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Making Probiotic *Trichoderma sp.* Using Rice Bran Media with Addition of Molasses**Author's Details:****⁽¹⁾Faisal Ahya Hartantyo* ⁽²⁾Novita Dewi Kristanti ⁽³⁾Agustbern Herychrist Benyamin FoEkh****⁽¹⁾⁽²⁾⁽³⁾Animal Husbandry and Animal Welfare Extension Program, Agricultural Development Polytechnic/Polbangtan Malang; Jl. Dr.Cipto 144A Bedali-Lawang, Malang, East Java****Received Date: 15-July-2019****Accepted Date: 31-July-2019****Published Date: 29-Aug-2019****Abstract**

Probiotics are one of the products that are beneficial for livestock. Giving probiotics to livestock can improve their metabolic processes. *Trichoderma* is one of the microbiology that has antagonistic properties, namely suppressing the presence of other microbiology (pathogens). Therefore the researchers wanted to know the effect of giving molasses to the growth of *Trichoderma sp* in probiotics.

This study aims to determine the effect of adding molasses to the growth of *Trichoderma sp.* in making probiotics using rice bran media. This study used a completely randomized design method (CRD) using four treatments and six replications. The treatment used is P0 = Rice Bran + 1% *Trichoderma sp.*, P1 = Rice Bran + 1% *Trichoderma sp.* + 1% Molasses, P2 = Rice Bran + 1% *Trichoderma sp.* + 3% Molasses, P3 = Rice Bran + 1% *Trichoderma sp.* + 5% Molasses. Giving Molasses in the manufacture of probiotics on rice bran media had a significant effect ($P > 0.05$) on the growth of *Trichoderma sp.* but did not provide a significant difference in the decrease in pH ($P < 0.05$). with the best results on P2 with $5.22 \times 10^{10}/g$ media spores

Keywords: *Trichoderma sp*, pH, molasses, rice bran media, probiotic.

INTRODUCTION

The feed is an important factor that can support success in livestock. Good feed quality will increase the productivity value of livestock. In addition to the addition of feed, other supplements can also help success in the livestock business. One supplement that can help increase success is called probiotics.

Probiotics can be single or mixed-culture of microorganisms that improve the gut microbial environment by displacing harmful bacteria that are often detrimental to the sustenance of living beings (Dhama et al., Without Years). According to the fuller (1989) the way probiotics work is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. So by giving probiotics to animal feed, it can balance the microbes in the animal's body so that the metabolic process can run well.

One of the microbes that can be used as a starter for probiotics is *Trichoderma sp.* *Trichoderma* is known to have antagonistic ability to pathogenic fungi (Alfizar et al., 2013). These fungi are beneficial micro-organisms, avirulent to host plants, and can parasitize other fungi (Harman et al., 2004). Thus it is expected that probiotics *Trichoderma sp* can have a good impact on livestock.

Rice bran is one of the media that can be used as a growth medium for *Trichoderma sp.* Gusnawaty et al. (2017) state that the amount of conidia *Trichoderma sp.* formed on each medium shows that the amount of conidia *Trichoderma sp.* the highest was found in bran media with $104,125.10^3/g$ media conidia which were significantly different from other media. Rice bran is a good growing medium because rice bran contains many nutrients needed for the growth of *Trichoderma sp.* The growth of *T. harzianum* in bran media is faster and thicker than other treatments, this is because bran has a high carbohydrate content (27.01%), high content of P, K each 0.69% and 1.92% and low pH (6.16) compared to sago ela compost and husk (Uruilal et al., 2012).

Carbohydrates and proteins include macro nutrients for metabolic processes by diffusion which is transported into fungal cells using carrier molecules (Juliana et al., 2017). According to Kusmiati et al. (2007) molasses contains nutrients that are high enough for bacterial needs so that it is used as an alternative material as a carbon source in fermentation media. Pujianingsih (2007) inside Ismi et al. (2017) States that The content contained in molasses includes 20% water, 3.5% protein, 58% carbohydrate, 0.80% Ca, 0.10% pospor and 10.50% other mineral ingredients. Molasses have the ingredients needed for the metabolism of living *Trichoderma sp.* Therefore this study aims to determine the effect of the addition of molasses to the growth of *Trichoderma sp.* on rice bran media as probiotics.

MATERIALS AND METHOD

This research was conducted at Animal Feed and Nutrition Laboratory, Agricultural Development Polytechnic (Polbangtan) Malang. The tools used in this study are stationery, cameras, drop pipettes, micropipets, analytical scales, 10 ml measuring cups, Bunsen, test tubes, test tubes, microscopes, autoclaves, Haemocytometers, handcounters,

International Journal of Agriculture and Biological Sciences- ISSN (2522-6584) July & Aug 2019

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spatulas, and lighters. The ingredients used are rice bran, *Trichoderma sp.*, Aquades, Molasses, label paper, plastic bags, sterile markers, 70% alcohol, gloves, masks, and tissues.

The method used is a completely randomized design (CRD) method with four treatments and six replications. The treatments used in the study are as follows: P0 = Rice Bran + *Trichoderma sp.*, P1 = Rice Bran + *Trichoderma sp.* + 1% Molasses, P2 = Rice Bran + *Trichoderma sp.* + 3% Molasses, P3 = Rice Bran + *Trichoderma sp.* + 5% Molasses.

Probiotic production begins by mixing rice bran with water as much as 60% of the weight of the media that has been added to molasses according to the treatment specified. then the media is weighed 100 grams each and put in a clear, heat-resistant plastic bag. Then the media is sterilized by autoclave with a temperature of 121°C for 15 minutes. The sterilized media is left until the temperature drops. Media that has been cooled is then inoculated with *Trichoderma sp.* Each media is given a sign according to the treatment, and the inclusion process is carried out for seven days.

RESULTS AND DISCUSSION**A number of *Trichoderma sp.* Spores**Table 1. An average number of *Trichoderma sp.* Spores. on multiplication media (/gram of media)

Composition of probiotic treatments	The average number of <i>Trichoderma sp.</i> Spores on multiplication (10^{10} /g media)
P0 (Rice bran + <i>Trichoderma sp.</i> 1%)	2,32 ^a
P1 (Rice bran + 1% <i>Trichoderma sp.</i> + 1 % molasses)	3,07 ^b
P2 (Rice bran + 1% <i>Trichoderma sp.</i> + 3 % molasses)	5,22 ^d
P3 (Rice bran + 1% <i>Trichoderma sp.</i> + 5 % molasses)	3,91 ^c

Data obtained from Table 1 is for the estimated number of P2, which is equal to 5.22×10^{10} and for the lowest results at P0 which is equal to 2.32×10^{10} . Based on the results of the research given molasses which has a large benefit on the growth of *Trichoderma sp.* on bran media. Pujianingsih (2007) inside Ismi et al. (2017) States that The content contained in molasses includes 20% water, 3.5% protein, 58% carbohydrate, 0.80% Ca, 0.10% pospor and 10.50% other mineral ingredients. This is related to molasses containing nutrients needed for the maintenance process of *Trichoderma sp.* This is due to the nutrients contained in molasses needed for the metabolic process of the fungus *Trichoderma sp.* This is in accordance with the opinion of Juliana et al. (2017) which states that carbohydrates and proteins include macro nutrients for metabolic processes through diffusion which are transported into fungal cells using carrier molecules. And the chemical elements also support the growth and development of *Trichoderma sp.* like phosphorus, potassium, calcium, and sodium.

although the addition of molasses has a significant effect on spore growth in *Trichoderma sp.* giving molasses at the level of 5% decreases the number of spores of *Trichoderma sp.* As explained by Fifendy et al. (2013) which states that the increase in the number of microbes is not always directly proportional to the addition of sugar levels in the fermentation process. This is also reinforced by Nainggolan (2009) which states that microbial growth in a media is not always directly proportional to the addition of sugar levels in the fermentation process, because in the fermentation process produced alcohol, organic acids, and other substances. With the increase in the amount of molasses given to the growth medium of *Trichoderma sp.* the higher the alcohol content found in the media. Alcohol is a compound that can inhibit the growth of *Trichoderma sp.* because alcohol has antibacterial properties that can inhibit the growth of *Trichoderma sp.* This is in accordance with the opinion of Pelczar and Chan (1988) inside Yuningsih (2007) which states that the main chemical compounds that have antibacterial properties are phenols, halogens, phenolic compounds, alcohols, halogens, heavy metals, detergents, and aldehydes. Alcohol reacts by denaturing proteins by dehydrating and dissolving fat so the cell membrane is damaged, and enzymes will be activated by alcohol (Susatyo, 2016). so that the higher the alcohol content contained in the fermentation process, the amount of *Trichoderma sp.* spores in the media will decrease due to the process of protein denaturation and fat dissolution.

Probiotic PH

Table 2. Average pH of probiotics

Komposisi perlakuan probiotik	pH probiotik
P0 (Dedak Padi + 1% <i>Trichoderma sp.</i> 1%)	6
P1 (Dedak Padi + 1% <i>Trichoderma sp.</i> + 1 % Molasses)	6
P2 (Dedak Padi + 1% <i>Trichoderma sp.</i> + 3 % Molasses)	6
P3 (Dedak Padi + 1% <i>Trichoderma sp.</i> + 5 % Molasses)	6

International Journal of Agriculture and Biological Sciences- ISSN (2522-6584) July & Aug 2019

August 31, 2019

The results showed that the average value of pH of probiotics at P1 - P4 was 6. Factors that influenced the development of the population of *Trichoderma sp.* in soil including pH, aeration, and nutrition (Amaria et al., 2016). Based on the results of this study, it can be seen that giving different levels of molasses does not affect pH conditions on bran media. The pH conditions produced in each treatment are somewhat acidic (pH 6). This pH condition is a suitable condition for the growth of *Trichoderma sp.* This is in accordance with the opinion of Wahyudi et al. (2004) which states that neutral pH conditions which are suitable for the growth of *T. harizianum* are pH neutral (7) and slightly acidic (6.2).

CONCLUSION

Addition of molasses in the manufacture of probiotics *Trichoderma sp.* gives a significant difference to the growth of *Trichoderma sp.* but does not affect pH in the media. addition of molasses at the level of 3% has the best results with the number of spores 5.22×10^{10} .

SUGGESTION

Further research is needed about the application of probiotics *Trichoderma sp.* in livestock to determine the effect of probiotics *Trichoderma sp.* on livestock productivity.

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