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Effectiveness Soaking Duration of Rice (*Oryza sativa* L.) Seed with *Trichoderma* sp. for Controlling Seed-Borne Pathogenic Fungi

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Abstract

The quality of the seeds can suffer a decrease caused by the attack of pathogenic fungi carried by the seeds both at the time of planting and storage, which will have an impact on the disruption of plant growth. Biopriming using Trichoderma sp. be an effort to control because it is antagonistic to pathogens. The success of biopriming is also influenced by the duration of seed priming. The study was conducted by providing seed soaking treatment using Trichoderma sp. suspension for 6 hours, 12 hours, and 24 hours with PDA medium incubation test and Growing on Test. The parameters tested are the percentage of infection rate and the percentage of germination. Identification results showed that pathogenic fungi carried by seeds included Rhizopus sp., Fusarium sp., Aspergillus sp., and Mucor sp. The results showed for incubation PDA medium, the duration of soaking rice seeds with Trichoderma sp. more effective in decreasing the infection rate is at a 12-hour. For growing on test, the duration of soaking rice seeds more effective in decreasing the infection rate is at a 24-hour. However, the duration of soaking rice seeds with Trichoderma sp. there was no noticeable difference for the percentage parameter of seed germination.

Keywords: *Biopriming, Infection Rate, Rice seeds, Seed-Borne Pathogenic Fungi, Trichoderma sp.*

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INTRODUCTION

Rice (*Oryza sativa* L.) is a very important food commodity in Indonesia, because it is the main source of food. Based on data from the Central Statistics Agency (2023), Indonesian rice production or rice consumption decreased in 2023 with an estimated amount of 30.90 million tons, while in 2022 rice production was 31.54 million tons.

The decline in rice production has not been able to meet the demand for higher consumption. Along with the increasing population of Indonesia, the demand for rice production also increased.

Decreased quality of rice seeds due to pathogenic fungal attack carried by seeds can be one of the reasons for reducing rice production. Seed borne pathogenic fungi can cause physical damage to seeds, decreased seed viability, and transmission or host pathogens when grown in the field. based on the statement of Harahap et al., (2015) that pathogenic infections carried by seeds can cause a decrease in viability, death of plant seeds, changes in chemical components, and cause a decrease in crop yields. Several studies have found pathogenic fungi carried by rice seeds, included: *Aspergilus* sp., *Fusarium* sp., *Alternaria* sp., *Mucor* sp., *Curvularia* sp., *Rhizopus* sp., *Penicilium* sp., *Pyricularia* sp., and *Alternaria* sp. (Ariyanto et al., 2021). Pathogenic fungi *Fusarium sp., Alternaria* sp., *Curvularia* sp., *dan Pyricularia* sp. included in the seed-borne pathogens, while the fungus *Mucor* sp., *Rhizopus* sp., *Aspergilus* sp., and *Penicilium* sp., including fungal contaminants in rice seed storage (Fatihah et al., 2022).

Biopriming using Biological Control Agents one of them *Trichoderma* sp. is one of the environmentally friendly control techniques that can be used to control seed pathogen infection. The purpose of seed biopriming is to increase seed growth, accelerate metabolic processes by balancing the water potential so that seeds can germinate faster (Hasanuddin et al., 2016). Addition of biological agents *Trichoderma* sp. during the seed priming process aims that antagonistic fungi can colonize the seed so that the growth of pathogens that infect the seed can be controlled. *Trichoderma* sp. has antagonistic properties against pathogenic fungi and fungal contaminants. Isolate *Trichoderma* sp. the origin of Jember soil has the potential as a biological agent because it has been able to reduce the intensity of disease in cocoa and chili seeds. Based on research by Rumandani (2022), soaking cocoa seeds with *Trichoderma* sp. for 3 hours with a spore density of 10^6 CFU /ml can reduce infection of *Phytophtora palmivores* up to 0%. Research conducted by Sari (2022) Isolate *Trichoderma* sp. obtained from the soil of Jember can suppress the intensity of Wilt chili plants caused by *R. solanacearum*. *Trichoderma* sp. the origin of Jember soil has a fairly high antagonistic ability both for soil borne and water borne pathogens, so it has the potential to be used as seed biopriming material with the aim of controlling seed-borne pathogens.

The success of biopriming techniques on seeds with the addition of biological control agents influenced by colony density and duration of immersion. Soaking duration that is too short can cause less than optimal APH ability, on the contrary, if it is too long, it will cause a negative influence on seed germination. Intensity of severity of rice leaf blight disease by 35% after seed soaked with *Trichoderma* sp. for 6 hours (Sandy, 2019). Soaking rice seeds with *Trichoderma* sp. for 24 hours can increased the number of *P. palmivores* infection by 18.71% (Sinay et al., 2022). Soaking rice seeds for 24 hours can reduce the intensity of blast disease up to 1% attack percentage rate, increase germination 96%, and vigor index 94% (Hidayat et al., 2014). This study aims to determine the effective duration of soaking rice seeds with *Trichoderma* sp. isolates from Jember soil as an effort to control seed-borne pathogens.

METHOD

Time and Place

This research was conducted in July to september 2024 at The Plant Health Laboratory and Greenhouse of the Faculty of Agriculture, Pembangunan Nasional "Veteran" Jawa Timur University, East Java.

Tools and Materials

Materials used in this study include: Potato Dextrose Agar media, Potato Dextrose Broth media, rice seed varieties Inpari 32 from Sukodadi district, Lamongan Regency, East Java, petri dish diameter 11 cm, tweezers, needle ose, bunsen, drill T, sterile soil, polybag, water, Isolat *Trichoderma* sp. from the land of Jember isolate collection of Dr. Ir. Arika Purnawati, MP.

Suspension of *Trichoderma* sp. & Seed Treatment

Suspension *Trichoderma* sp. with conidia density 10^6 spore/ml was made from adding 0.5 mm the disc of *Trichoderma* sp. colony wich cutting by cork borer in 150 ml potato dextrose broth media. Then the suspension is homogenized using vortex for 10 minutes. The calculation of spore density by taking 1 ml suspension to heamocytometer type nauberer. Rice seeds soaked with 50 ml suspension *Trichoderma* sp. with spore density 10^6 spore/ml each treatment soaking time (6 hours, 12 hours, and 24 hours). For negative control the seeds are soaked with sterile aquadest. While the positive control, rice seeds soaked with fungicide propineb 0.03 gr dissolved with 50 ml of water.

Incubation with PDA (*Potato Dextrose Agar*)

One of the methods of detection of seed-borne pathogens is using incubation with PDA agar media. The test was conducted by planting or placing rice seeds in a petri dish containing solid PDA medium. The placement of seeds is spaced from each other, so that when pathogens grow, they do not overlap each other. Based on research by Fatihah et al., (2022) Incubation of seeds on PDA medium was carried out for 7 days.

Growing on Test

Seed health testing method growing on test using sterile soil is intended to determine the symptoms of seed-borne fungi after germination. This method can detect pathogens that have a longer incubation period and are not detected in tests using PDA (Potato Dextrose Agar). Based on research by Sari et al., (2019), growing on test using sterile soil. The soil used for the test is pre-sterilized using 5% formalin. Sterilization is carried out by spraying, and covered for 7 days

Research Methods

This study used a one-factor randomized complete design (RAL). There are 3 treatments of long duration soaking rice seeds with *Trichoderma* sp., T1 (6-hours), T2 (12-hours), and T3 (24-hours). While T (control- sterile aquadest), and TF (control + fungicide propineb) only used as a comparison were not included in the experimental design. Each experiment was repeated 6 times, resulting in 18 experimental units for PDA incubation testing and 18 experimental units for Growing on Test.

Parameters

Identification of Seed Borne Pathogenic Fungi

Morphology of pathogenic fungi carried by rice seeds obtained from PDA incubation test was identified macroscopically and microscopically using binocular microscope olympus CX23. Macroscopic observation is done directly with the eye to observe the colony in the form of color and shape or texture of growth (Putu et al., 2018). Microscopic characteristics were observed based on the identification book Illustrated genera of imperfect fungi (Barnett & Hunter, 1972), by looking at the structure of hyphae and reproductive structure.

Infection Rate of Seed-Borne Patogenic Fungi

The calculation of the infection rate in the PDA test was carried out on the 7th day after incubation, while the growing on test was carried out on the 14th day after planting the seeds. Calculation of seed infection in the growing on test method is done by looking at the symptoms of infection. Based on research by Harahap et al., (2015) The symptoms of infection seed borne pathogenic fungi such as seeds do not grow, seeds overgrown with mycelium, rotten seeds, fallen seedlings, spots on the leaves of seedlings, dry seedlings, stunted, and dead. The Infection rate can be calculated using the following formula (Pujiarto et al., 2018):

$$\text{IT} = \frac{\sum Number\ of\ infected\ seed}{\sum Number\ of\ observed\ seed} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Percentage of Control Effectiveness

The control effectiveness can be calculated using the following formula (Supriati & Djaya, 2015) :

$$\text{E} = \frac{\text{Control infection rate} - \text{Treatment infection rate}}{\text{Control infection rate}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Effectiveness category are E > 69% (high effective), E = 50% - 69% (effective), E = 30% - 49% (moderately effective), and E < 30% (ineffective).

Rice Seed Viability

The calculation of the germination of sprouted seeds is carried out by counting seeds that germinate normally on the 5th and 7th day after seedlings. The percentage of germination is calculated according to the formula (Utami, 2018) :

$$IT = \frac{\sum KN1 + KNII}{\sum Planted\ seed} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Data Analysis

The data obtained were analyzed statistically using one way Analisis of Variance (ANOVA) at 5% ($P \leq 0.05$) and continued with Honestly Significant Different (HNJ) 5% test, calculation using software Statistic Package for the Social Science (SPSS) 24 version.

RESULT AND DISCUSSION

Identification of Rice Seed-Borne Pathogenic Fungi

Rhizopus sp.

The results of seed infection observation in the PDA incubation method (Figure 1a), showed an infection characterized by the growth of fungal mycelium on the surface of the seed, grayish with black spots on the surface of the mycelium. The mycelium of such mushrooms looks fibrous like cotton wool. Meanwhile, in microscopic observations (Figure 1b), the fungus is rhizoid with branched sporangiophores. Spores and their columns are spherical. Based on macroscopic and microscopic character, fungi that grow including the genus *Rhizopus* sp. The statement is in line with Sobianti et al., (2020), *Rhizopus* sp. it has white to gray colonies, its microscopic character is that it has rhizoids connected to sporangiophores, at the end of which there is a brownish sporangium, the columns are round and blackish brown.

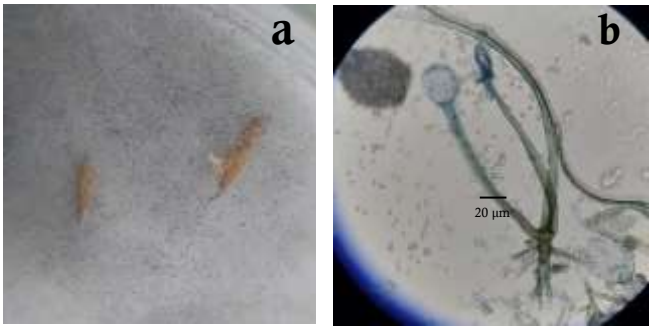

Figure 1. Morphology of *Rhizopus* sp. (a) Colony of *Rhizopus* sp. on seed surface (1 : 37 mm) (b) Microscopy of *Rhizopus* sp. (400x Magnificient)

Fusarium sp.

The results of seed infection observation (Figure 2a) showed the growth of colonies or fungal mycelium on the surface of the White seed with a texture that looks like Fine Cotton. But over time the color of the colony becomes slightly pink (Figure 2B). On microscopy observations, this fungus has macroconidia and microconidia. The microconidia are cylindrical or slightly rounded with a bulkhead inside. The macroconidia can be longer to curved, with a bulkhead inside. From the characteristics obtained, this fungus is included in the genus *Fusarium* sp. The statement is in line with Irmayanti (2023), the results of his research *Fusarium* sp.

identified in rice varieties Inpari 33 has a pink colony color with a texture like cotton. Based on the statement by Hassan & Chang (2022), the microconidia of *Fusarium* sp. are cylindrical to ellipsoidal, while the macroconidia are falcate with 1-5 septa.

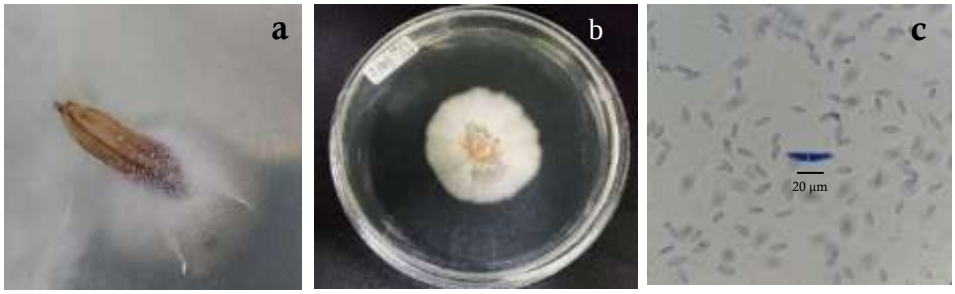

Figure 2. Morphology of *Fusarium* sp.
(a) Colony of *Fusarium* sp. on seed surface (1:37 mm); (b) Colony of *Fusarium* sp. on Potato Dextrose Agar Medium; (c) Microscopy of *Fusarium* sp. (400x Magnificient)

***Aspergillus* sp.**

The results of seed infection observation (Figure 3a) showed that the surface of the seed was enveloped by a colony of light green fungi with a powdery colony shape. While microscopically (Figure 3b), the fungus has a long conidiophore, vesicles and conidia are round. Based on the characters obtained, the fungus is *Aspergillus flavus*. The identification results are in line with the statement Zahara & Pamekas (2022), *Aspergillus flavus* has colonies that are light yellowish green, with granular and dense colony forms. Aspergillus flavus microscopically has a long, cylindrical conidiophore, and has vesicles and conidia of a spherical shape (Kapli et al., 2022).

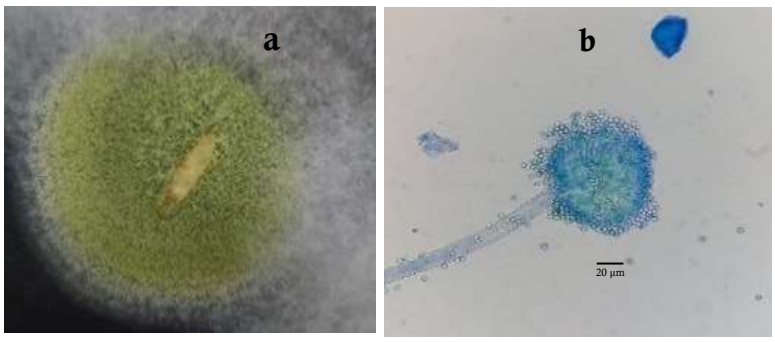

Figure 3. Morphology of *Aspergillus flavus*. (a) Colony of *Aspergillus flavus* on seed surface (1:37 mm) (b) Microscopy of *Aspergillus flavus* (400x Magnificient)

Other fungi have also been detected infecting the seed (Figure 3c), characterized by the growth of blackish-brown colonies on the surface of the seed. The growing colonies look round in shape, as well as the texture of the colonies is soft. Microscopically (Figure 3d), this fungus has an unbranched conidiophore, not septate, as well as hyaline. This fungus has vesicles or heads of spherical conidia, as well as conidia, which are also spherical. Based on these characteristics, this fungus belongs to the genus *Aspergillus* but the type *Aspergillus niger*. This is in line with the statement Sobianti et al., (2020) Colonies of Aspergillus niger fungi are round like granules, their

surface is soft and flat, and black. Microscopic observations showed *Aspergillus niger* has a round and black conidia, conidiophores about 400 - 3000 µm long, and not seperated.

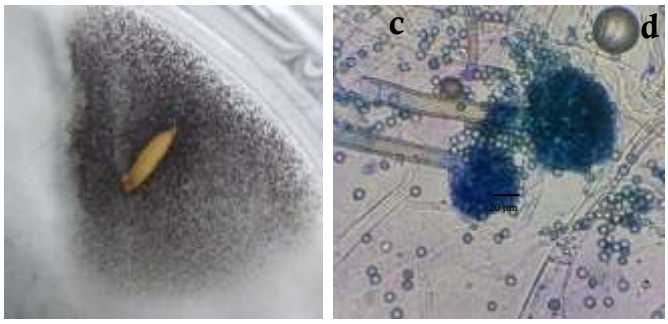

Figure 3. Morphology of *Aspergillus niger*. (c) Colony of *Aspergillus niger* on seed surface (1 : 37 mm) (d) Microscopy of *Aspergillus niger* (400x Magnificient)

***Mucor* sp.**

The results of macroscopic observations (Figure 4a) indicate the presence of seed infection, which is characterized by the growth of mycelium or colonies of white fungi on the surface of the seed. Colonies of these fungi have a rough and thick fiber-like texture. Microscopically (figure 4b) shows this fungus sporangiophores are branched, have Columella and spores are round. Based on these characteristics, this fungus belongs to the genus *Mucor* sp. The identification results are in line with the statement Adiwena et al., (2021), *Mucor* sp. it has a white colony color but will turn grayish, its texture is like thick fibrous cotton, and rapid growth. Microscopic observations show that the conidiophores are upright with branching and not septate, the columella and conidia are round.

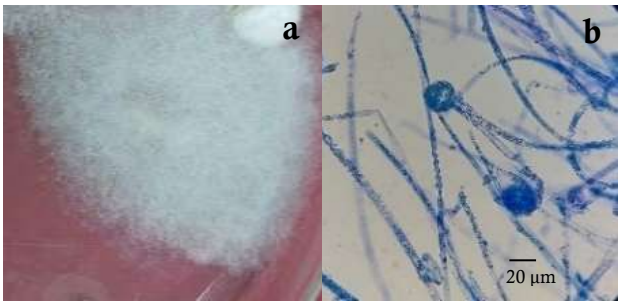

Figure 4. Morphology of *Mucor* sp. (a) Colony of *Mucor* sp. on seed surface (1 : 37 mm) (b) Microscopy of *Mucor* sp. (400x Magnificient)

Infection Rate and Control Effectiveness

Based on the analysis of variants (Table 1) shows the long duration of soaking rice seeds with *Trichoderma* sp. has a significantly different effect both on incubation testing using PDA media and Growing on Test methods. In the incubation method using PDA, rice seeds soaked *Trichoderma* sp. for 12 hours and 24 hours had a

significant difference in infection rates compared to rice seeds soaked for 6 hours. Rice seeds soaked *Trichoderma* sp. for 12 hours and 24 hours did not show any infection with a percentage of 0%, while at 6 hours of immersion the percentage of infection was 5%. Soaking rice seed with *Trichoderma* sp. for 12 hours have the best results compared to 6-hours soaking and control with highest percentage of effectiveness is 100 %.

In the growing on test method, rice seeds soaked for 24 hours have significant differences in infection rates compared to seeds soaked with *Trichoderma* sp. for 6 hour. However, 12-hours soaking of seeds has a less significant infection rate than the other two soaking durations. Soaking rice seed with *Trichoderma* sp. for 24 hours had the lowest percentage of infection rate of 8.33 %, while the 12-hours and 6-hours soaking percentage of infection rate of 25 % and 28.33 %. Soaking rice seed with Trichoderma sp. 24 hours had the best results compared to 6 hours, 12 hours, and control.

Table 1. Percentage Infection Rate of Seed-Borne Pathogenic Fungi

Treatment	Incubation with PDA		Growing on Test	
	IR (%)	E (%)	IR (%)	E (%)
T (Control Negative)	51.67	-	36.6	-
TF (Control Positive)	26.6	-	28.33	-
T1 (*Trichoderma* sp. 6 hours)	5.00 b	90.32	28.33 b	22.6
T2 (*Trichoderma* sp. 12 hours)	0.00 a	100	25.00 ab	31.69
T3 (*Trichoderma* sp. 24 hours)	0.00 a	100	8.33 a	77.24

Based on the results of the calculation of the infection rate, it can be seen that the treatment of long duration soaking rice seeds with *Trichoderma* sp. can lower the infection rate of seed-borne pathogens. This is suspected because the longer the duration of soaking the seeds with biological agents can provide more opportunities *Trichoderma* sp. to colonize the seeds. Based on research Alamsjah et al., (2023), viability test after rice seed biopriming treatment, colony count of *Trichoderma* sp. the obtained increases with the long duration of soaking. A longer duration of biopriming can help microbes more effectively colonize seeds. *Trichoderma* sp. able to reduce the rate of seed infection due to its antagonistic properties in the form of space and nutrient competition. The duration of seed soaking can affect the amount of colonization of *Trichoderma* sp. so there will be dominance in the competition of nutrients and growing space. In addition, another antagonistic trait produced is mycoparasitism. Based on the statement Loc et al., (2020), mycoparasitism *Trichoderma* sp. occurs by producing enzymes such as chitinase and glucanase which are produced 24 hours after inoculation.

Seed Viability

Based on the analysis of variants (Table 2), shows the long duration of soaking rice seeds with *Trichoderma* sp. no real difference between each treatment. However, the germination of seeds that have been treated with long soaking with *Trichoderma* sp. for 6 hours, 12 hours, and 24 hours showed a relatively high percentage of above 90 %, compared with sterile aquadest immersion control with lower

germination power. The percentage of germination in the seed soaking for 24 hours has the best results with a percentage of germination value of 98.33 %. This is because, with the immersion of *Trichoderma* sp. can stimulate the growth of rice seeds. *Trichoderma* sp. produce growth hormone to improve the growth performance of rice seedlings, as well as produce compounds that stimulate seed metabolism. This is in line with the statement Dalame et al., (2019) *Trichoderma* sp. produces phenolic compounds useful for improving seed viability. Growth hormone produced by *Trichoderma* sp. to stimulate the growth of shoots and roots is auxin and gibberellin. Based on the statement Qarni et al., (2021) *Trichoderma* sp. can produce auxin and gibberellin hormones that can improve the performance of rice seeds during germination.

Table 2. Percentage Germination Power Growing on Test Method

Treatment	Germination Power Growing on Test (%)
T (Control Negative)	75.00
TF (Control Positive)	85.00
T1 (*Trichoderma* sp. 6 hours)	90.00a
T2 (*Trichoderma* sp. 12 hours)	91.67a
T3 (*Trichoderma* sp. 24 hours)	98.33a

CONCLUSION

The results of the identification there are several kinds of pathogenic fungi carried by the seed when testing the incubation method using PDA media such as *Rhizopus* sp., *Fusarium* sp., *Aspergillus* sp., and *Mucor* sp. Results of rice seed soaking duration test on growing on test using *Trichoderma* sp. to reduce the infection rate is more effective at 24 hours with a percentage of 8.33 %. For incubation with potato dextrose Agar Medium Test, rice seed soaking duration using *Trichoderma* sp. to reduce the infection rate is more effective at 12 hours with a percentage of 0 %. Meanwhile, for the percentage of germination duration of soaking with *Trichoderma* sp. showed no noticeable difference between 6-hours, 12-hours, and 24-hours soaking.

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