptibility

There appeared to be no significant difference in susceptibility of cockroaches to deltamethrin at different time of insecticide application (Table 7). However, a propoxur susceptibility rhythm was shown with the adult males. Based on the five different application times, the cockroaches were most susceptible at 2000 hours, while their minimum susceptibility occurred at 0800 hours. It was reported earlier that German cockroaches susceptibility to KCN was highest at two hours after the beginning of scotophase (dark phase) and gradually decreased at the beginnning of light phase [13]; this was quite similar to that found in the present study. In fact, this also corresponded well with the oxygen consumption and activity rhythm of the insect [34,35].

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