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The application of Trichoderma asperellum on various media types increases peanut stem rot's resistance (*Sclerotium rolfsii Sacc.*)

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Abstract
The peanut plant is a valuable commodity in West Aceh, with peatlands showing great potential for cultivation. However, peanut farming on peatlands faces challenges, particularly stem rot disease caused by Sclerotium rolfsii Sacc. Controlling this disease with synthetic fungicides raises environmental and sustainability concerns. Biological control offers an eco-friendly alternative by reducing fungicide use and providing lasting protection when environmental conditions favor the biological agents. This study employed a Factorial Completely Randomized Design with two factors: the type of Trichoderma asperellum media substrate (six levels: rice, bran, corn, rice + bran, rice + corn, and rice + bran + corn) and the dose of substrate (four levels: 25, 50, 75, and 100 grams per plant). Each treatment had three replications with three plants each. Results showed rice-based media and rice mixtures promoted the best growth of T. asperellum colonies. Substrate type did not affect the pathogenicity of S. rolfsii on peanut seeds in PDA agar media. Higher doses of T. asperellum (75 and 100 grams) across substrates significantly increased peanut resistance to S. rolfsii during germination. These findings endorse biological control using optimized substrate types and doses for sustainable stem rot management.
Keywords: *Resistance, Stem Rot, Trichoderma Asperellum, Sclerotium Rofsii, Peanut.*

Introduction

Peanuts (*Arachis hypogaea* L.) are one of the strategic food commodities in Indonesia, which have an important role as a source of vegetable protein, vegetable oil, and raw materials for the food and animal feed industries. This crop not only contributes to national food security but also serves as a source of income for smallholder farmers in various regions. Domestic demand for peanuts is relatively stable and even tends to increase with the development of the peanut-based processing industry. However, peanut productivity at the farmer level still faces various obstacles, especially attacks by soil-borne diseases, which can significantly reduce crop yields.

One of the main limiting factors is the attack of stem rot disease caused by the fungus *Sclerotium rolfsii* Sacc., a polyphagous pathogen that can attack many types of plants and

survives for a long time in the soil through the formation of sclerotia. S. rolfsii infection causes seedling wilt, stem rot, and even plant death (Punja, 1985).

Stem rot disease control efforts have largely relied on the use of chemical fungicides. While effective in the short term, fungicide use has several drawbacks, including the emergence of pathogen resistance, environmental pollution, and negative impacts on human health and non-target organisms. Furthermore, the cost of purchasing fungicides is relatively high and burdensome for smallholder farmers. Therefore, a more environmentally friendly, sustainable and economical control alternative is needed. One approach that is increasingly being developed is the use of biological control agents, particularly antagonistic fungi from the genus *Trichoderma* sp.

Trichoderma asperellum is one species that has proven effective as a biocontrol agent against various soil-borne pathogens. Several studies have shown that T. asperellum has antagonistic abilities through the mechanisms of nutritional competition, mycoparasitism, antibiosis, and the production of hydrolytic enzymes such as chitinase and glucanase, which damage the pathogen's cell walls (Harman et al., 2004; Vinale et al., 2008). The liquid formulation of *T. asperellum* MSU007 consistently suppressed the growth of *S. rolfsii*, even after 45 days of storage (Sutthisa et al., 2024). Meanwhile, it was also reported that Trichoderma spp. application can suppress disease incidence by up to 85.93% in tomatoes, approaching the effectiveness of chemical fungicides (Aljabali et al., 2025). Furthermore, other research also shows that T. asperellum can inhibit the growth of Rhizoctonia solani and other pathogens in rice with an effectiveness of up to 68.40% (Arianti, 2025).

The success of *Trichoderma* application as a biocontrol agent is greatly influenced by the planting media used for propagation and application. Cereal-based media, such as corn and rice, have been shown to support the growth and sporulation of *Trichoderma* spp. well due to their high carbohydrate and nutrient content. Rice husk also has the potential to be an alternative medium because of its cellulose and lignin content, which supports mycelium development (Darotin et al., 2024; Mufida et al., 2023). Differences in nutrient content and media texture can affect the viability, colonization, and antagonism of *Trichoderma* against

pathogens. Therefore, selecting the right media is a crucial factor in determining the effectiveness of biological control.

Based on the description, research on the use of *Trichoderma asperellum* in various types of planting media (corn, rice, and husks) to increase peanut resistance to *Sclerotium rolfsii* stem rot disease is of high urgency. This research is expected to provide scientific information on the best media for *Trichoderma* application, thus resulting in more efficient, affordable, and sustainable biological control technologies. The research results are also expected to support increased peanut productivity and contribute to the development of environmentally friendly and sustainability-oriented agricultural systems.

The purpose of this study was to determine the effectiveness of using Trichoderma asperellum in various types of planting media, namely corn, rice, and husks, in increasing the resistance of peanuts to stem rot disease caused by *Sclerotium rolfsii* Sacc.

Research Methods

This research is located at the University farm of Teuku Umar University. The materials used in this study include pathogen inoculum, namely *Sclerotium rolfsii*, Trichoderma asperellum inoculum from peat land, potato dextrose agar/PDA media, rice substrate media, bran, rice husks, and corn, dolomite, compost, alcohol, chlorine, aquadest, and other materials. The tools needed in this study are autoclaves, laminar airflow, magnetic hotplate stirrers, microscopes, a spirit lamp, an inoculating loop, and other tools. This study used a Factorial Completely Randomized Design using two factors. The first factor is the type of Trichoderma asperellum media substrate, consisting of six levels: rice substrate, bran substrate, corn substrate, rice + bran substrate, rice + corn substrate, and rice + bran + corn substrate. The second factor is the dose of Trichoderma asperellum media substrate consisting of four levels, namely 25 grams/plant, 50 grams/plant, 75 grams/plant, and 100 grams/plant. Each treatment consisted of 3 replications, each of which consisted of 3 plants.

Potato Dextrose Agar (PDA) media was prepared by mixing 10 grams of PDA powder with 250 ml of distilled water, then boiling it. It was then sterilized using an autoclave at 121°C for 30 minutes. It was then poured into 100 mm diameter Petri dishes and allowed to

set until the PDA media hardened. Subsequently, it was inoculated with pathogenic and antagonistic fungi.

The *S. rolfsii* inoculum was obtained from infected peanuts and then purified, then multiplied on sterile rice husk media until sclerotia formed. The antagonistic fungus, *T. asperellum*, was multiplied in sterile media substrate according to the treatment. The *T. asperellum* fungus was then incubated until the entire medium was filled with culture. The antagonistic fungal substrate was given to the plants according to the treatment dose.

a. The growth potential and germinability of peanut seeds
b. Colony growth and number of Trichoderma asperellum spores
c. damping off, including pre and post emergence.
a. Incubation period
b. Disease severity (Chiang et al., 2017)
c. Disease Incidence
d. yields.

Results and Discussion

Pathogenicity of Sclerotium rolfsii on peanut seed germination

The analysis of variance showed that the pathogenicity of *S. rolfsii* on the growth potential and germinability of peanut seeds in various media substrate solutions grown with *T. asperellum* was not significantly different (Table 1).

Table 1. Pathogenicity of *Sclerotium rolfsii* on the growth potential and germinability of peanut seeds in various media substrate solutions grown with *Trichoderma asperellum*.

Substrate Media	Growth Potential	Germinability
without substrate media	84.44 ns	67.78 ns
Rice	91.11 ns	66.67 ns
Corn	86.67 ns	61.11 ns
Bran	88.89 ns	64.81 ns

Rice + Corn	80.00 ns	62.96 ns
Rice + Bran	91.11 ns	66.67 ns
Rice + Corn + Bran	84.44 ns	64.81 ns

Table 1 shows that T. asperellum can grow well on various media, both PDA agar media and PDA agar media enriched with treatment substrates. This is because T. asperellum is not only antagonistic to *S. rolfsii* but also saprophytic, so it does not affect the type of media as long as the nutrients needed for the growth of *S. rolfsii* are sufficient.

Colony growth and number of spores of Trichoderma asperellum

Based on the analysis of variance, there are significant differences in the type of media substrate on the growth of colonies and the number of T. asperellum spores (Table 2).

Table 2. The effect of media substrate on colony growth and the number of spores of *T. asperellum*.

Substrate Media	Colony growth (%)			Spore Count
	1 Weeks	2 Weeks	3 Weeks	(x10^5/gram)
Rice	24 bc	58 c	98 ns	60.00 c
Corn	23 bc	50 abc	96 ns	56.33 b
Bran	17 a	44 a	90 ns	50.33 a
Rice + Corn	23 bc	56 bc	96 ns	58.33 b
Rice + Bran	19 ab	48 ab	90 ns	52.33 b
Rice + Corn + Bran	27 c	58 c	98 ns	61.00 c

[1] Numbers followed by the same letter in the same column are not significantly different at the 5% level (Duncan's test). Ns = non-significant

Table 2 shows that the Rice+Corn+Bran media gave the highest colony growth results one week after inoculation, but were not significantly different from the use of Rice, Corn, and Rice+Corn media substrates. Meanwhile, the lowest colony growth at 1 MSI was found in the bran media substrate treatment, which was not significantly different from the

Rice+Bran treatment. After 2 weeks of inoculation, the Rice and Rice+Corn+Bran media substrate treatments gave the highest T. asperellum colony growth results, while the lowest colony growth was also found in the bran media substrate. Three weeks after inoculation, the entire medium was almost filled with T. asperellum colonies, so there was no significant difference between colonies. Higher colony growth in Rice and Rice+Corn+Bran media was also followed by higher spore density than other media. The lowest spore density was also found in the bran media.

Damping Off

The results of the analysis of variance showed that there was a significant difference in the combination of the Dosage and Type of Trichoderma media substrate on the resistance of peanut plants in the germination phase to damping-off disease caused by *S. rolfsii* (Table 3)

Table 3. Average damping off of peanut seedlings induced by Trichoderma asperellum with various doses and types of growth media substrates.

Media Substrate Dosage	**Rice**	**Corn**	**Bran**	**Rice + Corn**	**Rice + Bran**	**Rice + Corn + Bran**
Pre-Emergence Damping Off						
25 g	35.42hij	29.86f-i	32.99g-j	19.88c-f	37.51ij	36.38ij
50 g	34.24g-j	23.84d-g	25.57e-h	42.85j	29.40f-i	29.38g-j
75 g	15.49b-e	14.13a-d	18.62cde	13.72a-d	16.85b-e	7.44ab
100 g	16.79b-e	24.78efg	9.50abc	7.43ab	11.01abc	4.73a
Post-Emergence Damping Off						
25 g	20.42efg	14.86c-f	17.99def	4.88a	22.51fg	21.38fg
50 g	19.24d-g	8.84abc	10.57a-d	27.85g	14.40b-f	14.38b-f
75 g	6.65abc	7.23abc	12.40a-e	5.34abc	5.18a	4.25a
100 g	5.03a	5.11a	5.92ab	4.80a	6.74abc	4.18a

			Total Damping Off			
25 g	55.84hi	44.73fgh	50.98gh	24.77hi	60.02hi	57.75a-e
50 g	53.47ghi	32.67def	36.15efg	70.69i	43.80fgh	43.75fgh
75 g	22.13a-e	21.36a-e	31.02c-f	19.07a-e	22.03a-e	11.69ab
100 g	21.83a-e	29.89b-f	15.43a-d	12.23abc	17.75a-e	8.91a

[1] numbers followed by the same letter indicate numbers that are not significantly different at the 5% level (Duncan's test).

Table 3 shows that induction of *T. asperellum* with 100 grams of rice+corn+bran media substrate can provide maximum resistance to peanut germination in the pre-emergence phase with a damping off percentage of 4.73%. In the post-emergence phase, the lowest damping off was found in the 100-gram substrate dose treatment on all types of substrates given, except for the bran and rice+bran substrates, which were slightly higher but not significantly different. Giving rice+bran and rice+corn+bran media substrates with a dose of 75 grams also increased peanut resistance in the post-emergence phase. Overall, peanut plants in the germination phase were more resistant to damping off caused by *S. rolfsii* if induced with T. asperellum with rice+corn+bran media substrates with a dose of 100 grams, which was not significantly different from other types of media at the same dose, except for corn media. Likewise, with a dose of 75 grams in all media substrate treatments, there was no significant difference with the rice + corn + bran media dose of 100 grams, except for the bran media.

Peanut plants' resistance to damping off caused by *S. rolfsii* can be induced using T. asperellum using a high dose of media substrate (75 or 100 grams). High biomass of T. asperellum can be obtained at a high dose because it provides sufficient nutrients and is available as a starter for its initial growth.

Pathogenicity to Peanut Plants

The results of the analysis of variance showed that the type of media substrate had a significant effect on the incubation period of *S. rolfsii* on peanuts, but did not have a

significant effect on the incidence of disease and the severity of disease caused by *S. rolfsii* (Table 4)

Table 4. The effect of *T. asperellum* media substrate type on the incubation period of *S. rolfsii* in peanut plants.

substrate media	Incubation Period (days)
Rice	41.25ab
Corn	33.42a
Bran	32.58a
Rice + Corn	32.92a
Rice + Bran	36.42ab
Rice + Corn + Bran	48.75b

[1] Numbers followed by the same letter indicate numbers that are not significantly different at the 5% level (Duncan's test).

Applying *T. asperellum* in rice+corn+bran media substrate can provide the best results in slowing down the incubation period of pathogens against peanut plants. Thus, it will allow plants to survive from *S. rolfsii* attacks so that pathogens find it challenging to infect peanut plants. In contrast, if T. asperellum is applied in corn, bran, or rice+corn media, the incubation period of pathogens against peanut plants becomes shorter. Table 5 below presents the effect of T. asperellum media substrate dose on *S. rolfsii* pathogenicity, including incubation period, disease incidence, and disease severity.

Table 5. Effect of *T. asperellum* substrate dose on incubation period, disease incidence, and disease severity caused by *S. rolfsii* in peanut plants.

substrate media	Incubation Period (days)	Disease Incidence (%)	Disease Severity (%)
25 g	18.67 a	5.79 b	3.27 b
50 g	23.28 a	5.79 b	2.97 b
75 g	54.28 b	1.55 a	1.31 a
100 g	54.00 b	1.84 a	1.47 a

[1] Numbers followed by the same letter in the same column indicate numbers that are not significantly different at the 5% level (Duncan's test).

Peanut Plant Growth

The analysis of variance showed that the activity of *S. rolfsii* as a cause of peanut stem rot disease did not significantly affect the growth of peanut plants if applied with the administration of *T. asperellum* on various types and doses of media substrates. There was no interaction between the type and dose of T. asperellum media substrate. The observed plant growth was plant height and stem base diameter.

Yields

Based on the results of the analysis of variance, the activity of *S. rolfsii* as the cause of peanut stem rot disease significantly affects the number of pods and the percentage of shriveled peanut seeds when applied with the provision of T. asperellum at various doses of media substrates, but has no significant effect on various types of media substrates, as well as no interaction between the two. For other production parameters such as pod weight, filled pods, seed weight, and bunch weight, there is no significant difference in the type of media substrate, media substrate dose, or interaction between the two (Table 6).

Table 6. The effect of T. asperellum media substrate dosage on the control of *S. rolfsii* on the number of pods and shriveled seeds of peanuts.

substrate media	Number of Pods	Shrunk Seeds (%)
25 g	103.00 b	19.88 a
50 g	100.22 ab	30.90 b
75 g	89.11 ab	25.67 ab
100 g	81.89 a	23.62 ab

[1] Numbers followed by the same letter in the same column indicate numbers that are not significantly different at the 5% level (Duncan's test).

The selection of a growth medium is crucial in determining the success of the cultivation process of microorganisms, including Trichoderma fungi. One of the most frequently used and proven effective media is rice-based media, whether in the form of whole rice, broken rice, or a mixture of rice and other organic materials. The main advantage of rice media lies in its complete nutritional content, particularly complex carbohydrates, protein, and several essential minerals that support optimal Trichoderma growth and development.

Rice media provides sufficient nutrients for Trichoderma growth, allowing this fungus to grow rapidly and produce large amounts of biomass. The resulting biomass is crucial, especially in the context of Trichoderma's application as a biological agent to control plant diseases or as a biofertilizer. The greater the biomass produced, the greater its effectiveness in agricultural applications (Haristia & Pribadi, 2021).

Furthermore, rice offers advantages in terms of availability and cost. As a staple food in many countries, including Indonesia, rice is readily available and relatively inexpensive compared to synthetic or chemical-based media. This makes rice an economical and practical choice for mass production of Trichoderma, both on a laboratory and industrial scale. Rice is a convenient substrate for mass multiplication of Trichoderma, because its texture supports colonization and even distribution of mycelium (Longa et al., 2009).

Using a mixed medium consisting of rice, corn, and bran significantly increased the growth of Trichoderma asperellum. This substrate combination provides complete and balanced nutrition, including carbohydrates from rice, protein from corn, and fiber and minerals from bran, synergistically supporting biomass formation and sporulation of T. asperellum. In the context of biological control of *Sclerotium rolfsii*, this increase in biomass is crucial because the greater the number of propagules (such as conidia and mycelium) available, the greater the ability of T. asperellum to compete with and suppress pathogens in the peanut root zone.

High doses of T. asperellum inoculation (75–100 grams) can trigger a systemic defense response in peanut plants. This response involves the activation of defense genes, increased production of enzymes such as peroxidase and polyphenol oxidase, and the regulation of plant hormones such as salicylic acid and jasmonate. As a result, plants showed increased

resistance to *S. rolfsii* infection, both in the pre-emergence and post-emergence phases (Wang et al., 2024). Rice bran is a substrate that strongly supports the growth and sporulation of this antagonistic fungus. This is important because rice bran is often used as a mixed media component. Although some treatments showed slightly higher damping-off rates, this was not statistically significant compared to other mixed media (Chahyunisa & Anhar, 2024). Application of Trichoderma metabolites significantly suppressed disease symptoms and increased plant vigor. Nutrient-rich substrates used in high doses can produce large amounts of bioactive metabolites, which are crucial in biological pathogen control (Abd El-Hai & Ali, 2019).

Trichoderma inoculation on peanuts suppressed disease symptoms and maintained stable vegetative plant growth. Plant height and stem diameter consistently increased, even under pathogen-induced stress. This indicates that Trichoderma functions not only as a biocontrol agent but also as a growth stimulant by activating plant hormones and increasing nutrient uptake efficiency (Wang et al., 2024). Biocontrol treatments against *S. rolfsii* did not inhibit peanut plant growth. Parameters such as plant height and stem diameter continued to increase significantly, indicating that the presence of the pathogen is not always negative if biological agents are used effectively (Chang et al., 2025).

The results of the study showed that the type of substrate and the dosage of Trichoderma asperellum substrate did not significantly affect the production parameters of peanut plants infected with *Sclerotium rolfsii*, such as pod weight, number of filled pods, seed weight, and bunch weight. This finding is in line with several previous studies showing that the effectiveness of biological agents in controlling diseases is not always directly proportional to increased yields. Although biocontrol agents could suppress the severity of the disease, no significant differences were found in pod weight or seed yield between treatments. Resistance to stem rot disease does not always correlate with yield components such as pod weight or seed number (Bera et al., 2016; Li et al., 2023). Even at the molecular level, it was found that the activation of defense pathways in resistant genotypes was not directly related to increased pod or seed biomass (Verma et al., 2025).

Conclusion

Rice-based media—including mixtures with corn and rice bran—provide complete nutrients that support the growth and sporulation of Trichoderma asperellum. High inoculation doses can trigger a systemic defense response in peanut plants, suppress disease symptoms, and enhance vegetative growth. However, the effectiveness of biocontrol does not always translate directly to increased yields.

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