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The Applications Flour Formulation *Beauveria bassiana* Balls (Vuill.) Local Entomopathogen Fungi with Bran Flour Carrier against *Oryctes rhinoceros* L. Larvae in the Laboratory

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Abstract: ***The entomopathogenic fungi B. bassiana local is an effective biological agent to control pests among of them Oryctes rhinoceros. O. rhinoceros is a pest of palm oil plantation in Indonesia which causing any problem therefore need to control. Increases the mortality level of entomopathogenic fungi B. bassiana local can be done by forming a better formulation, for example flour formulation with the addition of bran flour as a carrier. Bran flour can encourage the entomopathogenic fungi B. bassiana to germinate faster and produce a higher number of conidia so that it can control O. rhinoceros larvae more quickly. This research was conducted using a completely randomized design (CRD) consisting of 5 treatments and 4 replications obtained 20 experimental units each infested ten O. rhinoceros larvae in star 3. The treatment is additional bran flour into 30 g B. bassiana starter made into flour formulation. Additional some of level bran flour to one kilogram empty fruit bunch (EFB) were 0 g. kg $^{-1}$ EFB, 0.45 g. kg $^{-1}$ EFB, 0.6 g. kg $^{-1}$ EFB, 0.75 g. kg $^{-1}$ EFB, and 0.9 g. kg $^{-1}$ EFB. The result showed of this research B. bassiana flour formulation and additional bran flour 0.90 g. kg $^{-1}$ EFB causing total mortality of O. rhinoceros larvae increasing starter from is 77, 50% becomes flour formulation 82, 5%.***

Keyword: Flour formulation, local *Beauveria bassiana*, *Oryctes rhinoceros*

1. Introduction

Oil palm is a plant originating from the African continent, and outside of its origin it is able to produce high production (Fauzi, 2012). Palm oil plants produce products in the form of palm oil which is the largest contributor to foreign exchange for the Indonesian economy.

The production of palm oil in Riau Province has increased every year, in 2017 the production reached 7, 591.20 thousand tons, in 2018 it reached 8, 496.00 thousand tons, and in 2019 it reached 9, 127.60 thousand tons (Central Bureau of Statistics, 2020). Increasing the yield of oil palm production needs to be maintained, one of which is by controlling the attack of plant pest organisms.

The pest that attacks oil palm plantations is *Oryctes rhinoceros* L. Sudharto and Guritno (2003) said *O. rhinoceros*s attack oil palm plants in the vegetative phase to the generative phase. Symptoms of this pest attack are indicated by the presence of a "V" shape at the growing point of the oil palm plant. *O. rhinoceros* attack can reduce the production of fresh fruit bunches in the first year of harvest by 60% and cause death in young plants up to 25% (Apriyaldi, 2015). Efforts can be made to save the production of oil palm plants by controlling the *O. rhinoceros* pest in the larval stage.

Pest control is still mostly done by using synthetic chemical insecticides. According to Untung (2001), the continuous and excessive use of synthetic chemical insecticides can have negative impacts, including environmental pollution, poisoning of non - target organisms, secondary pest explosions, resistance and resurgence. Therefore, an environmentally friendly control is needed, including control using the local entomopathogenic fungi local *Beauveria bassiana*.

Utilization of entomopathogenic fungi as biological agents has potential because entomopathogenic fungi have a high reproductive capacity, short life cycle, are relatively safe, selective, relatively easy to produce, and are very unlikely to cause resistance to pests (Prayogo *et al*., 2005). Entomopathogenic fungi are reported to be effective in controlling several species of insect pests including termites, mealy bugs, and several types of beetles such as *Beauveria bassiana* (Soetopo and Indrayani, 2007). The isolates of the fungi B. *bassiana* used came from the Riau Province or local lands. Local isolates were chosen because they are easier to adapt to the local ecosystem, so they do not cause disturbances in the balance of the ecosystem (Trizelia *et al*., 2015).

The results of the study by Salbiah *et al*. (2013) stated that a dose of the entomopathogenic fungi B. *bassiana* 30 g. kg $^{-1}$EFB was able to control *O. rhinoceros* with a total mortality of 77.50%. The results of this study can still be improved its effectiveness by forming entomopathogenic fungi into flour formulations. Entomopathogenic fungi in the form of flour formulations have advantages such as being able to be stored for a long period of time, practical, and easy to apply (Suwahyono, 2010).

Hasyim (2006) stated that the use of entomopathogenic fungi *B. bassiana* with rice flour as a carrier material can cause 90% mortality of the banana weevil borer. Flour formulations can be made by adding a carrier, such as bran flour. Nuryanti (2012) said the addition of bran flour can increase the ability of *B. bassiana* to cause mortality in insect pests. Bran flour contains carbohydrates, protein, and B vitamins which are sources of nutrients and energy that are needed for the growth of the entomopathogenic fungi *B. bassiana* local. In this study, the addition of 1.5% bran to rice media.

This research bran flour not added to the rice propagation medium, but is bran flour as a carrier for starters that have become flour formulations. The bran flour is easy to find and the price is not too expensive which is the reason for using bran flour in this study. The aim of this study was to obtain the best capability of the local entomopathogenic fungi B. *bassiana* flour formulation to control the larvae of the *O. rhinoceros* in the laboratory.

2. Research Method

Place and time: This research was conducted at the Laboratory of Plant Pests, Faculty of Agriculture, Riau University, Pekanbaru. The study started from January to March 2021.

Materials and tools: The material used was a local *B. bassiana* isolate Desita Salbiah collection. *O. rhinoceros* larvae third instar, bran flour, rice, oil palm empty fruit bunch, aquades, and 70% alcohol. The tools used were 40 cm x 25 cm x 15 cm treatment box, thermohygrometer, bunsen lamp, loop needle, pin set, petridish, blender, refrigerator, laminar air flow cabinet (LAFC), microscope, analytical scale, and digital camera.

Research methods: This research was used a completely randomized design (CRD) consisting of 5 treatments and 4 replications obtained 20 experimental units each infested ten *O. rhinoceros* larvae in star 3. The treatments are additional bran flour into 30 g *B. bassiana* starter made into flour formulation. Additional some of level bran flour to one kilogram empty fruit bunch (EFB) were 0 g. kg^{-1} EFB, 0.45 g. kg^{-1} EFB, 0.6 g. kg^{-1} EFB, 0.75 g. kg^{-1} EFB, and 0.9 g. kg^{-1} EFB.

Research Implementation: The provision of *O. rhinoceros* larvae was found in the community's garden. Treatment box refers to the research of Salbiah (2013) with a size of 40 cm x 25 cm x 15 cm. Organic matter as a place to live for *O. rhinoceros* larvae was obtained from the experimental garden area and then chopped.

Reisolation local entomopathogenic fungi B. *bassiana* propagated on PDA media next made of starter form with rice media. Entomopathogenic fungi B. *bassiana* was propagated by washing the rice thoroughly and then steaming the rice until it was half cooked (15 minutes). After that, the rice media was put into plastic as much as 30 g. Then the plastic is glued with a 3 cm pipe. Furthermore, aseptic inoculation local *B. bassiana* isolate in LAFC. Then the pipe was closed using cotton and aluminum foil then the entomopathogenic fungi was incubated for 10 days.

Preparation of local entomopathogenic fungi B. *bassiana* flour formulations using the method of Suwahyono (2010). The starter of the local entomopathogenic fungi B. *bassiana* was carried out in the refrigerator at 5^0C for 12 days. And then mashed by means of a blender until smooth into flour containing spore biomass. The bran carrier material was previously sterilized using an oven at 80^0C for 2 hours. After that, mixed bran flour into 30 g *B. bassiana* starter made into flour formulation.

The application of the mixed local entomopathogenic fungi B. *bassiana* with bran flour to the treatment on empty fruit bunch in the treatment box. Then the infestation of *O. rhinoceros* larvae was carried out by inserting ten *O. rhinoceros* larvae into a treatment box. The treatment box is stored in the Plant Pest Laboratory, Faculty of Agriculture, Riau University.

Observation: Consists of initial time of death, lethal time 50, total mortality, and temperature and humidity. Data on initial mortality, total mortality, and lethal time 50 were statistically analyzed using variance. The results of the variance were further tested with the Least Significant Difference Test (LSD) at the 5% level.

3. Results and Discussion

This research was conducted at the Laboratory of Plant Pests, Faculty of Agriculture, University of Riau, with a temperature of 23.92^0C and a humidity of 65.05%. The results of the study are as follows:

Early Time of Death: The results of variance showed that the application of the local entomopathogenic fungi *B. bassiana* flour formulation with the addition of bran flour was not significantly different from the time of early death of *O. rhinoceros* larvae. The results of further tests with LSD at the 5% level can be seen in Table 1.

Table 1: Average Early Time of Death of *O. rhinoceros* larvae after application local entomopathogenic fungi *B. bassiana* flour formulation with the addition of bran flour

Addition of Bran Flour (g. kg^{-1} EFB)	Time of Beginning of Death (Hours)
0, 00	135.0a
0.45	109.5a
0.60	82.50a
0.75	75.00a
0.90	54.00a

The numbers in the column followed by lowercase letters that are not the same are significantly different according to the smallest significant difference test (LSD) at the 5% level.

Table 1 shows that the application of the local entomopathogenic fungi B. *bassiana* flour formulation with the addition of 0.9 g. kg^{-1} EFB bran flour caused the earliest time for *O. rhinoceros* larvae to die at 54 hours after application. This is because the addition of bran flour can increase the ability of the local entomopathogenic fungi B. *bassiana* in mortality *O. rhinoceros* larvae. The bran flour itself contains carbohydrates, protein, and B vitamins which are a source of nutrients and energy that are needed for the growth of the local entomopathogenic fungi B. *bassiana*. According to Kansrini (2015) carbohydrates and proteins are needed by fungi to encourage vegetative growth and spore formation. The carbohydrate and protein content in bran flour helps accelerate vegetative growth and spore formation in the local entomopathogenic fungi B. *bassiana*, so that the conidia density becomes high and provides a great opportunity for conidia to attach to the cuticle, germinate and penetrate into the body of *O. rhinoceros* larvae so that time the early death of *O. rhinoceros* larvae occurs more quickly.

The local entomopathogenic fungi *B. bassiana* flour formulation with the addition of 0.9 g. kg $^{-1}$ EFB bran flour killed the *O. ehinoceros* larvae the fastest 54 hours after application, so that the initial time of death obtained was faster than the results of Salbiah (2013) study. stated that the administration of the local entomopathogenic fungi B. *bassiana* 30 g. kg $^{-1}$ EFB starter form total mortality the *O. rhinoceros* larvae faster, 90 hours after application. This was due to the addition of bran flour as a carrier which encouraged the germination of the local entomopathogenic fungi B. *bassiana* to develop faster.

The local entomopathogenic fungi *B. bassiana* attacks insects by contact poison and digestion. The local entomopathogenic fungi *B. bassiana* works as a contact poison entering through the cuticle and natural holes of insects. According to Soetopo and Indrayani (2007) stated that the local entomopathogenic fungi B. *bassiana* produces beauvericin toxin which can cause tissue damage due to infection as a whole.

The local entomopathogenic fungi *B. bassiana* works as a digestive poison when the conidia of the local entomopathogenic fungi B. *bassiana* enter the body of the *O. rhinoceros* larvae along with the tkks that have been eaten. Then the conidia enter the digestive tract and secrete the baeuvericin toxin which can cause overall tissue damage, causing *O. rhinoceros* larvae to have no appetite, decreased activity and finally *O. rhinoceros* larvae die. This is in line with the opinion of Saleh *et al*., (2000) which stated that the initial symptoms of local entomopathogenic fungi *B. bassiana* infection in insects are insects that do not want to eat, their movements slow down, then they die.

***Lethal time* 50:** The results of variance showed that the application of the local entomopathogenic fungi B. *bassiana* flour formulation with the addition of bran flour was not significantly different from the LT50. The results of the LSD further test at the 5% level can be seen in Table 2.

Table 2: Average LT50 of *O. rhinoceros* larvae after application local entomopathogenic fungi B. *bassiana* flour formulation with the addition of bran flour

Addition of Bran Flour (g. kg $^{-1}$ EFB)	Lethal Time 50 (LT50) (Hour)
0, 00	274.50a
0.45	231.00a
0.60	235.50a
0.75	225.00a
0.90	171.00a

The numbers in the column followed by lowercase letters that are not the same are significantly different according to the smallest significant difference test (LSD) at the 5% level.

Table 2 shows that the application of the local entomopathogenic fungi B. *bassiana* flour formulation with the addition of 0.9 g. kg $^{-1}$ EFB bran flour caused a faster time to mortality 50 *O. rhinoceros* larvae, which was 171 hours after application.

The local entomopathogenic fungi B. *bassiana* flour formulation the addition of bran flour took longer to mortality LT50 *O. rhinoceros* larvae, which was 274.5 hours after application.

The local entomopathogenic fungi B. *bassiana* flour formulation with the addition of 0.9 g. kg $^{-1}$ EFB of bran flour got a faster time to mortality LT50 *O. rhinoceros* larvae by 171 hours after application than the results of Salbiah's (2013) study which stated that of the fungi local entomopathogen *B. bassiana* 30 g. kg $^{-1}$ EFB starter form took 193.5 hours after application to mortality LT50 *O. rhinoceros* larvae. This is because the addition of a carrier material for bran flour can help increase the mortality of local entomopathogenic fungi *B. bassiana* by pushing faster than the germination of local entomopathogenic fungi *B. bassiana*, then high beauvericin toxin released to accelerate mortalityLT50 *O. rhinoceros* larvae. This is in accordance with the opinion of Brousseau *et al*., (1996) in Rustama; Melanie; Budi., (2008) larval death rate is caused by damage to the inside of the larval body due to toxins released by the fungi.

The total mortality of *O. rhinoceros* larvae: The results of variance showed that the treatment of several doses of the local entomopathogenic fungi B. *bassiana* flour formulation with the addition of bran flour was significantly different to the total mortality*O. rhinoceros* larvae. The results of the LSD further test at the 5% level can be seen in Table 3.

Table 3: Average total mortality of *O. rhinoceros* larvae after application local entomopathogenic fungi B. *bassiana* flour formulation with the addition of bran flour

Additional of Bran Flour (g. kg $^{-1}$ EFB)	Total Mortality (%)
0, 00	55.00b
0.45	70.00ab
0.60	75.00a
0.75	80.00a
0.90	82.50a

The numbers in the column followed by lowercase letters that are not the same are significantly different according to the smallest significant difference test (LSD) at the 5% level after being transformed by the formula (y) + 0, 5.

Table 3 shows that the local entomopathogenic fungi *B. bassiana* flour formulation with the addition of 0.9 g. kg $^{-1}$ EFB bran flour caused the highest mortality of *O. rhinoceros* larvae of 82.50%. This also occurred for the initial time of death of 54 hours and the lethal time of 50 for 171 hours after application when compared to other flour formulations.

The local entomopathogenic fungi *B. bassiana* flour formulation with additions of bran flour was significantly different from the local *B. bassiana* entomopathogenic fungi flour formulation without the addition of bran flour. Bran flour is a source of nutrition and energy that is needed for the growth of the local entomopathogenic fungi *B. bassiana*.

The local entomopathogenic fungi *B. bassiana* flour formulation without the addition of 0, 00 g. kg $^{-1}$ EFB bran flour caused the lowest total mortality of *O. rhinoceros* larvae at 55% and the same with the initial time of death of 135 hours, and the lowest lethal time 50 by 274.5 hours after application. The local entomopathogenic fungi *B. bassiana* flour formulation with the addition of 0.90 g. kg $^{-1}$ EFB bran caused a total mortality of 82.50%, the initial time of death was 54 hours after application, and the lethal time 50 hours was 171 hours after application. The results of Salbiah's research (2013) stated that the highest mortality was found at the local entomopathogenic fungi *B. bassiana* 30 g. kg $^{-1}$ EFB starter form of 77.50%, the initial time of total mortality was 90 hours after application, and the lethal time 50 hours was 193 hours after application.

O. rhinoceros larvae die undergo hardening in the larva's body or the so - called mummification process, this is due to local entomopathogenic fungi *B. bassiana* colonization in the larval body, and the fluid contained in the larval body is used by the local entomopathogenic fungi *B. bassiana* to grow and breed. Wowiling *et al*., (2015) stated that insects that died due to infection with the local entomopathogenic fungi *B. bassiana* were mummified or the body became hardened and stiff. Morphological changes of *O. rhinoceros* larvae due to infection with the local entomopathogenic fungi *B. bassiana* can be seen in Figure 2.

Figure 2: Morphological changes of *O. rhinoceros* larvae attacked by local entomopathogenic fungi B. *bassiana* (a) live larvae, (b) larvae that have died, (c) larvae change color to blackish, (d) larvae were mummified (Research documentation, 2021).

Environmental factors such as temperature and humidity also affect the effectiveness of the local entomopathogenic fungi B. *bassiana*. The average ambient temperature at the time of the research in the laboratory was 23.92^{0}C and the humidity of the environment at the time of the research in the laboratory was an average of 65.05%. According to Lecuona *et al*. (2001) in Rosfiansyah (2009) said the local entomopathogenic fungi B. *bassiana* was able to grow in the temperature range of 15 - 35^{0}C with humidity below 95.5%.

The local entomopathogenic fungi *B. bassiana* flour formulation with the addition of 0, 9 g. kg $^{-1}$ EFB of bran flour was to cause total mortality in *O. rhinoceros* larvae of 82.50%.

4. Conclusion

The application of the local entomopathogenic fungi B. *bassiana* flour formulation with the addition of bran flour to *O. rhinoceros* larvae in the laboratory concluded:

The local entomopathogenic fungi B. *bassiana* flour formulation with the addition of 0, 9 g. kg $^{-1}$ EFB of bran flour is to increasing total mortality to *O. rhinoceros* larvae with total mortality from 77, 5% become 82.50%.

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