FIELD EFFICACY OF ANTICOAGULANT RODENTICIDES AGAINST RAT INFESTATION IN OIL PALM PLANTATION

ARIFF ATEED MOHD NOH[1]; MUHAMMAD SYAFIQ MOHD ZALUDIN[1]; WAN MOHD HAFEZUL WAN ABDUL GHANI[2]; ABU HASSAN AHMAD[1] and HASBER SALIM[1,3]*

ABSTRACT

Field efficacy of anticoagulant rodenticides against rat infestation in oil palm plantation was carried out in a comparative study between five rodenticide baits. They were first-generation anticoagulant rodenticide baits, coumatetralyl, chlorophacinone and warfarin, and second-generation anticoagulant rodenticide baits, flocoumafen and brodifacoum. A control plot was left untreated for comparison purposes. In general, all treatments were effective to reduce the fresh rat damage on oil palm fresh fruit bunch (FFB) below the threshold level (5.0%) except for warfarin bait treatment. In the final round of baiting (5th round), the flocoumafen bait recorded the lowest fresh rat damage on FFB at 1.54 ± 0.14%. Coumatetralyl, chlorophacinone and brodifacoum recorded 2.95 ± 0.73%, 2.42 ± 0.57% and 4.30 ± 0.53% of fresh rat damage on FFB at the end of the study. The least effective was warfarin treatment which recorded 5.40 ± 0.18% in the final round of baiting. Only flocoumafen, coumatetralyl and chlorophacinone baits recorded more than 70.00% of rat damage reduction throughout the study as compared to pre-treatment. The brodifacoum treatment recorded rat damage reduction up to 58.70% and warfarin treatment was in the range of 13.58%-33.62%. All the rodenticide treatments managed to reduce the relative abundance of the estimated rat population in the field at the end of the study, except in the control plot.

INTRODUCTION

Rats have been considered an agricultural problem in Malaysia for many years, particularly in relation to oil palm cultivations (Chung, 2012; Turner and Gillbanks, 2003; Wood, 2001). The three main rodent

[1] Barn Owls and Rodent Research Group (BORG), School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.

[2] Plant Protection Department, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

[3] Vector Control Research Unit (VCRU), School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.

* Corresponding author e-mail: hasbersalim@usm.my

pests in Peninsular Malaysia, namely, Malayan wood rat (*Rattus tiomanicus*), rice-field rat (*Rattus argentiventer*) and Malaysian house rat (*Rattus rattus diardii*) have been reported as important rodent pests in oil palm plantations (Hafidzi and Saayon, 2001). Rat activities in mature oil palm plantations can cause crop damage on palm fruit bunch ranging from 8%-20% (Abidin *et al.*, 2021). Damage by rats on oil palm is inflicted throughout the growth of the palm regardless of age, leading to losses of seedlings through the attack in nurseries, damage to inflorescences and both ripe and unripe fruits, causing substantial oil yield losses up to 10% in oil palm plantations (Wood, 2001). Several control measures have been practised for controlling rats such as cultural, mechanical, biological and chemical control with varying degrees of effectiveness (Chung, 2012; Khoo *et al.*, 1982).

Chemical control by using anticoagulant rodenticides (ARs) has become the main control

measure of rat populations in oil palm plantations as it is effective to reduce pest numbers in such a short time and is also more practical to handle in the field (Chung, 2012; Wood and Chung, 2003). ARs can be grouped into two classes; first-generation anticoagulant rodenticides (FGAR) and second-generation anticoagulant rodenticides (SGAR), based on the number of doses to cause death to target animals (Valchev *et al.*, 2008). SGARs were developed and used following the increasingly widespread development of resistance amongst rodents to FGARs, in particular warfarin (Berny, 2011; Cowan *et al.*, 1995; Erickson and Urban, 2004).

Regardless of their classification, ARs interfere with vitamin K in the production of clotting factors in the liver, disrupt normal blood-clotting mechanisms and induce capillary damage (Rattner *et al.*, 2014; Valchev *et al.*, 2008). Death results from haemorrhage and exposed animals may exhibit increasing weakness prior to death. Rodents typically die five to 10 days after ingesting a lethal dose of ARs (Erickson and Urban, 2004). Both groups of ARs have different levels of toxicity against animals. SGARs are much more acutely toxic than FGARs; SGARs require only a single feeding to reach the lethal dosage to the target animals (Fisher, 2005; Pitt *et al.*, 2010). Meanwhile, FGARs need to be consumed multiple times by animals such as rats to reach the lethal dosage (Fisher, 2005). The most commonly used and registered FGARs in oil palm plantations in Malaysia are warfarin, chlorophacinone and coumatetralyl, while SGARs include brodifacoum and flocoumafen. Depending on the toxicity, the active ingredient for SGARs ranges from 0.005% to 0.050%, and from 0.025% to 0.050% for FGARs (DOA, 2021). Cereal-based baits are the most widely used formulation in oil palm plantations and the baits are available as wax block baits due to their durability in the tropical condition of Malaysian oil palm plantations (Chung, 2012).

Six-monthly baiting campaigns or two campaigns per year routine are commonly practised by oil palm plantations to deal with rat infestation. In each campaign, two to five rounds of baiting are carried out depending on the threshold level (below 5%) of fresh rat damage on oil palm fresh fruit bunch (FFB). FGAR baits are usually replaced at three to four days intervals while SGAR baits, which are more lethal, are replaced at seven to eight days intervals (Sime Darby Plantation, 2010). Nowadays, the application of AR bait is a routine activity in controlling rat populations in oil palm plantations (Chung, 2012; Turner and Gillbanks, 2003). However, the application and continued use of this chemical control for a long period have raised concerns about the development of resistance in rats in oil palm plantations. For commensal rat species such as Norway rat (*Rattus norvegicus*) and Roof rat (*Rattus rattus*) development of warfarin resistance

has been well documented and reportedly caused ineffective management of the rat population in the field (Buckle and Smith, 2015). The only available report of the development of resistance in Malaysian oil palm was reported in the 1980s against FGAR, warfarin in three different localities (Klang, Teluk Intan, Renggam, Malaysia) and SGARs such as brodifacoum and flocoumafen, which are more toxic than FGARs, were used and reportedly effective against warfarin-resistant rats (Wood and Chung, 1990). Besides this relatively old report, the field efficacy and resistance status of ARs in oil palm plantations in controlling rat infestation are scarce and have not been well documented. Thus, the main objective of this study is the evaluation of the field efficacy of selected ARs against rat infestation in oil palm plantations through key parameters such as fresh fruit damages and relative abundance of rats to determine the efficacy of different treatments against the target pest.

MATERIALS AND METHODS

Study Sites and Experimental Design

The study was carried out in an oil palm plantation in Besout, Sungkai, Perak, Malaysia from 15 September 2017 until 14 June 2018. The plantation is located at 03.48°N, 100.17°E. The total area of the plantation is 1000.10 ha, and the age of the palm ranges from six to 12 years old after planting. A single campaign of rat baiting using FGARs either warfarin, chlorophacinone or coumatetralyl was annually carried out to control rat pest populations at the plantation. The usual baiting campaign was last carried out about one year before the start of this study.

A total of five AR baits were used in this study. The first three treatments were FGAR baits consisting of coumatetralyl, chlorophacinone and warfarin. Two types of SGAR baits, *i.e.,* brodifacoum and flocoumafen were also tested. Field efficacy of each treatment was conducted in 3 ha study areas and was replicated three times in a randomised complete block design (RCBD). A control plot that was not treated with any rodenticide was also included for comparison purposes. The total plot area of the study site for all the treatments in the plantation was 54 ha. The distance between each study plot was at least 2 km apart since the home range of the rodent pest is about 1 km (Buckle *et al.*, 1997; Oyedele *et al.*, 2015; Pryde *et al*, 2005). The sites were sufficiently far apart to prevent rats from migrating across different plots. The experimental layout description of all treatments is summarised in *Figure 1*.

The replacement round baiting technique, which is the standard baiting technique where

replacement of the baits takes place after every round of baiting according to the replacement interval, was adopted as the method of bait application. Replacement of the bait only took place when the bait was missing, spoilt (*e.g.,* fungus, disfigured, attacked by insects, *etc*.) or 50% of the baits were consumed, which was evaluated by the observer. The bait replacement was an important step to maintain the bait quality throughout the study. For FGAR treatments, the bait replacement interval was set at four days while SGAR treatments were at seven days. All the baiting programs were set up for five rounds of baiting. It took 20 days for FGAR and 35 days for SGAR treatments to complete five rounds of baiting. All the rodenticide baits were applied one bait per palm on the ground near the oil palm weeding circle, which is approximately 1 m away from the palm base, as typically practised by plantations. The details of treatments rounds and replacement rounds of rodenticide baits are summarised in *Table 1*.

Rat Damages on Fruit Bunch

Pre-treatment assessment on FFB was carried out in order to determine the rat damage incidence in each plot prior to the baiting program. Post-treatment assessment was carried out following the round and interval of each rodenticide application. The assessment was conducted prior to the routine of harvesting by the plantation operator. A total of 100 palms (10 x 10 rows of fixed palms) were systematically selected in the centre of each plot (a total of 300 palms were selected for each treatment). A fresh rat damage score was used to quantify and compare the severity of damage between plots (Buckle, 2015). The scoring assessment was conducted and calculated at the end of each replacement round, before baiting application of the consequent round commenced. Only fresh damage was included in the quantification while old damages were excluded from the assessment.
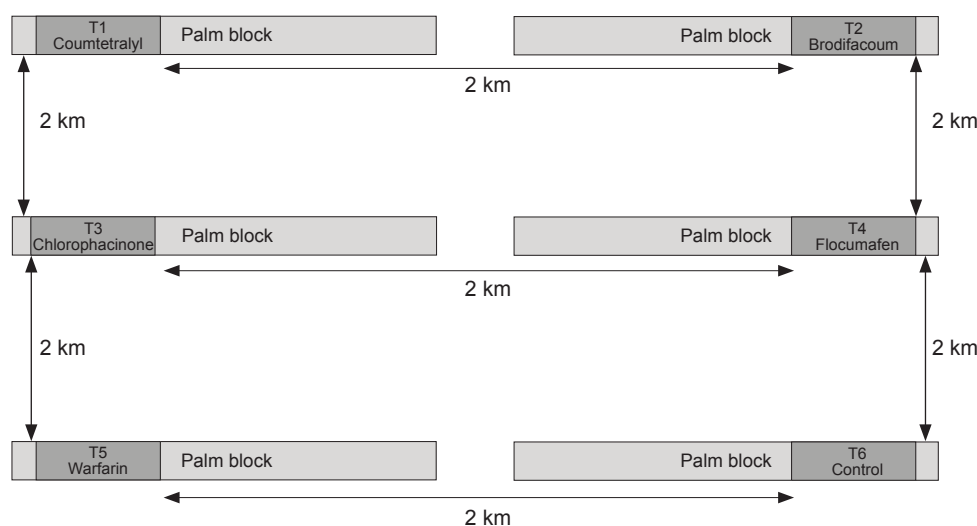

Figure 1. Experimental layout of first replicate for all treatments.

TABLE 1. DETAILS OF ROUND AND REPLACEMENT OF RODENTICIDE TREATMENTS

Treatment ID	Treatments	Bait weight (g)	*Average total bait used (g)	Total replacement round	Replacement interval (day)	Active ingredient (%)
T1	Coumatetralyl	5.0	6 897	5	4	0.0375
T2	Brodifacoum	10.0	14 623	5	7	0.0050
T3	Chlorophacinone	12.5	23 592	5	4	0.0050
T4	Flocoumafen	4.0	8 833	5	7	0.0050
T5	Warfarin	10.0	16 297	5	4	0.0500
T6	Control	-	-	-	-	-

Note: *Average total bait used from pre to final round of baiting per replicate.

Fresh damages were identified by the condition where the wound is moist and has a bright colour while the old damaged wound is dry and discoloured. The severity of the rat damages was determined according to the presence of rat damage on unripe and ripe oil palm fruits on the trees during the sampling. The final fresh damage score was calculated using Buckle (1994) in Equation (1) as follows:

$$\text{Fresh damage score} = \frac{(1 \times a) + (2 \times b) + (3 \times c)}{3\,N} \times 100\% \qquad (1)$$

where a = No. of the bunch with light damage (1-3 damaged fruit/bunch)

      b = No. of the bunch with medium damage (4-6 damage fruit/bunch)

      c = No. of the bunch with heavy damage (>6 damaged fruit/bunch)

      N = Total number of bunches assessed

To determine the impact of a particular treatment, the percentage reduction of fresh rat damage after each replacement baiting round was calculated as follows Equation (2):

$$\text{Reduction of fresh damage} = \frac{\text{Pre-treatment fresh damage - Current fresh damage}}{\text{Pre-treatment fresh damage}} \times 100\% \qquad (2)$$

Estimated Rat Population (Relative abundance)

Rat populations were estimated in pre- and post-treatment to measure the impact of the treatments on rat populations in each plot. Rat populations were assessed using the relative abundance of the trapping success. A total of 100 standard commercial live traps measuring 28 cm (length) x 13 cm (width) x 13 cm (height) were used. The live traps were set up in each treatment plot at 50 points at 50 selected palms (5 x 10 palms) in the middle of the plots following a grid-based configuration where the distance between each trap point was the same as the distance between palm trees at approximately 9 m (9 x 9 m). Two traps were placed at each point with a loose ripe palm fruit used as bait. Both traps were placed on the weeding circle, and the distance between each trap was approximately 2 m. The trapping program was carried out for four consecutive nights. To maintain the quantity of the baits, the replacement of absent bait in any trap was conducted every day. Meanwhile, to maintain the quality of the bait throughout the four nights of trapping, the old bait in every trap was replaced with

fresh bait during the second night of the trapping program. All traps were checked for captures daily from 8.00 am to 10.30 am until the last day of the trapping. Rats caught were recorded, marked, and subsequently released near the trapping point. Identification of caught rats was conducted based on physical features such as the size of the rat's body, size of the hindfoot, colour of fur and belly as stated by Francis (2008); Harrison (1974) and Payne *et al.* (1985). After trapping sessions were completed, the traps were removed from the trapping site. Trapping sessions were conducted twice during the study; once during pre-treatment and once post-treatment. Once trapping sessions were completed for the pre-treatment assessment, there was a lag period of seven days without the interference of any experiment on the trapping site to prevent the development of neophobic behaviour among the rats during the baiting program. The relative abundance or trapping success of rats (Puan *et al.*, 2011) before and after the treatment was calculated using the following Equation (3):

$$\text{*Trap success (relative abundance)} = \frac{\text{Number of individual rats captured}}{(2 \times \text{Number of trap points}) \times \text{Number of trap nights}} \qquad (3)$$

Statistical Analysis

Prior to analysis, data were tested for normality using the Shapiro-Wilk test. One-way ANOVA was performed to detect any statistical significance in fresh rat damage and the percentage of damage reduction between the treatments at each round of baiting. When there was a statistically significant difference, *post hoc* analysis, the Tukey's test was performed. Additionally, an independent sample t-test was used to analyse for a significant difference in relative abundance of rat population between treatment plots. All statistical analysis was conducted using the statistical package for social sciences (SPSS) for windows version 26.0 (IBM Corp., 2019).

RESULTS

Fresh Rat Damage on FFB

The results of average fresh rat damage per round of baiting on FFB at all treatments are shown in *Figure 2*. All treatments were effective and able to reduce fresh rat damage on oil palm FFB below the threshold level (5%) after three rounds of baiting except for warfarin bait (5.60 ± 0.29%) and control plot (7.42 ± 1.21%). At this stage, the result of all

treatments except warfarin bait and control had fallen below the threshold level in the range of 1% to 4%. Warfarin bait treatment score in fresh rat damages remained above the threshold level even after three rounds of baiting while the control plot scored the highest fresh rat damages at this stage compared to other treatments. According to one-way ANOVA analysis, at this stage, there was a significant difference ($F_{5,12} = 6.91$, P = 0.00) between treatments and the control plot. At the final round of baiting (5th round), flocoumafen bait recorded the lowest fresh rat damage compared to other treatments at $1.54 \pm 0.14\%$. Apart from flocoumafen bait, chlorophacinone and coumatetralyl baits recorded close results at $2.42 \pm 0.57\%$ and $2.95 \pm 0.73\%$, respectively. Meanwhile, brodifacoum bait recorded fresh rat damage at $4.30 \pm 0.53\%$ at the end of the study. There was a significant difference ($F_{5,12}= 11.29$, P= 0.00) detected in all treatments as compared to warfarin and control. Warfarin bait treatment produced poor control towards fresh rat damage and failed to reduce the fresh rat damage on FFB below the threshold level ($5.4 \pm 0.18\%$) even after five rounds of a rodenticide application. Meanwhile, the control plot showed a fluctuating trend of fresh rat damages in the range of 5.05%-7.54% throughout the study. The highest fresh rat damage in the control plot was recorded in the last round of baiting at $7.54 \pm 1.17\%$, which was higher than fresh rat damage recorded in pre-treatment ($7.24 \pm 1.05\%$) and this indicated the rat activities increased at the site.

Percentage of Rat Damage Reduction

The results of the percentage reduction of fresh rat damages at pre-application and post-application

of rodenticides are shown in *Figure 3*. There was no significant difference ($F_{5,12}= 0.99$, P = 0.47) indicated by one-way ANOVA of the percentage reduction of fresh rat damage in the first rounds of baiting. There was also no significant difference ($F_{5,12}= 1.70$, P= 0.21) in the second round of baiting. As early as the third round of baiting, flocoumafen and chlorophacinone baits recorded more than 70% reduction, indicating the high efficacy of both treatments. The flocoumafen bait treatment maintained more than 70% damage reduction at $75.22 \pm 2.72\%$ until the last round at $79.00 \pm 3.88\%$ reduction from pre-treatment damages while chlorophacinone bait treatment maintained more than 70% reduction from the third ($73.73 \pm 10.19\%$) to the fourth round of baiting ($73.75 \pm 6.06\%$) and later slightly reduced to $64.46 \pm 13.35\%$. Coumatetralyl bait treatment reached 70% damage reductions in the last round of baiting at $71.08 \pm 5.73\%$. Brodifacoum bait treatment recorded $2.76 \pm 15.53\%$ of fresh rat damage reduction throughout the study. At this stage, a significant difference ($F_{5,12} = 20.12$, P = 0.00) among all the rodenticide treatments was detected as flocoumafen, chlorophacinone and coumatetralyl baits recorded a significantly higher reduction of fresh rat damages compared to warfarin bait and control plot. Warfarin bait treatment recorded poor results with the reduction ranging from 13.58% to 33.78%. The control recorded fluctuating trend of damage, with reductions up to $28.88 \pm 5.17\%$ in the first round of baiting and the reduction dropped to $-5.90 \pm 12.64\%$ in the last round of baiting, indicating an increase in rat activities since there were no rodenticides applied in the plot. At the final round of baiting, the impact of flocoumafen, coumatetralyl and chlorophacinone baits was also significantly different ($F_{5,12} = 8.39$, P = 0.00) compared to control plots.
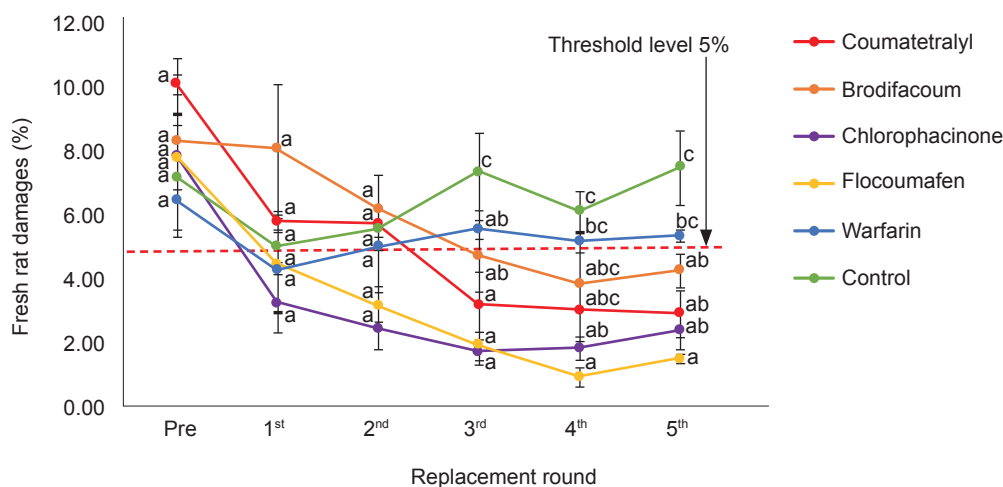

Figure 2. Fresh damages on oil palm fruit bunches in pre- and post-treatments where means in column with different letters are statistically significant different (p<0.05) by Tukey's test.
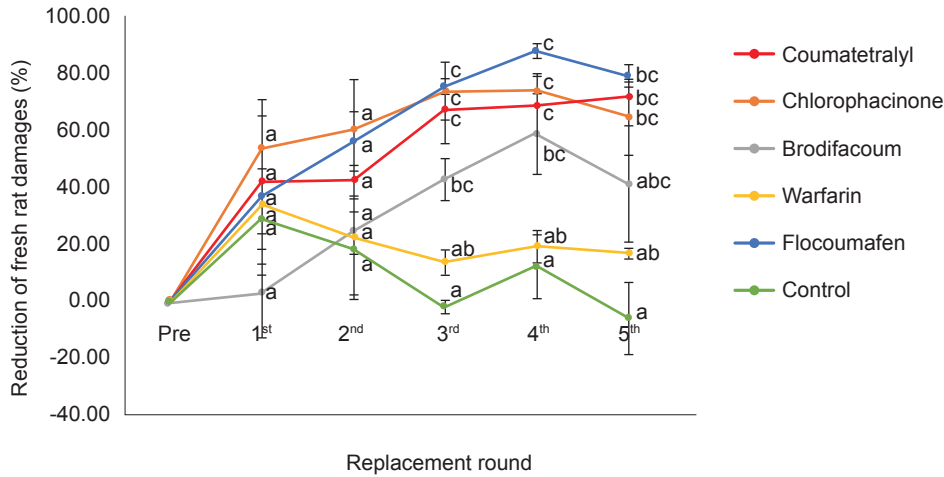
Figure 3. Percentage reduction of fresh rat damage before and after application of rodenticide baits where means in column with different letters are statistically significant different (p<0.05) by Tukey's test.

Relative Abundance

The results of the estimated rat abundance in this study are shown in *Figure 4*. Based on the trapping results, all treatments recorded reduced rat populations except for the control plot. The post-trapping results show there was a reduction in the relative abundance of rats captured in the treated plots. The difference in reduction of relative abundance in pre- and post-treatments ranged from 0.02 to 0.05 rats per trapping. Flocoumafen bait recorded the highest relative abundance reduction up to 0.05 rats per trapping. The treatment was able to reduce the relative abundance of rats from 0.07 ± 0.03 (pre-treatment) to 0.02 ± 0.01 rats per trapping (post-treatment). The reduction of relative abundance of rat population by chlorophacinone (reduced from 0.07 ± 0.03 to 0.03 ± 0.01 per trapping) and coumatetralyl (reduced from 0.08 ± 0.03 to 0.04 ± 0.01 per trapping) bait treatments where both treatments recorded a reduction of 0.04 rats per trapping, indicated the high efficacy of both rodenticides to reduce rat population in the study site.

Similarly, warfarin bait scored a similar reduction of relative abundance as chlorophacinone and coumatetralyl treatment baits. The treatment effectively reduced the relative abundance of the rat population by 0.04 rats per trapping, which was a drop in the relative abundances recorded during pre-treatment from 0.14 ± 0.03 rats per trapping to 0.09 ± 0.02 rats per trapping. Brodifacoum bait treatment recorded the least reduction compared to other treatments. The relative abundance of the rat population recorded during pre-treatment in the brodifacoum bait treatment plot was 0.09 ± 0.03 rats per trapping and dropped by 0.02 to score 0.07 relative abundance at the end of the study. The control plots showed no reduction and instead recorded an increment by +0.04 of relative abundance from 0.08 ± 0.03 to 0.12 ± 0.03 rats per trapping, indicating the rat population in the control plot increased. According to independent sample T-test analysis, there was no significant difference (*p*>0.05) in the relative abundance of rats recorded between pre and post-trapping in all the rodenticide bait and control plots.
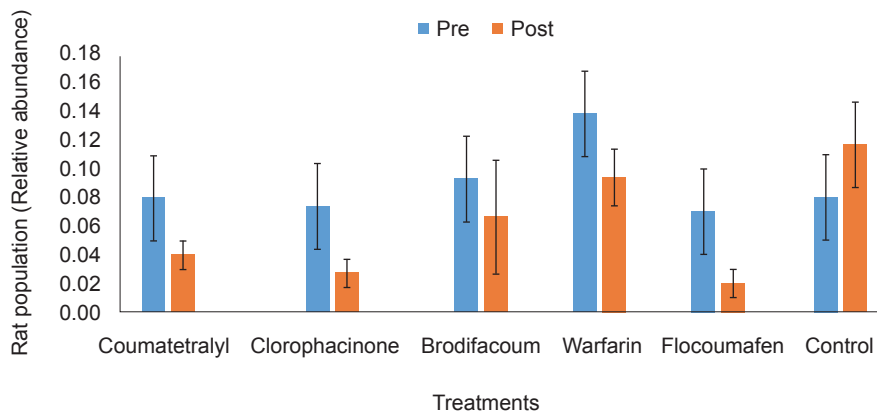

Figure 4. Relative abundance of R. tiomanicus *populations.*

DISCUSSION

In general, all rodenticide baits in this study were effective in reducing the fresh rat damage in the study sites. The high efficacy of flocoumafen bait has generally been reported in several previous research against many species of rat pests. Flocoumafen has recorded almost complete control against mice in the field (European Commission, 2009; Rowe *et al.*, 1985) and Norway rats, *R. norvegicus* (Buckle, 1986; European Commission, 2009) from trials conducted in infested farm buildings. A similar result was reported by Woo (1987), who reported high efficacy of flocoumafen wax block bait in controlling the rat population in oil palm plantations. In a laboratory no-choice feeding trial, Parshad (1988) reported flocoumafen (0.005% a.i) recorded 100% mortality against the Indian bush rat, *Golunda ellioti,* the Indian gerbil, *Tatera indica* and the Soft-furred field rat, *Rattus meltada.* Similarly, Srivinas *et al.* (2019) reported that flocoumafen (0.005% a.i) recorded 100% mortality of Lesser bandicoot rat, *Bandicota bengalensis* in choice and no-choice feeding. Flocoumafen bait also recorded high efficacy on wild warfarin-resistant mice, *Mus domesticus* (Gill, 1992; Johnson, 1988) and wild warfarin-resistant *R. norvegicus* (Gill, 1992).

Brodifacoum is a SGAR and one of the most potent of ARs (Pitt *et al.*, 2010; Prescott *et al.*, 2007). However, in the current study, brodifacoum bait was less effective compared to flocoumafen bait and other FGARs tested in the study. The current result contrasts with previous findings by Chia and Visvalingam (1990) who reported high efficacy of brodifacoum wax block bait against rat population in oil palm plantations. However, to the best of our knowledge, there has been no recent published report on the efficacy of brodifacoum baits against specific rat species in oil palm plantations. Unpalatable and poor bait formulation could be a possible factor for the relatively lower efficacy of brodifacoum bait used in the current study (Whisson, 1996). In contrast, several published data present a high degree of field efficacy of different formulations of brodifacoum baits against commensal rodents, both in susceptible and warfarin resistant populations (Blazic *et al.*, 2018; Buckle *et al.*, 2012). A recent field study by Frankova *et al.* (2019) reported the highly palatable bait of a low dose of brodifacoum (0.0025%) showed 95.7% and 99.8% efficacy against house mice, *Mus musculus* from an agriculture building in Prague, Czech Republic. Unpalatable bait formulation would cause ineffective results due to little or no consumption of the applied bait in the field (Cowan *et al.*, 1995). The repercussion of poor bait formulation would also be a misunderstanding that the treatment might experience resistance issues, as stated by Quy *et al.* (1992) regarding the resistance of brodifacoum in an earlier report by Greaves *et al.* (1982) in Hamsphire, United Kingdom area was premature due to the possibility of poor bait consumption. Naturally, rats prefer high-quality foods and do not favour less attractive food items (Syahputri and Priyambodo, 2020; Whisson, 1996). The result of the poor formulation of bait will lead to poor acceptance by rats in the field. Similarly, Johnson and Collier (2001) stated that in the situation where a more palatable diet is present, the rats tend to choose the more palatable diet and consume less of the least palatable diet. Rodriguez *et al.* (2006) stated that the reason for the low efficacy of rat control is probably due to the low palatability of bait used and the seasonal availability of rat's normal food in the field which reduce the consumption of bait by the target pest. According to Brown and Singleton (1998), brodifacoum was effective to control the population of *M. domesticus* in Southern Australia. High palatable brodifacoum bait at 1 kg ha^{-1} and 2 kg ha^{-1} applied aerially recorded reduction of mice population up to 99% after seven days of treatment application.

In addition, the current study reported the high efficacy of two FGAR baits, namely coumatetralyl and chlorophacinone, to control rat infestation in the field study. Both compounds were able to reduce fresh fruit damage on FFB as early as the first and third rounds of rat baiting. A field study conducted by Ocampo and Lontoc (2016) showed that coumatetralyl bait (0.0375% a.i) was highly effective in reducing *R. tanezumi* activities in rice fields based on the percentage of the damaged tiller of paddy at harvest in the Philippines. A significant reduction in rat activities based on tracking or feeding census was also noted. Coddou *et al.* (2014) reported the baiting program of coumatetralyl bait had a mean efficacy of 76.5% against rat populations in Christmas Island, Australia. A more recent study carried out by Shahwar (2017) reported that coumatetralyl (0.0375% a.i) produced excellent control of up to 82.0% of rodent activity in a poultry farm in Rawalpindi-Islamabad, Pakistan. Laboratory testing by Chopra and Parshad (1985) recorded 100.0% mortality of coumatetralyl treated *B. bengalensis* (n=10) and *R. rattus* (n=10) after single and 10-day feeding testing. Additionally, Kowalski *et al.* (2006) demonstrated high efficacy of chlorophacinone against California ground squirrel (*Spermophilus beecheyi*) up to 94.2%. Similarly, Salmon *et al.* (2007) showed that chlorophacinone effectively controlled California ground squirrel (*S. beecheyi*) by more than 80.0% in cattle-grazed rangeland areas in Sierra Nevada foothills and Monterey County, California. The laboratory study by Marsh *et al.* (1977) reported that 0.005% of chlorophacinone was able to achieve complete control against deer mice, *Peromyscus maniculatus* in four days of free choice

feeding test. A comparative study by Mathur and Prakash (1984) showed high efficacy of up to 90.5% of brodifacoum, 83.2% of chlorophacinone and 81.1% of coumatetralyl against desert rodents near Bikaner, Rajasthan, India.

The poor result of warfarin bait treatment aligned with past literature which reported that the poor efficacy of the compound that contributed to poor control in the field was largely due to resistance (*e.g.,* Wood and Chung, 1990; Wood and Liau, 1977). Warfarin resistance of major rat species of oil palm plantation, *R. tiomanicus* was reported six to seven years after systematic rodent control was introduced in Malaysia (Wood and Liau, 1977). Wood and Chung (1990) reported high resistance of warfarin against *R. tiomanicus* in Kluang, Johor. The authors reported that two baiting campaigns using warfarin conducted in March-May 1983 and November 1984-January 1985 failed to completely control damage even after 13 and 16 rounds of baiting were conducted. The conclusion was also supported by an increase in the number of rats trapped during post trapping sessions in both campaigns. A laboratory study by Lam *et al.* (1982) reported that five out of 14 samples of *R. rattus diardii* that were captured from a cocoa plantation in Kuala Bernam Estate, Teluk Anson (now known as Teluk Intan), Perak showed high tolerance to 0.025% warfarin in six days exposure in a no-choice feeding test. Despite the historically poor performance against the rats, warfarin is still in production as it is still reliable to control the rats in certain plantation areas where barn owl conservation is practised, and also the compound provides additional alternatives to existing FGARs in the market such as chlorophacinone and coumatetralyl.

There are no other reports of warfarin-resistant rats in oil palm, however, there are vast reports on the emergence of warfarin resistance in other species of commensal rats all over the world. The first incidence of warfarin resistance in the commensal rat of house rats, *R. rattus* and Norway rats, *R. norvegicus* was reported in the late 1950s (Lasseur *et al.,* 2005) and later house mice, *M. musculus* (Pelz *et al.,* 2005). The majority of warfarin resistance reports were made in Europe (Buckle, 2013; Kohn *et al.,* 2000), however, the issue was also raised in North America (USA, Canada), Asia (Japan, China) and Australia (Marquez *et al.,* 2019). A field study by Pelz and Klemann (2004) reported warfarin resistance of live-trapped rats up to 89% in dairy cattle and pig fattening farms in North-West Germany through a blood clot resistance (BCR) test. Warfarin resistance of buff-breasted rat, *Rattus flavipectus* was documented in Zhanjiang in Guandong, China by Huang *et al.* (2010). The researchers reported that four of 36 samples of rats fed with 0.005% warfarin in a nine-day no-choice feeding survived. Further mutation screening analysis via DNA sequencing showed that three of four surviving *R. flavicpectus* carried Tyr139Cys mutation, which is a mutation of the VKORC1 gene. The mutation of the gene was reported to be responsible for anticoagulant resistance in many species of rodents across Europe (Pelz *et al.,* 2005; Rost *et al.,* 2005).

CONCLUSION

All ARs used in the study, except warfarin, were effective in controlling rat damage in the study site by reducing damage on oil palm bunches below the threshold level of 5%. All treatments also recorded a reduction in rat abundance at the sites (except for the control plot). The relatively low effectiveness of warfarin bait was suspected to be due to the resistance development of the rat populations towards the compound that has been used for a long period in oil palm plantations. Thus, further studies are needed to confirm the presence of AR-resistant, especially warfarin-resistant, rats in oil palm plantations in Malaysia to ensure more effective and efficient rat management.

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REFERENCES

Abidin, C M R Z; Noor, H M; Hamid, N H; Ravindran, S; Puan, C L; Kasim, A and Salim, H (2021). Comparison of effectiveness of introduced barn owls, *Tyto javanica javanica*, and rodenticide treatments on rat control in oil palm plantations. *J. Pest. Sci., 95*: 1009-1022. DOI: 10.1007/s10340-021-01423-x.

Berny, P (2011). Challenges of anticoagulant rodenticides: Resistance and ecotoxicology. *Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment, 20*: 441-468. DOI: 10.5772/19916.

Blazic, T; Jokic, G; Gotz, A; Esther, M; Vuksa, M and Dedovic, S (2018). Brodifacoum as a first choice rodenticide for controlling bromadiolone-resistant *Mus musculus*. *J. Stored Prod. Res., 79*: 29-33.

Brown, P R and Singleton, G R (1998). Efficacy of brodifacoum to control house mice, *Mus domesticus*,

in wheat crops in Southern Australia. *Crop. Prot., 17(4)*: 345-352.

Buckle, A P (1986). Field trials of flocoumafen* against warfarin-resistant infestations of the Norway rat (*Rattus norvegicus* Berk.). *J. Hyg., 96*: 467-473.

Buckle, A P (1994). Rodent control methods: Chemical. *Rodent Pests and their Control* (Buckle, A P and Smith, R H eds.). CAB International. Wallingford, Oxon. pp. 127-160.

Buckle, A P; Chia, T H; Fenn, M G P and Visvalingam, M (1997). Ranging behaviour and habitat utilisation of the Malayan wood rat (*Rattus tiomanicus)* in an oil palm plantation in Johore, Malaysia. *Crop. Prot., 16(5)*: 467-473.

Buckle, A P; Klemann, N and Colin, P (2012). Brodifacoum is effective against Norway rats (*Rattus norvegicus*) in tyrosine139cysteine focus of anticoagulant resistance in Westphalia, Germany. *Pest Manag. Sci., 68(12)*: 1579-1585.

Buckle, A (2013). Anticoagulant resistance in the United Kingdom and a new guideline for the management of resistant infestations of Norway rats (*Rattus norvegicus* Berk.). *Pest Manag. Sci., 69(3)*: 334-341.

Buckle, A P (2015). Damage assessment and damage surveys. *Rodent Pests and their Control* (Buckle, A P and Smith, R H eds.). *2nd edition*. CAB International. Wallingford, Oxon. p. 209-230.

Buckle, A P and Smith, R H (2015). *Rodent Pests and Their Control*. 2nd edition. CAB International. Wallingford, Oxon. 432 pp.

Chia, T H and Visvalingam, M (1990). Methods for evaluation of rodenticides in oil palm plantations (Malaysia). *Proc. of 3rd International Conference on Plant Protection in the Tropic: Volume IV.* Malaysian Plant Protection Society. Kuala Lumpur. p. 19-26.

Chung, G F (2012). Rat management in oil palm. *Fourth MPOB-IOPRI International Seminar: Existing and Emerging Pests and Diseases of Oil Palm Advances in Research and Management*. 52 pp.

Chopra, G and Parshad, V R (1985). Evaluation of coumatetralyl against two predominant murid species. *J. Hyg., 94(3)*: 327-330.

Coddou, A; Mills, H; Hamilton, N and Algar, D (2014). Baiting effectiveness for introduced rats (*Rattus* sp.) on Christmas Island. *Raffles Bull. Zool., 30*: 54-59.

Cowan, D P; Dunsford, G; Gill, J E; Jones, A; Kerins, G M; MacNicoll, A D and Quy, R J (1995). The impact of resistance on the use of second-generation anticoagulants against rats on farms in Southern England. *Pestic. Sci., 43*: 83-93.

DOA (2021). List of registered agricultural pesticides in Malaysia. Department of Agriculture Malaysia (DOA). http://www.doa.gov.my/index.php/pages/view/388?mid=235, accessed on 16 May 2022.

Erickson, W and Urban, D (2004). Potential risks of nine rodenticides to birds and nontarget mammals: A comparative approach. Washington, DC, USA, United States Enviromental Protection Agency, Office of Pesticides Programs Environmental Fate and Effects Division. 225 pp.

European Commission (2009). Directive 98/8/EC concerning the placing biocidal products on the market. European Commission Netherland. 83 pp.

Fisher, P (2005). Review of house mouse (*Mus musculus)* susceptibility to anticoagulant poisons. *DOC Science Internal Series, 198*: 19.

Francis, C M (2008). *A Field Guide to the Mammals of South-East Asia.* New Holland Publication. 392 pp.

Frankova, M; Stejskal, V and Auliky, R (2019). Efficacy of rodenticide baits with decreased concentration of brodifacoum: Validation of the impact of the new EU anticoagulant regulation. *Sci. Rep., 9*: 16779.

Gill, J E (1992). Laboratory evaluation of the toxicity of flocoumafen as a single-feed rodenticide to seven rodent species. *Int. Biodeterior. Biodegradation, 30*: 65-76.

Greaves, J H; Shepherd, D S and Quy, R (1982). Field trials of second-generation anticoagulants against difenacoum-resistant Norway rat populations. *J. Hyg., 89*: 295-301.

Hafidzi, M N and Saayon, M K (2001). Status of rat infestation and recent control strategies in oil palm plantations in Peninsular Malaysia. *Pertanika J. Trop. Agric. Sci., 24(2)*: 109-114.

Harrison, J (1974). *An Introduction to the Mammals of Singapore and Malaya.* Malayan Nature Society. 340 pp.

Huang, B H; Feng, Z Y; Yue, L F; Yao, D D; Gao, Z X; Wang, D W and Liu, X H (2010). Warfarin resistance test and polymorphism screening in the VKORC1 gene in *Rattus flavipectus. J. Pest Sci., 84*: 87-92.

IBM Corp. (2019). IBM SPSS Statistics for Windows, Version 26.0. IBM Corp, Armonk, New York.

Johnson, R A (1988). Performance studies with the new anticoagulant rodenticide, flocoumafen, against *Mus domesticus* and *Rattus norvegicus*. *Bulletin OEPP/ EPPO Bulletin, 18*: 481-488.

Johnson, D F and Collier, G (2001). Taste, intake rate, and food choice in rats. *Physiol. Behav., 72*: 37-44.

Khoo, K C; Lam, Y M; Teoh, C H; Lim, W H and Mohamad, B M (1982). *Rodent Pests of Agricultural Crops in Malaysia*. Malaysian Plant Protection Society, Kuala Lumpur, Malaysia. 69 pp.

Kohn, M H; Pelz, H J and Wayne, R K (2000). Natural selection mapping of the warfarin-resistance gene. *Proc. Natl. Acad. Sci., 97(14)*: 7911-7915.

Kowalski, V J; Long, R; Sullins, J; Garcia, S and Salmon, T P (2006). *Grower Evaluation of California Ground Squirrel* (Spermophilus beecheyi) *control using Anticoagulant Baits.* (Timm, R M and O'Brien J M eds.). *Proc. 22nd Vertebr. Pest Conf.* University of California, Davis, USA. p. 142-147.

Lam, Y M; Lee, A K; Tan, Y P and Mohan, E (1982). A case of warfarin resistance in *Rattus rattus diardii* (Jentink). *MARDI Res. Bull., 10(3)*: 378-383.

Lasseur, R; Longin-Sawageon, L; Videmann, B; Billeret, M; Berny, P and Benoit, E (2005). Warfarin resistance in a French Strain of rats. *J. Biochem. Mol., 19(6)*: 379-385.

Mathur, R P and Prakash, I (1984). Field trials of brodifacoum, chlorophacinone and coumatetralyl against desert rodents in India. *Trop. Pest Manag., 30(1)*: 59-61.

Marquez, A; Khalil, R A and Fourel, I (2019). Resistance to anticoagulant rodenticides in Martinique could lead to inefficient rodent control in a context of endemic leptospirosis. *Sci. Rep., 9*: 13491. DOI: 10.1038/s41598-019-49661-5.

Marsh, R E; Howard, W E and Cole, R E (1977). The toxicity of chlorophacinone and diphacinone to deer mice. *J. Wildl. Manag., 41(2)*: 298-301.

Ocampo, P O and Lontoc, B M (2016). On-farm pulsed baiting with coumatetralyl paste for the control of rice field rats. *Philipp. Entomol., 20(1)*: 81-93.

Oyedele, D T; Mohd Sah, S A; Kairuddin, L and Ibrahim, M W (2015). Range measurement and a habitat suitability map for the norway rat in a highly developed urban environment. *Trop. Life Sci. Res., 26(2)*: 27-44.

Parshad, V R (1988). Laboratory and field evaluation of flocoumafen for Rodent control. *Int. Biodeterior., 24*: 475-480.

Payne, J; Francis, C M and Phillipps, K (1985). *A Field Guide to the Mammals of Borneo.* Sabah Society, Malaysia. 332 pp.

Pelz, H J; Rost, S; Hunerberg, M; Fregin, A; Heiberg, A C; Baert, K; MacNicoll, A D; Prescott, C V; Walker, A S; Oldenburg, J and Muller, C R (2005). The genetic basis of resistance to anticoagulants in rodents. *Genetics, 170*: 1839-1847. DOI: 10.1534/ genetics.104.040360.

Pelz, H J and Klemann, N (2004). Rat control strategies in organic pig and poultry production with special reference to rodenticide resistance and feeding behaviour. *NJAS, 52(2)*: 173-184.

Pitt, W C; Driscoll, L C and Sugihara, R T (2010). Efficacy of rodenticide baits for the control of three invasive rodent species in Hawaii. *Arch. Environ. Contam. Toxicol., 60(3)*: 533-542. DOI: 10.1007/ s00244-010-9554-x.

Prescott, C V; Buckle, A P; Hussain, I and Endepols, S (2007). A standardised BCR resistance test for all anticoagulant rodenticides. *Int. J. Pest Manag., 53*: 265-272.

Pryde, M; Dilks, P and Fraser, I (2005). The home range of ship rats (*Rattus rattus*) in beech forest in the Eglinton Valley, Fiordland, New Zealand: A pilot study. *N Z J Zool., 32*: 139-142.

Puan, C L; Goldizen, A W; Zakaria, M; Hafidzi, M N and Baxter, G S (2011). Relationships among rat numbers, abundance of oil palm fruit and damage levels to fruit in an oil palm plantation. *Integr. Zool, 6*: 130-139. DOI: 10.1111/j.1749-4877.2010.00231.x.

Quy, R J; Shepherd, D S and Inglis, I R (1992). Bait avoidance and effectiveness of anticoagulant rodenticides against warfarin and difenacoum-resistant populations of Norway rats (*Rattus norvegicus*). *Crop Prot., 11*: 14-20.

Rattner, B A; Lazarus, R S; Elliott, J E; Shore, R F and van den Brink, N (2014). Adverse outcome pathway and risks of anticoagulant rodenticides to predatory wildlife. *Environ. Sci. Technol., 48*: 8433-8445. DOI: 10.1021/es501740n.

Rodriguez, C; Torres, R and Drummond, H (2006). Eradicating introduced mammals from

a forested tropical island. *Biol. Conserv., 130*: 98-105.

Rost, S; Fregin, A and Hunerberg, M (2005). Site-directed mutagenesis of coumarin-type anticoagulant-sensitive *VKORC$_1$*: Evidence that highly conserved amino acids define structural requirements for enzymatic activity and inhibition by warfarin. *J. Thromb. Haemost., 94(4)*: 780-786.

Rowe, F P; Bradfield, A and Swinney, T (1985). Pen and field trials of a new anticoagulant rodenticide flocoumafen against the house mouse *(Mus musculus* L.). *J. Hyg. Cambridge, 95*: 623-627.

Salmon, T P; Whisson, D A; Berentsen, A R and Gorenzel, W P (2007). Comparison of 0.005% and 0.01% diphacinone and chlorophacinone baits for controlling California ground squirrels (*Spermophilus beecheyi*). *Wildl. Res., 34*: 14-18.

Sime Darby Plantation (2010). Pioneers and innovators of integrated pest management in oil palm. Sime Darby Plantation Sdn. Bhd. Subang Jaya, Selangor.

Shahwar, D E (2017). Review on development of cereal baits and comparative field efficiency of some additives as bait carrier for zinc phosphide and coumatetralyl against rodent pests of poultry farms. *J. Pestic. Biofert.* p. 1-4. DOI: 10.2017/1.10001.

Syahputri, D A A and Priyambodo, S (2020). Flavor ingredients in the rodenticide formulation to improve consumption rate and mortality of house rat (*Rattus tanezumi* L.). *IOP Conf. Ser.: Earth Environ.* IOP Publishing. 468 pp.

Turner, P D and Gillbanks, R A (2003). Oil Palm Cultivation and Management - 2nd edition. The Incorporated Society of Planters, Kuala Lumpur, Malaysia. 915 pp.

Srivinas, R N; Anusha, B; Narasimha Rao, C H V and Tripathi, R S (2019). Evaluation of flocoumafen and difenacoum against lesser bandicoot rat, *Bandicota bengalensis* (gray) in rice. *J. Entomol. Zool. Stud., 7(3)*: 907-910.

Valchev, I; Binev, A R; Yordanova, V; Ordanova, A and Nikolov, Y (2008). Anticoagulant rodenticide intoxication in animals - A review. *Turkish J. Vet. Anim., 32(4)*: 237-243.

Whisson, D (1996). Rodenticides for control of Norway rats, roof rats and house mice. *Poultry Fact Sheet No. 23, Cooperative extension*, 7. Retrieved from http://animalscience.ucdavis.edu/avian/pfs23.htm, accessed on 20 January 2020.

Woo, Y K (1987). Efficacy against oil palm rats in Malaysia. Flocoumafen, a new second generation anticoagulant rodenticide. National Crop Protection Centre Library (The Philippines).

Wood, B J and Liau, S S (1977). Preliminary studies on the toxicity of anticoagulants to rats of oil palms, with special reference to the prospect of resistance. *International Development in Oil Palms* (Earp, D A and Newall, W eds.). Incorporated Society of Planters, Kuala Lumpur.

Wood, B J and Chung, G F (1990). Warfarin resistance of *Rattus tiomanicus* in oil palms in Malaysia and the associated increase of *Rattus diardii*. *Proc. of the 14th Vertebrate Pest Conference 1990*. p. 129-134.

Wood, B J (2001). Rat control in oil palms and rice fields. *Pestic Outlook, 4*: 71-74.

Wood, B J and Chung, G F (2003). A critical review of the development of rat control in Malaysian agriculture since the 1960s. *Crop Prot., 22*: 445-461.