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Control of insecticide-resistant German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) in food-outlets with hydramethylnon-based bait stations

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Abstract. Field and laboratory performance of a commercially available hydramethylnon-based bait station (Combat®) were conducted against insecticide-resistant German cockroach, *Blattella germanica* (Linn.) populations in five coffee-shops in Penang, Malaysia. Bioassay screening on F1 generation of the field collected cockroaches using a modified W.H.O. glass jar method detected moderate to high levels of propoxur, chlorpyrifos and cypermethrin resistance. Synergism studies suggested possible involvement of monooxygenase and esterase in the resistance in these strains. Laboratory assays of the bait stations against all five strains showed similar results when compared to that of the laboratory susceptible strain. Field efficacy study was done by placing 15 bait stations in the kitchen of each premise where heavy infestation of German cockroaches had previously been found. At 4-week post-treatment, a reduction of >90% was achieved in all premises. Suppression of the cockroach population remained high up to 12 weeks with >80% reduction in all populations.

INTRODUCTION

Insecticide resistance in the German cockroach, *Blattella germanica* (L.) is a prevalent problem to the pest control industry world-wide (Lee *et al.*, 1996; 1997a). In Malaysia, this species is the most important insect pests in hotels and food-preparative industries (Lee *et al.*, 1993). Current control strategies which rely heavily on the use of neurotoxic insecticides in residual formulations have been found to be ineffective against multiple resistant German cockroaches (Cochran, 1995). Many cases of control failures by residual insecticides due to insecticide resistance have been reported earlier (Atkinson *et al.*, 1991; Zhai & Robinson, 1991; Lee *et al.*, 1996). A recent review on this subject had been reported by Lee (1997). With the increase in the number of German cockroach resistance to insecticides lately, there is a serious need to address the problem with the use of chemicals with novel mode of actions.

Baiting has become a popular mode for control of German cockroach nowadays (Rust, 1986; Milio *et al.*, 1986; Appel, 1990; Appel, 1992; Appel & Benson, 1995) and no longer play a complimentary role to residual sprays (Ogg & Gold, 1993). In addition, the use of baits in the form of containerized station, or gel can also lessened the likelihood of pesticide misapplication (Robinson, 1988). Use of toxicant baits against insecticide-resistant German cockroaches have been found effective in several laboratory studies. Cochran (1990) reported good efficacy of abamectin-based baits against pyrethroid-resistant German cockroaches. Koehler & Patterson (1991) demonstrated the performance of hydramethylnon baits against insecticide resistant German cockroach, but cautioned that low level of hydramethylnon resistance may evolve in the field populations. This present study reports the field and laboratory performance of a commercially available hydramethylnon-based bait station (Combat®), against five populations of insecticide-resistant German cockroaches in food outlets in Malaysia.

MATERIALS AND METHODS

Test sites
All test sites used in this study were Chinese coffee shops located in George Town, Penang, Malaysia, namely HH, CS, BA, AK and WT (names of the shops are withheld as it has been mutually agreed earlier that only initials will be used). The treatment area for the test sites ranged from 12.4 – 19.2 m^2. All shops received pest control services on a quarter yearly basis. The insecticides used in the operation were unknown. During this study, all premise owners had been advised to stop all their usual pest control services on the quarter yearly basis.

Bait stations
A hundred hydramethylnon-based (active ingredient: 1.65% hydramethylnon) bait stations (Combat®) were purchased directly from three local supermarkets. A total of 15 bait stations were then randomly chosen and assigned to each premise.

Collection of cockroaches
Prior to baiting, pre-treatment sampling was conducted in all test sites using ten glass jar traps, left for 48 hours in the food-preparation area. Each trap was baited with a piece of white bread soaked earlier in local beer. During the collection of traps, all cockroaches were counted. The cockroaches were then brought back to the laboratory, reared for 1 – 2 generations and the F1 – F2 generations were tested for their susceptibility to the baits and other commonly used insecticides.
Cockroaches caught during pre-treatment were not released back into the population will not affect results of subsequent trappings. This is because German cockroaches have a very short life cycle (about 40 days) and this would allow ample time for population recovery.

Laboratory bait evaluation
For laboratory bait efficacy evaluation, box arenas each measuring 35 x 30 x 10 cm were used. Bait station and food were each provided at adjacent corners of the box, opposite to where harborage (folded paper) was placed. Water was placed close to the harborage. A total number of 20 adult males (aged 7 – 14 days) were used in this study. A susceptible strain (ICI) was used for comparison. Mortality of the cockroaches was assessed daily up to 14 days. Control experiments were done by introducing cockroaches into test arenas with similar condition as above except without bait station. No mortality was recorded in the control during the test period. Each experiment was replicated three times and conducted for all strains. Data were pooled and subjected to probit analysis according to procedure described by Robertson & Preisler (1992) using a computer software, POLO-PC (LeOra Software, 1997).

Baseline insecticide susceptibility and synergism tests
Baseline insecticide susceptibility of the cockroaches to commonly used insecticides (propoxur, chlorpyrifos and cypermethrin) was determined using W.H.O. glass jar method, as described in Lee *et al.* (1997a). Ten adult males (aged 2 – 10 days) were introduced into 0.45 l glass jars, each treated earlier with an insecticide (technical grade diluted in acetone) at 20 $\mu g/cm^2$ and their knockdown activities were recorded at selected time interval. A minimum of three replicates was done for all strains. Synergism test was conducted by applying topically 100 $\mu g/\mu l$ of piperonyl butoxide, or 30 $\mu g/\mu l$ of DEF® (*S,S,S*-tributylphosphorotrithioate) on the first abdominal segment of adult males, about two hours prior to W.H.O. glass jar tests. A susceptible strain (ICI) was used for comparison. Control for insecticide susceptibility tests were performed by exposing the cockroaches into glass jars treated earlier with acetone. Control for synergism test was performed by treating the cockroaches with synergists and then exposed them into acetone-treated glass jars. No mortality or knockdown was recorded for both

insecticide susceptibility and synergism controls during the test period.

Data were pooled and subjected to probit analysis according to similar procedure described above. Resistance ratios were calculated by dividing the LT_{50} values of the resistant strain with the corresponding lethal time of the susceptible strain.

Field bait evaluation

Baiting was done by placing 15 bait stations in the food-preparation area. Baits were placed in locations such as cabinets, under the sink, preparation table, behind the boiler, behind the refrigerator, etc. Post-treatment trapping were done using similar procedure to pre-treatment sampling at 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks post-baiting. No control house was done in this study due to ethical reason and inability to obtain consent from premise owner. The cockroaches trapped were counted and percentage population reduction at each sampling time interval was determined by comparing the number of cockroach trapped at each sampling date with the pre-treatment results. All cockroaches trapped at post-treatment were released back into the population after counting. Mean percentage reduction in cockroach count per trap between different premises was compared with Kruskal-Wallis (KW) one-way analysis of variance and separated with KW multiple range test (KWMRT) (Siegel & Castellan, 1988).

RESULTS AND DISCUSSION

Bioassays of the five strains using glass jar method revealed low to high resistance to propoxur (1.7 – 9.8X), low to moderate resistance to chlorpyrifos (1.1 – 4.3X) and low resistance to cypermethrin (1.2 – 1.7X) (Table 1). The WT strain demonstrated the highest resistance level to both propoxur and chlorpyrifos. Synergism studies using PBO and DEF suggested the possible involvement of both monooxygenase and elevated esterase in the resistance in all strains tested. Earlier, Lee *et al.* (1996) had reported moderate to high levels of propoxur resistance and low levels of chlorpyrifos resistance in twelve strains of field collected German cockroaches from hotels and restaurants in Kuala Lumpur and Penang, Malaysia. They also reported a similar involvement of both resistance mechanisms in the resistance in these strains. This may indicate that these two mechanisms of resistance are wide-spread among various field populations of German cockroaches in the country. High levels of elevated esterase also have been reported in other Malaysian strains of the German cockroach (Lee *et al.*, 1997a; Lee *et al.*, 1997b)

Laboratory assays of bait stations against all strains showed comparable LT_{50} values when compared to that of the ICI susceptible strains (Table 2). This indicated that all field strains tested were susceptible to hydramethylnon-based baits. This results indicated that hydramethylnon-based bait stations can perform well against carbamate- and organophosphate-resistant German cockroaches. Hydramethylnon is an inhibitor of mitochondrial respiration (Hollingshaus, 1987). Thus, due to different mode of action when compared to carbamate and organophosphate insecticides, this may explain why good efficacy was obtained when tested against these resistant insects.

Initial pretreatment trap counts ranged from 35 – 233 cockroaches per trap per night. The bait stations demonstrated excellent performance against all field strains. At the first week post-treatment, the bait performance differed significantly among each other with BA having the highest reduction rate (BA > HH = CS = AK > WT; $P < 0.05$, KWMRT). However at 4-week post-treatment, a reduction of >90% was observed in all premises (Figure 1) and did not differ significantly from each other ($P > 0.05$). Earlier studies by Milio *et al.* (1986) also reported >85% reduction in German cockroach population at 4 weeks post-treatment of using similar bait stations. Appel (1990) however, found a lower reduction rate of about 50% in his studies in Tallassee and Opelika, Alabama, USA which concurs with the studies by Ogg & Gold

Table 1. Insecticide resistance and synergism of field populations of insecticide-resistant German cockroaches from food-outlets to commonly used insecticides using modified W.H.O. glass jar method.

Insecticide Strain	Alone				+ PBO				+ DEF			
	n	LT_{50} (95 % FL) (min)	Slope	RR_{50}[1]	n	LT_{50} (95 % FL) (min)	Slope	RR_{50}[1]	n	LT_{50} (95 % FL) (min)	Slope	RR_{50}[1]
Propoxur												
ICI (sus.)	50	13.1 (12.7 – 13.5)	12.1	–	50	10.7 (9.1 – 12.4)	18.9	–	50	11.3 (10.2 – 12.4)	17.5	–
HH	50	73.4 (69.2 – 77.7)	12.0	5.6	50	12.8 (11.5 – 14.2)	17.8	1.2	30	10.2 (11.1 – 13.8)	15.7	0.9
CS	50	22.3 (21.0 – 23.6)	11.9	1.7	30	11.8 (10.6 – 13.0)	15.2	1.1	30	12.4 (11.1 – 13.8)	13.0	1.1
BA	50	30.1 (27.5 – 32.7)	7.9	2.3	30	12.8 (11.2 – 14.4)	9.2	1.2	30	14.7 (12.9 – 16.5)	22.2	1.3
AK	30	28.8 (26.3 – 31.4)	10.5	2.2	30	11.8 (10.7 – 13.0)	20.7	1.1	30	17.0 (14.2 – 20.1)	20.3	1.5
WT	30	128.4 (123.5 – 133.3)	6.3	9.8	30	36.4 (34.0 – 38.8)	10.1	3.4	30	21.5 (19.8 – 23.2)	13.5	1.9
Chlorpyrifos												
ICI (sus.)	50	37.2 (36.1 – 38.3)	6.8	–	50	35.4 (33.8 – 37.0)	7.8	–	30	32.3 (30.8 – 33.9)	10.5	–
HH	50	55.8 (53.7 – 57.9)	7.5	1.5	50	42.5 (40.2 – 44.8)	9.2	1.2	30	33.5 (31.2 – 35.8)	9.8	1.0
CS	50	40.9 (39.6 – 42.2)	5.4	1.1	50	38.9 (37.4 – 40.4)	7.7	1.1	30	28.4 (27.4 – 29.5)	9.1	0.9
BA	30	70.7 (69.0 – 72.4)	6.1	1.9	30	53.1 (52.2 – 54.0)	8.5	1.5	30	31.9 (30.3 – 33.5)	8.5	1.0
AK	30	85.6 (83.5 – 87.8)	6.7	2.3	30	60.2 (58.7 – 61.7)	6.9	1.7	30	42.2 (40.9 – 43.5)	11.2	1.3
WT	30	160.0 (157.5 – 162.5)	4.8	4.3	30	56.6 (55.4 – 57.8)	7.1	1.6	30	43.1 (41.6 – 44.7)	7.5	1.3
Cypermethrin												
ICI (sus.)	50	8.5 (8.1 – 8.9)	10.9	–	30	5.3 (4.9 - 5.7)	13.5	–	30	9.0 (8.6 - 9.4)	10.2	–
HH	50	10.6 (9.9 – 11.3)	8.4	1.2	30	5.2 (5.0 - 5.4)	11.1	1.0	30	11.9 (11.3 – 12.6)	12.5	1.3
CS	50	9.9 (9.2 - 10.7)	9.2	1.2	30	6.0 (5.5 - 6.5)	14.2	1.1	30	12.4 (11.3 – 13.5)	10.9	1.4
BA	30	10.8 (10.2 – 11.4)	11.5	1.3	30	5.5 (4.8 - 6.2)	12.9	1.0	30	10.8 (10.1 – 11.4)	9.8	1.2
AK	30	12.1 (11.8 – 12.5)	7.2	1.4	30	5.8 (5.7 - 6.0)	10.6	1.1	30	12.5 (12.3 – 12.7)	7.3	1.4
WT	30	14.7 (13.9 – 15.5)	8.8	1.7	30	6.4 (5.9 - 6.8)	12.4	1.2	30	18.2 (16.8 – 19.6)	7.6	2.0

[1]Resistance ratio was calculated by dividing the LT_{50} value of the resistant strain with the corresponding lethal time of the susceptible strain ((ICI).

Table 2. Laboratory efficacy of a hydramethylnon-based bait station against adult males of five field populations of the German cockroach.

Strain	n	LT_{50} (95% fiducial limit) [in days]	Slope	RR[1]
ICI	60	3.1 (2.8 – 3.4)	4.5	–
HH	60	3.2 (2.9 – 3.5)	4.9	1.0
CS	60	3.0 (2.7 – 3.3)	5.1	1.0
BA	60	3.7 (3.2 – 4.2)	3.9	1.2
AK	60	3.2 (2.8 – 3.6)	5.3	1.0
WT	60	3.9 (3.7 – 4.1)	5.2	1.3

[1]RR was calculated by dividing the LT_{50} value of the resistant strain with the corresponding lethal time of the ICI (susceptible) strain.

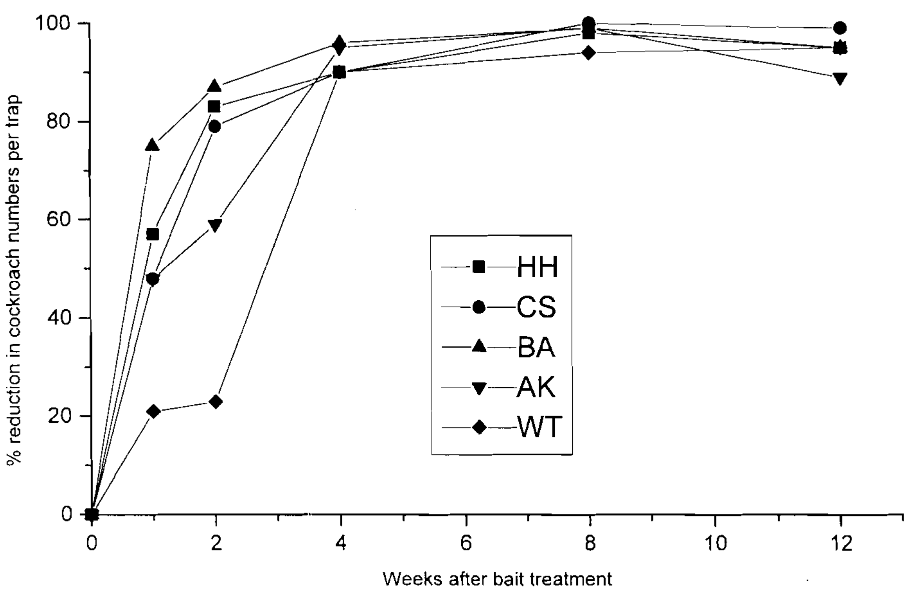

Figure 1. Performance of a hydramethylnon-based bait station against five field populations of insecticide-resistant German cockroaches in food-outlets in Penang, Malaysia.

(1993) in Nebraska, USA.

Up to three months post-treatment, suppression of cockroach population was sustained with a reduction of >80% in cockroach population in all premises. Reierson *et al.* (1983) reported that hydramethylnon-based bait stations reduced more than 85% of German cockroach population up to 12 weeks in urban Los Angeles. Their results were supported by Milio *et al.* (1986) where the authors reported >90% reduction in population at 12-week post-treatment. However, these authors found that the bait station numbers influenced the performance of the formulation. In this study, 15 bait stations were used to avoid lack of control due to low bait numbers.

Besides bait numbers, another important aspect which warrants attention during baiting is premise sanitation. From the author's experience when baiting in suburban squatters, a good sanitation practice by the premise owner is of paramount importance for fast reduction of cockroach population, usually within 7 weeks post-treatment (CY Lee, unpublished data). In the current study, all owners had been advised to get rid of accumulation of cardboard boxes, clutters and prevent food debris on the floor. Other factors influencing the performance of bait stations include bait placement, bait repellency (Reierson 1995) and bait age (Milio *et al.* 1986).

The current study demonstrated good performance of a bait formulation against insecticide-resistant German cockroaches, both in the laboratory and field. More studies have to be conducted to further substantiate current findings. Overall, the outcome of this study suggested an effective alternative method to combat insecticide-resistant German cockroaches in Malaysia.

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