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Proceeding Paper

Effects of Oleander Leaves (*Nerium oleander*) against Metabolism, Activity Pattern, and the Leaves Potency as Rice-Field Rat Repellent (*Rattus argentiventer*) †

Ichsan Nurul Bari [1,*], Nur'aini Herawati [2] and Syifa Nabilah Subakti Putri [1,*]

1 Pest Laboratory, Vertebrae Sub-Division, Department of Pest and Plant Disease, Faculty of Agriculture, Universitas Padjadjaran, Sumedang 45363, West Java, Indonesia
2 Indonesian Center for Rice Research, Jalan Raya 9, Sukamandi, Subang 41256, West Java, Indonesia; nherawati.yuwono@gmail.com
* Correspondence: ichsan@unpad.ac.id (I.N.B.); syifa.nsp05@gmail.com (S.N.S.P.)

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Abstract: *Nerium oleander* historically has been known worldwide as a poisonous plant that can be used to control pests. However, studies on the effects of oleander leaves against *Rattus argentiventer* as a major agricultural rodent pest are limited. This research aimed to probe the potency of oleander leaves extracted in methanol as a rice-field rat repellent. The experiments involved a choice test (T-maze arena) and a no-choice test (metabolic cage) that were analyzed by the *T*-test using three replications for 6 days. The results showed that the rats on the T-maze avoided consuming food and water near the oleander treatment. The same results occurred in the metabolic cage, which was indicated by a decrease in the average of food and feces, and also by an increase in the average of water and urine. Additionally, the treatment also caused a disruption in daily activity patterns, which was significantly indicated by an increase of 22.84% in average time for resting activities and a decrease in time for locomotion and nesting activities (by 9.71% and 13.13%, respectively). Overall, oleander leaves have the potential to provide a repellent effect against rice-field rats, especially in the choice test.

Keywords: extract; plant-based; repellence; *Nerium oleander*; *Rattus argentiventer*; metabolic disorder; apocynaceae

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1. Introduction

Rice-field rats (*Rattus argentiventer*) have been classified as important pests in rice cultivation since 1986 [1]. The rate of rice-field rat attacks on rice plantations in Indonesia averaged 161,000 ha/year, equivalent to the loss of 555 million kg of rice, which was enough to feed 6.3 million citizens for one year [2]. Synthetic chemical control methods can be used to reduce problems from rat pests. Although it works quickly and well, synthetic chemical-based pest control has disadvantages, such as the development of pesticide resistance in rats and the risk of accidental poisoning of non-target species [3], and also adversely impacts the ecosystem. Alternatively, plant-based repellents are one of a variety of environmentally friendly methods that are suitable for pest control, especially against rice-field rats whose lives depend on their sense of smell.

Indonesia is a tropical country with a variety of plant species that have not been widely used, one of which is *Nerium oleander* [4]. Oleander is widely known as a poisonous plant that can affect the mortality of the Wistar rat species Rattus norvegicus [5]. In another study, it was reported that oleander plants contain terpenoid metabolites that can have a repellent effect on the pests *Ixodes rinicus*, *Plutella xylostella* [6], *Culex tritaeniorhynchus*, and *Cx. gelidus* [7]. A terpenoid compound study [8] showed that this compound has the potential to repel rats.

An important requirement of repellents is that they repel rats by interfering with their sense of smell [9]. Repellent control, if used appropriately in the environment with the target biology, has a high likelihood of being successfully applied against rodents by manipulating their behavior. For instance, in one study, oil of sandalwood, patchouli, and vetiver had a repellent effect on rice-field rats [10]. However, the potency of oleander plants as a source for a rice-field rat repellent is still unknown. Therefore, based on its observed effects, in this study, on the metabolism and daily activity patterns of male and female rice-field rats and its potency as a rat repellent, the application of plant secondary metabolite (PSM) odor mixtures from the extract of oleander leaves holds promise as a pest control tool.

2. Experiments

2.1. Study Site

The experiments were conducted at Laboratory of Pests, Division of Pests Vertebrate, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran (6°35′32.72″ S and 107°64′55.73″ E) and Laboratory of Rats, Indonesian Center for Rice Research (6°55′33.0″ S & 107°46′24.6″ E), from February to June 2019.

2.2. Rice-Field Rats

Rice-field rats originated from Sukamandi rice-field, Indonesian Center for Rice Research, and were collected using the linear trap barrier system (LTBS). The 24 rice-field rats used as test animals were selected based on health status, sex (12 mature males and 12 non-pregnant females), and body weight (weight range between 90–130 g). The collected rats were adapted to food (brown rice) and water (tap water) for 1 week in aluminum-framed cages.

2.3. Plant Materials and Extraction

Young oleander leaves were collected from plants growing along the Irigasi roadway, Bandung, West Java, Indonesia. The collected leaves were washed with tap water and air-dried for about 2 weeks, then blended into a powder. The powder was then finely soaked with methanol (75%) in a ratio of 100 g/L for over 4 h. The sample was then filtered with Whatman 2 mm filter paper, and distilled using automatic steam distillation (±80 °C) for about 1.5–2 h. The 500 mL extract sample was then transferred into a spray bottle for the treatment.

2.4. Choice Test in T-Maze

The choice test research on the repellent effect of oleander leaves on rice-field rats in the T-maze (Figure 1) was implemented at the Rat Laboratory, Indonesian Center for Rice Research. Rats first entered from the start pipe, then chose between two pipe arms leading to food and water. The left-hand pipe led to a treatment room with extract of oleander leaves (a), and the right-hand pipe led to a control room without extract of oleander leaves (b). The extract was sprayed onto 2 cloth sheets (10 × 10 cm) with a hand sprayer (5 mL/sheet) and placed inside the treatment room (a), close to the food and water containers. Rice-field rats were individually kept in the T-maze arena and provided with food (brown rice, 10% of body weight) and water (100 mL) in the same amounts in both the left- and right-hand rooms in the afternoon on every day of the 6-day experiment. Observations were carried out every morning and included measurements of food consumption (g) and water consumption (mL).

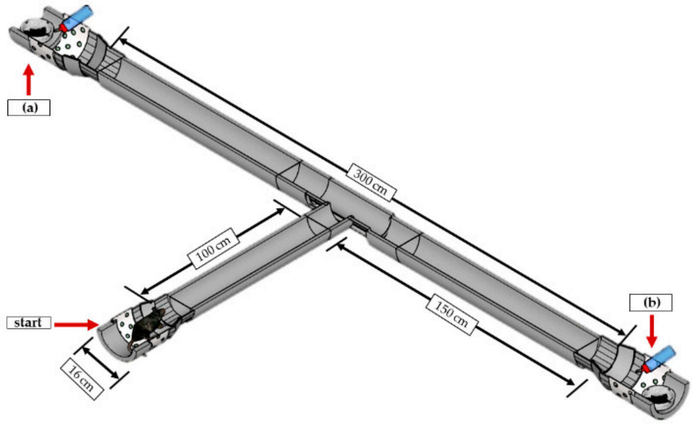

Figure 1. T-maze arena prototype and pipe size. (**a**) Treatment room containing extract of oleander leaves and (**b**) control room without extract of oleander leaves.

2.5. No-Choice Test in Metabolic Cages

No-choice tests on the effect of oleander leaves on metabolic parameters were carried out at the Pest Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The extract was sprayed onto 2 cloth sheets (10 × 10 cm) with a hand sprayer (5 mL/sheet) and placed inside a treatment room, close to food and water containers (Figure 2). Rice-field rats were individually placed in each metabolic cage and provided with food (brown rice, 10% of body weight) and water (100 mL) every day in the afternoon during the 6-day experiment. Observations were carried out every morning and included measurements of food consumption (g), water consumption (mL), feces production (g), and urine production (mL). Furthermore, the test was carried out using closed circuit television (CCTV) in the metabolic cages to observe the daily activity of the rice-field rats. The parameters included time spent in movement activities (locomotion), eating and drinking activities (foraging), and resting and sleeping activities (resting). The activities were viewed every minute for 12 h (18.00–06.00) each day, and the results were tabulated and divided by 6 (days of observation), then converted into percentages.

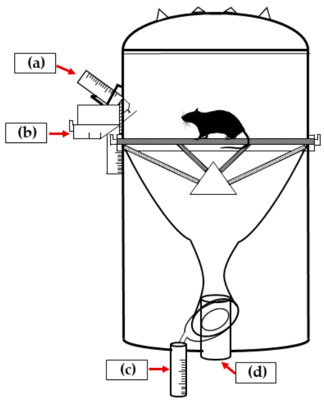

Figure 2. Metabolic cage prototype. Containers of (**a**)water, (**b**) food, (**c**) urine, and (**d**) feces.

2.6. Data Processing and Analysis

The treatment was replicated 3 times for both female and male rice-field rats. The experimental data were compared using the average difference test method with two independent data sets (independent sample *T*-test) for the extract and control treatments. Statistical results and the significance assessed at 95% confidence level to compare the differences between treatments were analyzed using Statistical Package for the Social Sciences (SPSS) software version 25.0 in Windows.

3. Results

3.1. Choice Test in T-Maze Arena

The choice test results below indicate the average repellency of oleander leaves in terms of food and water consumed each day by rice-field rats in the T-maze after 6 days of observations (Table 1). The rice-field rats tended to consume more in the control room (B) than treatment room (A), as evidenced by the significant differences in the average amounts of food consumed daily, both by female (3.42 g difference) and male rats (2.88 g difference). Of note, the rice-field rats also drank more water in the control room than in the treatment room, as shown by the significant differences in the average amounts of water consumed daily, both by females (4.55 mL) and males (5.55 mL).

Table 1. Average daily amounts of food and water consumption between female and male rice-field rats after 6 days of the choice test in the T-maze.

Sex	Treatment	Average per Day	
		Food Consumed (g)	Water Consumed (mL)
Female	Extract	2.44 *	4.56 *
	Control	5.86	9.11
Male	Extract	3.29 *	4.56 **
	Control	6.17	10.11

Asterisk (*) indicates a significant difference between the treatments. * $p < 0.05$; ** $p < 0.001$ highly significant.

3.2. No-Choice Test in Metabolic Cage

3.2.1. The Effect of Oleander Leaves on Rice-Field Rat Metabolism

Based on the results shown in Table 2, both male and female rice-field rats tended to consume significantly more food and water in the metabolic cage without extract of oleander leaves (control). On the other hand, smaller amounts were consumed when the rice-field rats were placed in the metabolic cage containing the extract of oleander leaves. For comparison, the average amounts of food and water consumed by rats in the treatment and control cages differed by 3.59 g and 6.34 mL, respectively, for females and 2.94 g and 3.73 mL, respectively, for males. The metabolism-disrupting effects of the extract of oleander leaves were also observed in the amounts of feces and urine produced by male and female rice-field rats in the metabolic cages (Table 2). The study using the metabolic cage without extract of oleander leaves showed that both male and female rice-field rats produced significantly higher quantities of feces and urine, which were correlated with the quantities of food and water they had consumed.

Table 2. Average daily amounts of food and water consumption and feces and urine production by female and male rice-field rats after 6 days of the choice test in the metabolic cage.

Sex	Treatment	Average per Day			
		Food Consumed (g)	Water Consumed (mL)	Feces (g)	Urine (mL)
Female	Extract	6.13 *	4.44 **	0.52 **	1.78 *
	Control	9.72	10.78	1.56	3.5
Male	Extract	7.08 *	8.00 *	1.05	3.33
	Control	10.02	11.72	1.75	4.11

Asterisk (*) indicates a significant difference between the treatments. * $p < 0.05$; ** $p < 0.001$ highly significant.

3.2.2. The Effect of Oleander Leaves on Rice-Field Rats' Daily Activities

The bar graph below depicts the percentages of time spent by rice-field rats in daily various activities for 12 h in a metabolic cage (Figure 3). It is found that both female and male rice-field rats tended to spend more time in resting activities (female 55.8% and male 40.9%) in the metabolic cage that had the extract of oleander leaves in it. Due to the extract treatment, the rats spent significantly less time engaged in locomotion and foraging activities than in the control. However, rice-field rats in the control treatment spent most of their time on foraging, followed by locomotion. Rice-field rats of either sex tended not to rest during the observation period (18.00–06.00).

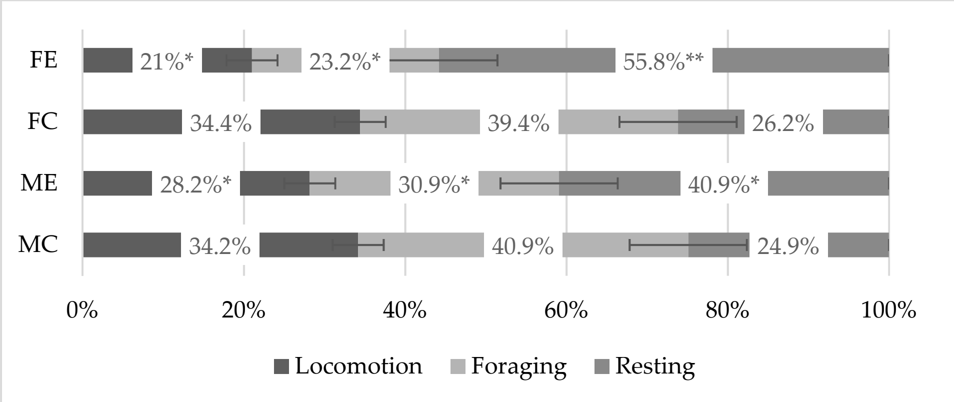

Figure 3. Comparison of average daily activity patterns in 12 h (18.00–06.00) using CCTV for 6 days of observation in the metabolic cage. Female extract (FE); female control (FC); male extract (ME); male control (MC). Extract implies the rat is in the metabolic cage with oleander extract treatment, whereas *control* implies without any treatment in the metabolic cage. The standard error (SE) of the mean is shown by a vertical bar. The asterisk (*) indicates a significant difference between the treatments. * $p < 0.05$; ** $p < 0.001$ highly significant.

4. Discussion

Rice-field rats have advantages with a sense of smell that is perfectly developed [11] and used to identify prey, avoid danger, and search for food [12]. The experiments showed that rice-field rats tended to eat and drink in the control room compared to those placed near the oleander extract treatment of the choice-test in T-maze arena. This phenomenon occurred because the rats had two options to choose from for access to food and water: the most pleasant-smelling control room, or the uncomfortable-smelling oleander extract treatment room. During 6 days of research, the rats increasingly tended to go to the same place because they had learned which place was safer. The results of our observations are supported by research [13] showing that the rat brain is perfectly developed for learning and remembering something well.

Consistent results were also obtained with the no-choice test in the metabolic cage. Even though the rats consumed higher amounts of food and water than in the choice test, this situation could be considered reasonable because the rats had no choice over the location of the food and water they consumed. However, the amount consumed by the rats in cages with the oleander extract treatment differed significantly from that of rats in the control. Food and water are the most important sources of energy in the growth and development of rats. The results of this experiment are thought to be due to neophobic behavior that caused stress actions. Rats that experience stress eat less food than unstressed rats [14].

Stress conditions can determine the amount of energy that is consumed, and thus can reduce food motivation and intake. This behavior was shown by rats due to the influence of oleander extract repellent at an applied dose of 5 mL. A substance can only be considered a repellent if it causes an organism to orient its motion away from that source [15]. Less eating behavior in the treatment cage is thought to be influenced by stress reactions that orient the rats to stay away because of the unusual and uncomfortable smell from oleander extract.

Food intake is related to feces production. Based on the observations, rice-field rats that were in oleander extract treatment conditions produced less feces on average than the controls. Lower urine production in rice-field rats also may be due to stress from the aroma of oleander extract, related to stress-related displacement associated with sympathetic nerves that work more actively with the help of bladder contractions [16]. This phenomenon is considered reasonable based on the research results because the less food and water rats consume, the less feces and urine they will produce.

Most of the normal activities of rice-field rats start from dusk and last until before dawn. This is because rats are active nocturnal mammals. Research observations were conducted between 18.00–06.00 to see if the behavior of the rats changed because of the oleander extract treatment. The study showed that both female and male rice-field rats in the control cage tended to spend their time normally engaged in foraging and locomotion activities. In contrast, rats in the treatment cage tended to spend more time sleeping or resting rather than engaging in other activities (foraging or locomotion), presumably due to the influence of the repellent. The aroma from oleander extract is thought to create uncomfortable conditions for rice-field rats in metabolic cages, thus disrupting the activity of the rats. Rats engage in defensive behavior when under stress or in uncomfortable conditions [17].

In addition, both of the tests showed that male rice-field rats tend to consume more food and water compared to female rats. This is supported by research [18] showing that male rats exhibit a strong determination to survive and are more predisposed to risk-taking than female rats. Biologically, female rats tend to be more sensitive to new things; thus, it is suspected that the ability to adapt takes longer in the female rat than the male rat [19]. Females tend to reduce their activity more than males when under stress [20]. Thus, the stress felt by the rats due to the influence of oleander extract resulted in significant differences between the treatment and control. Gender differentiation can also make a difference in the measured patterns of rice-field rat activity.

5. Conclusions

We concluded that extract of oleander leaves has a repellent effect that disrupts the metabolism and daily activity patterns of both female and male rice-field rats. The rats tended to show reduced interest in consuming food or water, which in turn affected the amount of urine and feces they produced. Moreover, oleander extract also affected the rice-field rats' daily activity patterns, confirmed by changes in their habitual behavior during the observation period (18.00–06.00), from active foraging or locomotion to becoming more passive (resting). It was also found that male rice-field rats tended to accept more risk than females in foraging for more food and water. In view of the experimental findings, further investigation is warranted regarding the isolation of bioactive compounds in oleander leaves as a repellent against rice-field rats, or for investigations into their effects on other species.

Supplementary Materials: The poster and video presentation is available online.

Author Contributions: Conceptualization, I.N.B. and S.N.S.P.; methodology, I.N.B., N.H. and S.N.S.P.; analysis tools, S.N.S.P.; validation, I.N.B. and N.H.; formal analysis I.N.B. and S.N.S.P.; investigation, I.N.B., N.H. and S.N.S.P.; experiments, S.N.S.P. and N.H.; resources, I.N.B., N.H. and S.N.S.P.; data curation, S.N.S.P.; writing—draft preparation, S.N.S.P.; review, I.N.B. and N.H.; writing—editing, S.N.S.P.; visualization, S.N.S.P.; supervision, I.N.B. and N.H.; funding acquisition, I.N.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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