BIOEDUSCIENCE
ISSN: 2614-1558
http://journal.uhamka.ac.id/index.php/bioeduscience

Potential *Trichoderma* sp. from Peat Soil in Controlling Seed-Borne Pathogens and Growth Stimulator in Soybean (*Glycine max L.*)

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Abstract

Background: Seed-borne pathogens threaten plants because they can survive in seed tissues for long periods. Efforts that can be made include treating the seeds before planting them in the field with biological agents, one of which is *Trichoderma* sp. It is often found in soil with high organic matter, namely peat soil. This research aims to determine the potential of *Trichoderm*a sp. isolates. from peat soil on pathogen infection of soybean seeds and germination of soybean seeds. **Method:** This research used a factorial method. The first factor was sick and healthy seeds, and the second was Trichoderma sp. isolates, consisting of TP1 and TP2. The control treatment uses the active ingredient fungicide mancozeb 80%. Seed treatment was carried out by soaking the seeds in a suspension of *Trichoderma* sp. with a spore density of 106 for 24 hours. The seeds were then tested using the growing-on-test method in sterile soil. **Results:** Identifying pathogens in soybean seeds found two genera, *Aspergillus* sp., and Fusarium sp., in the immersion treatment on *Trichoderma* sp. isolates. TP2 gave the best results, where the lowest infection power was 46.6% for healthy seeds and 73.3% for symptomatic seeds. In addition, treatment with isolates of *Trichoderma* sp. TP2 can increase the germination capacity of soybean seeds by 60.0%. However, seed treatment with *Trichoderma* sp. does not affect soybean plant height and root length growth. **Conclusion:** The two isolates of *Trichoderma* sp. from peat soil positively protect seeds against soybean seed pathogens, seed germination, and soybean growth.

Keywords: Pathogens; Seed; Treatment; *Trichoderma* sp.

Article history
Received: 08 Mar 2024
Accepted: 12 Dec 2024
Published: 31 Dec 2024

Citation:
Diasi et al., 2024. Potential *Trichoderma* sp. from Peat Soil in Controlling Seed-Borne Pathogens and Growth Stimulator in Soybean (*Glycine max* L.). BIOEDUSCIENCE, 8(3), 41-47. doi: 10.22236/jbes/14563

Introduction

Soybean (*Glycine max* L.) is an essential crop in Indonesia and is the third food crop after rice and corn. The content of soybeans consists of 40% protein, 20% oil, 35% carbohydrates, and 5% ash (Krisnawati, 2017). In 2025, soybean consumption in Indonesia will reach 3.35 million tons or an increase of 19% per year over the 2009-2025 period (BPS, 2019), explains that in the 2014-2018 period, there were fluctuations in soybean productivity in Indonesia, where soybean production in Indonesia only met around 33% of needs, the rest of domestic needs were met through imports.

The problem with soybean production in Indonesia is that it is estimated to continue to decline due to the lack of Availability of quality seeds. The cause is the presence of seed-borne pathogens. According to Rahayu (2018), pathogens occupy different positions in the seed, namely attaching, entering the seed or seed fragment, and penetrating the embryo. Pathogens in soybean seeds are dominated by fungal groups, including Alternaria sp., *Colletotrichum* sp., and *Cercospora kikuchii* (Hapsari, 2022). Seed-borne fungi identified from soybean seeds are *Cescospora* sp., *Fusarium* sp., and *Collectotrichum* sp. (Wain-Tassi et al., 2012). Ramesh et al. (2013) identified the most critical fungi on soybean seeds as *Aspergillus flavus*, *Aspergillus niger*, *Phoma* sp., *Fusarium oxyporium*.

Pathogenic fungi in the seeds can be the initial inoculation of pathogens in the field, which causes the seeds to experience a decline in production. To overcome this, farmers often use fungicides. However, treating seeds with fungicides can reduce germination, this is because the effect of treating seeds with fungicides is influenced by the condition of the seeds, which has an impact on the phytotoxicity of the fungicide used (Tanzil & Purnomo, 2021). Efforts to minimize the use of fungicides on seeds are carried out continuously, namely by using biological agents, one of which is the fungus *Trichoderma* sp. *Trichoderma* sp is used as a biological agent capable of controlling seed-borne pathogens and as a plant growth stimulator.

Use of Trichoderma sp. Many things have been done to control pathogens, including Trichoderma sp. Able to control pathogens both in vivo and in vitro. In vitro, Trichoderma sp. can control the pathogens *Rhyzoctonia solani*, *Sclerotium rolfsii*, and *Fusarium oxysporum* (Kalay et al., 2018). Meanwhile, in vivo, it can suppress the growth of *S. rolfsii* on soybean plants with a damage percentage of 40% (Oktaviawati et al., 2022). Apart from that, *Trichoderma* sp can be used as a soil growth stimulator. Research results from *Trichoderma* sp. isolate. It can improve seed quality with its effect on germination, root length, and seed vigor index of soybean (Tancic et al., 2013), mustard (Lalitha et al., 2012), corn and beans (Okoth et al., 2011).

Habitat *Trichoderma* sp. is in plant roots (Entesari et al., 2013) and soil with high organic matter. One is peat soil, mainly composed of organic material (Barchia, 2006). Peat soil, consisting of organic material, causes many microorganisms to live and play an important role, including *Trichoderma* sp. Peat soil generally has a low pH level, a high cation exchange capacity, low base saturation, and a low content of N, P, K, Ca, and Mg elements. Also, it has a low content of microelements (such as Cu, Zn, Mn, and B) (Sasli, 2011). In 2023, the Palangka Raya City Environmental Service stated that peat soil in Indonesia is often found in Central Kalimantan, especially in Palangka Raya City, with an area of 115,873 hectares.

Peat soil in Central Kalimantan is quite old, so it has a maturity level that is capric (mature); this causes the organic material to decompose more (completely) so that the living microorganisms are more diverse (Page et al., 2002). *Trichoderma* sp. from Palangka Raya peat soil can act as an entomopathogen (Risbianti, 2015) and produce the hormone IAA to encourage plant growth and development (Astriani et al., 2014). However, research has not been carried out on the ability of *Trichoderma* sp. from peat soil to control pathogens in seeds. Therefore, this research aims to determine the ability of Trichoderma sp. from peat soil to control pathogens transmitted through seeds and soybeans to determine Trichoderma sp.'s ability to increase the germination capacity of soybean seeds.

Methods

This research was conducted at the Plant Health Laboratory, Department of Agrotechnology, Faculty of Agriculture, National Development University "Veteran" East Java, from August to September 2023. The equipment used was an autoclave, laminar air flow, erlenmeyer, lighter, beaker, analytical balance, and glass. Preparation tools cover glass, microscope, media bottle, spatula, test tube, petri dish, bunsen, tweezers, tube needle, pipette, cork borer, hemocytometer, and vortex. The materials used are Willis variety soybean seeds, PDA media, EKG media, 70% alcohol, distilled water, spirit wrap, aluminum foil, and heat-resistant plastic. The active ingredient fungicide for treatment is 80% mancozeb, *Trichoderma* sp.

Isolation of *Trichoderma* sp. from Peat Soil

Isolation techniques can be carried out using the spreading method, namely by taking 1 gram, putting it into a petri dish containing PDA media, and then spreading it evenly.

Preaparation of *Trichoderma* sp. Suspension

Trichoderma sp. 30 plongs were taken, 100 ml of Sugar Potato Extract media was added and vortex shaken for 10 minutes to multiply the fungal cells, then to calculate the conidia density, this was done by taking 1 ml of the suspension using a pipette, then dropping it on a hemocytometer and counting the density of the conidia to get the density. 10^6 (Risyda, 2019).

Seed Treatment

Soybean seed treatment was carried out with 100 ml of *Trichoderma* sp suspension. Each treatment soaked 30 seeds. The seeds are soaked for 24 hours and then air-dried in laminar air flow (Livia, 2017)

Identification of Soybean Seedborne Pathogens

Soybean seeds were isolated on PDA media and observed every day. After that, the fungal colonies that had grown on each seed were identified based on the identification key of Barnett & Hunter (1972).

Growing on Test

Growing on Test is intended to imitate or represent field conditions. The media used is soil that has been sterilized with a dose of 2.5 ml/kg formalin and stirred until evenly distributed, then the soil is wrapped in plastic for 7 days and opened for 7 days (Musafa et al., 2015). Planting soybean seeds was carried out in polybags, each containing five seeds. Seeds planted were observed at 7 Days and 14 Days (Illahi et al., 2023).

a. Seed Germination

The percentage of seed sprouting is done using a formula (ISTA, 1996):

$$DB = \frac{\sum Germinating\ Seeds}{\sum Germinated\ Seeds} \text{ x } 100\%$$

b. Plant Growth

Observations were made by observing the growth of plant height and root length. Observation activities were conducted on the 14th day following Malinda's research (2015). Plant height is measured from the base of the stem to the tallest leaf. Meanwhile, root observation was carried out destructively by dismantling the polybag and then measuring the length of the roots from base to tip.

This study was conducted using a factorial method. The first factor was symptomatic soybean seeds, whose color and shape were not normal or healthy, and the second factor was the isolate of *Trichoderma* sp., which consists of TP1 and TP2. Meanwhile, the control treatment used the active ingredient fungicide 80% mancozeb.

Result

Identification of *Trichoderma* sp. isolates. from Peat Soil

The results of the identification of peat soil isolation are presented in Figure 1 and Figure 2.

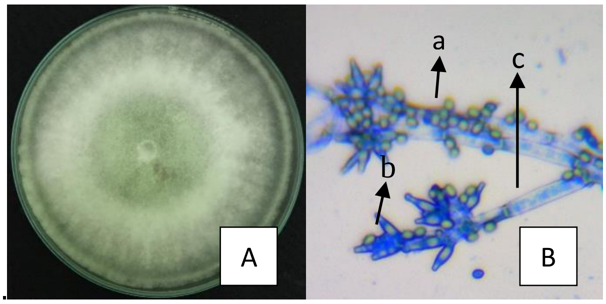


Figure 1. Macroscopic morphology of *Trichoderma* sp. TP1 isolate 100x magnification (A) and microscopic *Trichoderma* sp. TP2 isolate 100x magnification (B). *a. conidia; b. fialid*; *c. conidiophores.*

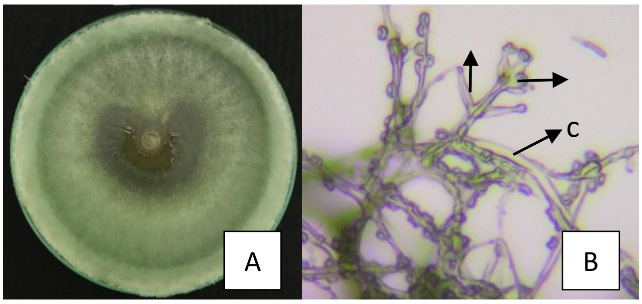


Figure 2. Macroscopic morphology of *Trichoderma* sp. TP2 isolate 100x magnification (A) and microscopic *Trichoderma* sp. TP2 isolate 100x magnification (B). *a. Conidia; b. fialid; c. conidiophores.*

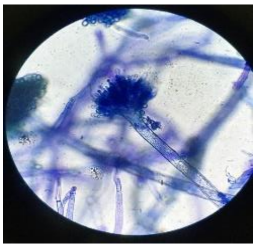
The results of isolating peat soil from Palangkaraya, East Kalimantan, found two isolates of the fungus *Trichoderma* sp. with codes TP1 and TP2, where the two isolates have different morphological characteristics. (Figure 1) Isolate TP1 has a morphology that is initially white, and after 7 days, it has a round, circular shape with a green center and white edges. Microscopically, isolate TP1 has the structure of conidia, phyalids, and conidiophores. Conidia are round and produced in large numbers, and fields are short and filled. Conidiophores are long, branched, and transparent (Figure 2). Isolate TP2 has a macroscopic morphology in the form of dark green colonies and proliferates on the 7th day, visible up to the lid of the cup. Microscopically, isolate TP2 has round conidia with microconidia inside. It has long and slender phialids and branched and unseparated conidiophores.

Identification of Soybean Seed Pathogens

The results of isolating soybean seeds on PDA media found two genera of pathogenic fungi: *Aspergillus* sp. and *Fusarium* sp. (Table 1). *Aspergillus* sp. Macroscopically, the fungus colony is yellowish olive green, and the whole will become green as the fungus ages (Hapsar et al., 2022) with fasciculate white edges (Makhlouf et al., 2019). Microscopically, *Aspergillus* sp. has a complete structure, including conidia, fialid, metula, vesicles, conidiophores, and hyphae. The conidia are round, thin-walled, and have long, cylindrical phialids that grow on the metula. The metula are covered by vesicles and radiate in all directions; the conidiophores are long and contain insulated vesicles and hyphae (Khan et al., 2021).

Fusarium sp. infects soybean seeds and causes the seeds to rot and turn black. Infected seeds show white to black mycelia covering the entire seed. Table 1. shows *Fusarium* sp., which infects the seeds, causing them to be covered in white mycelia on the 7th day. Microscopically, the fungus Fusarium sp. has macroconidia and microconidia. Microconidia are oval to kidney-shaped septates with 1-2 septa, and macroconidia usually have three septates; conidiophores are monophialid, and chlamydisopores are round to ellipsoid (Jeon et al., 2013).

Table 1. Morphology of pathogenic fungi on soybean seeds

Species	Morphology		Figure
	Macroscopic	Microscopic	
Aspergillus sp.	Fungal colonies are green with white edges	The visible morphology is Conidia, Fialid, Metula, and conidiophores.	
Fusarium sp.	Colonies are white-like cotton.	The visible morphology is hyphae, 1-2 partitioned macroconidia, and 3-5 partitioned microconidia.	

Soybean Seed Borne Pathogenic Infection Power

Table 2. Results of Pathogenic Infection Power of Soybean Seeds

Treatment	Infection Power (%)	
	Healthy Seeds	Seeds of Symptoms
Mancozeb fung	73.3 abc	86.6 a
Trichoderma sp. TP 1	53.3 bc	80.0 ab
Trichoderma sp. TP 2	44.6 c	73.3 abc

The analysis of variance (ANOVA) showed a significant effect between treatments on the infectiousness of soybean seed-borne pathogens (Table 2). The highest pathogen infection in soybean seeds occurred in healthy seeds, namely in the mancozeb fungicide treatment of 73.3%, and the lowest in the *Trichoderma* sp. TP2 was 44.6%, which is not very different from the *Trichoderma* sp treatment. TP1 of 53.3%. Meanwhile, soaking the seeds with symptoms of infection was the highest in the mancozeb fungicide treatment at 86.6% and the lowest in the *Trichoderma* sp. TP2 was 73.3%. The difference in the percentage of infectious power in symptomatic seeds is because symptomatic seeds can be a potential source for spread to growing plants. In addition, in seeds, the symptoms of the presence of pathogens are already present before the plants grow, with this condition making the plants vulnerable to pathogens because their defense system is weakened.

Low infection power in seed treatment with Trichoderma sp. because the antagonistic microorganisms come from peat soil. The characteristics of microorganisms in peat soil are different, especially in anaerobic conditions due to waterlogging, acidic (very low pH ranging from 2 - 4), and high organic compound content due to low decomposition activity of microorganisms. The high levels of organic compounds, especially humic acid in peat soil, can open up great opportunities to find microorganisms that have the potential to be antagonists. In addition, Saputra et al. (2022) research states that Trichoderma sp. in peat soil has speedy growth in petri dishes. This is one of the main factors that determine the ability of antagonistic fungi to control diseases that attack plants so with this ability, antagonistic fungi can compete in maintaining space and nutrients so that they can inhibit the growth of pathogenic fungi (Gusnawaty et al., 2013).

Seed Germination of Soybean

Table 3. Results of Germination of Soybean Seeds

Treatment	Seed Germination (%)			
	Healthy seeds		Seeds of symptoms	
	7 Days	14 Days	7 Days	14 Days
Mancozeb Fungicide	2.0 ab	20.0 b	6.67 b	20.0b
Trichoderma sp. TP1	33.3 a	33.3 ab	20.0 a	33.3 ab
Trichoderma sp. TP2	46.6 a	60.0 a	33.3ab	40.0 ab

The results of seed treatment with *Trichoderma* sp. showed a real influence on the germination indicators of soybean seeds on the 7th days and 14th days (Table 2). In soybean plants aged 7 days, healthy seeds treated with Trichoderma sp had the highest germination rate. TP2 was 46.6% but was not much different from the treatment of healthy seeds with *Trichoderma* sp. TP1 was 33.3% and had the lowest germination value in all symptomatic seed treatments. Meanwhile, when observing the germination of soybean plants aged 14th days, the highest value was in healthy seeds treated with *Trichoderma* sp. TP2 was 60.0%; the lowest was in symptomatic seed treatment with *Trichoderma* sp. TP1 and control treatment with 80% mancozeb fungicide, namely 20.0%. From these results (Table 3), it was found that fungicide treatment on both healthy seeds and symptomatic seeds produced the lowest germination value compared to therapy with *Trichoderma* sp isolates. The research results explain that the application of seed treatment fungicides can inhibit or prevent the penetration of pathogenic fungi but can reduce seed germination, which can be caused by the condition of the seeds when they are treated, thus having a phytotoxic impact on the fungicide used (Tanzil & Purnomo, 2021).

Seed treatment with *Trichoderma* sp. can break seed dormancy, so germination occurs quickly. This is supported by research by Rozen et al. (2011), who stated that the *Trichoderma* fungus can break sugar palm seeds from 8 months to 3 months because the *Trichoderma* fungus is antagonistic. From this antagonistic nature, the fungus can renovate the seed coat, resulting in tears in the seed coat, so the imbibition process can take place, causing the seeds to germinate quickly. Another factor is that it is helped by the hormone IAA (Indole Acetic Acid) contained in the *Trichoderma* fungus. The IAA hormone can break seed dormancy and stimulate seed germination, encouraging seeds to germinate (Haerani & Nurdin, 2021).

Plant Growth

The results of observing plant growth, including plant height and root length, did not show a real effect on the treatment of soybean seeds with *Trichoderma* sp suspension. And fungicide. The highest average results in plant height parameters occurred when healthy seeds were treated with fungicide at 40.2 cm, and the lowest average was when diseased seeds were treated with fungicide at 13.5 cm. The root length parameter obtained the highest average when treating diseased seeds with *Trichoderma* sp. TP1 was 25.3 cm, while the lowest root length in treating diseased seeds with fungicide was 6.9 cm. Results showing no treatment effect on plant height and root length can also be indicated by the initial seed health level, which may have been optimal without additional improvement. Besides that, Trichoderma sp. does not affect plant height and root length caused by isolates of *Trichoderma* sp., which is considered less effective in acting as a plant growth stimulator.

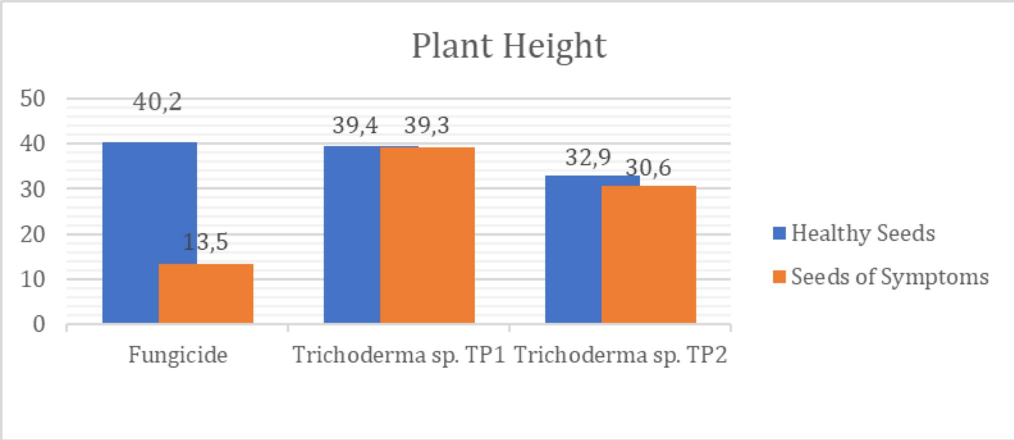


Figure 3. Effect of Trichoderma sp. on soybean plant height

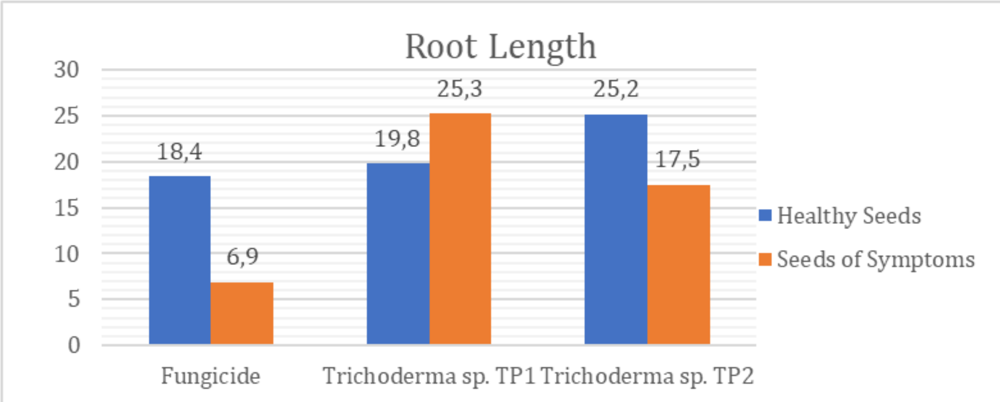


Figure 4. Impact of Trichoderma sp. on soybean root length

Discussion

Treatment of *Trichoderma* sp. applied to soybean seeds did not show a real effect on plant height and root length, which was thought to be influenced by the Availability of food in the growing medium. In contrast, in research using the growing-on-test method, the planting medium used was soil without a fertilizer mixture. According to Fitria et al. (2021), the Availability of food sources for *Trichoderma* sp. on plant media dramatically determines the effectiveness of *Trichoderma* sp. in increasing plant growth. According to Baihaqi et al. (2013), insufficient food sources, rainfall, and air humidity affect the growing space for the development of *Trichoderma* sp. They will also affect the efficiency of the application of *Trichoderma* sp., which has an impact on planting. The length of treatment can also affect the role of *Trichoderma* sp. Research by Haerani & Nurdin (2021) shows that seed treatment with *Trichoderma* sp. for 24 hours could influence germination capacity, vigor index, and growth simultaneity but did not affect plant length and height.

Conclusions

Based on the results of research on *Trichoderma* sp suspension. It significantly affects the pathogenic infectivity and germination of soybean seeds but has no real impact on the growth of soybean plants. Soaking treatment on *Trichoderma* sp isolates. TP2 is the best treatment for reducing infection and increasing soybean seed germination.

Acknowledgments

Thank you to the Plant Health Laboratory, Faculty of Agriculture, and National Development University "Veteran" East Java for providing facilities during the research.

Declaration statement

The authors reported no potential conflict of interest.

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