MARDI Res. Bull., (1983) 11, 2: (132–135)

LABORATORY EVALUATION OF 0.025% WARFARIN AGAINST *RATTUS TIOMANICUS*

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Keywords: Laboratory evaluation, Warfarin, *Rattus tiomanicus*, Susceptibility, Fiducial limit.

RINGKASAN

Kajian pemakanan bagi penentuan keupayaan 0.025% Warfarin membunuh tikus *Rattus tiomanicus* yang diperangkap di ladang koko dan kelapa MARDI di Hilir Perak, telah dijalankan di makmal. Pemakanan cara tiada pilihan bagi jangkamasa 2, 4, 6, 8 dan 10 hari mengakibatkan kematian 25, 45, 80, 95 dan 100% bagi binatang yang diuji. Terdapat variasi-kepekaan yang nyata di antara individu-individu tersebut terhadap warfarin. Jangkamasa yang berkesan membawa maut (LFP 50) ialah 3.20 hari bagi tikus betina dan 3.77 hari untuk tikus jantan. Tiada perbezaan yang nyata di antara jantina didapati dan analisa 'probit' ke atas data tersebut memberi satu garis asas untuk kepekaan *R. tiomanicus* terhadap warfarin. LFP 95 (upper 95% fiducial limits) menunjukkan keupayaan hidup bagi tikus selepas diberi makan umpan mengandungi 0.025% warfarin selama 14 hari adalah cara yang sesuai untuk menguji ketahanan terhadap racun tersebut.

INTRODUCTION

Rattus tiomanicus is the predominant rodent pest in most oil palm (WOOD, 1969a) and cocoa-coconut (LEE, 1981; HAN and BOSE, 1980) growing areas in Peninsular Malaysia. Their widespread importance has led to the formulation, and subsequently the recommendation of an effective method of control using warfarin at 0.025% concentration in maize based paraffin wax cubes (WOOD, 1969b). The use of warfarin since then had led to the prospect of warfarin resistance in oil palm plantations (WOOD and LIAU, 1977) even though there was limited information available pertaining to the susceptibility of this species. This paper presents the results of the evaluation of 0.025% warfarin against *R. tiomanicus* in the laboratory.

MATERIALS AND METHODS

Rattus tiomanicus were live-trapped from cocoa-coconut fields within Mardi Research Station in Hilir Perak. These fields have never been baited with any type of rodenticides.

Captured rats were caged individually (45 x 45 x 30 cm cages) in the laboratory and kept under observation for two weeks before they were tested. They were maintained on a laboratory diet of mouse pellets, fresh copra and ripe bananas with water *ad libitum*. Rats that were weak, injured, diseased and/or pregnant were discarded. Animals were weighed before the start of each tests and animals below 70g body weight were excluded from the tests. The animals were divided into five groups, each comprising 10 males and 10 females.

Warfarin bait was prepared by first dispersing the technical grade active ingredient in rice bran to form a master-mix containing warfarin at 0.5%. The final bait formulation of 0.025% warfarin was prepared by adding 5% of the master-mix to 70% finely ground maize, 5% refined sugar, 15% ground fish fry heads and prawn dust and 5% coconut oil (use as a sticker).

No-choice feeding tests were conducted. Animals were prebaited for two days. The five groups of animals were then allowed unrestricted feeding of poison bait for 2, 4, 6, 8, and 10 days respectively. Bait

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consumption was measured (to the nearest 0.1g) daily for each animal and mortality was recorded. Rats were considered to have survived a test if they were alive 28 days after the beginning of the test period. The above test protocol follows that established by WHO (1975).

RESULTS AND DISCUSSION

WOOD and LIAU (1977) noted that fine grain materials recommended as bases elsewhere were unsuitable for use and reported that banana mash *ad lib* at 0.01% or maize base bait at 0.025% warfarin offer the best means for standard tests against rats. Commercially, warfarin at 0.025–0.05% in maize base is used as a rodenticide. Hence the use of the latter rather than banana mash would provide a better standard diet for laboratory screening tests on rats.

For both poison and unpoison bait uptake, a slight difference noted in all the tests were insignificant *(Table 1)*. Over the various feeding periods the lowest amount of bait eaten was 5.8 g while the highest was 11.8 g. Considerable variation in susceptibility to warfarin poisoning among the individuals was found in the feeding tests. The lowest fatal dose was 24 mg/kg and the animals concern was a female weighing 100 g, ate only 9.6 g of bait over a two days feeding period and it died on the 6th day. In contrast the highest survived dose was 196 mg/kg by a male weighing 104 g and eating 81.5 g of the bait over an eight day feeding period. Such high variation was also noted by WOOD and LIAU (1977).

The data in *Table 1* was examined by the probit analysis (FINNEY, 1971). The median lethal feeding period (LFP_{50}) with 95% fiducial limits were 3.2 days (2.04–4.17 days) for females and 3.77 days (2.95–4.83 days) for males *(Figure 1)*. The difference between the mean of the two sexes were found to be statistically insignificant. The data of both sexes is then pooled for probit analysis *(Figure 2)*. The LFP 50 was found to be 3.47 days (2.7–4.17 days).

Similarly the LFP 95 and 98 calculated within 95% fiducial limits for all the animals tested were 9.11 days (7.16–14.01 days) and 11.58 days (8.68–19.91 days) respectively. These values are more meaningful in contrast to 134.9 days (females) and 7.17 days (males) for LFP 95 established by WOOD and LIAU (1977); and could be used as a baseline for the monitoring of warfarin resistance in this species. Taking the upper 95% fiducial limits, these data suggests that a 14–day or a 20–day feeding period would be a suitable test for resistance, depending on the stringency adopted.

TABLE 1. RESULTS OF NO. CHOICE FEEDING TESTS IN WHICH *R. TIOMANICUS* WERE GIVEN 0.025% WARFARIN IN MAIZE-BASE BAIT FOR VARIOUS NUMBER OF DAYS

No. of days feeding	Sex	Mean body weight (g)	Mortality (dead/ treated)	Mean bait intake last day plain (g)	Mean bait intake 1st day poison (g)	Lethal dose of warfarin (mg/kg) Mean	Lethal dose of warfarin (mg/kg) Range	Survived dose of warfarin (mg/kg) Mean	Survived dose of warfarin (mg/kg) Range	Days to death Mean	Days to death Range
2	M	104.2	2/10	9.0	8.7	50	(36– 60)	41	(20– 60)	7	6– 8
	F	95.7	3/10	7.8	8.9	33	(24– 58)	40	(20– 60)	7	6– 8
4	M	114.5	4/10	11.8	11.3	60	(28–127)	69	(40– 86)	5.8	4– 8
	F	93.5	5/10	8.2	8.4	73	(62–126)	64	(46– 80)	6	4– 8
6	M	110.4	8/10	10.4	10.1	100	(46–142)	104	(86–122)	6.3	3– 9
	F	100.5	8/10	7.5	7.0	90	(30–140)	64	(62– 67)	6.6	5– 8
8	M	108.3	9/10	6.7	6.2	87	(25–140)	196		5.4	3– 8
	F	110.1	10/10	7.2	6.4	92	(40–150)	–		6.3	4– 8
10	M	115.9	10/10	6.0	5.8	60	(29–116)	–		6.8	6–11
	F	110.6	10/10	8.0	7.7	59	(38–103)	–		7.6	6–10

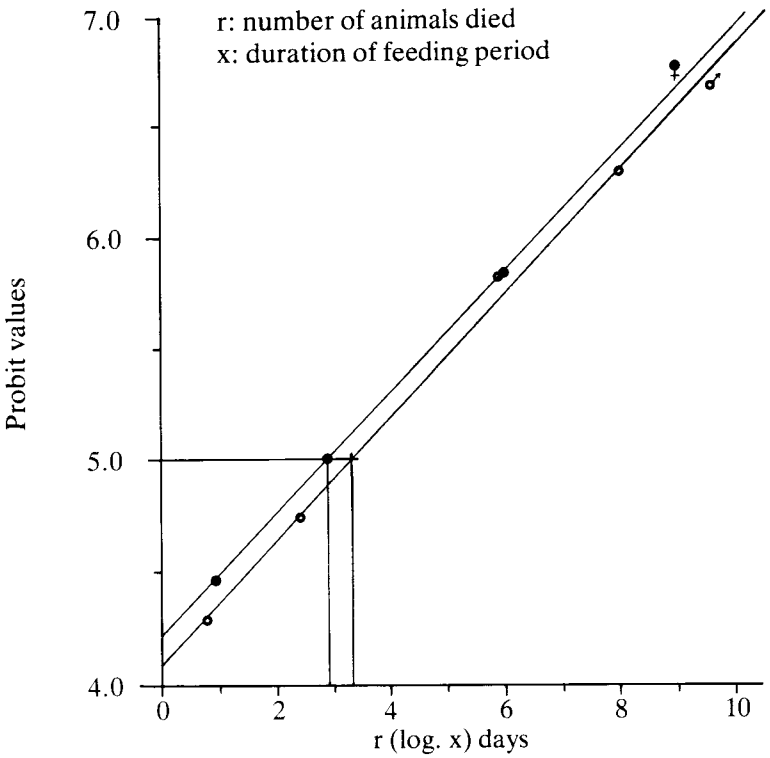

Figure 1. Graph of probit (% mortality) against r (log. x)

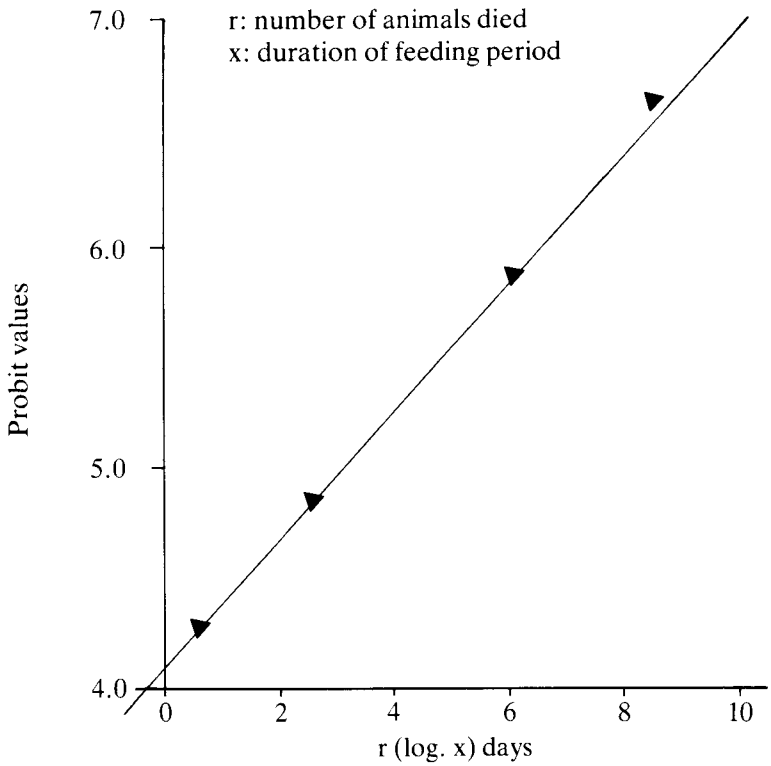

Figure 2. Graph of probit (% mortality) against duration of feeding period for all the animals tested.

Warfarin is effective for the control of *R. tiomanicus*. However, prolonged feeding on the poison bait is required to achieve an acceptably high kill. Intermittent feeding is likely to occur in most cocoa-coconut growing areas as the cacao tree fruits all the year round and together with the rich floor fauna, they provided an alternative food source. Baiting rounds should be undertaken during low crop season where food is scarce as this would ensure a higher acceptance of the baits.

The group of five individuals that survived a feeding period of 6–8 days on 0.025% warfarin is not an exception in terms of the range in the 'wild' population. Alternatively this indicated that intermittent sustained application of warfarin in the field may lead to the emergence of warfarin resistant strains. This possibility is foreseeable in the near future (LAM, 1980). by then other rodenticides labelled as the 'second generation rodenticide' probably would offer a solution to the warfarin resistant rats.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Sharif Ahmad (Pengarah Tanaman Saka), Mr. Ramadasan Krishnan, Dr. Mohd. Musa Mohd. Jamil, and Mr. Kamal A. Kamaruddin (all of MARDI) for their suggestions and comments in the preparation of this manuscript.

SUMMARY

Laboratory feeding tests were conducted to determine the efficacy of 0.025% warfarin against *Rattus tiomanicus* trapped from cocoa-coconut fields in Mardi Research Station in Hilir Perak. No-choice feeding periods of 2, 4, 6, 8 and 10 days gave mortalities of 25, 45, 80, 95 and 100% respectively for the pooled data. Mark variation in susceptibility to warfarin among the individuals was noted. The lethal feeding period (LFP_{50}) with 95% fiducial limits were 3.20 days for females and 3.77 days for males. No significant difference was found between the mean of the two sexes and the data was pooled for probit analysis. This provided a base-line on the susceptibility of *R. tiomanicus* to warfarin. The upper 95% fiducial limit calculated for the LFP 95 indicated that survival following a 14-day feeding period on 0.025% warfarin bait is a suitable test for detecting warfarin resistance.

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Accepted for publication on 2nd December, 1982.