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A case of increased tolerance of *Rattus tiomanicus* (Miller) to brodifacoum and bromadiolone

[Peningkatan tolerans *Rattus tiomanicus* (Miller) terhadap brodifakum dan bromadiolon]

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Key words : tolerance, *Rattus tiomanicus*, brodifacoum, bromadiolone

Abstrak

Tikus belukar *(Rattus tiomanicus)* dari kawasan koko-kelapa di Estet Kuala Bernam, Hilir Perak, yang dahulunya dikawal dengan menggunakan bromadiolon atau brodifakum telah diperangkap hidup-hidup kerana kaedah pengawalan dahulu kurang berkesan. Tikus-tikus ini telah ditempatkan di makmal dan diberi air serta makanan tanpa had. Enam kumpulan (20 jantan dan 20 betina setiap kumpulan) diberi makan umpan beracun bromadiolon 0.005% tanpa pilihan selama 1, 2, 3, 4, 5 dan 6 hari. Empat kumpulan lagi diberi makan umpan yang mengandungi brodifakum 0.003% selama 1, 2, 3 dan 4 hari manakala satu kumpulan lagi sebagai kawalan diberi makan umpan tanpa racun selama 6 hari. Tiada tikus yang mati dalam kumpulan kawalan dan kematian yang dicatat bagi ujian bromadiolon dalam keenam-enam kumpulan ialah 30, 52.5, 72.5, 87.5, 97.5 dan 100% manakala bagi keempat-empat kumpulan dalam ujian brodifakum ialah 57.5, 82.5, 90 dan 100%.

Dalam kedua-dua ujian terhadap bromadiolon dan brodifakum, tikus-tikus tersebut didapati mati pada takaran yang lebih tinggi dan mengambil masa yang lebih lama berbanding dengan tikus-tikus yang telah dilaporkan sebelum ini. Jangka masa purata pengambilan makanan berkesan bagi membawa maut (LFP_{50}) ialah 1.74 (1.42-2.02) hari bagi bromadiolon, dan 0.92 (0.56-1.18) hari bagi brodifakum. Pada puratanya, tikus-tikus mati selepas 8.1 hari diberi makan umpan yang mengandungi racun bromadiolon dan 8.4 hari selepas diberi makan umpan yang mengandungi racun brodifakum. Penemuan ini menunjukkan peningkatan tolerans tikus terhadap brodifakum dan bromadiolon. Beberapa ekor tikus didapati masih hidup walaupun kadar racun yang dimakan adalah tinggi. Pemakanan umpan yang mengandungi racun bromadiolon (0.005%) dan brodifakum (0.003%) selama 6 dan 4 hari telah menyebabkan semua tikus yang diuji mati. Keadaan ini menunjukkan bahawa racun-racun tersebut masih berkesan untuk membunuh tikus-tikus ini.

Abstract

Rattus tiomanicus were live-trapped from some cocoa-coconut fields in Kuala Bernam Estate, Hilir Perak with unsatisfactory bromadiolone or brodifacoum control programmes. The rats were brought back to the

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laboratory and conditioned for 2 weeks with food and water ad libitum. Six groups (20 males and 20 females in each group) were fed 0.005% bromadiolone in maize-based wax cubes for 1, 2, 3, 4, 5 and 6 days no-choice feeding; four groups were fed commercially prepared 0.003% brodifacoum baits for 1, 2, 3 and 4 days, and another group, as control, was fed non-poison wax baits for 6 days. No animal death was observed in the control while mortalities recorded in the bromadiolone tests were 30, 52.5, 72.5, 87.5, 97.5 and 100% and for the brodifacoum tests, they were 57.5, 82.5, 90 and 100% respectively.

Rats that succumbed to the bromadiolone and brodifacoum tests required a higher dose and a longer duration to death compared with that of susceptible rats reported previously. The mean lethal feeding period (LFP_{50}) was 1.74 (1.42-2.02) days for bromadiolone and 0.92 (0.56-1.18) day for brodifacoum. The average mean duration to death of the animals after having consumed a lethal dose was 8.1 days for bromadiolone and 8.4 days for brodifacoum. These findings showed increased tolerance of *R. tiomanicus* to brodifacoum and bromadiolone. Several of the animals that survived the bromadiolone and brodifacoum feeding tests consumed relatively high doses of the poisons. These rats would survive a pulse baiting programme using brodifacoum or bromadiolone which accounts for unsatisfactory rat control in the field. Total mortality in the 6-day feeding period on 0.005% bromadiolone baits or the 4-day feeding period on 0.003% brodifacoum baits showed that these poisons are still effective against such problem rats.

Introduction

Poison-baiting is an integral part of field management practices for reducing rat population in plantations. Warfarin-based baits have been frequently used in most plantation crops, especially oil palm since the 1960s (Wood 1969). However, with reports of warfarin-resistant rats in some cocoa-coconut fields in Hilir Perak (Lam et al. 1982; Lee et al. 1983), available potent anticoagulants like brodifacoum and bromadiolone are welcome alternatives.

Brodifacoum and bromadiolone are single-feed poisons with a short duration to death (mean <7 days) (Lam 1982, 1985; Lee and Mastor 1984; Lee and Kamarudin 1988). Rennison and Dubock (1978) recommended that pulse baiting be carried out with these kind of poisons. Khoo (1980) showed that pulse baiting with 0.003% brodifacoum was effective for rat control in oil palm plantations. Altogether four baiting-rounds at 7-day intervals between baiting rounds were required. Lee and Arikiah (1984) showed that in cocoa-coconut plantations, effective rat control was achieved with three baiting rounds of 0.005% bromadiolone baits also at 7-day intervals between rounds. This rat-baiting technique has shown success in most cocoa-coconut areas in Hilir Perak. However, unsatisfactory rat control with bromadiolone and brodifacoum baiting programmes was recently noted in some cocoa-coconut fields of Kuala Bernam Estate. This paper presents laboratory evidence of increased tolerance to bromadiolone and brodifacoum in a population of *Rattus tiomanicus*, which was the only species live-trapped from the affected cocoa-coconut fields.

Materials and methods

Field pod damage assessment

In every field, pods were harvested by the estate management fortnightly. At every harvesting round, pod damage in selected field blocks was assessed. This enabled the determination of fields requiring rat control via baiting programmes and also the monitoring of the control programme

implemented.

Laboratory screening

Rattus tiomanicus were live-trapped from cocoa-coconut field no. 13, 14 and 34 of Kuala Bernam Estate in Hilir Perak. These areas had unsatisfactory rat control with bromadiolone or brodifacoum after 6 years of effective baiting control programmes. The rats were brought back to the laboratory and caged (45 cm x 45 cm x 30 cm cages) individually, maintained on a diet of laboratory rat pellets, fresh copra and ripe bananas with water ad libitum for 2 weeks. Eleven groups of rats were then used (each group comprising 20 males and 20 females) for the no-choice feeding tests. The test regimes followed that of the World Health Organisation (Anon. 1982) test procedures.

The bait formulation of 0.005% bromadiolone was prepared in maize-based wax cubes. Two percent of the technical liquid (0.25% bromadiolone in solution) was added to 53% mesh grind maize, 5% refined sugar, 10% fish fry heads and prawn dust, 5 g coconut oil (used as a sticker) and 25% melted paraffin. Brodifacoum wax baits at 0.003% concentration were obtained commercially.

All rats were given similarly prepared non-poison wax baits for 2 days prior to the feeding tests. Six groups of rats were allowed unrestricted feeding on 0.005% bromadiolone wax maize baits for 1, 2, 3, 4, 5 and 6 days; four groups were allowed unrestricted feeding on 0.003% brodifacoum baits for 1, 2, 3 and 4 days; and the remaining group (control) was given non-poison wax baits for 6 days. At the end of the prescribed feeding period for each group, laboratory pellets, fresh copra and ripe bananas were given as food and the rats were observed for a further 30 days (inclusive of the first day of feeding upon the poison baits). Bait consumption was measured (to the nearest 0.1 g) daily and mortality recorded. Dead rats were autopsied for signs of poisoning. The rats were considered to have survived the test if they were alive after the 30th day. The mean lethal feeding period (LFP_{50}) was determined by probit analysis from the results of the feeding tests.

Results

Field results

Field no. 13, 14 and 34 showed high pod damage *(Table 1)* before and even after implementation of the rat baiting programme *(Table 2)*. Following this, live rat trapping was commenced a week later. A total of 492, 347, 645 rats were removed following a 15, 20 and 23-day trapping period from field no. 13, 14 and 34 respectively.

Laboratory results

In the laboratory, the difference in the mean bait intake between plain and poison baits given was small *(Table 3)* and statistically insignificant ($p > 0.05$). The daily bait intake, however, indicated good acceptance. No death was recorded in the control treatment while in the others, rats were found to die with typical anticoagulant poisoning symptoms. Mortality rates in the tests increased with longer feeding periods.

In the bromadiolone no-choice feeding tests, total mortality was noted in rats subjected to 6 days of feeding *(Table 3)*. The least fatal dose was 3.27 mg/kg body weight (a 110-g male rat ate 7.2 g of the baits in the 1-day feeding period and died on the eighth day). In contrast, the highest dose survived was 15.63 mg/kg (a female rat of 104 g ate 32.5 g of the baits in the 4-day feeding period). Most of the rats succumbed to the poison between 4 and 14 days, with a mean death duration of 8.1 days. One female was noted to die only after the 22nd day following a 5-day feeding period. Probit analysis of the data showed that the LFP_{50} within 95% fiducial limits for male rats fed 0.005% bromadiolone baits was 1.80 (1.31-2.21) days, and 1.79 (1.31-2.21) days for females *(Table 4)*. The pooled data gave an LFP_{50} of 1.74 (1.42-2.02) days.

In the brodifacoum no-choice feeding tests, all rats given a 4-day feeding period

Table 1. Cocoa pod damage and rat baiting programmes in Kuala Bernam cocoa-coconut field no. 13, 14 and 34

	Cocoa-coconut field no.			
	13	14	34	Remarks
1st harvesting round				High damage,
No. pods examined	2 080	3 350	1 326	implement
No. rat-damaged pods	605(29.1)	1 050(31.3)	260(19.6)	rat baiting
Control programme	Bromadiolone	Brodifacoum	Brodifacoum	1st and 2nd baiting
2nd harvesting round				Increased
No. pods examined	1 635	2 766	3 061	damage in
No. rat-damaged pods	605(37.0)	646(23.4)	881(28.8)	2 areas
Complete rat control programme				3rd and 4th baiting
3rd harvesting round				Increased
No. pods examined	862	974	3 394	damage
No. rat-damaged pods	520(60.3)	384(39.4)	1 554(45.8)	
4th harvesting round				Unsatisfactory
No. pods examined	2 146	2 466	2 269	rat control
No. rat-damaged pods	1 196(55.7)	626(25.4)	989(43.6)	programme

Values in brackets denote % of damage

Table 2. Rat baiting programme implemented in Kuala Bernam Estate cocoa-coconut field no. 13, 14 and 34

Field no.	13	14	34
Area (ha)	10.2	11.3	20.2
Bait used	Bromadiolone	Brodifacoum	Brodifacoum
No. baits used			
1st round	1 830	1 912	2 497
2nd round	1 026 (56)	1 300 (68)	2 022 (81)
3rd round	833 (46)	1 070 (56)	2 322 (93)
4th round	658 (36)	611 (32)	1 323 (53)

Values in brackets denote % of baits replaced

succumbed to the treatment *(Table 3)*. The least fatal dose was 1.50 mg/kg body weight (a male rat of 82.0 g ate 4.1 g of the bait in the 1-day feeding period and died on the sixth day). In contrast, the highest dose survived was 10.35 mg/kg body weight (a male rat of 120 g ate 41.4 g of the baits in the 3-day feeding period). Most of the rats died between 4 and 8 days, and 12 individuals, however, died between 10 and 28 days. The mean duration to death was 8.4 days. The LFP_{50} within 95% fiducial limits for males was 0.91 (0.32-1.29) day, and 0.97 (0.44-1.32) day for females *(Table 4)*. The pooled LFP_{50} was 0.92 (0.56-1.18) day.

Discussion

The problem rats were evaluated with 0.005% bromadiolone and 0.003% brodifacoum waxed baits since both poisons were commercially used at such formulations for rat-baiting programmes. The LFP_{50} of normally susceptible *Rattus tiomanicus* to both these poisons was also established using the above formulation (Lee and Mastor 1984; Lee and Kamarudin 1988). This enabled comparison of the poisons' potency to the problem rats with normal susceptible rats.

This study on bromadiolone and brodifacoum, both derivatives of hydroxycoumarin, showed that brodifacoum

Table 3. No-choice feeding tests on *Rattus tiomanicus* treated with maize-based paraffin poison baits at various number of days

Poison	Feeding period (days)	Sex	Mean body wt. (g)	Mortality	Mean bait intake		Mean lethal poison dose (mg/kg)	Mean survived poison dose (mg/kg)	Mean duration to death (days)
					Last day plain (g)	1st day poison (g)			
Control	6	M	98.6	0	9.4	-	No poison baits offerred		
		F	89.2	0	8.7	-	No poison baits offerred		
Bromadiolone 0.005%	1	M	110.4	6	9.0	8.8	4.52 (3.27-5.56)	4.20 (3.20-6.64)	6.8 (4-10)
		F	102.0	6	11.0	10.6	4.86 (3.40-6.36)	5.20 (3.69-7.20)	7.6 (6-14)
	2	M	105.3	10	9.8	10.0	7.80 (4.05-12.80)	7.52 (4.18-8.46)	8.0 (6-14)
		F	98.0	11	9.2	8.9	8.92 (3.28-15.15)	6.72 (3.80-10.50)	9.2 (6-14)
	3	M	115.6	15	10.5	10.0	9.02 (4.23-13.20)	6.38 (5.29-10.60)	8.2 (4-14)
		F	104.2	14	9.6	9.0	10.30 (5.38-15.23)	8.64 (6.21-11.20)	8.0 (4-12)
	4	M	100.0	18	8.9	9.5	15.20 (8.82-20.83)	11.58 (8.54-14.62)	7.8 (7-13)
		F	92.0	17	9.0	9.2	17.60 (7.85-22.42)	12.30 (8.45-15.63)	9.0 (6-13)
	5	M	124.0	19	11.5	10.6	14.90 (8.92-26.50)	13.62	8.4 (5-12)
		F	98.5	20	9.2	9.8	18.40 (5.84-28.90)	-	8.6 (4-22)
	6	M	102.1	20	8.7	9.2	22.35 (11.73-27.80)	-	7.6 (5-13)
		F	86.0	20	7.8	8.2	20.45 (8.63-37.42)	-	8.0 (6-13)
Brodifacoum 0.003%	1	M	100.0	12	9.5	10.2	4.21 (1.50-6.95)	4.35 (2.93-6.56)	11.6 (7-20)
		F	88.0	11	8.8	9.4	4.35 (2.35-7.52)	4.94 (3.63-6.26)	8.9 (4-19)
	2	M	125.6	16	10.5	9.8	5.18 (2.05-8.72)	5.43 (2.86-7.30)	10.6 (6-28)
		F	94.4	17	9.8	8.7	5.75 (3.21-9.36)	5.84 (3.54-8.72)	7.1 (5-21)
	3	M	120.0	18	11.0	9.8	7.52 (2.85-10.94)	8.24 (6.12-10.35)	6.5 (4-14)
		F	104.8	18	9.2	10.0	8.48 (2.62-12.86)	7.92 (5.98-9.86)	7.3 (4-10)
	4	M	121.0	20	10.6	10.2	9.06 (6.29-14.52)	-	8.5 (5-15)
		F	103.0	20	8.9	7.8	9.49 (4.99-18.65)	-	6.9 (5-8)

Values in brackets denote range
Each treatment comprised 20 rats

Table 4. Probit analysis of no-choice feeding tests with bromadiolone and brodifacoum baits against *Rattus tiomanicus*

Treatment	Sex	Regression equation	Chi-sq.	d.f.	LFP_{50} (95% fiducial limits) (days)
Bromadiolone 0.005%	M	y = 3.50x + 4.11	2.12	4	1.80 (1.31-2.21)
	F	y = 3.48x + 4.12	4.00	4	1.79 (1.31-2.21)
	Pooled	y = 3.38x + 4.18	5.41	4	1.74 (1.42-2.02)
Brodifacoum 0.003%	M	y = 2.76x + 5.12	1.26	2	0.91 (0.32-1.29)
	F	y = 3.11x + 5.04	1.12	2	0.97 (0.44-1.32)
	Pooled	y = 2.90x + 5.11	2.18	2	0.92 (0.56-1.18)

is more potent than bromadiolone at their respective recommended formulations. Similar findings have been reported in studies with normally susceptible *R. tiomanicus* populations (Lee and Mastor 1984; Lee and Kamarudin 1988). Brodifacoum is a potent anticoagulant that has been used in the final screening for brown rats resistant to several anticoagulants in Denmark (Lund 1984). It was noted that rats resistant to two or more of these anticoagulants (i.e. warfarin, coumatetralyl, bromadiolone and difenacoum) failed to survive a 6-day feeding period on 0.005% brodifacoum baits (Lund 1984).

These feeding tests showed that more animals survived compared with that reported earlier for *R. tiomanicus* normally susceptible to bromadiolone (Lee and Mastor 1984) and brodifacoum (Lee and Kamarudin 1988). The longer interval to death for rats consuming a lethal dose indicated signs of increased tolerance towards the poisons. Furthermore, the LFP_{50} values of 1.74 days for bromadiolone and 0.92 day for brodifacoum were both 35% higher than the 1.29 days reported for bromadiolone (Lee and Mastor 1984) and the 0.68 day given for brodifacoum (Lee and Kamarudin 1988) of normally susceptible *R. tiomanicus*. Compared with warfarin-resistant *R. tiomanicus* whose LFP_{50} is 1.38 days for brodifacoum (Lee and Kamarudin 1988), these field rats are definitely from a more susceptible population.

Field rats being more tolerant, would consume more baits including those made available in the replacement rounds. The longer duration before death would also enable higher pod damage to be inflicted. In a baiting programme laid out for these animals, most of the baits would probably be consumed by a small number of rats present, culminating with others inflicting pod damage. This would lead to low animal kill together with high pod damage, and could thus account for the unsatisfactory rat control.

Following the detection of warfarin-resistant *Rattus rattus diardii* in Kuala Bernam Estate (Lam 1980), pulse baiting with bromadiolone and brodifacoum was introduced. This had brought about effective rat control for almost 7 years. In contrast, with brown rats *(R. norvegicus)* in Denmark, the effectiveness of bromadiolone is rather short-lived because bromadiolone-resistant rats were encountered after a year of introduction (Lund 1984). In Canada, increased tolerance to these compounds was noted for house mice *(Mus musculus)* a few years after their introduction (Siddiqi and Blaine 1982). These findings indicated that the effectiveness of rodenticides varied between species.

In accordance to WHO (Anon. 1982), determination of the resistance of an animal species should be based on the estimated feeding period that is lethal to at least 99% of normally susceptible animals. For *R. tiomanicus*, the LFP_{99} of normally susceptible rats to 0.005% bromadiolone

baits is 9 (5-25) days (Lee and Mastor 1984) and to 0.003% brodifacoum is 3 (2-99) days (Lee and Kamarudin 1988). This study showed increased tolerance and no resistance to bromadiolone as the rats did not survive beyond the 6-day feeding period. Resistance to brodifacoum was not noted as all rats succumbed to the poison in a 4-day feeding period. However, tolerance to brodifacoum is noted as four out of 40 rats survived a 3-day feeding period.

Total death of all the rats was achieved following a 4-day feeding period on 0.003% brodifacoum baits or a 6-day feeding period on 0.005% bromadiolone baits. This showed that these poisons are still effective against these tolerant rats, provided the baiting technique used ensured multiple feeding. Instead of utilising pulse baiting, conventional baiting which enables multiple feeding as advocated by Wood (1969) may be followed. Siddiqi et al. (1983) showed that multiple baiting with bromadiolone and brodifacoum brought about effective control of warfarin-resistant house mice. Multiple feedings were also reported to have brought about mortality of mice tolerant to bromadiolone (Siddiqi and Blaine 1982).

Subsequently, field baitings undertaken by the Kuala Bernam Estate management using commercial brodifacoum bait at four rounds, with an interval of 4-5 days between rounds, brought about effective rat control in these fields. This is achieved with the removal of a large number of rats from these fields by trapping and baiting undertaken for whatever number of rats (presumably small) that remained.

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