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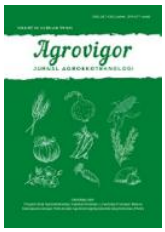

Article

Damping-off Defense: A Comparative Analysis of *Trichoderma* spp. (Hypocreales: Hypocreaceae) Isolates Against *Sclerotium rolfsii* (Polyporales: Atheliaceae) in Groundnut

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ABSTRACT

Arachis hypogaea L. (Fabales: Fabaceae), a vital *palawija* commodity in Indonesia, has seen declining production in Jawa Timur in recent years. Among agricultural challenges, biotic factors like pest and disease infestations significantly impact yield. Damping-off, caused by the fungal pathogen *Sclerotium rolfsii*, is a major disease impacting groundnut cultivation. Biocontrol agents offer a promising alternative to synthetic fungicides for managing plant pathogens. This study evaluated the effectiveness of various *Trichoderma* spp. isolates in controlling *S. rolfsii* damping-off disease in Bison groundnut variety based on four key parameters: incubation period, disease incidence and severity, and control efficacy. The results demonstrated that all tested *Trichoderma* spp. isolates had the ability to suppress damping-off disease. Notably, isolate TB01 exhibited the best performance significantly prolonging the pathogen's incubation period, reducing disease incidence and severity in both pre- and post-emergence phases, and demonstrating the highest control efficacy 47.95% "sufficiently capable". This study highlights the potential of *Trichoderma* spp. as a sustainable and eco-friendly biocontrol agent for managing *S. rolfsii* damping-off in groundnut cultivation. The successful application of isolate TB01 offers a promising approach for farmers seeking effective disease control strategies with minimal environmental impact.

INTRODUCTION

Arachis hypogaea L. (Fabales: Fabaceae) or groundnut is an important legume crop with a high economic value. It contains 25-30% protein, making it a good source of plant protein (Sastrahidayat, 2019). According to data from the Ministry of Agriculture of the Republic of Indonesia, (2022), groundnut production in East Java has declined for three consecutive years. In 2019, groundnut production decreased to 131,161 tons. This decline continued in the following years, with production falling to 124,784 tons in 2020. Groundnut production fell again in 2021 to 108,913 tons.

The decline in groundnut production can be attributed to a several of factors, including biotic and abiotic stresses. Damping-off disease is one of the major biotic diseases that can affect groundnut plants (Kalay et al., 2018). It is caused by the soil-borne pathogen *S. rolfsii* (Prasad et al., 2017). This pathogen can infect groundnut plants at the seedling and young plant stages. Symptoms of damping-off disease include wilting, yellowing, and rotting of the stem base (Billah et al., 2017). The stem base of infected plants may also have white mycelium and brown irregular spherical bodies called sclerotia (Hawaladar et al., 2022).

Infection by *S. rolfsii* can reduce groundnut production by 25-50%, with yield losses of up to 80% (Ma'ruf & Munif, 2020). This pathogen is difficult to control because sclerotia can survive in the soil for long periods of time (Bhamra & Borah, 2022). Chemical pesticides are the most common method used to control damping-off disease. However, the use of chemical pesticides can have a number of negative impacts, including environmental pollution, development of resistance in pathogens, and harm to beneficial microorganisms and soil fauna (Naufal & Purwantisari, 2020). Biological control using microbial antagonists is an alternative approach to controlling damping-off disease. (Anggraeni & Usman, 2015).

Trichoderma spp. is a type of microbial antagonist that has been shown to be effective in controlling disease in a variety of crops (Adnan et al., 2019). *Trichoderma* spp. can suppress the growth of pathogen through a number of mechanisms, including antibiosis, parasitism, and competition (Ayyandurai et al., 2021). Research on *Trichoderma* spp. has been widely carried on *Rhizoctonia solani*, *Fusarium oxysporum*, and *S. rolfsii* that can inhibit producing lytic enzymes such as chitinase, which can affect the cell membrane of the pathogenic fungi, causing the hyphae exocytosis (Elsharkawy et al., 2022; Kalay et al., 2018; Putri & Majid, 2019; Tantawizal & Rahayu, 2017).

Application of *Trichoderma* spp. can be done through a variety of methods, including seed treatment, foliar sprat, and soil application. Biological seed treatment is a method of seed treatment that uses beneficial microorganisms, such as fungi and bacteria, to protect seeds from pathogens (Lamichhane et al., 2022). One common method of biological seed treatment is to soak seeds in a microorganism solution. The active ingredients in the solution will adhere to the seed surface or be absorbed through the process of imbibition (Supriadi, 2018). The microorganisms that adhere to the seed surface will colonize the seed and protect it from pathogen (Wohel et al., 2022).

Based on the potential benefits of *Trichoderma* spp. and biological seed treatment. This study aims to systematically evaluate the effectiveness of different *Trichoderma* spp. isolates against *S. rolfsii* damping-off in Bison groundnut variety seeds using the seed treatment method. The findings of this research will contribute valuable knowledge towards implementing a sustainable and eco-friendly approach to managing *S. rolfsii* damping-off, potentially benefiting both farmers and the environment.

MATERIALS AND METHODS

The research was conducted at the Microbiology Laboratory of the Department of Biology Education and the Green House of the Faculty of Teacher Training and Education, University of Jember, from May to September 2023.

***S. rolfsii* isolate.** Fungal isolate was obtained from groundnut plants that showed symptoms of the disease in the field (Lat-8.167509º - Long 113.654628º). The groundnut plants were surface sterilized, cut 5-10 mm in size, inoculate on PDA media, and characterized (Mindarsusi et al., 2015)

***Trichoderma* spp. isolates.** Fungal isolates were obtained from four sources: Laboratory of Mycology, BBPPTP Surabaya (KN03); Laboratory of Biological Control, LPHP TPH Tanggul (BP01, JS02); Laboratory of Microbiology, Education Biology FKIP UNEJ (TB01); Exploration bamboo plantation in Sumbersari District, East Java, Indonesia (CN01). The isolates were renewed

on potato dextrose agar (PDA) media for 7 days and characteristics were observed.

Pathogenicity test. Five days before planting, pieces of PDA media that had been grown with *S. rolfsii* were placed on sterile potting media. Three groundnut seeds were planted in each polybag, with four replications. Observations were conducted until the plants showed symptoms of the disease.

Antagonism test. The antagonism test was conducted using a dual culture method. The method involves growing the *Trichoderma* spp. isolates and *S. rolfsii* on the same plate of PDA media.

***Trichoderma* spp. spore density.** The density of *Trichoderma* spp. spores can be calculated using a serial dilution method and observed with Hemocytometer Neubauer Improved type (Ruliyanti & Majid, 2020) under a microscope Olympus CX21LED.

Soil preparation. The soil used for the experiment was a mixture of soil and compost in a ratio of 2:1. The soil was sterilized using a steam sterilizer at 121°C for 20 minutes. The sterilized soil was then placed in trays (Febriyanti et al., 2015).

Selection and surface sterilization of groundnut Seeds. Groundnut seeds are selected for their quality and viability. The seeds are then surface sterilized to remove any pathogens or other contaminants (Temesgen & Chala, 2020).

Pathogen inoculation. The pathogen was inoculated 5 days before planting by placing colonies of the pathogen on the culture medium using a cork borer. Two pieces of culture medium containing *S. rolfsii* mycelium were placed in each planting hole in the seedling tray (Sofiani et al., 2016).

Seed treatment with suspension *Trichoderma* spp. isolates. The *Trichoderma* suspension was at a density of 10^6 spores/ml (Turnip et al., 2015; Wahdania et al., 2016). Seeds treatment with immersion for 30 minutes (Wulandari et al., 2022).

Groundnut planting and maintained. Each treatment was planted with 30 groundnut seeds, where 1 groundnut seed was planted in each hole of the seedling tray. The maintain includes watering and weeding.

Research design. The research design used in this study was a Completely Randomized Design (CRD) with 6 treatments and 4 replications. The treatments were used to soak the groundnut seeds. Treatment (P0) dH2O sterile/ control, (P1) KN03 isolate, (P2) BP01 isolate, (P3) JS02 isolate, (P4) TB01 isolate, (P5) CN01 isolate. Observed parameter are incubation period, disease incidence, disease severity, control efficacy.

Incubation period. The timeframe between planting and the appearance of disease symptoms, categorized as pre-emergence or post-emergence damping-off (Bedine Boat et al., 2020); **Disease Incidence.** The percentage of plants exhibiting pre-emergence or post-emergence damping-off symptoms (Bedine Boat et al., 2020); **Disease Severity.** The degree of disease symptoms on affected plants according to an equation Rajput et al., (2019), scored using a standardized scale by Muslim et al., (2014); **Control Efficacy.** The percentage reduction in disease severity compared to the control group without *Trichoderma* spp. application (Putri & Majid, 2019), with rating category: 0% not capable, 1-20% very poor, >20-40% less capable, >40-60% sufficiently capable, >60-80% capable, >80% very capable.

Statistical Analysis. Analysis of variance (ANOVA) was employed to identify statistically significant differences. If ANOVA indicated a significant effect, Duncan's Multiple Range Test (DMRT) was performed at the 95% confidence level.

RESULTS AND DISCUSSION

Sclerotium rolfsii as a pathogen of groundnut

Characteristic. *S. rolfsii* isolates were obtained from infected groundnut plants in the field (Figure 1.). The infected plant parts were isolated and observed for their macroscopic and microscopic morphology. The isolates were multiplied by inoculating sclerotia that grew on PDA media. Macroscopic observations showed that *S. rolfsii* had a white colony that resembled fine threads like cotton. The appearance of white dots on the surface of the colony that then turned brown to dark brown occurred on day 12 after inoculation. Sclerotia that were inoculated on PDA medium could germinate to produce mycelium through the sides of the sclerotia. Microscopic observations showed that *S. rolfsii* had hyaline hyphae and clamp connections. This is in accordance with the identification according to Hawaladar et al., (2022); Watanabe, (2002), that *S. rolfsii* has a white colony that is tightly arranged, sclerotia-shaped brown to dark brown that spread on the surface

of the colony, and hyphae with a clamp connection structure.

Pathogenicity. Pathogenicity tests showed that the fungus *S. rolfsii* was pathogenic to groundnut plants. *S. rolfsii* can cause symptoms in the pre-emergence and post-emergence phases. Symptoms in the pre-emergence phase are characterized by the presence of mycelium and sclerotia on the soil surface and groundnut seeds (Figure 2.) Infected seeds then rot and cannot germinate. Symptoms in the post-emergence phase are characterized by the wilting condition of the plants and the presence of mycelium and sclerotia around the base of the groundnut plant stem. In addition, brown lesions were found on the base of the plant stem. In addition, brown lesions were found at the base of the plant stem.

Groundnut seeds that were infected in the post-emergence phase at the base of the stem had brown lesions. These symptoms are in accordance with those described by Lamichhane et al., (2017), namely groundnut seeds that are infected in the pre-emergence phase will become soft and then rot, so they fail to germinate, and seeds that are infected in the post-emergence phase at the base of the plant stem have lesions and can be accompanied by stem rot, which causes plant growth to be inhibited.

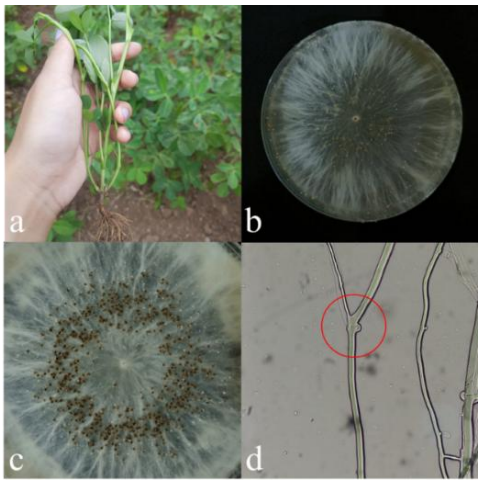

Figure 1. Characteristics of *S. rolfsii* fungus isolates. a) Symptomatic plants, b) Fungal colonies, c) Sclerotia that appear on fungal colonies, and d) Clamp connections (400× magnification).

Figure 2. Pathogenicity test of *S. rolfsii* on groundnut plants. a) Pre-emergence damping-off symptoms, b) Post-emergence damping-off symptoms, and d) Lesions at the base of the plant stem.

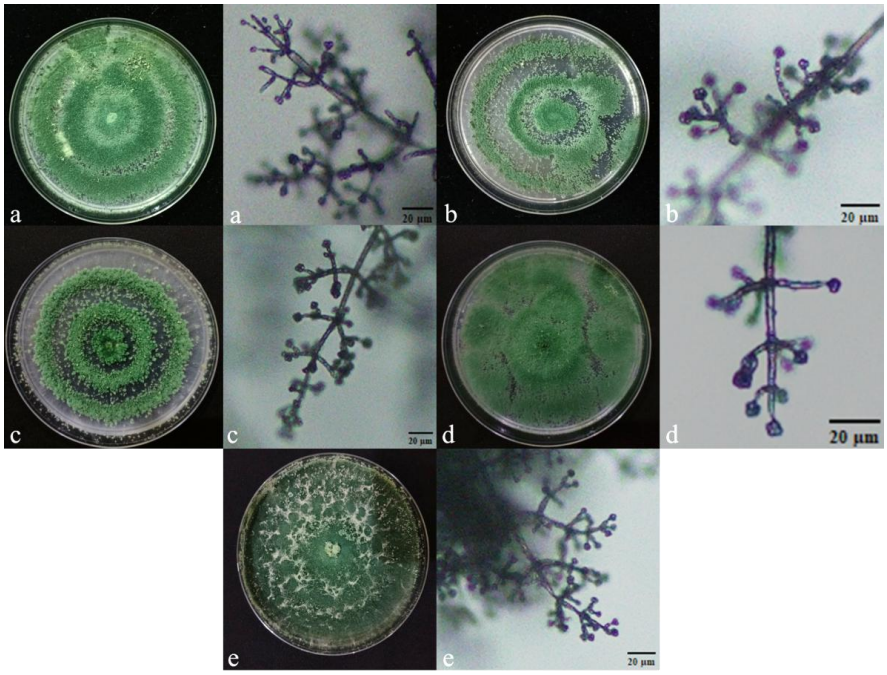

Figure 3. Morphological characteristics of *Trichoderma* spp. a) Isolate KN03, b) Isolate BP01, c) Isolate JS01, d) Isolate TB01, and e) Isolate CN01. (100x magnification)

Table 1. Morphological characteristics of *Trichoderma* spp isolates.

Trichoderma isolate	Colony	Conidiophore Branching	Phialide Form	Conidia Form	phialide size (µm)	Conidia Size (µm)
KN03	White to green is concentric and dense	Branching	Thin and pointy	Globose	7.22	3.45
BP01	White to green concentric	Branching	Thin and long	Globose	7.73	3.20
JS02	Green and concentric	Branching	Short thickened	Subglobose	8.16	3.35
TB01	Irregularly shaped green	Branching	Thin and long	Globose	6.90	3.29
CN01	White to dark green and dense	Branching	Short thickened	Globose	6.43	2.54

***Trichoderma* spp. as a biocontrol agent**

Characteristic. *Trichoderma* spp. isolates obtained from the collections and exploration were reisolated and observed for their macroscopic and microscopic morphology (Figure 3.). The macroscopic observations of each isolate showed that they had green colonies that formed concentric circles, spread, or were dense. Microscopic observations showed that each isolate had branched conidiophores, slender or short-thickened phialides, and subglobose and globose conidia that were located at the tips of phialides. This is in accordance with the morphological characteristics of *Trichoderma* spp. as described by Iqbal et al., (2022); Nurhayati et al., (2021), that *Trichoderma* spp. has a green colony that forms concentric circles, hyaline hyphae, pyramidal conidiophores with branched phialides, and green spherical conidia.

Isolate JS02 has a longer phialide size than the other isolates, which is 8.16 µm. Isolate KN03 has a larger conidial size than the other isolates, which is 3.45 µm. Isolate CN01 has a shorter phialide size and smaller conidia than the other isolates, which are 6.43 µm and 2.54 µm, respectively (Table 1). According to Kumar et al., (2020), *T. harzianum* has a phialide size of 7.2-11.2 µm and a conidial size of 2.8-3.1 µm, *T. asperellum* has a phialide size of 6.2-10.6 µm and a conidial size of 2.8-3.1 µm, and *T. virens* has a phialide size of 5.3-11.6 µm and a conidial size of 3.0-3.2 µm.

Antagonism in vitro. Five *Trichoderma* spp. isolates were found to have antagonistic capabilities against the pathogenic fungus *S. rolfsii* (Figure 4.). Based on the results of the study, all five *Trichoderma* spp. isolates were able to suppress the pathogenic fungus *S. rolfsii* in vitro (Table 2.). Treatment P4 (isolate TB01) had the highest inhibition value of 61.18%. The results of the observation of antagonistic mechanisms showed that *Trichoderma* spp. hyphae were seen attaching to and coiling around *S. rolfsii* hyphae (Figure 5.). The observations also showed that *Trichoderma* spp. could cause *S. rolfsii* hyphae to become abnormal (curled). This proves that *Trichoderma* spp. can suppress the pathogenic fungus *S. rolfsii* by the mycoparasite mechanism. The results of the observation of antagonistic mechanisms showed that *Trichoderma* spp. hyphae were seen attaching to and coiling around *S. rolfsii* hyphae. According to Bedine Boat et al., (2020), *Trichoderma* spp. can produce degrading enzymes that can destroy the cell wall of pathogenic fungi such as cellulase

Incubation period in vivo. The early symptoms of *S. rolfsii* infection in groundnut plants can be divided into two phases: pre-emergence and post-emergence (Figure 6.) The early symptoms that appear in the pre-emergence phase are the presence of mycelium and sclerotia grains on the plant seeds or soil surface, which can cause the seeds to rot. In the post-emergence phase, the symptoms are characterized by wilted plants with mycelium and sclerotia grains at the base of the plant stem. These symptoms are consistent with those described by Lamichhane et al., (2017).

The treatment of groundnut seeds with *Trichoderma* spp. did not have a significant effect on the pre-emergence phase. Treatment P0 (control) had the shortest incubation period of 6 days after sowing (DAS) in the post-emergence phase. The longest incubation period occurred in treatment P4 (isolate TB01), which was 12 DAS in the post-emergence phase (Table 3.). The longer incubation period of the pathogen was due to the ability of isolate TB01 to produce high spore density, which can protect groundnut plants from the pathogen. This is supported by the research of Erdiansyah & Anugerah, (2023), the results of a correlation between spore density and antagonistic activity of *Trichoderma* spp. showed that the higher the spore density of *Trichoderma* spp., the higher its antagonistic activity.

Disease Incidence

Pre-Emergence Damping-off. Seeds that are infected with pre-emergence damping-off are characterized by the presence of mycelium that envelops the surface of the seed (Figure 7.). The percentage of seeds infected with pre-emergence damping-off increased in all treatments over the 6 days of observation. Treatment P0 (control) showed the highest percentage of infected seeds, at 24.17%, and treatment P4 (isolate TB01) showed the lowest percentage of infected seeds, at 1.67% (Table 4.).

The incidence of disease in the pre-emergence phase in treatment P0 (control) had a higher percentage of infected seeds than the other treatments. The high percentage of infected seeds in treatment P0 (control) was due to the absence of *Trichoderma* spp. as the treatment, so the pathogenic fungi could more easily infect the plant seeds. (Table 4.)

Treatment P4 (isolate TB01) had the lowest percentage, this is thought to be because the spores carried by the seeds during the soaking time more quickly colonize the seeds so that the seeds can be protected from pathogen attacks in the soil. This is in accordance with the statement of Shams et al., (2023), that *Trichoderma* spp. can colonize the surface of the seed and release bioactive compounds around the seed to protect the seed from pathogens. The presence of infected seeds in each treatment is thought to be due to the small amount of *Trichoderma* spp. spores carried on the surface of the seeds during the soaking time. This is supported by the statement of Shofiyani & Budi, (2013), the number of *Trichoderma* spp. spores carried during soaking is less, so the seeds become not fully protected from pathogen attacks. Soaking seeds with *Trichoderma* spp. can also help plants to activate plant growth hormones so that plants can germinate faster (Mukhopadhyay & Kumar, 2020).

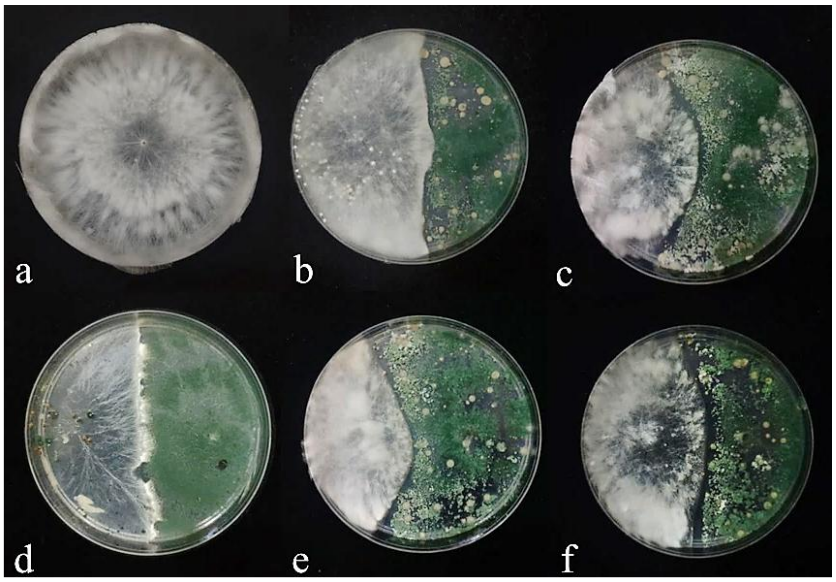

Figure 4. Inhibition test. a) Control, b) Isolate KN03, c) Isolate BP01, d) Isolate JS01, e) Isolate TB01, and f) Isolate CN01.

Table 2. Inhibitory power and spore density of *Trichoderma* sp isolates.

Treatment	Pathogen Colony Radius (cm)	Inhibitory (%)	Spore Density (10^8)
Control	4.25	-	-
P1	2.9	31.76 a	21.25 a
P2	2.5	41.18 b	26.33 a
P3	2.4	43.53 b	37.58 b
P4	1.65	61.18 c	38.58 b
P5	2.55	40.00 b	28.67 ab

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the Duncan test at the 95% confidence level.

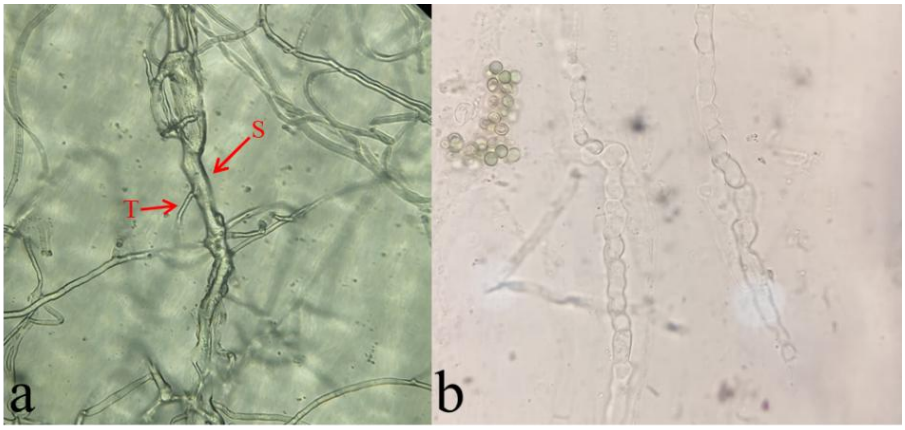

Figure 5. Antagonistic mechanism of *Trichoderma* spp. Isolate TB01 a) Winding of *S. rolfsii* hyphae by *Trichoderma* sp hyphae. and b) Abnormal *S. rolfsii* hyphae (400× magnification).

Figure 6. Early symptoms of *S. rolfsii* damping-off disease in groundnut plants. a) Pre-emergence, b) Post-emergence.

Figure 7. Symptoms of infected groundnut seeds in pre-emergence.

Figure 8. Symptoms of infected groundnut plants post-emergence

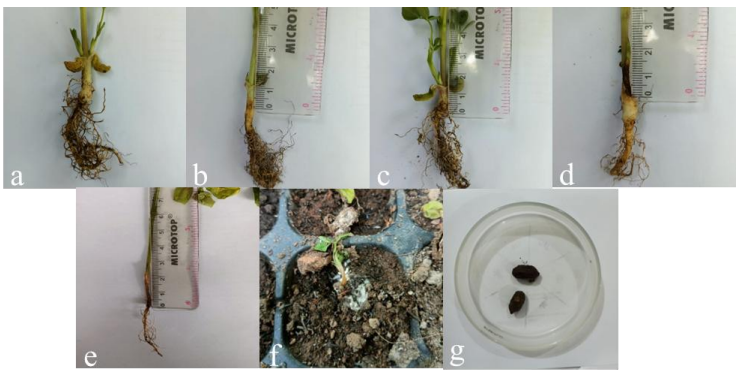

Figure 9. Symptoms of *S. rolfsii* damping-off disease in groundnut plants based on scoring values. a) Score 0, b) Score 1, c) Score 2, d) Score 3, e) Score 4, and f-g) Score 5.

Post-Emergence Damping-off. Seeds that are infected with post-emergence damping-off are characterized by the presence of mycelium and sclerotia clusters at the base of the plant stem (Figure 8.). The percentage of seeds infected with post-emergence damping-off increased in all treatments. Treatment P0 (control) showed the highest percentage of infected seeds, at 32.97%, and treatment P4 (isolate TB01) showed the lowest percentage of infected seeds, at 18.65% (Table 5.).

The incidence of disease in the post-emergence phase in treatment P0 (control) had a higher percentage of infected seeds than the other treatments (Table 5.). Treatment P4 (isolate TB01) had the lowest percentage of infected seeds. The low percentage of infected seeds compared to the control is thought to be due to *Trichoderma* spp. being able to compete antagonistically with the pathogenic fungus *S. rolfsii* in the soil. This is supported by the statement of Raja et al., (2023), *Trichoderma* spp. can colonize plant roots, which can reduce the growth of *S. rolfsii* by producing enzymes such as chitinase, β-1,3-glucanase, and protease.

Disease Severity. The calculation of the severity of damping-off disease is seen from the severity of the symptoms in each groundnut plant. The symptoms calculated by observing the development on plants using a scoring system (Figure 9). Disease severity continued to increase in all treatments. Each treatment showed different increases in disease severity. Treatment P0 (control) showed the highest disease severity value compared to other treatments, at 37.67%. The lowest disease severity was shown by treatment P4 (isolate TB01), at 12.63% (Table 6).

The severity of disease in the plants in treatment P0 (control) had a higher value than the other treatments. Treatment P4 (isolate TB01) had the lowest severity of disease. This is supported by the statement of Bedine Boat et al., (2020), that seed treatment with *Trichoderma* spp. can prevent plants from being infected and delay the onset of disease symptoms caused by pathogens.

Treatment P4 (isolate TB01) had the lowest severity of disease. This is supported by the statement of Bedine Boat et al., (2020), that seed treatment with *Trichoderma* spp. can prevent plants from being infected and delay the onset of disease symptoms caused by pathogens. The symptoms that appear on groundnut plants are caused by the hyphae of *S. rolfsii*, which produce cellulolytic and oxalic acid enzymes that can soften plant tissues, making it easier for the hyphae to penetrate the plant tissues (Masnilah et al., 2023).

Control Effectiveness. The application of *Trichoderma* spp. with the seed treatment method had different efficacy values for controlling damping-off disease (Table 7). Treatment P4 (isolate TB01) had an efficacy value of 47.95% for damping-off disease, which is classified as "sufficiently capable". Efficacy was obtained from the value of disease severity that was observed previously.

The difference in efficacy values for each *Trichoderma* spp. isolate can be caused by the growth ability and competitiveness of *Trichoderma* spp. against the pathogen *S. rolfsii*. This is supported by the statement of Karim et al., (2020), that one of the factors that can affect the ability of antagonistic microorganisms to control plant pathogens is having rapid growth so that they are able to compete.

CONCLUSION

Based on the results of this study, all isolates of *Trichoderma* spp. were able to suppress *S. rolfsii* damping-off disease in Bison groundnut variety. The best isolate obtained from this study was isolate TB01. Treatment of groundnut seeds with isolate TB01 was able to extend the incubation period of the pathogen by 6 days longer than the control, reduce the incidence of disease in the pre-emergence phase by 1.67% and in the post-emergence phase by 18.65%, reduce the disease severity by 12.63%, and the efficacy of control by 47.95% with the category "sufficiently capable".

Table 3. Incubation period of *S. rolfsii* damping-off disease on groundnut plants

Treatment	Incubation Period (DAS)	
	Pre-emergence	*Post-emergence*
P0	2a	6a
P1	3a	9abc
P2	3a	8ab
P3	5a	10bc
P4	5a	12c
P5	3a	9abc

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the Duncan test at the 95% confidence level.

Table 4. Percentage of infected seeds in pre-emergence damping-off at 6 DAS

Treatment	Percentage of Infected Seeds in Pre-emergence Damping-off (%)
P0	24.17c
P1	10.83b
P2	8.33ab
P3	4.17a
P4	1.67a
P5	6.67ab

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the Duncan test at the 95% confidence level.

Table 5. Percentage of infected seeds in post-emergence damping-off at 20 HST

Treatment	Percentage of Infected Seeds at Post-emergence Damping-off (%)
P0	32.97c
P1	24.34b
P2	23.68b
P3	21.69ab
P4	18.65a
P5	22.37b

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the Duncan test at the 95% confidence level.

Table 6. Severity of damping-off disease at 20 DAS

Treatment	Severity of Damping-off Disease (%)
P0	37.67c
P1	22.17b
P2	19.00b
P3	14.21a
P4	12.63a
P5	13.50a

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the Duncan test at the 95% confidence level.

Table 7. Assess the effectiveness of controlling damping-off disease

Treatment	Effectiveness Value (%)	Category
P1	35.05	Less capable
P2	28.83	Less capable
P3	39.21	Less capable
P4	47.95	Sufficiently capable
P5	38.68	Less capable

Note: Numbers followed by the same letter in the same column indicate that they are not significantly different in the Duncan test at the 95% confidence level.

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AUTHORS CONTRIBUTIONS

EC and RM considered as research concept and design. EC carried out the characteristic *Sclerotium rolfsii*, and pathogenicity. EC and ZFNH caried out the characteristic *Trichoderma* spp., antagonism in vitro, incubation period in vivo, disease incidence, disease severity, and control effectiveness. EC, and ZFNH perform data collection and/or assembly of data. EC, and RM perform data analysis and interpretation. RM perform critical revision of the article. EC, and ZFNH perform writing the article. RM perform final approval of the article. The authors provided responses and comments on the research flow, data analysis, and interpretation as well as the shape of the manuscript. All the authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adnan, M., Islam, W., Shabbir, A., Khan, K. A., Ghramh, H. A., Huang, Z., Chen, H. Y. H., & Lu, G. (2019). Plant defense against fungal pathogens by antagonistic fungi with Trichoderma in focus. *Microbial pathogenesis*, *129*, 7–18.

Anggraeni, D. N., & Usman, M. (2015). Uji Aktivitas Jamur Rhizosfer pada Tanah Perakaran Tanaman Pisang (Musa paradisiaca) Terhadap Jamur Fusarium. *Jurnal Biologi Lingkungan, Industri, dan Kesehatan*, *1*(2), 89–98.

Ayyandurai, M., Akila, R., Manonmani, K., Mareeswari, P., Mini, M. L., Kalpana, K., Kalaimathi, D., & Brindhadevi, S. (2021). Upregulation of defense enzymes in groundnut (Arachishypogaea L.) by combined application of Trichoderma spp. and Mahua oil cake against Sclerotium rolfsii. *The Pharma Innovation*, *10*(10), 1912–1916. https://doi.org/10.22271/tpi.2021.v10.i10aa.8469

Bedine Boat, M. A., Sameza, M. L., Iacomi, B., Tchameni, S. N., & Boyom, F. F. (2020). Screening, identification and evaluation of Trichoderma spp. for biocontrol potential of common bean damping-off pathogens. *Biocontrol Science and Technology*, *30*(3), 228–242. https://doi.org/10.1080/09583157.2019.1700909

Bhamra, G. K., & Borah, M. (2022). A review on collar rot disease of soybean caused by Sclerotium rolfsii sacc. *International Journal of Economic Plants*, *9*(3), 235–239.

Billah, K. M. M., Hossain, M. B., Prince, M. H., & Sumon, M. M. P. (2017). Pathogenicity of Sclerotium Rolfsii on Different Host, and Its over Wintering Survival; A Mini Review. *International Journal of Advances in Agriculture Sciences*, *2*(7), 1–6.

Elsharkawy, M. M., Kuno, S., Hyakumachi, M., Mostafa, Y. S., Alamri, S. A., & Alrumman, S. A. (2022). PCR-DGGE Analysis Proves the Suppression of Rhizoctonia and Sclerotium Root Rot Due to Successive Inoculations. *Journal of Fungi*, *8*(2), 0–11. https://doi.org/10.3390/jof8020133

Erdiansyah, I., & Anugerah, E. R. (2023). Karakteristik Trichoderma Harzianum Asal Tanah Latosol dan Sifat Antagonisnya Terhadap Penyakit Busuk Batang Kacang Tanah. *Proceedings: Penguatan*

Potensi Sumberdaya Lokal Guna Pertanian Masa Depan Berkelanjutan, 94–103.

Febriyanti, L. E. F., Martosudiro, M., & Hadiastono, T. (2015). Pengaruh Plant Growth Promoting Rhizobacteria (PGPR) Terhadap Infeksi Peanut Stripe Virus (PSTV), Pertumbuhan Dan Produksi Tanaman Kacang Tanah (Arachis Hypogaea L.) Varietas Gajah. *Jurnal Hama dan Penyakit Tumbuhan*, *3*(1), 84–92.

Hawaladar, S., Nandan, M., Vinaykumar, H. D., Hadimani, R. H., Hiremath, S., Venkataravanappa, V., Basha, C. R. J., & Reddy, C. N. L. (2022). Morphological and molecular characterization of Sclerotium rolfsii associated with stem rot disease of groundnut (Arachis hypogaea L.). *Indian Phytopathology*, *75*(1), 25–36. https://doi.org/10.1007/s42360-021-00419-y

Iqbal, S., Ashfaq, M., Malik, A. H., Inam-ul-Haq, M., & Khan, K. S. (2022). Morpho-Molecular Characterization of Trichoderma Isolates from Rhizospheric Soils of Vegetables in Pakistan. *International Journal of Phytopathology*, *11*(3), 253–266. https://doi.org/10.33687/phytopath.011.03.4309

Kalay, A. M., Talahaturuson, A., & Rumahlewang, W. (2018). Uji Antagonisme Trichoderma harzianum Dan Azotobacter chroococcum Terhadap Rhyzoctonia solani, Sclerotium rolfsii dan Fusarium oxysporum secara in-vitro. *Agrologia*, *7*(2), 71–78. https://doi.org/10.30598/a.v7i2.764

Karim, A., Rahmiati, & Fauziah, I. (2020). Isolasi dan Uji Antagonis Trichoderma Terhadap Fusarium oxysporum Secara In Vitro. *Jurnal Biosains*, *6*(1), 18–22. https://doi.org/https://doi.org/10.24114/jbio.v6i1.16839

Kumar, G., Singh, A., Pandey, S., Singh, J., Chauhan, S. S., & Srivastava, M. (2020). Morphomolecular Identification of Trichoderma sp . and their Mycoparasitic Activity Against Soil Borne Pathogens. *International Journal of Bio-resource and Stress Management*, *11*(6), 613–627.

Lamichhane, J. R., Corrales, D. C., & Soltani, E. (2022). Biological seed treatments promote crop establishment and yield: a global meta-analysis. *Agronomy for Sustainable Development*, *42*(3), 42–45. https://doi.org/10.1007/s13593-022-00761-z

Lamichhane, J. R., Dürr, C., Schwanck, A. A., Robin, M. H., Sarthou, J. P., Cellier, V., Messéan, A., & Aubertot, J. N. (2017). Integrated management of damping-off diseases. A review. In *Agronomy for Sustainable Development* (Vol. 37, Nomor 2). https://doi.org/10.1007/s13593-017-0417-y

Ma'ruf, K., & Munif, A. (2020). Potensi Bakteri Endofit Asal Brotowali (Tinospora crispa) sebagai Pengendali Sclerotium rolfsii dan Pemacu Pertumbuhan Kacang Tanah. *Jurnal Fitopatologi Indonesia*, *16*(3), 95–104. https://doi.org/10.14692/jfi.16.3.

Masnilah, R., Dewi, I. I., & Pradana, A. P. (2023). Antifungal Efficacy Of Piper Betle L. And Azadiractha indica A. Juss Leaves Extract In Controlling Damping-Off Diseases In Groundnut. *Pakistan Journal of Phytopathology*, *35*(1), 145–154.

Mindarsusi, V. A. P., Djauhari, S., & Cholil, A. (2015). Eksplorasi Jamur Endofit Daun Kacang Tanah Arachis hypogaea L. Dan Uji Antagonis Terhadap Patogen Scleretium rolfsii Sacc. *Jurnal HPT (Hama Penyakit Tumbuhan)*, *3*(3), 9–15.

Ministry of Agriculture of the Republic of Indonesia. (2022). *Statistik Pertanian 2022*. Pusat Data dan Sistem Informasi Pertanian Kementerian Pertanian Republik Indonesia.

Mukhopadhyay, R., & Kumar, D. (2020). Trichoderma: a beneficial antifungal agent and insights into its mechanism of biocontrol potential. *Egyptian Journal of Biological Pest Control*, *30*(1), 1–8.

Muslim, A., Palimanan, K., Hamidson, H., Salim, A., & Anwar, N. (2014). Evaluasi Trichoderma dalam Mengendalikan Penyakit Rebah Kecambah Tanaman Cabai. *Jurnal Fitopatologi Indonesia*, *10*(3), 73–80. https://doi.org/10.14692/jfi.10.3.73

Naufal, M. F. Q., & Purwantisari, S. (2020). Viabilitas Biofungisida Produk Lokal dan Aplikasinya untuk Penundaan Gejala Penyakit Hawar Daun Tanaman Kentang. *Jurnal Bioma*, *22*(2), 188–195.

Nurhayati, Y., Suryanti, S., & Wibowo, A. (2021). In Vitro Evaluation of Trichoderma asperellum Isolate UGM-LHAF against Rhizoctonia solani Causing Sheath Blight Disease of Rice. *Jurnal Perlindungan Tanaman Indonesia*, *25*(1), 64. https://doi.org/10.22146/jpti.65290

Prasad, M. R., Sagar, B. V., Devi, G. U., & Rao, S. R. K. (2017). n vitro Evaluation of Fungicides and Biocontrol Agents Against Damping Off Disease Caused by Sclerotium rolfsii on Tomato. *International Journal of Pure & Applied Bioscience*,

5(4), 1247–1257. https://doi.org/10.18782/2320-7051.5144

Putri, S. K., & Majid, A. (2019). Efektivitas Pelapisan Benih (Seed Coating) Berbahan Aktif Cendawan Antagonis Untuk Mengendalikan Penyakit Rebah Kecambah (Damping Off) Kacang Tanah. *Jurnal Pengendalian Hayati*, *2*(1), 23–33. https://doi.org/10.19184/jph.v2i1.17136

Raja, M., Sharma, R. K., Jambhulkar, P. P., Thava Prakasa Pandian, R., & Sharma, P. (2023). Comparative evaluation of native Trichoderma species from groundnut rhizosphere against stem rot caused by Sclerotium rolfsii Sacc. *Indian Phytopathology*, *76*(2), 459–471.

Sastrahidayat, I. R. (2019). *Penyakit pada Tanaman Kacang-Kacangan*. UB Press.

Shams, A. H. M., Helaly, A. A., Algeblawi, A. M., & Awad-Allah, E. F. A. (2023). Efficacy of Seed-Biopriming with Trichoderma spp. and Foliar Spraying of ZnO-Nanoparticles Induce Cherry Tomato Growth and Resistance to Fusarium Wilt Disease. *Plants*, *12*(17), 3117.

Shofiyani, A., & Budi, G. P. (2013). Spesies Unggul Trichoderma sp. Indigenus Rizosfir Pisang sebagai Pengendali Penyakit Layu Fussarium pada Bibit Tanaman Pisang Mas Hasil Kultur In Vitro. *AGRITECH*, *15*(2), 25–40.

Singh Rajput, R., Singh, P., Singh, J., Vaishnav, A., Ray, S., & Singh, H. (2019). Trichoderma mediated seed biopriming augments antioxidant and phenylpropanoid activities in tomato plant against Sclerotium rolfsii. *Journal of Pharmacognosy and Phytochemistry*, *8*(3), 2641–2647.

Sofiani, M., Djauhari, S., & Aini, L. Q. (2016). Pengaruh Aplikasi Plant Growth Promoting Rhizobacteria (PGPR) dalam Menghambat Penyakit Rebah Kecambah yang Disebabkan oleh Jamur Sclerotium rolfsii pada Kedelai. *Jurnal HPT*, *4*(1), 32–38.

Supriadi. (2018). Inovasi Perlakuan Benih Dan Implementasinya Untuk Memproduksi Benih Bermutu Tanaman Rempah Dan Obat. *Jurnal Penelitian dan Pengembangan Pertanian*, *37*(2), 71–80. https://doi.org/10.21082/jp3.v37n2.2018.p71-80

Tantawizal, & Rahayu, M. (2017). Insidensi penyakit layu Sclerotium rolfsii pada beberapa varietas kacang tanah dan aplikasi agens pengendali hayati. *Jurnal PRIMORDIA*, *13*(1), 24–28.

Temesgen, T., & Chala, B. (2020). Isolation and Identification of Fungi from Peanut Samples Sold at Adama City Markets, Oromia Regional State, Ethiopia. *Int. J. Sci. Res. in Biological Sciences Vol*, *7*(3).

Turnip, A., Efri, & Prasetyo, J. (2015). Pengaruh Perlakuan Benih Dengan Trichoderma viride dan Pseudomonas fluorescens TErhadap Keterjadian Penyakit Bulai (Peronosclerospora maydis) Pada Berbagai Varietas Jagung (Zea mays L.). *Jurnal Agrotek Tropika*, *3*(2), 216–219. https://doi.org/10.23960/jat.v3i2.2000

Wahdania, I., Asrul, & Rosmini. (2016). Uji Daya Hambat Aspergillus niger Pada Berbagai Bahan Pembawa Terhadap Phytopththora palmivora Penyebab Busuk Buah Kakao (Theobroma cacao L.). *J. Agrotekbis*, *4*(5), 521–529.

Watanabe, T. (2002). *Pictorial Atlas of Soil and Seed Fungi*. CRC Press LLC.

Wohel, C. M., Kalay, A. M., & Talahaturuson, A. (2022). Efek Perendaman Benih dengan Pupuk Hayati terhadap Pertumbuhan Bibit dan Serangan Penyakit Rebah Semai pada Tomat (Solanum lycopersicum). *Jurnal Agroekoteknologi*, *14*(1), 93–107. https://doi.org/10.33512/jur.agroekotetek.v14i1.15255

Wulandari, N. M., Yusnawan, E., & Widyaningsih, T. D. (2022). Biopriming and Biofertilizer of Trichoderma asperellum and Trichoderma virens on Cowpea Bioactive Compounds. *Jurnal Teknologi Pertanian*, *23*(3), 203–214.