

Etiology, Intensity, and Use of *Trichoderma* Spp. to Control Budok Disease in Patchouli Plants

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ABSTRACT

Patchouli (*Pogostemon cablin* Benth) began to be cultivated in 2020 in the Tompaso Baru region and then spread to several districts in North Sulawesi province, Indonesia. Since 2020, the area under patchouli cultivation has increased dramatically, estimated to already cover thousands of hectares. Budok disease has been found in several patchouli plantations in Tompaso Baru. Typically, a new disease in one area will quickly spread to other plantations because natural enemies are not yet familiar with it. This spread is accelerated because patchouli seedlings to be planted in other regions' plantations may already contain the budok pathogen. The objectives of this study were to calculate the incidence and severity of budok disease in the field, to identify the fungus that causes budok disease, and to obtain information on effective and efficient doses of *Trichoderma harzianum* in controlling the budok pathogen. The results of this study indicate that the incidence of the new infection process begins to occur in the second week after inoculation. Entering 21 days, the disease progression increased quite significantly, indicated by an increase in incidence in treatments 1 to 3 which reached 50%, while treatment 4 still showed the lowest incidence of 16.66%, and the control remained unaffected according to its function as a negative control. *Trichoderma* treatment did not have a significant effect

on the severity value of budok disease in patchouli plants ($p > 0.05$).

Keywords: Budok Disease, *Pogostemon cablin*, *Trichoderma harzianum*.

INTRODUCTION

Patchouli (*Pogostemon cablin* Benth) began cultivation in 2020 in Tompaso Baru, South Minahasa, Indonesia. It is now planted in surrounding areas such as Southeast Minahasa, Minahasa, Tomohon, North Minahasa, and Bitung. Since 2020, the area under patchouli cultivation has increased dramatically, already thousands of hectares. Patchouli was introduced to North Sulawesi from Java and Sumatra without quarantine, making it possible for the seedlings brought into our region to carry the budok pathogen caused by *Synchytrium pogostemonis*. Ecologically, a plant and pathogen introduced to a new area still need a long time to adapt and survive. This is especially true with monoculture planting patterns and the intensive use of synthetic fertilizers and pesticides. Budok disease has been found in several patchouli plantations in the Tompaso Baru area, and typically, a new disease in one area will quickly spread to other plantations because its natural enemies are unfamiliar. The spread of this disease is accelerating because patchouli seedlings destined for planting in gardens in other areas may already contain the budok pathogen (Enus, 2022).

The budok pathogen can survive in the form of spores and mycelia, surviving in dead patchouli tissue, zoospores, and possibly in the rhizosphere. Consequently, it is difficult to control with fungicides, especially those that survive in the soil. Fungi sprayed on the soil are neutralized by the physical, chemical, and microbiological properties of the soil (Rondonuwu, 2021).

Solutions for controlling budok disease include the introduction of natural control agents, such as the fungus *Trichoderma harzianum* (Hasari et al., 2018). This fungus can directly kill *S. pogostemonis* or pre-colonize plant parts, preventing the pathogen from reaching the plant. This indirectly impacts plant resistance because the relationship between *T. harzianum* and patchouli plants can activate dormant resistance genes, making them more susceptible to attack by the budok pathogen. Patchouli plants already produce these anti-pathogenic compounds.

Controlling new pathogens in newly introduced plants is difficult using current methods because the planting pattern is still monoculture and the use of fertilizers and pesticides is still haphazard or not environmentally friendly. One solution is to introduce natural enemies or natural controls of the budok pathogen, including *T. harzianum*. This study aims to calculate the incidence and severity of budok disease in the field, to find the cause of budok disease, and to obtain information on the effective and efficient dosage of *T. harzianum* to control the budok pathogen.

MATERIALS & METHODS

Disease Intensity Assessment

A disease incidence and severity survey was conducted on five plantations in the Tompaso Baru District. The plantations will be selected using purposive sampling, and the survey will be conducted only once. The following formula is used to calculate disease incidence:

$$IP = n \times 100\%$$

Description:

IP: Disease Incidence

n: Number of infected plants

N: Total number of plants observed

Calculation of the severity of budok disease

The severity of budok disease is calculated using the formula:

$$SP = \sum (v \times n) / N \times Z \times 100 \%$$

Description:

SP: Disease severity

v: Numerical value of each damage category

n: Number of patchouli trees in a damage category

N: Total number of patchouli trees observed

Z: Number of highest damage scales or categories

To determine the severity of the disease-causing pathogen, use the categories and descriptions of budok pathogens as shown in Table 1.

Table 1. Category and description of budok pathogen (Raharjo & Suhardi, 2008)

Score	Description
0	No infection (0%)
1	The area of the affected plant reaches 0–25.0%
2	The area of the affected plant reaches 25–50.0%
3	The area of the affected plant reaches 50–75.0%
4	The area of the affected plant reaches 75%

Experimental Design

This study used a randomized block design with 5 *Trichoderma spp.* dosage treatments and 6 replications, resulting in 30

experimental units. The experimental design was as follows:

Treatment P0 = 0 g *Trichoderma spp.* dosage/0.2 kg manure

Treatment P1 = 0.5 g *Trichoderma* spp. dosage/0.2 kg manure

Treatment P2 = 1.0 g *Trichoderma* spp. dosage/0.2 kg manure

Treatment P3 = 1.5 g *Trichoderma* spp. dosage/0.2 kg manure

Treatment P4 = 2.0 g *Trichoderma* spp. dosage/0.2 kg manure

The experimental units were patchouli plants in polybags. Each polybag contained 5 kg of growing medium with a composition of soil: sand: manure (1:1:1), resulting in 1.67 kg of manure per polybag.

Research Procedure

1. Sampling

Patchouli plant samples showing disease symptoms will be taken from patchouli plantations in Maluku Village, East Amurang District, South Minahasa Regency. Purposive sampling was used to collect the samples.

2. Planting Media Preparation

The planting media used in this study consisted of a mixture of soil, sand, and manure in a 1:1:1 ratio. The manure for the treatment was mixed with a powdered biocontrol agent, *Trichoderma* spp., from a commercial product under the brand name MARFU-P. The doses of *Trichoderma* spp. were applied according to the experimental design, namely 0.5 g, 1.0 g, 1.5 g, and 2.0 g for every 0.2 kg of manure. After mixing, the manure containing *Trichoderma* spp. was incubated for two weeks to allow the biological agents to develop properly. The prepared planting media was then placed in 35 x 35 cm planter bags and arranged in a greenhouse for more controlled environmental conditions.

3. Planting Patchouli Seedlings

Healthy patchouli seedlings, approximately one month old and 15–20 cm tall, were planted in planter bags filled with the appropriate treatment medium. During cultivation, the plants were watered twice daily, in the morning and evening, to

maintain humidity, and weeding was performed to prevent weed growth.

4. Preparing Pathogen Inoculum

Pathogen inoculum was obtained from patchouli leaves showing signs of budok collected from farmers' gardens in Sonder District. Twenty-five grams of collected leaves were weighed and washed with sterile water to remove any dirt. Next, the leaves were chopped into small pieces and ground in a sterilized blender, adding 250 ml of sterile water. The resulting mixture was then filtered through cheesecloth to obtain a filtrate suspension containing *S. pogostemonis* spores, ready for inoculation.

5. Pathogen Inoculation

Inoculation was performed on healthy, three-week-old patchouli plants. The surface of the plant leaves was first lightly wounded using a pin or fine needle to facilitate pathogen entry. Afterward, the inoculum suspension was sprayed evenly onto the leaf surface using a hand sprayer until thoroughly wet. The inoculated plants were then covered with transparent plastic for 24 hours to maintain humidity and support the pathogen infection process.

6. Application of *Trichoderma* spp.

The application of the *Trichoderma* spp. biological agent was carried out during the media preparation stage by mixing *Trichoderma* spp. powder from the MARFU-P product with manure according to the treatment dosage. Thus, *Trichoderma* spp. actively colonized the media from the beginning of patchouli growth. During the study, the plants were maintained without the addition of chemical fungicides, allowing the interaction between the pathogen, the plant, and *Trichoderma* spp. to be observed naturally without the influence of synthetic chemicals.

Observation Parameters

Observations were conducted weekly after the plants were inoculated with the pathogen until the end of the study. The main

parameters observed included disease incidence, disease severity, and the incubation period.

1. Incubation Period

Observations for the incubation period were conducted daily after planting until symptoms of the disease appeared on the lower leaves and base of the stem. Observations were conducted on all test plants.

2. Plant Growth

The effect of *Trichoderma spp.* fungus application on plant growth was determined by observing plant height, number of leaves, and stem diameter.

STATISTICAL ANALYSIS

Observation data were analyzed using SPSS version 21 with Analysis of Variance (ANOVA) at a 5% significance level. The results of this analysis were then used as a basis for drawing conclusions regarding the effectiveness of the biological control tested in the study.

RESULT AND DISCUSSION

Etiology

Infected leaves are characterized by the presence of warts on their abaxial or adaxial surfaces. Infected leaves are cut into pieces of about 1 cm each, which are then soaked in 1% sodium hypochlorite solution for one minute, rinsed with sterilized water, and finally incubated in sterilized water in bottles for two days. Under a dissecting microscope, slide preparations are made from softened leaf pieces and the slides are then observed under a compound microscope. The structure of the *Synchytrium* present is recorded, measured, and characterized. The existing fungal structures are categorized and named according to the system proposed by Karling (2024). The results of observations in the field and in the laboratory show that Budok Disease that attacks Patchouli plants in the field can show symptoms as shown in Figure 1 and microscopic observations can be seen in Figure 2.



Figure 1. Symptoms of budok pathogen attack on patchouli plants at the base of the roots (A) and on the branches (B)

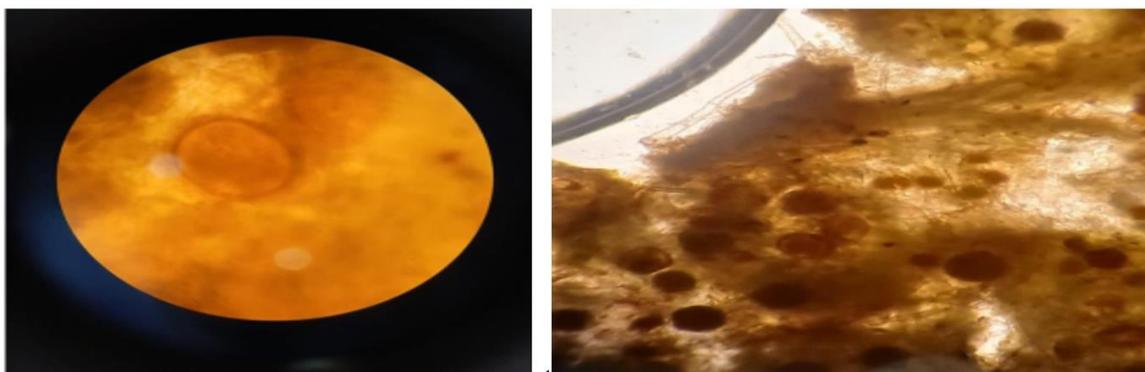


Figure 2. Microscopic observation of the budok disease pathogen on patchouli plants (200x magnification)

Incidence of Budok Disease in Patchouli Plants

Observations of the incidence of budok disease in patchouli plants over three

periods showed varying percentages of disease occurrence in each treatment. This can be seen in Table 2.

Table 2 Percentage of incidence of budok disease on patchouli plants caused by *S. pogostemonis*

Treatment	7 days	14 days	21 days
P0 (0 g <i>Trichoderma</i> + 0,2 g manure)	0%	0%	0%
P1 (0.5 g <i>Trichoderma</i> + 0,2 g manure)	0%	33,33%	50%
P2 (1.0 g <i>Trichoderma</i> + 0,2 g manure)	0%	33,33%	50%
P3 (1.5 g <i>Trichoderma</i> + 0,2 g manure)	0%	16,66%	50%
P4 (2.0 g <i>Trichoderma</i> + 0,2 g manure)	0%	0%	16,66%

Based on Table 2 above, the incidence of budok disease in plants began to appear 14 days after inoculation. The first symptoms appeared in treatments P1 and P2, with an incidence of 33.33% each, and in P3, at 16.66%. P0, the negative control, and P4 still showed 0% values. This indicates that the infection process only began in the second week after inoculation. Entering day 21, disease progression increased significantly, as indicated by an increase in incidence in P1, P2, and P3, reaching 50%. P4 maintained the lowest incidence at 16.66%, and P0 remained uninfected, serving as a negative control. This pattern indicates that the dynamics of pathogen infection increase over time, particularly in less effective treatments, while P4 had a better ability to suppress disease incidence until the final observation. Thus, treatment P4 has the greatest potential to inhibit disease spread compared to other treatments.

The results of the study, as shown in Table 2, also show that the application of *Trichoderma* and manure can affect the incidence of Budok disease in patchouli plants. In particular, the highest dose (P4: 2.0 g *Trichoderma* + 0.2 g manure) showed a relatively low incidence compared to the medium dose at 14 and 21 days. This pattern is in line with the understanding that *Trichoderma* spp. works as a biological agent that effectively suppresses pathogens through various mechanisms of space/nutrient competition, direct antagonism, and induction of systemic resistance in plants, thus not only inhibiting

pathogens but also increasing plant resistance (Sriwati et al., 2022). Furthermore, Marhama et al. (2021) stated that the results of research using *Trichoderma* spp. Pellet Suspension Formulation in Inhibiting the Fungus *S. pogostemonis* stated that the application of *Trichoderma* spp. in pellet form to patchouli seedlings was able to inhibit the pathogen that causes budok and significantly increase plant growth parameters such as plant height, number of leaves, and number of shoots. This supports your findings: that *Trichoderma* application not only has the potential to suppress the disease but also supports plant vigor, which is crucial for healthy and productive patchouli production. Furthermore, Liana et al. (2023) and Ruhama et al. (2020) stated that studies of antagonistic microbes (including *Trichoderma harzianum* and *T. asperellum*) and essential oils significantly reduced the severity of budok disease. The lowest reported severity was around 10%. These findings support the idea that integrating organic compounds (compost/manure), biological agents, and good agronomic practices can provide effective and environmentally friendly disease control, without relying on chemical fungicides.

The research results confirm that *Trichoderma* has a dual role: pathogen biocontrol and plant growth promotion through improved nutrient efficiency, resistance induction, and mutualistic interactions with plant roots. Therefore, high doses of *Trichoderma* and manure tend to reduce the incidence of budok,

conceptually consistent with the mechanism and effectiveness of *Trichoderma* on various host plants. Based on this synthesis, it can be concluded that the application of *Trichoderma* spp., especially in optimal formulations and doses, and integration with organic fertilizers or compost/manure, is a promising approach for controlling budok disease in patchouli. This biocontrol approach is not only effective in terms of reducing disease incidence, but also in line with the principles of sustainable and environmentally friendly agriculture.

Effectiveness of *Trichoderma* spp. Towards Suppressing the Development of

Budok Disease Pathogens in Patchouli Plants

Based on observation data on the effectiveness of *Trichoderma* spp. in suppressing the development of budok disease in this study, which is expressed in the average severity percentage. Symptoms of budok disease in patchouli plants that were first given the appropriate dose of *Trichoderma* spp., the severity of the budok disease was observed. In the first week of observation, there were no attacks. Attacks appeared during the second and third weeks after application. The results of the observations can be seen in Table 3.

Table 3. Analysis of variance of average percentage severity of budok disease in patchouli plants caused by *S. pogostemonis*

Treatment	7 days	14 days	21 days
P0 (0 g <i>Trichoderma</i> + 0,2 g manure)	0,00	0,000 a	0,00 a
P1 (0,5 g <i>Trichoderma</i> + 0,2 g manure)	0,00	0,33 a	1,23 a
P2 (1,0 g <i>Trichoderma</i> + 0,2 g manure)	0,00	1,62 a	4,89 a
P3 (1,5 g <i>Trichoderma</i> + 0,2 g manure)	0,00	2,31 a	7,29 a
P4 (2,0 g <i>Trichoderma</i> + 0,2 g manure)	0,00	3,71 a	8,76 a

*) Numbers followed by the same letter indicate no significant difference.

Table 3 shows the effect of *Trichoderma* spp. on the severity of budok disease in patchouli plants. Analysis of variance for *Trichoderma* spp. application at various doses did not significantly affect the severity of budok disease in patchouli plants. The treatment factor had an F-value of 0.601 with a p-value of 0.666, which was not statistically significant at the 5% level. The group factor also had no significant effect ($p = 0.819$). The R^2 value was 0.186, and the adjusted R^2 was even negative (-0.180), indicating that the statistical model was unable to adequately explain variations in severity.

Therefore, statistically, it can be concluded that the application of *Trichoderma* spp. at doses of 0.5–2.0 g did not cause significant changes in the development of budok disease severity compared to the control. Although not statistically significant, the data pattern indicates that at 7 days after infection, all treatments showed low virulence values to no visible symptoms.

Furthermore, at 14 and 21 days after infection, disease virulence increased in some treatments.

The treatment with the highest dose (2 g of *Trichoderma*) showed a trend toward lower virulence than the 1–1.5 g treatment, although this was not significant. This indicates that the patchouli plant's response to *S. pogostemonis* attack is rapid, while *Trichoderma* requires a longer colonization period to provide protection.

In relation to the results of this study, the causal factors did not significantly differ in the treatment effects due to the nature of the *S. pogostemonis* pathogen. Budok disease is a systemic disease that causes gall formation (swelling) in patchouli plant tissue. Boedijn (1934) stated that infection occurs in young tissue and rapidly progresses to internal tissues. This pathogen is difficult to control once the infection has already established because it resides within the tissue, so biological agents acting in the rhizosphere, such as *Trichoderma*, are unable to

optimally inhibit pathogen development. Furthermore, *Trichoderma* colonization takes time. According to Howell (2003) and Harman (2004), *Trichoderma* requires 2–3 weeks to develop in the rhizosphere, produce antibiosis metabolites, stimulate plant resistance, and parasitize the pathogen. Because observations were conducted weekly, the treatment may not have shown its full effect at the beginning of the infection.

In this study, 0.2 g of manure was used, a relatively low amount. Woo et al. (2014) and Benítez et al. (2004) stated that *Trichoderma* requires sufficient organic matter (organic carbon) to support its antagonistic activity. Low doses are thought to inhibit the activity of antagonistic microbes in the soil. These results align with several previous studies, stating that *Trichoderma*'s effectiveness is highly dependent on conditions. Harman (2004) and Vinale et al. (2008) explain that *Trichoderma*'s success is influenced by several key factors, such as humidity, soil pH, organic matter, competition from other microorganisms, and application time. They further explain that if any of these factors are suboptimal, its effectiveness in suppressing pathogens decreases.

Systemic pathogens are more difficult to control because several studies have shown that biological agents are more effective against necrotrophic pathogens such as *Fusarium* and *Rhizoctonia* (Howell, 2003; Yedidia et al., 2001). Conversely, biotrophic or obligate pathogens such as *Synchytrium* are more difficult to control with antagonists because they reside within the tissue. This explains why in this study, changing the *Trichoderma* dose did not significantly reduce virulence.

CONCLUSION

1. The incidence of the infection process only began to occur in the second week after inoculation. By day 21, disease progression had increased significantly, as evidenced by a 50% increase in incidence in treatments 1, 2, and 3.

Treatment 4 maintained the lowest incidence at 16.66%, while the control remained uninfected, serving as a negative control.

2. *Trichoderma* spp. treatment did not significantly impact the severity of budok disease in patchouli plants ($p > 0.05$).

Declaration by Authors

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