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The population of red flour beetle (*Tribolium casteneum*) imago and crude protein of rice bran with an addition of lemongrass (Cymbopogon citratus) flour

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Abstract. Rice bran is an agricultural waste that is widely used as animal feed because it is cheap and easy to obtain. The main obstacle to using rice bran is the damage during storage, especially its protein content due to the presence and growth of insects such as the Red Flour Beetle (Tribolium castaneum). Lemongrass (Cymbopogon citratus) is a plant often used as a natural insecticide. The objective of this study was to determine the ability of Lemongrass to inhibit the growth of the Red Flour Beetle and maintain protein content in rice bran during storage. This study was performed according to a completely randomized design with five treatments and four replications. Treatments consisted of P0: rice bran, P1: rice bran infected with a beetle, P2: rice bran infected with beetle + 1% lemongrass flour, P3: rice bran infected with beetle + 2% lemongrass flour, and P4: rice bran infected with beetle + 3% lemongrass flour. The results showed that the use of Lemongrass flour (Cymbopogon citratus) had a significant effect (P<0.05) on lowering the population of Tribolium casteneum imago, while crude protein content had no significant effect. From this study, it can be concluded that the addition of lemongrass flour can reduce the population of Tribolium caestaneum and maintain the crude protein content of rice bran during storage.

1. Introduction

Lowland rice production in South Sulawesi reached 5,292,152 tons and upland rice reached 179,654 tons [1], high rice production reflects high bran production. In South Sulawesi, the poultry industry increases every year. In 2016, the population of broilers in South Sulawesi reached 48,203,640 heads, and in 2017 increased to 50,613 heads [2], this causes the need for bran to also increase. The use of bran in poultry rations reaches 35% in rations [3], to meet these needs, the bran is stored so that it is always available. However, the problem is the damage to the bran due to storage, for example, a decrease in nutrient content, one of which is protein. The decrease in protein content is usually caused by the storage itself and the presence of insect contaminants.

Insect often attacks the rice bran is red flour beetle (Tribolium castaneum) which can secrete Bunzokuinon, a toxic compound that is harmful to livestock. Besides, this beetle has a major contribution to both physical and chemical damage to feed ingredients. Physical damage occurs due to contamination of feed ingredients by faeces, nets, body parts, and the smell of faeces. Beetles live and eat the bran component and cause damage to the structure of feed materials, such as holes, crumbling and trigger the growth of other microorganisms i.e., fungi and moulds. The feeding activity carried out by the beetles causes the feed ingredients to lose their nutrient content

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The type of plant that has a great opportunity as an insecticide is lemongrass. Lemongrass (*Cymbopogon citratus*) is a plant used as a botanical insecticide that contains essential oils. Lemongrass essential oil consists of citral compounds, citronella, geraniol, mirsena, nerol, Pharmacol methyl heptanol and dipentene. The presence of these compounds in the lemongrass plant is thought to kill or reduce the growth of *Tribolium castaneum* beetles [4].

Ashes from the leaves and stalks of lemongrass contain 49% silica [5]. This compound can cause desiccation (continuous discharge) on the skin of insects so that the insects will die from drying out. Citronellol and geraniol are active ingredients that are not preferred and avoided by insects, therefore this study will examine the effect of lemongrass addition to rice bran to determine the ability of lemongrass to inhibit the growth of the beetles (*Tribolium castaneum*) and maintain protein content in rice bran during storage. This research is expected to be a source of information for the public, especially breeders in utilizing lemongrass as an alternative natural insecticide for red flour beetles.

2. Materials and methods

The research was conducted at the Non-ruminant Nutrition Laboratory and Animal Food Chemistry Laboratory, Faculty of Animal Science, Universitas Hasanuddin, Makassar.

The tools used in the study were digital scales, filters, loop applications, blenders, knives, trays, tweezers, plastic jars, stationery, digital temperature humidity meter clocks and cameras as well as tools for analyzing rice bran crude protein. The materials used in the study were 40 kg of rice bran, *Tribolium castaneum* (20 imagos each unit), label paper, rope, plastic, gauze and lemongrass flour (*Cymbopogon citratus*), and materials for analysis of crude protein content.

The study was arranged based on a completely randomized design with five treatments and four replications. The sample bags used were 20 experimental units and each unit consisted of two kg of rice bran, as the following P0: bran without being infected with beetles; P1: bran infected with beetles; P2: bran infected with beetles + 1% lemongrass flour; P3: bran infected with beetles + 2% lemongrass flour; P4: bran infected with beetles + 3% lemongrass flour.

All samples were stored for 30 days to allow the development of the beetles. The time needed for Tribolium beetles development in rice flour was 24–28 days [6]. A digital thermo-hygrometer was prepared to measure temperature and humidity in the storage room. After 30 days, observation and sampling were carried out by separating the beetles and bran to calculate the beetles' population for each replication. Another sample of 100 grams per unit of the experiment was taken and then analyzed for protein content. The analysis was carried out in the chemical feed laboratory using the Kjeldahl and Lowry method [7]. The parameters observed in the study were the population of the Tribolium beetles imago in each experimental unit, and the protein content of rice bran.

The data obtained were analyzed based on a general linear model for variance using the SPSS version 16.0 program. the Duncan test was performed to determine the differences among the experimental unit mean.

3. Results and discussion

3.1. The population of Tribolium beetles imago

The use of lemongrass flour has a significant effect (P < 0.05) on lowering the *Tribolium* beetles imago population (table 1). It is also revealed that although the infected bran treatments have a higher population of imago in comparison to the non-infected bran, the addition of lemongrass flour up to 3% can inhibit the growth rate of the beetles. This condition was indicated by the lower population number of imago in bran samples during storage with 2 or 3% lemongrass compare to other infected groups.

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Treatments	Imago population	Protein content (%)
PO	3.00±2.16 ^a	9.73±0.99
P1	128.25±67.44°	7.79 ± 3.71
P2	74.00 ± 20.70^{bc}	9.69 ± 0.26
Р3	53.00±19.44 ^{ab}	10.13 ± 1.14
P4	69.50±39.21 ^b	10.98 ± 1.78

Table 1. The population of *Tribolium* beetles imago and protein content of the rice bran.

^{abc}Superscript on the same columns shows a significant difference (P < 0.05).

The result of this study is in line with a previous study [8] that revealed the mortality of lice increases by increasing the concentration of lemongrass. This is related to the natural compounds of lemongrass such as citral citronellal, geraniol, citroneol, nerol, and farnesol found in lemongrass plant tissue. In high concentrations, these compounds can kill insects and in lower doses can inhibit insect growth and development. The biotic substances found in lemongrass have dehydrating toxicity (desiccant) and contact poison which can kill insects and can also inhibit bacterial activity [9].

In addition to the characteristic of lemongrass compounds as contact poison to the insect, [10] reported that some lemongrass substances can function as a fumigant, an insecticide that easily evaporates into a gas and enters the insect's body through the respiratory system or tracheal system which is then circulated throughout the body, and causes the death of the insect. Citral and linalool compounds in lemongrass were reported to have properties either as contact poison or fumigant that actively suppress the growth of *Tribolium castaneum* beetles [11,12]

Another mode of action of lemongrass essential oil compounds was as an anti-fungal by inhibiting ergosterol synthesis (the main sterol forming fungal cell membranes), damaging membrane protein structures, and increasing membrane permeability [13]. Moreover, terpenoid compounds also reported cause disruption of the fungal cell respiration chain.

Table 1 shows that in the non-infected treatment (P0), there were still visible beetles growing during the study. This can be due to the presence of built-in pests from the raw material and also the temperature of the storage area which allows beetles imago to breed. Based on [14] the presence of warehouse pests is caused by pest introduction, the interaction of pests from raw materials, or the conditions of the storage room. The ideal room temperature for insect growth is between 25°C and 30°C [15]. The temperature of the storage room at the time of the study was in the range of 28°C and humidity of 81% which caused the lice to thrive.

3.2. The protein content of rice bran

The use of lemongrass flour has no significant effect on the crude protein content of rice bran. The average crude protein content of rice bran during storage ranged from 9.69–10.98%. The crude protein content of rice bran usually influenced by *Tribolium castaneum* imago using protein in the bran for their survival and reproduction, according to Chapman (2013) that most insects get amino acid protein from the feed, because insects cannot synthesize their amino acids. Amino acids is used for structural purposes such as enzyme activity for transport and storage and as molecular receptors, various amino acids are also used as energy for respiration and energy when insects fly [16].

Lemongrass flour can control the growth of *Tribolium caestaneum*. This is because fewer insects consume protein. [17] also added that protein is an essential element needed by female imago to produce eggs. Diets that have low protein content can affect egg production produced by female imago insects. The results of this study indicate that the addition of lemongrass flour can indirectly affect the crude protein content of rice bran during storage because the role of lemongrass flour is more to suppress the population of *Tribolium caestaneum* imago. active substances in lemongrass can suppress appetite in *Tribolium caestaneum* imago so that it automatically reduces the consumption of protein feed. In general, the mechanism of action of vegetable pesticides is directly inhibiting the reproduction process of insect pests, especially female insects, reducing appetite, causing insects to refuse food, damaging

the development of eggs, larvae and pupae so that insect pest reproduction is disturbed, and inhibiting skin change [18].

4. Conclusion

The conclusion of the study can be concluded that giving lemongrass flour at a level of 1-3% in rice bran can reduce the population of red flour beetle (*Tribolium caestaneum*) imago during storage and maintain crude protein content.

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