Flavor ingredients in the rodenticide formulation to improve consumption rate and mortality of house rat (*Rattus tanezumi* L.)

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**Abstract.** House rat (*Rattus tanezumi* L.) is an urban pest that are disturbing in the settlements habitat. It can cause destruction and as a reservoir of pathogen to human. Control efforts have been conducted, both non-chemical and chemical. A flavoring ingredient in the formulation of rodenticide is required for chemical control. The objective of this study was to investigate the preference of house rat to flavor ingredients i.e. egg, sugar, monosodium glutamate, cooking oil, and soy sauce. The benefit of this research is to provide information and knowledge on the preference of house rat to the flavor type combined with rodenticide. These ingredients could be attractive enough to combine with acute rodenticide (zinc phosphide) and chronic rodenticide (coumatetralyl) for application in the field. Research conducted at Vertebrate Pest Laboratory, Dept. of Plant Protection, IPB Univ. for 6 month. Preliminary trial conducted to test the different concentration of several flavoring ingredients. Then, it mixed with 2 rodenticides, acute and chronic. The preliminary trial indicated that 10% of egg was the most preferable bait. The daily consumption on acute rodenticide (1.29 g) was significantly different to positive control (0.24 g). On the other hand, the consumption on chronic rodenticide was very high (3.34 g) and significantly different to negative control (2.18 g) and positive control (0.21 g). Mortality rate of test animal on acute and chronic rodenticide was 80% and 90%, respectively.

1. **Introduction**

Settlement pest is a disturbing organism in residential habitat, whose presence is risky for human being due to some negative impacts [1]. These pests can cause losses by damaging to building materials, alarming environmental cleanliness, and the transmission of diseases that interfere to human health. Types of settlement pests often found are cockroaches, termites, ants, mosquitoes, flies, and those from the mammal group namely rats [2]. Rats often observed in the habitat of houses, yards, and warehouses are *Rattus tanezumi*, *R. norvegicus*, and *Mus musculus*. House rats like food derived from whole grains, fruits, vegetables, nuts, tubers, meat, fish, and eggs. House rats have excellent climbing and grooming abilities. The activity of gnawing in rodents is the behavior of rats to reduce the growth of incisor, so that it could destruct many things included food and feed [3].

Efforts to control house rats have been performed through non-chemical and chemical techniques. The non-chemical control ranges from sanitation, constructing of barriers, setting of traps, and expulsion by electromagnetic waves [6]. When the rat population is quite high and lead to a destruction, the effective and efficient control is by applying chemical poisonous bait [7]. The poisonous compound is usually mixed with a particular bait which attract the rats. Type of bait and flavoring material that can hold (arrestant) so that the rat wants to eat more bait [3].
The reason for adding such flavoring ingredients to the bait is to improve its taste and aroma which attract the rats. Moreover, this addition is also to reduce the distasteful taste of the poisons used [6]. The type of flavoring ingredients must be attractive compared to the diet in the vicinity. The bait is different from the ordinary diet, which mainly ranges from raw corn, grain, and rice. Therefore, some other flavoring ingredients such as shrimp paste, sugar, oil, caramel, and preservatives need to be added. A previous study reported that adding these flavoring materials increase the consumption of rats to the bait [7]. The effectiveness of using rodenticides in rat control can be predicted from the attractiveness of the bait used. The attractiveness of the bait is very important to the rat feeding behavior, because the toxic material used as rodenticide is not preferred by rats [3]. The aim of this study is to determine the success of adding flavoring mixed with rodenticide to the consumption of rats in poisoned bait and death, and controlled the population of rats.

2. Methods

2.1. Duration and location of research
The study was conducted from Oct 2017 to Feb 2018. The research was carried out at the Vertebrate Pest Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB University.

2.2. Materials and tools
The materials used in this study included house rats (R. tanezumii), rice, unhulled rice, drinking water, eggs, monosodium glutamate, sugar, soy sauce, and cooking oil. Rodenticides used are acute poisons with active ingredients of zinc phosphide and chronic poisons with active ingredients coumatetralyl. The tools used include electronic top loading balance for animals (scales), rat cages, bamboo roofs, cups, and glass water containers.

2.3. Research methods

2.3.1. Test animal preparation. Test animals used were house rats (R. tanezumii) obtained from housing around Darmaga District, Bogor Regency. Rats from the field were adapted in a maintenance cage in the laboratory for 3 to 7 d by feeding grain and drinking water every day (ad libitum) [8]. Rats that have been adapted, then moved into individual cages and fed with rice for 24 hr. Before being transferred to the individual cage the rats were weighed to find out the initial weight. Rats were provided with drinking water only to make them hungry. It was intended to make the animals hungry and consume more bait mixed with flavoring ingredients and rodenticides to be tested [9].

2.3.2. Bait, flavoring materials, and rodenticides preparation. Rice bait was obtained from a feed shop in Darmaga, Bogor. The ingredients used as flavoring were egg, monosodium glutamate, sugar, soy sauce, and cooking oil, which bought at Darmaga local market, Bogor. Rodenticides used were acute poison with active ingredients of zinc phosphide in the form of black powder and chronic poison with active ingredients of coumatetralyl in the form of blue flour. Standard concentration for the application of the zinc phosphide and coumatetralyl was 1% and 5%. Rodenticide powder was mixed with rice bait which have been mixed with vegetable oil to get them stick on rice [9].

2.3.3. Treatment and observation. Treatment was divided in 3 stages, each of them used choice test method with ten rats as replication. The first step was testing the concentration of the most effective flavoring ingredients on house rats. Each flavoring material was tested with 5 different concentrations, namely 1%, 2.5%, 5%, 7.5%, and 10%. One type of flavoring ingredients with 5 concentrations was mixed with rice bait and put different color to differentiate every single concentration. The bait was provided daily about 20% of animal body weight in feed container. The position of this container was randomized every day for 7 d of treatment. The animal body weight was monitored at the beginning and last day of treatment to record their initial and final body weight.

Three flavoring ingredients with the most effective or most preferable concentrations in the first stage of the test were followed by the second phase. The most effective concentration from the second step was then selected as the one type to be tested in the third stage. The test was performed for 7
consecutive days with the position of the feed container changed every day. The initial and final body weight of animals were also recorded in this step.

Acute and chronic rodenticides were used in the third stage of the test. Three feed containers were provided in each cage, namely negative control (rice), positive control (rice mixed with rodenticides), and treatment (rice mixed with flavoring ingredients and rodenticides). The position of this container was randomized every day for 7 d of treatment. Observations were made for 7 consecutive days on the consumption of feed. Dead rats were weighed finally and observed for symptoms of death. After 7 d of observation, if test rats were still survived, the treatment was stopped and rats were given 20 g of grain feed for 7 more days of observation.

2.4. Data analysis

The experimental design used was a completely randomized design. The data were analyzed using SAS for Windows V.9 to detect if there is significant differences between treatments in the 1st, 2nd, and 3rd stages. A continual test were performed with Duncan's Multiple Range Test (DMRT) at α = 5% and 1%. All data obtained from the tested animals were converted to 100 g of rat body weight. Lethal dose of rodenticide against rat was gained from the following formula:

Feed conversion (g) = \[
\frac{\text{average feed weight (g)}}{\text{average body weight of rats (g)}}\times 100
\]

Average rat body weight (g) = \[
\frac{\text{initial weight (g)} + \text{final weight (g)}}{2}
\]

Lethal dose (ppm) = consumption of total toxin (mg)/average rat body weight (kg)

3. Results and discussion

3.1. Feed consumption to several concentrations of flavoring materials

Phase I testing was carried out using 5 flavoring ingredients (eggs, monosodium glutamate, sugar, soy sauce, and cooking oil) with concentrations of 1%, 2.5%, 5%, 7.5%, and 10%. Ten house rats were assigned in each flavouring treatment. This test was to obtain a combination of flavoring ingredients with the most effective concentration. The results of the Phase I test is presented in Table 1.

| Concentration (%) | Feed consumption on the type of flavoring (g)a | Eggs | Sugar | Soy sauce | Monosodium glutamate | Cooking oil |
|-------------------|-----------------------------------------------|------|-------|-----------|----------------------|-------------|
| 1                 | 0.29 cC                                       | 0.35 cC | 3.28 aA | 0.97 bB | 1.16 aA              |
| 2.5               | 0.43 cC                                       | 0.52 cC | 1.77 bAB | 1.10 bAB | 0.67 aA              |
| 5                 | 0.93 cC                                       | 0.79 cC | 0.99 bB | 1.60 abAB | 0.88 aA              |
| 7.5               | 2.16 bB                                       | 2.37 bB | 1.32 bB | 2.35 aA | 1.61 aA              |
| 10                | 5.42 aA                                       | 3.75 aA | 1.89 bAB | 2.75 aA | 1.12 aA              |
| Pr > F            | < 0.0001                                      | < 0.0001 | 0.0022 | 0.0056 | 0.2615              |

* Numbers in the same column followed by the same letter have no significant difference based on Duncan's Multiple Range Test at levels α = 5% (lowercase) and α = 1% (capital letters).

The results showed that the highest consumption of bait in a row is eggs 10% (5.42 g), sugar 10% (3.75 g), soy sauce 1% (3.28 g), monosodium glutamate 10% (2.75 g), and cooking oil 7.5% (1.61 g). Consumption of bait mixed with 10% eggs and 10% sugar differed significantly based on the different concentration tested (Duncan Test α = 5% and 1%). On the other hand, consumption of bait on 1% soy sauce was significantly different in different concentrations tested (Duncan Test α = 5%). Cooking oil consumption did not differ significantly in the 5 concentrations tested. Bait with monosodium glutamate flavoring differed significantly between the consumables tested, but the consumption was still lower compared to eggs, sugar, and soy sauce.

Bait mixed with eggs, sugar, and soy sauce is quite attractive for the rats to consume more. According to Komala [10], egg nutritional content are 73.7% water, 12.9% protein, 11.2% fat, and 0.9% carbohydrates. Sugar consists of nutritional components namely carbohydrates and fats [11]. Judoamidjojo [12] reported that sweet soy sauce contain 29.61%, water component, 1.46% crude
protein, 0.14% fat, 61.15%, carbohydrates and 6.27% salt. This is consistent with the statement of Priyambodo and Nazaretta [8] mentioning that house rats prefer to kind of baits or flavoring ingredients that contain protein nutrients and carbohydrates.

Sabri et al. [6] states that the addition of flavoring ingredients such as eggs, sugar, soy sauce, monosodium glutamate, and cooking oil can increase rat consumption to the bait. However, the addition of cooking oil to the bait lead to production of rancid which not really attractive to the rats [3]. According to Sudiarta [7], the addition of mono-sodium glutamate to the bait can also increase rat consumption. In contrast, monosodium glutamate in this current study was less favored by rats, because there were side effects in the form of hair loss in rats when consuming the bait.

3.2. Feed consumption for the most preferable flavoring material

Table 2 indicated the average daily consumption of animals to the bait mixed with the top 3 most preferable gained from stage I testing. Bait with eggs flavoring (10%) is the most preferable for the rats.

| Types of flavoring ingredients | Feed consumption (g) |
|-------------------------------|----------------------|
| Eggs 10%                      | 6.93 aA              |
| Sugar 10%                     | 1.41 bB              |
| Soy Sauce 1%                  | 0.51 bB              |

Pr > F < 0.0001

a Numbers followed by the same letters represent no significant difference based on Duncan’s Multiple Range Test at α = 5% (lowercase) and α = 1% (capital letters).

Generally, there was a top 3 of bait representing higher daily consumption by rats, namely bait with 10% egg flavoring is the highest, then followed by bait with 10% sugar flavoring, and the lesser bait consumed bait with 1% soy sauce. Based on the Duncan Test (α = 1%), consumption of egg baits (10%) differed very significantly compared to the consumption of bait combined with sugar (10%) and soy sauce (1%). This data indicates that house rats prefer bait with flavoring eggs at the highest concentration (10%).

There is ideal requirement for rat food including 12% protein, 5% fat, and roughly 5% crude fiber, and must contain enough vitamin A, vitamin D, linoleic acid, thiamine, riboflavin, pantothenic acid, vitamins B12, biotin, pyridoxine, and certain choline and minerals [13]. Adding eggs to the bait is preferable by house rats, because it improves the taste and aroma. Higher concentration of ingredients can also increase the aroma of the bait [7]. In addition, the nutritional content of eggs is one of the components in fulfilling the ideal diet of rats, which has higher fat and protein content than other flavorings.

3.3. Consumption in rodenticide vs flavoring vs rice ingredients and lethal dosage

Table 3 and 4 represents house rat daily consumption on the bait containing acute rodenticides (zinc phosphide) and chronic rodenticides (coumatetralyl) with lethal dose.

Consumption of mixed treatment with rodenticide and flavoring did not differ significantly from the consumption of positive controls (α = 5%). House rats tended to consume mixed treatments compared to positive controls, due to egg-flavoring ingredients. These ingredients lead to increase its aroma and taste creating more attractable bait for the animals. Egg flavoring provide additional taste and aroma to the bait and cover the unpleasant odor from the rodenticides. The highest consumption was achieved from the negative control, since we did not mix the rice with any rodenticide.

Rats consume more rice without mixed treatment. There are some rats that still have high suspicion to the tested rodenticides, so there is still leftover feed in several treatment containers. The remaining bait can be identified by the presence of leftover food found on the next day [14]. Decreased appetite is an indication of poisoned rats. This indication is thought to be physiological response of the animals to the poisonous effects of active substances found in rodenticides [15].
According to Fatmawati [16] rat mortality in rodenticide treatment was caused by rats consuming the rodenticide. The more the amount of poison ingested in the rat's body, the higher the mortality of the rat. Consumption of house rats in the treatment of chronic rodenticides is significantly different than in positive controls (α = 5% and 1%).

The addition of flavoring eggs is quite effective on covering the unpleasant odor. Toxic treatment with the addition of egg-flavoring ingredient increased rat consumption to the rodenticides. The greater the number of treatments, the less the chronic rodenticide does not release a pungent odor in the bait. The addition of flavoring eggs is significantly different than in positive controls (α = 5% and 1%).

Table 3. Daily consumption of house rats on acute rodenticides and lethal doses.

| Repetition | Food consumption (g)b | Average body weight (g) | Lethal dose (ppm) | Remarks |
|------------|-----------------------|-------------------------|-------------------|---------|
|            | Negative control      | Positive control        | Treatment         |         |
| 1          | 0.00                  | 0.00                    | 1.01              | 126.37  | 63.94 | Dead    |
| 2          | 0.00                  | 0.00                    | 1.13              | 149.09  | 60.63 | Dead    |
| 3          | 0.34                  | 0.00                    | 2.90              | 97.72   | 237.41| Dead    |
| 4          | 0.53                  | 0.00                    | 1.33              | 113.70  | 93.58 | Dead    |
| 5          | 6.77                  | 0.07                    | 0.39              | 102.41  | 35.94 | Surived |
| 6          | 5.03                  | 0.02                    | 0.47              | 108.39  | 36.16 | Survived|
| 7          | 3.49                  | 1.89                    | 2.66              | 129.50  | 281.08| Dead    |
| 8          | 1.78                  | 0.05                    | 1.59              | 76.45   | 17.16 | Dead    |
| 9          | 4.19                  | 0.05                    | 0.55              | 103.35  | 46.44 | Dead    |
| 10         | 0.12                  | 0.33                    | 0.89              | 138.47  | 70.48 | Dead    |
| Averageb   | 2.22 aA               | 0.24 bA                 | 1.29 aB           | 114.55  | 94.28 |         |

Pr > F = 0.0125

*a Numbers followed by the same letter showed no significant difference based on Duncan's Multiple Range Test at α = 5% (lowercase) and α = 1% (capital letters).

b Negative control (rice), positive control (rice + rodenticide), treatment (rice + rodenticide + egg).

Table 4. Daily consumption of house rats on control and treatment of chronic rodenticides and lethal doses.

| Repetition | Consumption of test animal (g)b | Average body weight (g) | Lethal dose (ppm) | Remarks |
|------------|---------------------------------|-------------------------|-------------------|---------|
|            | Negative control                | Positive control        | Treatment         |         |
| 1          | 5.73                            | 0.03                    | 0.54              | 95.63   | 2.24  | Survived|
| 2          | 0.05                            | 0.05                    | 5.51              | 97.15   | 21.46 | Dead    |
| 3          | 1.39                            | 0.09                    | 3.84              | 145.37  | 10.14 | Dead    |
| 4          | 2.97                            | 1.22                    | 1.29              | 120.02  | 7.84  | Dead    |
| 5          | 1.93                            | 0.42                    | 7.13              | 200.05  | 14.15 | Dead    |
| 6          | 4.11                            | 0.02                    | 0.91              | 134.62  | 2.59  | Dead    |
| 7          | 4.37                            | 0.09                    | 0.55              | 150.41  | 1.59  | Dead    |
| 8          | 1.36                            | 0.02                    | 4.53              | 117.07  | 14.58 | Dead    |
| 9          | 0.01                            | 0.03                    | 4.93              | 145.68  | 12.77 | Dead    |
| 10         | 2.03                            | 0.29                    | 2.56              | 164.35  | 6.50  | Dead    |
| Averageb   | 2.18aAB                         | 0.21 bB                 | 3.34 aA           | 137.04  | 9.39  |         |

Pr > F = 0.0191

*a Numbers followed by the same letter showed no significant difference based on Duncan's Multiple Range Test at α = 5% (lowercase) and α = 1% (capital letters).

b Negative control (rice), positive control (rice + rodenticide), treatment (rice + rodenticide + egg).

Table 4 showed that daily consumption in negative treatments and control is higher and significantly different than in positive controls (α = 5% and 1%). Consumption of house rats in the treatment of chronic rodenticides is higher than the treatment of acute rodenticides. This occurs due to the chronic rodenticide does not release a pungent odor in the bait. The addition of flavoring eggs is quite effective on covering the unpleasant odor. Toxic treatment with the addition of egg-flavoring ingredient increased rat consumption to the rodenticides. The greater the number of treatment feed consumed, the higher the amount of poison ingested in the rat's body and the higher the mortality of the rat [15].

Application of rodenticides in mixed treatments and positive control caused death in some animals. According to Fatmawati [16] rat mortality in rodenticide treatment was caused by rats consuming rodenticide at lethal dose, while rats survived after consumed rodenticide at a non-lethal dose (sub-lethal dose). However, some rats died in sub-lethal dose, it happened because some of them were in...
an unsuitable condition. Therefore, some rats died even though they consumed only a little bit of bait containing rodenticides. During the rodenticide treatment, some rats lost weight. This happens because toxic compounds contained in rodenticides can accumulate in the rat’s body. The longer of rats absorb the toxic compounds, the more obvious effect on the metabolism of the rat's body, which continue with their death.

Ten rats were used for every single rodenticide treatment. Two animals from acute rodenticide (zinc phosphide) and one rat from chronic rodenticide (coumatetralyl) treatment were survived, respectively (Table 5). According to Wiratno et al. [17], animals which survived are encouraged to eat more for obtaining additional energy. The energy is used to detoxify toxins contained in the rodenticide at sub lethal doses that enter the body.

Table 5. Mortality of house rats and its duration along the treatment period.

| Treatment       | Day(s) | Number of rats Death (individ) |
|-----------------|--------|--------------------------------|
| Zinc phosphide  | 6      | 1                              |
|                 | 1      | 7                              |
|                 | $\bar{x} = 1.63$ | $\sum = 8$ |
| Coumatetralyl   | 4      | 3                              |
|                 | 5      | 2                              |
|                 | 6      | 2                              |
|                 | 7      | 2                              |
|                 | $\bar{x} = 5.33$ | $\sum = 9$ |

The toxin from rodenticides have accumulated in the body of rats, so that the rats will become more greedy and cause a decrease the feeding process. Decreasing the amount of feed consumption caused rats no longer active in feeding process due to the influence of rodenticides which caused rats lose their appetite and can cause die [18]. House rats died faster after consuming bait containing zinc phosphide toxin (1.63 d) compared to coumatetralyl (5.33 d). This shows that zinc phosphide toxin acted faster than that of coumatetralyl poison. Buckle [19] reported that acute rodenticides can cause death in rats within 24 hr or less after giving a lethal dose.

Generally, after consuming bait containing acute rodenticides (zinc phosphide), rats express the same symptoms. Symptoms appear more quickly, such as decreasing on diet, dull eyes, and decreased their movement. Rats that still alive in this treatment were suspected to experience a deterrent due to the rodenticide. Acute rodenticides (zinc phosphide) cause bait shyness, due to its poisoning symptoms appear so quickly. Therefore, rats stop eating bait before the lethal dose is achieved [20]. Decreasing appetite and reluctant to move are such symptoms of rat detoxification [21].

The active ingredient of chronic rodenticides (coumatetralyl) works slowly in the body of rats compared to acute rodenticides (zinc phosphide). Therefore, rats do not die immediately on the spot after consuming poison. Symptoms observed from test animal are generally the same, namely nose bleeding, staggering paths, drooping eyelids, wet anal area, and blood scattered around the cage. The abdominal part of the dead rat is very soft. Additionally, there was visible blood on the teeth and nose of rats, shrinking body, and wet genital [15]. Further research is required for observing different combinations of flavoring and rodenticides with different species of rats.

4. Conclusion
Egg-flavoring ingredients in the bait provide a pleasant aroma and taste to the house rats. The addition of egg seasoning dincrease the consumption of rats to the bait containing particular tested rodenticides. The rate of death rats is faster in acute rodenticides compared to chronic rodenticides. The mortality rate of acute rodenticides was 80%, whereas chronic rodenticides were 90%.

Further research is required regarding different combinations of flavoring and rodent-cides with different species of rats.
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