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ENHANCEMENT OF PEANUT PLANT RESISTANCE TO DAMPING-OFF AND STEM ROT CAUSED BY *SCLEROTIUM ROLFSII* SACC. IN PEAT SOIL

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ABSTRACT

Peatlands have high agronomic potential for peanut cultivation but are susceptible to stem rot disease caused by *Sclerotium rolfsii*. The use of synthetic fungicides to control this pathogen poses ecological risks and is less effective in the long term due to the pathogen's ability to form sclerotia that are resistant to extreme conditions. As a sustainable alternative, biological control with antagonistic microorganisms such as *Trichoderma asperellum* and *Bacillus* sp. offers an environmentally friendly solution. This study aimed to evaluate the synergy between *Trichoderma asperellum*, *Bacillus* sp., and *Rhizobium* sp. in increasing peanut resistance to *S. rolfsii* in peat soil. The study used a Completely Randomized Design with eight treatments and three replications, analyzed using SPSS and Duncan's Multiple Range Test (DMRT) at the 5% significance level. The results showed that the application of a combination of biocontrol agents significantly reduced the incidence of damping-off, extended the incubation period, and reduced the incidence and severity of the disease, although it did not significantly affect the number of soil microbial colonies. The combination treatment of *Rhizobium* sp., *Trichoderma asperellum*, and *Bacillus* sp. provides the best results in suppressing pathogen attacks and increasing the resistance of peanut plants. The combined treatment yielded the best outcome in this pot experiment on peat soil, but results may vary under different environmental conditions. Field validation is needed to confirm consistency.

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INTRODUCTION

Peanuts are a leading commodity with specific characteristics in West Aceh Regency. Based on available data, peanut production in the region has reached approximately 2,000 tons per year, spread across four main sub-districts. The peanut market potential in West Aceh is promising, but the availability of raw materials is still unable to optimally meet market demand. Therefore, a more intensive cultivation development strategy is needed to increase production capacity and the sustainability of this commodity. West Aceh Regency is also recorded as the region with the largest peanut harvest area in Aceh Province, reaching 959 hectares. This position is followed by Nagan Raya Regency with a harvest area of 386 hectares (Statistics Indonesia, 2023). Peatlands have significant potential for development as peanut cultivation areas, primarily due to their physical properties, which allow them to retain water and organic nutrients. In Aceh Province, peatlands are spread across several districts/cities, covering a significant area of approximately 338,000 hectares (Ministry of Environment and Forestry, 2022). Utilizing peatlands for peanut cultivation in Aceh has the potential to significantly increase the production of this commodity. This not only supports local food security but also contributes to meeting regional and national peanut market demand.

Peanut cultivation on peatlands is not without its challenges, one of which is plant disease. One of the main diseases affecting peanuts is stem rot, caused by the soil pathogenic fungus *Sclerotium rolfsii* Sacc. This fungus is known to form colonies with white, cotton-like mycelium that are compact and dense. The resulting sclerotia have a dispersive germination pattern, resembling white cotton. Based on observations, the smallest colony diameter of *S. rolfsii* reaches approximately 0.61 cm, while the largest is approximately 1.71 cm (Magenda, 2011).

Controlling stem rot caused by *Sclerotium rolfsii* using synthetic fungicides often faces various limitations. Although chemical fungicides have been shown to suppress *S. rolfsii* growth both in vitro and in planta, their effectiveness is not always consistent across strains and environmental conditions (Das *et al*., 2024; Mishra *et al*., 2020). This is due to the pathogen's ability to form resting structures called sclerotia, which are watertight and resistant to extreme environmental conditions. These sclerotia can remain dormant in the soil and germinate again when conditions become favorable, making the use of fungicides ineffective in completely suppressing the pathogen inoculum. Furthermore, the continued use of synthetic pesticides has the potential to leave chemical residues in the soil and plants, which can pollute the environment and pose a risk to human health and non-target organisms (Hasna *et al*., 2022; Mishra *et al*., 2020).

Biological control is a strategic approach that is increasingly prioritized in sustainable agricultural systems, particularly to reduce dependence on synthetic fungicides. In addition to being environmentally friendly, biological control agents (BACs) have a sustainable protective effect as long as environmental conditions support their activity. In the context of peanut cultivation on peatlands, the use of local BACs should be prioritized because they have a higher level of adaptation to local soil characteristics and microclimates compared to introduced BACs. Several antagonistic microorganisms that have been identified as potential BACs in peat ecosystems include *Trichoderma asperellum* and *Bacillus* spp., which have been shown to suppress soil-borne pathogens and support plant growth through mechanisms of competition, antibiosis, and induction of systemic resistance (Guzmán-Guzmán *et al*., 2023; Mirsam *et al*., 2025).

The use of biological agents in plant disease control has long been practiced to reduce the use of synthetic fungicides. However, the effectiveness of inducing peanut plant resistance to stem rot disease in peatlands using indigenous biological agents is unknown, necessitating further research.

MATERIAL AND METHODS

The research was conducted in vitro at the Phytopathology Laboratory, Faculty of Agriculture, and in vivo in the greenhouse of the University Farm, Teuku Umar University, from June to September 2025. The goal was to establish a synergistic relationship between biological control agents and *Rhizobium* sp., thus developing inductive resistance in peanut plants.

The materials used included peanut seeds, a pathogen inoculum (*Sclerotium rolfsii*), an antagonistic fungal inoculum, and an antagonistic bacterium, namely *Trichoderma asperellum* from indigenous peatlands (Subandar *et al*., 2021a) and *Bacillus* sp. (Subandar *et al*., 2021b), *Rhizobium*, aquadest, alcohol, potato dextrose agar (PDA) media inoculum, nutrient agar (NA), planting media pots, peat soil media, dolomite, compost, alcohol, sodium hypochlorite, aquadest, 250 micron UV plastic, and other materials.

The tools needed in this research are a microscope, autoclave, oven, colony counter, haemacytometer, incubator, laminar air flow, spectrophotometer, hotplate magnetic stirrer, spirit lamp, needle, and other tools.

This study used a Completely Randomized Design with 8 treatments, namely no treatment (P0), Antagonistic Bacteria: *Bacillus* sp. (P1), Antagonistic Fungi: *Trichoderma asperellum* (P2), Antagonistic Fungi and Bacteria (P3), *Rhizobium* spp. (P4), *Rhizobium* spp. + antagonistic bacteria (P5), *Rhizobium* spp. + antagonistic fungi (P6), and *Rhizobium* spp. + antagonistic fungi + antagonistic bacteria (P7). Each treatment consisted of three replications, with four experimental pot units per replication.

The process of making Potato Dextrose Agar (PDA) media is by adding 10 grams of PDA powder to 250 ml of distilled water, then boiling it on a hotplate and using a magnetic stirrer so that the solution is stirred perfectly. After the PDA is ready, it is then sterilized in an autoclave at 121^{o}C for 30 minutes. Then poured into a petri dish with a diameter of 90 mm and left until the PDA media hardens. After the PDA media has hardened, it is ready to be inoculated with pathogenic or antagonistic fungi. The same treatment is also applied to NA media for inoculation of antagonistic bacteria.

The pathogenic fungus *S. rolfsii* was isolated from infested peanut soil using a baiting technique, which involves covering the infested soil to maintain moisture. The fungus was then grown on Potato Dextrose Agar (PDA)

media until a pure culture of *S. rolfsii* was obtained. The pure culture was then propagated on sterile rice husk media until sclerotia formed. The antagonist agent was obtained from previous research collections.

Before being mixed with the fungal culture medium, the peat soil was treated with dolomite, evenly mixed according to the treatment, for two weeks prior to the infestation of biological control agents. The peat soil was maintained at adequate moisture to prevent drying. The seedling medium consisted of pots with a diameter of 28 cm and a depth of 27.5 cm, each containing 10 planting holes per pot. Each replication comprised four pots, thus totaling 120 seedling holes per treatment. After the seedling period of 14 days, two plants were retained for further observation. Pathogen inoculation was carried out simultaneously with seed sowing, with each seedling hole receiving four pathogen sclerotia. Application of biological control agents was performed on the soil eight days before sowing.

Fungi of *Trichoderma* was applied to each pot at 50 grams of substrate in rice medium, with a colony density of 10^5 cfu/g of substrate. Soil moisture was maintained within the range of 70%–100% by watering in the morning and evening. During the germination phase, peanut seedlings were provided with 60% shading, which also served to stimulate the germination of pathogen sclerotia.

Bacteria of *Bacillus* sp. was cultured in Nutrient Broth (NB) medium and incubated on a rotary shaker for 24 hours at 28°C, achieving a cell density of 10^7CFU/mL before being applied at 50 mL per pot. *Rhizobium* sp. was initially cultured in Yeast Extract Mannitol Broth (YEMB) for 48 hours at 28°C to achieve a bacterial density of 10^8CFU/mL. This liquid culture was then inoculated into sterile compost medium and incubated for 28 days to establish a final carrier density of 10^6CFU/g of substrate, which was applied to each pot at 50 grams per pot.

Damping-Off

Damping off observations include pre-emergence damping-off, post-emergence damping-off, and total damping-off.

Incubation Period

Incubation period (days), calculated from the time the pathogen is inoculated into the plant until the plant shows the first symptoms.

Disease Incidence (%)

The incidence of disease is calculated using the formula KP = a/b x 100%, where, KP = Incidence of Disease, a = number of plants affected, and b = total number of plants.

Severity of Disease (%)

Disease severity (%), calculated using the formula (Chiang *et al*., 2017):

$$DS\% = \frac{\sum(class\ frequency\ x\ value\ of\ class\ rating)}{(total\ observations) \times (highest\ disease\ index)} \times 100$$

The scale index used to categorize attacks of disease severity uses a modified scale as follows. (Shokes *et al*., 1996):

0 = healthy plant,
1 = lesions on the stem until 25% of the plant shows symptoms (wilted, dead, or dying),
2 = 26-50% of the plant shows symptoms, and
3 = more than 50% of the plant shows symptoms.

Soil Microbial Activity (Colony Count)

The number of colonies was counted using the counting plate method. The plates selected for counting were Petri dishes with a colony count between 30 and 300. The number of colonies was calculated using the following formula. (Cappuccino and Sherman, 2014):

$$Colonies\ (\frac{cfu}{ml}) = \frac{comonies\ counted}{dilution\ factor\ \times inoculum\ volume}$$

RESULTS

Soil Chemical

The results of laboratory analysis of peat soil characteristics at the research site are summarized in Table 1.

Table 1. Results of laboratory analysis of peat soil chemical properties.

Elements of Analysis and Method	Unit	Basic Condition	After Liming
Soil Reaction			
pH (H_2O) (1:2.5) - Electrometric		4.11	5.65
pH (KCl) (1:2.5) - Electrometric		3.55	4.99
Organic C, Walkley & Black	%	34.53	33.47
Total N, Kjeldahl	%	1.17	1.18
Available P			
P Bray II (Bray II Extracted P)	mg kg^{-1}	26.85	24.10
Exchanged Ca	cmol kg^{-1}	3.74	19.23
Exchanged Mg	cmol kg^{-1}	0.40	0.41

Exchanged K	cmol kg^{-1}	0.23	0.31
Exchanged Na	cmol kg^{-1}	0.16	0.15
Cation Exchange Capacity (CEC)	cmol kg^{-1}	101.60	94.80
Base Saturation	%	4.46	21.20
Exchanged Al	cmol kg^{-1}	1.72	unmeasured
Exchanged H	cmol kg^{-1}	0.43	0.48
Electrical Conductivity (EC)	mS cm^{-1}	0.20	0.40

The analysis results showed that the peat soil at the research site had a very acidic reaction, with pH H_2O 4.11 and pH KCl 3.55. This condition is a major constraint in utilizing peatlands for agriculture. After liming, the pH increased significantly to 5.65 (H_2O) and 4.99 (KCl), thereby reducing acidity and improving soil fertility.

The organic carbon content was very high (34.53%), a typical characteristic of peat soils, though it slightly decreased to 33.47% after liming. Total nitrogen remained relatively stable (1.17–1.18%), indicating that liming did not significantly affect this element. Available phosphorus decreased slightly from 26.85 to 24.10 mg/kg, likely due to interaction with calcium from lime.

The most striking change occurred in base cations, especially calcium, which increased sharply from 3.74 to 19.23 cmol/kg. Potassium also rose from 0.23 to 0.31 cmol/kg, magnesium remained stable, while sodium slightly decreased. The cation exchange capacity (CEC) remained high, though it declined from 101.60 to 94.80 cmol/kg, showing that peat soil still has a strong ability to retain cations. Base saturation increased sharply from 4.46% to 21.20%, indicating improved soil fertility.

Exchangeable aluminum (1.72 cmol/kg) disappeared after liming, which is important since Al is toxic under acidic conditions. Exchangeable hydrogen remained relatively stable, while electrical conductivity increased from 0.20 to 0.40 mS/cm, indicating a rise in dissolved ions.

Analysis of variance results showed that the application of various types of biocontrol agents significantly affected the incidence of damping-off, the duration of the incubation period, the disease incidence rate, and the severity of the disease. However, these treatments did not significantly affect the number of soil microbial colonies.

Damping off

The analysis results showed that the application of the antagonistic fungus *Trichoderma asperellum* combined with *Bacillus*, *Rhizobium*, or a combination of both (*Bacillus* and *Rhizobium*) provided the highest effectiveness in suppressing damping-off events, both in the pre-emergence, post-emergence, and overall phases. In contrast, the application of a single biocontrol agent was not able to provide optimal results in controlling damping-off. The application of *Rhizobium* alone was not effective in suppressing *Sclerotium rolfsii* disease, even in the post-emergence and total emergence phases did not show significant differences compared to the control. However, if *Rhizobium* was applied simultaneously with antagonistic bacteria or fungi, there was a significant decrease in damping-off events. The application of *Bacillus* alone only provided mild suppression of damping-off and was not significant in the post-emergence or total emergence phases. The combination of *Bacillus* with *Rhizobium* showed a slight reduction in the incidence of damping-off, but the application of *Bacillus* with *Trichoderma asperellum*, or a combination of the three (*Trichoderma asperellum*, *Bacillus*, and *Rhizobium*), was significantly able to suppress damping-off due to *S. rolfsii* disease (Figure 1 & Table 2).



Figure 1. Germination of peanut under pre-emergence and post-emergence damping-off conditions.

Table 2. The effect of biocontrol agents on damping off of peanuts caused by *Sclerotium rolfsii.*

Biocontrol Agent Treatment	Pre-Emergence Damping off (%)	Post-Emergence Damping off (%)	Total Damping off (%)
Control	20.83 e	12.50 c	33.33 e
Antagonistic bacteria	15.83 d	11.67 bc	27.50 de
Antagonistic fungi	10.83 bc	8.33 abc	19.17 bc
Fungi + bacteria antagonist	7.50 ab	7.50 ab	15.00 ab
Rhizobium	16.67 d	11.67 bc	28.33 de
Rhizobium + Antagonistic bacteria	14.17 cd	10.00 abc	24.17 cd
Rhizobium + Antagonistic fungi	9.17 ab	7.50 ab	16.67 ab
Rhizobium + Antagonistic bacteria & Fungi	6.67 a	5.83 a	12.50 a

Numbers followed by the same letter in the same column do not show significant differences at the 5% level (DMRT 0.05).

Incubation Period

The application of *Trichoderma* indigenous fungi from peatlands has been shown to have a positive impact on extending the incubation period of the *Sclerotium rolfsii* pathogen in peanut plants (Table 3).

Table 3. The effect of biocontrol agent treatment on the incubation period of *Sclerotium rolfsii*.

Biocontrol Agent Treatment	Incubation Period (days)
Control	9.33 a
Antagonistic bacteria	14.00 a
Antagonistic fungi	35.00 b
Fungi + bacteria antagonist	35.00 b
Rhizobium	11.67 a
Rhizobium + Antagonistic bacteria	14.00 a
Rhizobium + Antagonistic fungi	35.00 b
Rhizobium + Antagonistic bacteria & Fungi	35.00 b

Numbers followed by the same letter in the same column do not show significant differences at the 5% level (DMRT 0.05).

All treatments involving *Trichoderma*, either alone or in combination with *Bacillus* sp., *Rhizobium*, or both, showed effectiveness in extending the pathogen's incubation period. Conversely, the application of *Bacillus* sp. alone had no significant effect on extending the incubation period. Similarly, the application of *Rhizobium* alone was unable to extend the pathogen's incubation period when not combined with *Trichoderma* (Figure 2).



Figure 2. Pathogen mycelial mass colonizing peanut plants at the early growth stage.

Disease Incidence

Stem rot disease in peanut plants caused by *Sclerotium rolfsii* was not detected in treatments using the antagonist fungus *Trichoderma asperellum*, whether applied alone or in combination with *Bacillus* sp., *Rhizobium*, or both. Conversely, the application of *Rhizobium* alone was not effective in suppressing the incidence of disease caused by *S. rolfsii*. Reduction in disease incidence only occurred when *Rhizobium* was combined with other biocontrol agents, such as *T. asperellum* or *Bacillus* sp. (Figure 3a, 3b).



Figure 3. Plant condition. (a) Condition of plants without treatment, (b) Condition of plants with Rhizobium + bacteria + *Trichoderma asperellum*.

Table 4. The effect of biocontrol agents on the incidence of stem rot disease in peanut caused by *Sclerotium rolfsii.*

Biocontrol Agent Treatment	Disease Incidence (%)			
	14 DAI	21 DAI	28 DAI	35 DAI
Control	25.00 c	33.33 c	41.67 c	29.17 c
Antagonistic bacteria	8.33 ab	8.33 ab	16.67 b	12.50 ab
Antagonistic fungi	0.00 a	4.17 ab	0.00 a	0.00 a
Fungi + bacteria antagonist	0.00 a	0.00 a	0.00 a	0.00 a
Rhizobium	16.67 bc	16.67 b	25.00 b	20.83 bc
Rhizobium + Antagonistic bacteria	12.50 b	12.50 ab	16.67 b	16.67 bc
Rhizobium + Antagonistic fungi	0.00 a	0.00 a	0.00 a	0.00 a
Rhizobium +bacteria + Fungi	0.00 a	0.00 a	0.00 a	0.00 a

DAI = Day after Inoculation

Numbers followed by the same letter in the same column do not show significant differences at the 5% level (DMRT 0.05).

Severity of Disease

The application of biocontrol agents has proven effective in reducing the severity of plant diseases. Specifically, the use of the antagonist fungus *Trichoderma asperellum* can eliminate disease symptoms, both when applied alone and in combination with *Bacillus* sp., *Rhizobium* sp., or both (*Bacillus* sp. and *Rhizobium* sp.) (Figure 4, Table 5).

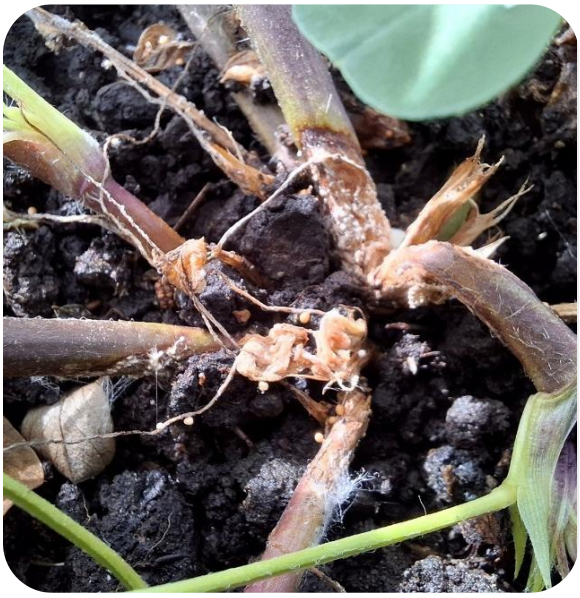


Figure 4. Lesions on peanut stems and the form of sclerotia.

Table 5. Effect of biocontrol agent treatment on the severity of peanut stem rot disease caused by *Sclerotium rolfsii*.

Biocontrol Agent Treatment	Severity of Disease (%)			
	14 DAI	21 DAI	28 DAI	35 DAI
Control	11.11 c	22.22 b	26.39 b	20.83 b
Antagonistic bacteria	2.78 ab	4.17 a	6.94 a	4.17 a
Antagonistic fungi	0.00 a	1.39 a	0.00 a	0.00 a
Fungi + bacteria antagonist	0.00 a	0.00 a	0.00 a	0.00 a
Rhizobium	5.56 b	6.94 a	9.72 a	6.94 a
Rhizobium + Antagonistic bacteria	4.17 ab	4.17 a	5.56 a	5.56 a
Rhizobium + Antagonistic fungi	0.00 a	0.00 a	0.00 a	0.00 a
Rhizobium +bacteria + Fungi	0.00 a	0.00 a	0.00 a	0.00 a

DAI = Day after Inoculation.

Numbers followed by the same letter in the same column do not show significant differences at the 5% level (DMRT 0.05).

DISCUSSION

Damping-off disease caused by *Sclerotium rolfsii* is a major challenge in crop cultivation, especially during the early growth phase. The use of biocontrol agents such as *Trichoderma asperellum*, *Bacillus* spp., and *Rhizobium* spp. has become an environmentally friendly alternative approach to reduce dependence on synthetic fungicides.

The fungus *Trichoderma asperellum* is known to possess potent antagonistic mechanisms against soilborne pathogens, including *S. rolfsii*. These mechanisms include competition for space and nutrients, mycoparasitism, production of pathogen cell wall-degrading enzymes (such as chitinase and glucanase), and secretion of antibiotic metabolites (Adielfina *et al*., 2021; Sutthisa *et al*., 2024). In vivo application of *Trichoderma* sp. can reduce damping-off events by up to 81.30% through the seed coating method (Adielfina *et al*., 2021).

Although *Bacillus* spp. has the ability to produce antibacterial and antifungal compounds such as surfactin and iturin, its single application only provides mild suppression of *S. rolfsii*. Similarly, *Rhizobium* spp., better known as nitrogen-fixing bacteria, has not shown significant effectiveness in controlling damping-off when used alone. (Guzmán-Guzmán *et al*., 2023; Sutthisa *et al*., 2024). However, the combination of the three showed strong synergy, with *Trichoderma* providing direct protection against pathogens, *Bacillus* enhancing antibiosis and increasing plant resistance, and *Rhizobium* improving nutritional status and supporting root growth. The combination of antagonistic microbes can significantly increase the effectiveness of disease control compared to single applications. (Vamshi *et al*., 2025).

Trichoderma spp., particularly indigenous isolates from peatlands, exhibit strong antagonistic potential against *S. rolfsii*. *Trichoderma harzianum* and *T. asperellum* are capable of producing lytic enzymes such as chitinase and β-1,3-glucanase, which play a role in inhibiting pathogen growth (Raja *et al*., 2023). This mechanism slows the infection process, thereby extending the pathogen's incubation period before symptoms appear. Furthermore, *Trichoderma* can trigger plant defense responses by increasing the expression of resistance-related genes and stabilizing plant cell walls by suppressing the expression of pectinesterase genes. (Wang *et al*., 2024). This effect strengthens plant resistance to pathogen penetration.

In biological control mechanisms, microorganisms such as *Trichoderma* and *Bacillus* utilize different bioactive compounds to suppress plant pathogens. Glucanase is a hydrolytic enzyme produced by antagonistic fungi such as *Trichoderma*. *Trichoderma* spp. produce cell wall–degrading enzymes such as chitinase and glucanase, which enable them to parasitize other fungi (Harman *et al*., 2004). In contrast, lipopeptides are secondary metabolites produced by bacteria, particularly *Bacillus subtilis* and *Bacillus amyloliquefaciens*. Lipopeptides act by disrupting the cell membrane of pathogens, exhibiting strong antifungal activity through interference with membrane integrity (Chowdhury *et al*., 2015). Thus, although both play roles in pathogen control, their modes of action differ: glucanase targets the fungal cell wall through enzymatic degradation, whereas lipopeptides target the cell membrane through chemical activity. The combination of these mechanisms can provide synergistic effects in controlling soil-borne pathogens such as *Sclerotium rolfsii*.

The combination of *Trichoderma* with *Bacillus* sp. or *Rhizobium* showed synergy in extending the incubation period of pathogens. *Bacillus* sp. is known to produce

antibacterial compounds and induce systemic resistance, while *Rhizobium* plays a role in nitrogen fixation and improving root health. However, the effectiveness of both in extending the incubation period was proven significant only when combined with *Trichoderma* (Cilliers *et al*., 2003; Wang *et al*., 2024). *Trichoderma* is a rapid colonizer in the rhizosphere. By utilizing root exudates, it dominates space and nutrient sources, thereby inhibiting the early colonization of *S. rolfsii*. This competition delays the formation of the pathogen's infective structures and prolongs the latent phase of the disease (Wang *et al*., 2024). The mechanism of mycoparasitism is a critical factor. *Trichoderma* produces lytic enzymes such as chitinase, β-1,3-glucanase, and protease that degrade the cell wall of *S. rolfsii* hyphae. This results in cytoplasmic degeneration and reduced pathogen viability, thereby slowing the transition from the latent to the aggressive phase (Vamshi *et al*., 2025). Root colonization by *Trichoderma* triggers systemic resistance in plants. Activation of plant growth hormones enhances the expression of defense-related genes and the production of phytoalexins. Consequently, pathogen penetration becomes more difficult and disease symptoms appear more slowly (Wang *et al*., 2024). *Trichoderma* also plays a role in modulating the soil microbial community. Its presence creates a rhizosphere environment less conducive to the development of *S. rolfsii*, suppresses pathogen populations, and provides an additional layer of protection for plants (Meena *et al*., 2023).

The application of *Bacillus* sp. or *Rhizobium* alone did not significantly affect the incubation period of *S. rolfsii*. This may be explained by the limitations of each microbe in producing lytic enzymes or triggering direct plant defense responses against necrotrophic pathogens like *S. rolfsii* (Raja *et al*., 2023; Wang *et al*., 2024). The combination of *T. asperellum* with *Bacillus* sp. or *Rhizobium* produces a synergistic effect in disease control. *Bacillus* species are known to synthesize fungistatic metabolites and to elicit induced systemic resistance (ISR) in host plants. (Vamshi *et al*., 2025). The application of Rhizobium alone has not been effective in suppressing the incidence of disease caused by *S. rolfsii*. This is due to *Rhizobium*'s limited ability to produce antifungal compounds or lytic enzymes that directly attack the pathogen. Reduction in disease incidence only occurs when *Rhizobium* is combined with *T. asperellum* or *Bacillus* sp., which have direct antagonistic mechanisms against the pathogen (Meena *et al*., 2024).

The combination of *Rhizobium*, *Trichoderma*, and *Bacillus* is more effective than single use. This synergy allows *Rhizobium* to continue functioning in nitrogen fixation, while *Trichoderma* and *Bacillus* help overcome environmental constraints typical of peat soils, such as low pH and high organic content. The use of *Rhizobium* as a single inoculant in peat soils is often ineffective. This is mainly due to the acidic nature of peat soils and their high organic matter content. Acidic conditions inhibit the activity of the nitrogenase enzyme required for nitrogen fixation, resulting in suboptimal root nodule formation. In addition, acidic soils usually contain high concentrations of aluminum and manganese, which are toxic to both symbiotic microbes and host plants (Bordeleau and Prévost, 1994). The high organic matter content of peat also produces phenolic compounds and organic acids. These compounds can suppress root colonization by *Rhizobium* and reduce the effectiveness of symbiosis. Consequently, although *Rhizobium* is able to associate with legume plants, its contribution to increasing soil nitrogen remains limited (Herridge *et al*., 2008).

In contrast, other microbes such as *Trichoderma* and *Bacillus* show higher tolerance to acidic conditions and can act as supporters of the peat ecosystem. *Trichoderma* functions as a lignocellulose decomposer, thereby helping to reduce the accumulation of inhibitory compounds and creating a more *favorable* environment for other microbes (Cavalcante *et al*., 2025; Zhu *et al*., 2022). Meanwhile, Bacillus is known as PGPR (plant growth-promoting rhizobacteria), capable of producing antibiotics, enzymes, and phytohormones, as well as *solubilizing* phosphate to increase nutrient availability for plants (Chowdhury *et al*., 2015; Kumar *et al*., 2011) .

CONCLUSIONS

The combination of *Rhizobium* spp., *Trichoderma asperellum*, and *Bacillus* sp. applied simultaneously to plants produced the best results in suppressing *S. rolfsii* infection while enhancing the resistance of peanut plants in peat soil. *Rhizobium* plays a role in nitrogen fixation, but its activity is inhibited by low pH and toxic compounds derived from organic matter. *Trichoderma* and *Bacillus* are able to overcome these constraints through lignocellulose decomposition, phosphate solubilization, and the production of antimicrobial metabolites, thereby supporting plant growth and microbial stability.

The best results from the combined treatment in this study apply specifically to the pot experiment conducted

on peat soil. Different environmental conditions may produce varying responses; therefore, field trials are required to ensure the consistency of these findings.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, I.S.; methodology, I.S.; software and formal analysis, I.S., C.C., and D.F.; investigation, I.S., C.C., and D.F.; writing original draft preparation, I.S.; writing review and editing, I.S., C.C., and D.F.; supervision, I.S.; project administration, I.S. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Adielfina, S., L. Sulistyowati, L. Q. Aini and A. Inayati. 2021. Antagonist test of endophytic fungus against pathogen *Sclerotium rolfsii* Sacc. causes of stem rot disease on peanut plants. Agrosainta, 6: 29-36.

Bordeleau, L. M. and D. Prévost. 1994. Nodulation and nitrogen fixation in extreme environments. Plant and Soil 161: 115-125.

Cappuccino, J. G. and N Sherman. 2014. Microbiology: a Laboratory Manual. Tenth edition. Pearson Education.USA.

Cavalcante, A. L. A., A. M. P. Negreiros, N. J. de A. Melo, F. J. Q. Santos, C. S. A. Soares Silva, P. S. L. Pinto, S. Khan, I. M. M. Sales and R. Júnior. 2025. Adaptability and sensitivity of *Trichoderma* spp. isolates to environmental factors and fungicides. Microorganisms, 13: 1689.

Chiang, K. S., H. I. Liu, and C. Bock. 2017. A discussion on disease severity index values. Part I: warning on inherent errors and suggestions to maximise accuracy. Annals of Applied Biology, 171: 139–154.

Chowdhury, S. P., A. Hartmann, X. Gao and R. Borriss. 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42–a review. Frontiers in Microbiology, 6: 780

Cilliers, A. J., Z. A. Pretorius and P.S. Van Wyk. 2003. Integrated control of *Sclerotium rolfsii* on groundnut in South Africa. Journal of Phytopathology, 151: 249-258.

Das, T., P. Maitra and M. S. Islam. 2024. Evaluation of chemicals fungicides and botanicals extract on the suppression of *Sclerotium rolfsii* causes wilt of sunflower (*Helianthus annuus* L.). Energy Environment & Economy, 2: 1-8.

Guzmán-Guzmán, P., A. Kumar, S. de Los Santos-Villalobos, F. I. Parra-Cota, M. del C. Orozco-Mosqueda, A. E. Fadiji, S. Hyder, O. O. Babalola and G. Santoyo. 2023. *Trichoderma* species: Our best fungal allies in the biocontrol of plant diseases—A review. Plants, 12: 432.

Harman, G. E., C. R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2: 43–56.

Hasna, M. K., J. Farthouse and K. M. E. Nabi. 2022. In vitro evaluation of fungicides and biocontrol agents against *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. Bangladesh Journal of Nuclear Agriculture, 36: 65-72.

Herridge, D. F., M. B. Peoples and R. M. Boddey. 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil, 311: 1-18.

Kumar, A., A. Prakash and B. N. Johri. 2011. Bacillus as PGPR in crop ecosystem, in: Bacteria in Agrobiology: Crop Ecosystems. Springer, Berlin, Germany. p. 37-59.

Magenda, S. 2011. Characteristics of *Sclerotium rolfsii* fungal isolates from peanut plants (*Arachis hypogaea* Linn.). Jurnal Bios Logos 1: 1-7.

Meena, P. N., A. K. Meena, R. K. Tiwari, M. K. Lal and R. Kumar. 2024. Biological control of stem rot of groundnut induced by *Sclerotium rolfsii* Sacc. Pathogens, 13: 632.

Meena P. K, R. S. Sharma and Y. Nain. 2023. Efficacy of bio-control agents against *Sclerotium rolfsii* causing collar rot disease of chickpea, under in vitro conditions. Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 25: 648-650.

Ministry of Environment and Forestry. 2022. National Peat Hydrological Unit . Ministry of Environment and Forestry. Jakarta. Indonesia.

Mirsam, H., Suriani, S. Kurniawati, O. D. Purwanto, Y. Nugraha, E. Uge, E. Yusnawan, M. F. Anshori and M. Azrai. 2025. Bioefficacy of *Trichoderma asperellum*

and P*enicillium raperi* filtrate culture consortium for suppressing bacterial stalk rot disease (*Dickeya zeae*) and improving the initial growth of corn seedlings. Egyptian Journal of Biological Pest Control, 35: 10.

Mishra, S., P. Mishra, R. Singh, J. Singh, A. Arya and A. Kumar. 2020. Screening of fungicides and bio-control agents against *Sclerotium rolfsii* (Sacc.) causing collar rot in Lentil. International Journal of Chemical Studies, 8: 2968-2973.

Raja, M., R. K. Sharma, P.P. Jambhulkar, R. T. P. Pandian and P. Sharma. 2023. Comparative evaluation of native *Trichoderma* species from groundnut rhizosphere against stem rot caused by *Sclerotium rolfsii* Sacc. Indian Phytopathology, 76: 459-471.

Shokes, F. M., K. Rozalski, D. W. Gorbet and D.A. Berger. 1996. Techniques for Inoculation of Peanut with *Sclerotium rolfsii* in the Greenhouse and Field. Peanut Science, 23: 124-128.

Statistics Indonesia. 2023. Planted Area, Crop Failure, Harvest, Production, and Productivity of Peanut Plants by Regency/City in Aceh Province. Aceh Central Statistics Agency. Aceh. Indonesia

Subandar, I., L. Hakim, I. Suliansyah and S. Syakur. 2021a. Screening of biological control agent fungi against peanut stem rot (*Sclerotium rolfsii* Sacc.) in the peatlands of Kuala Pesisir Nagan Raya, Aceh, Indonesia, in: IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 012007.

Subandar, I., L. Hakim, I. Suliansyah and S. Syakur. 2021b. Identification of antagonistic bacteria against peanut stem rot disease (*Sclerotium rolfsii* Sacc.) on the peatland of Kuala Pesisir-Nagan Raya, Indonesia, in: IOP Conference Series: Earth and Environmental Science. IOP Publishing, p. 012063.

Sutthisa, W., W. Hompana and R. Yutthasin. 2024. Enhancing biocontrol potential: Development and efficacy assessment of a liquid formulation of *Trichoderma asperellum* MSU007 against *Sclerotium rolfsii*. Trends in Sciences, 21(4): 7550.

Vamshi, J., G. U. Devi, B. Somraj, K. Supriya and H. K. Sudini. 2025. Biocontrol efficacy of *Trichoderma* and *Bacillus* isolates against *Sclerotium rolfsii* under in vitro conditions. Legume Research: An International Journal. 48(11): 1846

Wang, X., Z. Zhao, H. Li, Y. Wei, J. Hu, H. Yang, Y. Zhou and J. Li. 2024. The growth-promoting and disease-suppressing mechanisms of *Trichoderma* inoculation on peanut seedlings. Frontiers in Plant Science. 15: 1414193.

Zhu, L., X. Zhao, C. Wang, J. Wang, P. Wang and C. Tian. 2022. *Trichoderma* affects plant growth and soil ecological environment: a review. Zemdirbyste-Agriculture, 109(4): 341-348.

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