

Preference *Rattus Tiomanicus* Mill. Against Various Rodenticide Formula Aroma and Taste in Storage Container

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Abstract

Rattus tiomanicus (Malayan field rat) attacks oil palm trees, and rodenticides without protective containers (bait stations) become stale due to rain and sunlight exposure. This study aims to identify a rodenticide preferred by *R. tiomanicus* using suitable bait stations. A multiple-choice test with 15 treatments and 3 replications was conducted, using bromadiolone, supplemented with peanut butter, cricket flour, cheese, salted fish, cocoa powder, and desiccated coconut powder. Bait station materials tested were Ivory paper (210 gsm), Duplex paper (230 gsm), and Wax paper (220 gsm). Data were analyzed using a Completely Randomized Factorial Design (CRFD). Results showed a significant influence of formula and bait station material on rodenticide consumption by *R. tiomanicus*, with a slight correlation between formula treatments and bait station materials. The best formula was a combination of salted fish, chocolate, peanut butter, and desiccated coconut powder in a Wax paper bait station, with a preference score 10.82 times higher than control. All bait station materials maintained the quality of the rodenticide during the treatment. In conclusion, the study identified that a rodenticide with salted fish, chocolate, peanuts, and desiccated coconut powder in a wax paper bait station effectively controls *R. tiomanicus* for use at oil palm plantations.

Keywords: Bromadiolone, Malayan Field Rat, Preference Test, Rodenticide Container, Rodenticide Flavour.

Introduction

The Malayan field rat (*Rattus tiomanicus* Mill.) is a detrimental pest in the oil palm plantation. *R. tiomanicus* can predominantly attack newly planted, immature plants or plants that have already produced palm crops (Priyambodo, 2009). In newly planted and immature plants, rats eat the base of the leaf sheath, resulting in stunted plant growth due to rat resistance to the growing point of up to 80% (Adidharma, 2009). While in plant production, *R. tiomanicus* can eat its fruits around 4.29 to 13.6 grams per day, which causes 5-10% production damage (Wood and Chung, 2003). The phenomenon is very troubling considering that Indonesia, as the world's biggest producer, has 14.62 million hectares and produces 34.6 million, which is expected to expand to meet the growing world consumption of palm oil (Indonesia Statista Research Department, 2022).

The use of poisoned baits or rodenticides is an effective control carried out to date by laying poisoned baits on the paths of rats. An attractant or component that can release aroma is required for the rodenticide to appeal to the rats' appetite in their habitat (Surono, 2016). In addition, the nutritional content contained in the bait must meet the rats' needs, including 12% protein, 5% fat, and 5% fiber (Smith and Mangkoewidjojo, 1988).

Additional ingredients in aroma and flavor are given in poisoned baits to attract rats and eliminate the toxic ingredients' lousy flavor. Thus, rats will eat a large amount of bait, so the amount of poison eaten will be significant in any way (Priyambodo, 2003). Coconut can be used as an attractant, where more coconut is consumed by *R. tiomanicus* than rice and grain (Aryata, 2006). In addition, coconut is one of the habitual natural foods of *R. tiomanicus* (Ikhsan et al., 2020). Cheese is a product produced from clumping protein in milk to form curd (Yuniwat et al., 2008). Cheese contains enough fat that it is often used as rat bait.

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Cricket flour has the potential to feed fish and pets and even as food for humans. Cricket flour contains protein, fat, crude fiber, and ash (Kim, 2019).

Chocolate is a processed food ingredient composed of chocolate paste, sugar, cocoa fat, and several additional flavors (Kelishadi, 2005). In this case, rats' appetites will be stimulated by chocolate since it has a complete composition and a pleasant flavor (Contini et al., 2018). Legumes contain minerals, B vitamins, complex carbohydrates, and dietary fiber. In addition, nuts are one of the preferred foods of *R. tiomanicus* (Franklin, 2013). Another additional ingredient used is fish. Fish in flour can be used as an attractant agent in rat baits to mask the unpleasant flavor of a given poisoned bait (Priyambodo, 2003).

To protect humans and non-targets, the United States Environmental Protection Agency (EPA) established stricter guidelines on consumer use of rodenticides on June 4, 2011. The EPA seeks to reduce the risk of exposure to rodenticides to consumers and other animals that do not target (Vantassel et al., 2012). Therefore, a bait station contains rodenticide baits currently used to control rats. Quy (2011) stated that using bait stations is an effort to reduce the risk or negative impact on wildlife and can also protect baits from weather disturbances and environmental pollution. According to Vantassel et al. (2012), using storage containers effectively increases rat control success.

A bait station must fulfill several requirements, such as shielding rodenticides from environmental conditions while still allowing the rodenticides' scent to be detectable (AEPMA, 2019). Furthermore, the rodenticide's active ingredient must have an antidote in case non-target animals ingest it. One of the active ingredients in rodenticides that has an antidote is bromadiolone. Bromadiolone is an anticoagulant poison with vitamin K as its antidote (Natawigena, 2013).

Innovations are needed in terms of its application to increase further the frequency of rats to rodenticide bait. One way to do this is to separate several aromas and flavors separated by partitions. In this way, the aroma and flavor of each formula will be intact and natural because it has much aroma, and rats will prefer it. In this study, a new innovation is offered in the presentation of rodenticides that differs from previous rodenticide formulas. The rodenticide innovation is placing four separate aromas and flavors using a partition but combined in a particular container ('bait station'). This approach is expected to make the new rodenticide formula more preferred by *R. tiomanicus* and has a longer shelf life due to the inclusion of the 'bait station'.

Materials and Methods

The experiment was conducted in the pest laboratory, Vertebrate division, Plant Pest and Disease Department, Faculty of Agriculture, Universitas Padjadjaran (6°35'32.72" S-107°64'55.73" E).

Rattus Tiomanicus Preparation

Healthy males of *R. tiomanicus* weighing 80-130 grams were obtained from the state's official oil palm plantation land at PT Perkebunan Nusantara Indonesia VIII (6° 33' 8.1883" S-106° 32' 19.79643"E). Rats are separated individually in adaptation cages, with ad libitum food and drink.

Rodenticides and Storage Preparation

The ingredients used to manufacture rodenticides are rice flour, white sugar, gelatin, water, and the active ingredient bromadiolone 0.005% (Table 1). Rodenticides are made using gelatin as an adhesive material to replace wax, thus, the aroma and flavor added to the rodenticide are not covered by wax. The rodenticides given in each treatment were a combination of 4 variations of rodenticides with multiple aromas and flavors (Figure 1).

The amount of each rodenticide is two blocks for each scent adjusted to the feeding needs of rats per day, which is 10% of the weight of the rats. Rodenticides are replaced once every three days. The rodenticide that has been made is placed in a flat block-shaped container with a size of 8 cm length x 4 cm width x 2

cm height. In order for the aroma released by the rodenticide to be smelled by the rats, each block-shaped container was given a hole with a diameter of 1 cm, totaling 12 holes, and evenly distributed in each bait station. (Figure 1). Overall, the materials tested were four different rodenticide formulas with three types of storage container materials, such as (Figure 2).

Table 1. The Composition of the Multi-Aroma and Flavor Rodenticide

Materials	Ingredients in 100 g of feed (%)
Rice	45%
Gelatin	10%
Sugar	10%
Active ingredient	5%
Aroma and flavor	30%

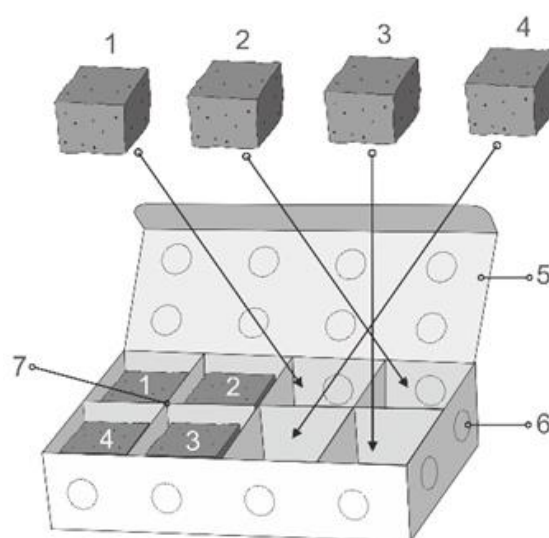


Figure 1. For Rat Poison, The Perspective View Is on Multi-Scented and Flavored Poisonous Bait with Perforated Containers. (1) Coconut Powder, Cheese, Cricket Flour, And Chocolate; (2) Salted Fish, Chocolate, Nuts, And Coconut Powder; (3) Cheese, Nuts, Salted Fish, And Coconut Powder; (4) Cricket Flour, Nuts, Chocolate, And Cheese, (5) Storage Container Material., (6) Perforation Holes Are Parts That Are Easily Perforated During Application Rodenticide., (7) Divider as A Barrier for Each Formula With a different aroma and taste.





Figure 2. Variations of Rodenticides Used in Preference Testing: (A) Coconut, Cheese, Cricket Flour and Chocolate (B) Salted Fish, Chocolate, Peanuts and Coconut (C) Cheese, Peanuts, Salted Fish and Coconut (D) Cricket Flour, Peanuts, Chocolate and Cheese (E) Control, I.E., Without the Addition of Aroma and Flavor.

Multiple Choice Test

The test consists of factor A (bait with variations in aroma and flavor) and factor B (storage container material). The treatment consists of fifteen treatments (Table 2) and three repetitions. Observations are made once every three days for fifteen days. Preference observations are determined by the weight of the rodenticide eaten by subtracting the feed's initial weight from the feed's remaining weight (g). After obtaining the data on the weight of the eaten feed, then calculate its preferences by the formula:

$$P = \frac{T}{C} \times 100\%$$

Note:

P = Preference (multiplied)

B = Weight of the rodenticide consumed in each treatment

K = Weight of rodenticide consumed at control treatment

Table 2. Treatment In Multi-Aroma and Flavor Rodenticide Testing and Storage Container Materials

Code Letter	Treatments	
	Variety of aroma and flavor (A)	Storage container material (B)
A ₁	Coconut powder, cheese, cricket flour, and chocolate	Ivory paper (210 gsm)
A ₂	Coconut powder, cheese, cricket flour, and chocolate	Duplex paper (230 gsm)
A ₃	Coconut powder, cheese, cricket flour, and chocolate	Wax paper (220 gsm)
B ₁	Salted fish, chocolate, nuts, and coconut powder	Ivory paper (210 gsm)
B ₂	Salted fish, chocolate, nuts, and coconut powder	Duplex paper (230 gsm)
B ₃	Salted fish, chocolate, nuts, and coconut powder	Wax paper (220 gsm)
C ₁	Cheese, nuts, salted fish, and coconut powder	Ivory paper (210 gsm)
C ₂	Cheese, nuts, salted fish, and coconut powder	Duplex paper (230 gsm)
C ₃	Cheese, nuts, salted fish, and coconut powder	Wax paper (220 gsm)
D ₁	Cricket flour, nuts, chocolate, and cheese	Ivory paper (210 gsm)
D ₂	Cricket flour, nuts, chocolate, and cheese	Duplex paper (230 gsm)
D ₃	Cricket flour, nuts, chocolate, and cheese	Wax paper (220 gsm)
E ₁	Control: without the addition of aroma and flavor	Ivory paper (210 gsm)

E ₂	Control: without the addition of aroma and flavor	Duplex paper (230 gsm)
E ₃	Control: without the addition of aroma and flavor	Wax paper (220 gsm)

Data Analysis

The experimental data were statistically processed with a Factorial Complete Randomized Design (FCRD) and Duncan's test at the level of 5% using the SPSS Version 25.0 program.

Results and Discussion

The analysis results in Table 3 showed that the consumption of multi-aroma and flavor rodenticides gives a noticeable difference between treatments. The consumption of *R. tiomanicus* against rodenticide formulations with variations of salted fish, chocolate, nuts, and coconuts was the most variation at 5.10 g. In contrast, the least consumed rodenticide is a rodenticide without adding aroma and flavor (control), which is 0.51 g.

Treatments with coconut and nut elements tend to have a higher bait average than others. Based on Sipayung et al. (1987), *Rattus tiomanicus* tends to consume cereals because of its high protein and carbohydrate content, and the aroma and taste of coconut are preferred because its natural habitat is oil palm. The results of the preference calculation showed that rodenticides with variations of salted fish, chocolate, peanuts, and coconut using wax paper had a preference value of 10.82 times compared to the control. Wax paper emits an aroma that attracts rats (Pardosi & Sukana, 2005). The lowest preference value was for rodenticides with variations of cricket flour, peanuts, chocolate, and cheese, 2.36 times compared to the control.

Moreover, it is suspected that palm oil is the natural habitat of these rats, so they tend to consume and store coconut aroma in their memory. Rats will consume the most preferred type of feed when there are many, even though there are other types of feed around it (Kasper, 2014). This is due to increased activity in the brain in the posterior pyriform cortex associated with flavor recognition memory underlying the weakening of the neophobic effect (Grau-Perales, 2019). Based on Elliot and Maier (2020), flavor and smell interact, which informs a multisensory flavor assessment that gives rise to additive interactions.

Table 3. The Average Rodenticide Formulation Consumed by *Rattus Tiomanicus*.

Treatment	Average feed eaten/3 days (g)
Coconut powder, cheese, cricket flour, and chocolate	2.91 d
Salted fish, chocolate, nuts, and coconut powder	5.10 e
Cheese, nuts, salted fish, and coconut powder	2.30 c
Cricket flour, nuts, chocolate, and cheese	1.36 b
Control: Without aroma and flavor	0.51 a

Note: the average value followed by the same letter in the same column shows no significant difference of 5% according to the Duncan Test.

The results of the preference calculation showed that the use of wax paper (220 gsm) applied to all treatments caused a higher preference value than duplex paper and ivory paper, even though statistically not significantly different (Table 4). The wax paper emits a scent that can attract rats' attention (Pardosi & Sukana, 2005). The wax is usually mixed with the bait because it has an aroma that attracts rats (Pacific Invasives Initiative, 2014). Damiri et al. (2021) tested wax as a mixture in rodenticide and proved better results than rodenticide without wax. Wax is an inert material that tends to be less toxic and tasty and does not cause neophobic effects on rats (Damiri et al., 2021).

Shukor et al. (2021) tested wax as a mixture in rodenticides and proved better results than rodenticides without wax. Wax is an inert ingredient that tends to be less toxic, palatable, and does not create a neophobic effect for rats (Shukor et al., 2021). Wax paper has the property of being able to hold water expletive; this follows the statement of Vantassel et al. (2012) that storage containers must be able to keep the feed from splashing water and dust, providing a place to store bait to be safer and more consumable.

Table 4. Preference of *Rattus Tiomanicus* on Several Rodenticide Formulas and Types of Bait Station.

Treatment	Preferences (times)
Coconut powder, cheese, cricket flour, and chocolate in ivory paper storage	5.12 c
Coconut powder, cheese, cricket flour, and chocolate in duplex paper storage	6.26 c
Coconut powder, cheese, cricket flour, and chocolate in wax paper storage	6.51 c
Salted fish, chocolate, nuts, and coconut powder in ivory paper storage	10.56 e
Salted fish, chocolate, nuts, and coconut powder in duplex paper storage	9.92 d
Salted fish, chocolate, nuts, and coconut powder in wax paper storage	10.82 e
Cheese, nuts, salted fish, and coconut powder in ivory paper storage	4.12 b
Cheese, nuts, salted fish, and coconut powder in duplex paper storage	4.88 b
Cheese, nuts, salted fish, and coconut powder with storage made from wax paper	4.93 b
Cricket flour, nuts, chocolate, and cheese in ivory paper storage	3.08 a
Cricket flour, nuts, chocolate, and cheese in duplex paper storage	2.36 a
Cricket flour, nuts, chocolate, and cheese in wax paper storage	2.74 a

Note: The preference indicates the multiple treatment values compared to the control; the value followed by the same letter in the same column shows no real difference at a rate of 5% according to the Duncan Test.

R. tiomanicus experienced weight loss during bromadiolone rodenticide treatment (Table 5). The weight loss is caused by a reaction in the rat's body after taking the rodenticide, where the rodenticide consumed has begun to react and interfere with physiological processes and death in the rat's body. The bromadiolone effect is supported by Valverde et al. (2020) against another vertebrate animal, *Falco tinnunculus*, which experienced an overtime weight loss.

Table 5. Weight Changes to An Average of *Rattus Tiomanicus* After Treatments

Observations to-	Initial Weight (g)	Final Weight (g)	Weight Loss (g)
1	63.965	61.986	1.979
2	64.498	62.311	2.187
3	64.232	62.412	1.820
4	62.335	60.543	1.792
5	63.500	61.543	1.957
Total	318.530	308.795	9.735
Average	63.706	61.759	1.947

Bromadiolone bait at low doses 0.005% showed lethal effectiveness of up to 99.8% in the wild-caught house rats (*Mus musculus* L.) populations (Frankova et al., 2022). Bromadiolone is a chronic anticoagulant that can result in fatal hemorrhage in test animals (Blazic et al., 2018). The main side-effect of anticoagulants

is bleeding that can occur in all body organs, including the eyes (Bagnis et al., 2008). The other observation results showed a difference in the shelf life of rodenticides between those using protective containers (bait stations) and those not using containers (control). Rodenticides stored in protective containers maintained their quality even after being stored for 2 months, as shown in (Figure 3). In contrast, rodenticides stored without protective containers experienced color changes (faded), decreased aroma, and damage due to fungal growth. Bait Station was able to maintain the quality of rodenticides during treatment.

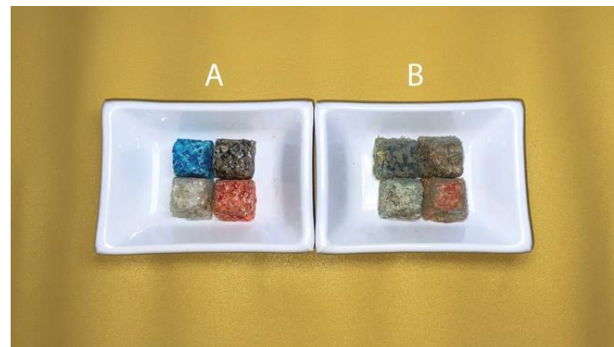


Figure 3. Condition Of Rodenticide After 1 Month of Storage. (A) Rodenticide With Protective Container (Bait Station); B: Control Rodenticide Without Protective Container.

Conclusion

The rodenticide application by placing four separate flavors and aromas in a protective container, a 'bait station,' increased the rats' preference compared to the control. Making a 'bait station' from Ivory paper (210 gr), Duplex (230 gr), and wax paper (220 gr) was able to maintain the quality of the rodenticide during treatment. The results of the study concluded that the innovation of presenting rodenticide with four separate flavors and aromas in a protective container 'bait station' could increase the preference of rats compared to the control. The best rodenticide formula was a combination of salted fish, chocolate, peanut butter, and dried coconut powder with a 'bait station' made of wax paper (220 gr). The preference value obtained was 10.82 times greater than the control.

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