Jurnal Biologi Tropis

Original Research Paper

Growth of *Trichoderma asperellum* with the Addition of *Ecoenzyme* to Red Glutinous Rice-Based Medium

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Article History
Received : December 23th, 2023
Revised : January 26th, 2024
Accepted : February 15th, 2024

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Abstract: *Ecoenzyme* is a fermented liquid product containing lactic acid bacteria in fruits and vegetables. Nutrients derived from *ecoenzymes* such as nitrogen are used for growth. This study evaluated the effect of *ecoenzyme* administration on the number of *Trichoderma asperellum spores* in brown glutinous rice medium. *Trichoderma asperellum* is known as a beneficial fungus in the decomposition of solid waste and the enhancement of plant growth. The use of medium made from brown glutinous rice was tested as an alternative to *Potato Dextrose Agar* (PDA), with the addition of *ecoenzyme* as an additional nutrient. This study used a Complete Randomized Design (RAL) with six treatments and five repeats. The treatment given is the addition of *ecoenzyme* concentrations of 0%, 20%, 40%, 60%, 80%, and 100%. Data was analyzed using fingerprints and follow-up tests with DNMRT at an error rate of 5%. Observation of the number of spores is carried out at the age of 60 days by dilution and hemasitometer. The results showed that the 60% *ecoenzyme concentration* had the highest average spore count and was different from the 0% control. In contrast, the 20% concentration had the lowest average and was the same as the controls. Giving *ecoenzyme* to brown glutinous rice medium can increase the number of *Trichoderma asperellum spores.*

Keywords: Brown glutinous rice, Ecoenzyme, Trichoderma asperellum

Introduction

Trichoderma asperellum is a saprophytic soil microorganism and naturally attacks fungi that become pathogenic and beneficial for plants (Dwiastuti *et al*., 2015). *Trichoderma* is one type of fungus included in the class Ascomycetes (Novianti, 2018) which is found in almost all types of soil (Yudha *et al*., 2016) and habitat, as well as one type of fungus that can be used as a biofertilizer to control soil pathogens (Gusnawaty *et al.*, 2014). Species *Trichoderma* consists of *Trichoderma harzianum, Trichoderma viride, Trichoderma asperellum,* and *Trichoderma koningii* (Cahyani *et al.*, 2021). *Trichoderma* Free-living filamentous fungi are also beneficial microorganisms and have been popular for their ability to decompose solid waste, disease control and promote growth of various types of plants (Anhar, Princess, *et al*., 2020). *Trichoderma asperellum* is one of the environmentally friendly fungicides that is expected to reduce the use of synthetic fungicides (Chatri *et al*., 2018). *Trichoderma asperellum* Widely found on almost all types of soil. Its wide distribution is one of the factors that cause *Trichoderma* It is easy to cultivate. *Trichoderma* can be used to improve plant growth (Anhar, Advinda, *et al*., 2020).

Trichoderma Sp. will grow on a medium that has elements of carbon, nitrogen, hydrogen, oxygen that are useful for its growth (Andriani *et al*., 2012). Propagation *Trichoderma* in the laboratory usually uses PDA medium (*Potato Dextrose Agar*) with the main source of carbohydrates coming from potatoes, but because the price is expensive and easily contaminated, another alternative medium is used, namely brown glutinous rice (Kurniawati *et al*., 2021). Brown glutinous rice (*Oryza sativa* L var glutinosa) has several advantages

compared to PDAs, namely that it contains many phenolic compounds (Azis *et al*., 2015). Brown glutinous rice contains high antioxidants that act as hydrogen donors. Brown glutinous rice also contains anthocyanins, carbohydrates and nutrients for growth *Trichoderma*. Brown glutinous rice can be used as a growing medium *Trichoderma*. Brown glutinous rice has a shelf life of six months at room temperature before being made into a medium. During the shelf life, the nutrients are still there but have decreased. For this reason, additional nutrients are needed for brown glutinous rice medium which can be obtained from the addition of *Ecoenzyme* (Monika *et al*., 2014).

Ecoenzyme is a fermented product liquid containing lactic acid bacteria in fruits and vegetables (Karila *et al*., 2022). *Ecoenzyme* has complex functions. Liquid *Ecoenzyme* It has a dark brown color and a strong fresh sour aroma. The principle of the manufacturing process *Ecoenzyme* Almost the same as the process of making compost, but it is necessary to add water as a growth medium so that the final product obtained is a liquid that is easy to use (Zirrazaq *et al*., 2022). Nutrients derived from *Ecoenzyme* Such nitrogen can be utilized by plants for the process of photosynthesis and produce photosyntate. In addition to affecting plant growth, the addition of *Ecoenzyme* also increased availability of P (*Phosfor*) in the soil as well as affecting soil pH (Djaya *et al*., 2014). *Ecoenzyme* contains ammonia converted into nitrate (NO3), a natural hormone and nutrient for plants as a provider of elements needed by plants such as organic carbon, nitrogen (N), *phosphore* (P), and potassium (K) thus increasing plant growth (Natasya *et al*., 2023).

Previous research on growth *Trichoderma* asperellum in bran medium only lasts up to 28 days (Hakim, 2019). Research on the addition of glycerol to talc medium only maintains viability over the shelf life (Amaria *et al*., 2016). Growth in the number of spores in studies with corn-based medium got low results for 14 days (Day, 2022). While this study reached the 60th day and this study aimed to determine the effect of adding several concentrations *Ecoenzyme* against the number of spores *Trichoderma asperellum*.

Material and Method

Types of Research

This research is an experimental study. This research will be carried out from August to October 2023 at the General Research and Biology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Padang State University.

Tools and Materials

The tools used in this study include: petri dishes, measuring cups, erlenmeyer, test tubes, test tube racks, *bunsen*, ose, *autoclave*, hot plate stirrer, *beaker glass*, Laminar Air Flow *(LAF)*, vortex, cutter, scissors, incubator, drip pipette, hemasitometer, micropipette, and tip. The materials used in this study were *aquadest*, brown glutinous rice, 70% alcohol, tissue, *aluminum foil*, cotton, gauze, plastic wrap, label paper, hypochlorite, *ecoenzyme*, Trichoderma asperellum *isolate* collected by Mr. Febri Doni, Ph.D.

Research Design

The study used a Complete Randomized Design (RAL) with six treatments and five repeats. The treatment given in this study is the addition of concentration *Ecoenzyme* 0%, 20%, 40%, 60%, 80%, and 100%. The study used a liquid medium of brown glutinous rice which was sterilized with *Autoclave* then poured 1 mL *Trichoderma asperellum* into the medium of brown glutinous rice and added concentration *Ecoenzyme* each test tube. *Trichoderma asperellum* This is observed once every fifteen days. Observation of the number of spores is carried out on the 60th day. The density is calculated by the formula (Rohmah & Alif, 2021):

$$C = \frac{t \, x \, d}{n \, x \, 0{,}25} \, 10^6 \qquad (1)$$

Information:
C = number of spores per ml of solution.
t = total number of spores in the observed sample box.
n = number of sample boxes (5 large boxes × 16 small boxes).
0,25 = correction factor for the use of small-scale sample boxes on hemocytometers.

d = dilution factor when it must be diluted (d=1 means undiluted d=10 means diluted 1:10).

106 = Good spore density standards reported from the Directorate of Plantation Protection of the Ministry of Agriculture in 2014.

Results and Discussion

Observation of Trichoderma asperellum *Spore Number* on Day 60

Research results indicates that concentration *Ecoenzyme* 60% had the highest average spore count and differed by the 0% control. In contrast the 20% concentration has the lowest average and is equal to the control, as seen in Table 1.

Table 1. Effect of *ecoenzyme concentration* on the number of Trichoderma *asperellum spores* at 60 days of age

No.	Treatment	Average Number of Spores in 1 mL
1.	60%	4.67×106 [a]
2.	80%	2.84×106 [b]
3.	100%	2.63×106 [b]
4.	0% (Control)	2.43×106 [b]
5.	40%	2.35×106 [b]
6.	20%	1.93×106 [b]

Description: Numbers followed by the same different lowercase letters are not real according to DNMRT 5%.

This trichoderma asperellum is observed on the 60th day. According to the East Kalimantan Plantation Office (2017), *Trichoderma* sp. will be seen growing after one to two weeks so that it can only be observed on the fifteenth day and the addition of *ecoenzyme* on the 5th day. Observations on day 15 of *Trichoderma asperellum* the highest number of spores produced remained at 60% concentration and on day 30 had no noticeable comparison between treatments. Based on research that has been done sum Each spore's concentration has a marked difference. The number of spores in studies that have been conducted is from 1.93×106 to 4.67×10^6. Gift *Ecoenzyme* with a concentration of 60% has an average sum The highest spore was 4.67×106 among concentrations *Ecoenzyme* others while concentration *Ecoenzyme* 20% have an average sum The lowest spore was 1.93×106.

The difference in average spore density indicates that there is a marked difference from the administration of some concentrations *Ecoenzyme* exerted on controls without concentration *Ecoenzyme* into the medium made from red glutinous rice has an averagesum spores are different from which concentrations are added *Ecoenzyme*, so that the grant *Ecoenzyme* Effect on growth *Trichoderma asperellum*. Sticky rice contains carbohydrates, proteins, beta-carotene, antioxidants and iron. The fat content in glutinous rice is not so high, which is an average of 0.7% and the highest content of fatty acids is oleic acid, linoleic acid, and palmitic acid. Some of the vitamins found in glutinous rice are mainly thimin, riboflavin and niacin. Some of the minerals found in glutinous rice are iron, calcium, phosphorus, magnesium (Syofian & Anhar, 2022). Brown glutinous rice has a higher potassium content than other glutinous rice (Sulfianti *et al*., 2021). It can be seen that various kinds of natural resources encourage researchers to make brown glutinous rice as a growth medium (Aini & Rahayu, 2021).

The medium of brown glutinous rice contains high antioxidants acting as hydrogen donors. Brown glutinous rice also contains anthocyanins, carbohydrates and nutrients for growth *Trichoderma*. Brown glutinous rice (*Oryza sativa* L var glutinosa) has several advantages compared to PDAs, namely that it contains many phenolic compounds (Azis *et al*., 2015). According to Monika *et al*., 2014 Brown glutinous rice has a shelf life of six months at room temperature before being made into a medium, but each month there is a decrease in levels of bioactive compounds and antioxidant activity. In research conducted by researchers by making liquid medium from brown glutinous rice can maintain growth *Trichoderma asperellum* for 60 days. According to Muljowati, Safitri &; Purnomowati (2010) White glutinous rice flour with a long storage time of less than 8 weeks with a decrease in the number of spores. This study proves that with the medium of brown glutinous rice and the addition of concentration *Ecoenzyme* may increase the number of spores. Nutrition on *Ecoenzyme* with a concentration of 60% has

precise and optimal coordinate points for growth *Trichoderma asperellum*. If the content *Ecoenzyme* Too little or too much can be *toxic* Share Growth *Trichoderma asperellum*.

Conclusion

This study showed that the administration of *ecoenzyme* to *Trichoderma asperellum* in brown glutinous rice medium affected the growth and number of spores. A 60% *ecoenzyme concentration gives the highest average* spore count, while a 20% concentration has the lowest average. Brown glutinous rice-based medium provides growth-supporting conditions with diverse nutrient content. Giving *ecoenzyme* as an additional nutrient can increase the number of *Trichoderma asperellum spores*.

Acknowledgments

Thank you to the supervisor who has directed me in conducting research, spending time, energy, and mind in providing guidance, advice, and motivation in completing this research and article.

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