Pertanika J. Trop. Agric. Sci. 49 (4): 1877 - 1895 (2026)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Influence of Culture Media on Growth and Conidial Quality of Two *Trichoderma* spp. Isolates with Preliminary Evaluation Against Downy Mildew in Maize

Elly Roosma Ria[1]*, Ida Nurhelawati[2], Agus Surya Mulya[1], and Nunung Sondari[1]

[1]*Department of Agrotechnology, Faculty of Agriculture, University of Winaya Mukti, Bandung, 40124 West Java, Indonesia*

[2]*The Plantation Protection Center, Bandung, 40616 West Java, Indonesia*

ABSTRACT

Downy mildew caused by *Peronosclerospora* spp. is a major disease of maize (*Zea mays* L.), and *Trichoderma* spp. are widely used as biological control agents against this pathogen. This study aimed to determine the effect of two *Trichoderma* spp. isolates propagated on different media on the incidence of downy mildew in maize. The study was conducted at the Biological Control Agent Laboratory of the West Java Plantation Protection Agency and in a field in Pasirbiru Village, Rancakalong District, Sumedang Regency, from April to May 2025. A Completely Randomised Factorial Design with two factors, isolate type (*Trichoderma* spp. from BBPOPT and BBPPTP) and propagation medium (cracked corn and rice bran), replicated five times, was used in the laboratory. At the same time, a Randomised Block Design was applied in the field to evaluate effectiveness against downy mildew. The propagation medium significantly affected the reduction in medium weight before and after inoculation, with rice bran reducing medium weight by up to 1.20 g. The BBPOPT and BBPPTP isolates grown on maize medium produced the lowest numerical AUDPC values (128.33 and 122.50, respectively) and the highest numerical disease suppression (42.8% and 45.40%, respectively), although differences among treatments were not statistically significant. These results indicate that *Trichoderma* spp. treatments delayed early-stage disease development but did not significantly suppress overall downy mildew progression. As field evaluation was conducted at only one location and season, further multi-location, multi-season studies are needed to confirm the consistency of these effects

Keywords: Bran, conidial viability, conidial density, corn, *Peronosclerospora* spp., *Trichoderma* spp. isolates

ARTICLE INFO

Article history:
Received: 28 May 2026
Accepted: 28 July 2026
Published: 14 August 2026

DOI: https://doi.org/10.47836/pjtas.49.4.14

E-mail addresses:
elly.roosma.ria@gmail.com (Elly Roosma Ria)
idanurhelawati14@gmail.com (Ida Nurhelawati)
agussurya1204@gmail.com (Agus Surya Mulya)
nunungsondari@gmail.com (Nunung Sondari)
* Corresponding author

e-ISSN: 2231-8542

INTRODUCTION

Downy mildew is one of the most important diseases affecting maize. This disease is caused by several species belonging to the genera *Peronosclerospora* and *Sclerospora* (Muis et al., 2016; Rashid et al., 2013). Yield losses caused by downy mildew in maize, particularly in susceptible varieties, may reach 90-100% (Nirwanto & Sutikno, 2024). Such high yield losses can result in significant economic damage if the disease is not properly managed.

The most common method for controlling downy mildew is the application of synthetic chemical fungicides. Fungicides containing metalaxyl as the active ingredient are widely used for controlling downy mildew (Prasetyo et al., 2021). However, continuous application without proper dosage consideration may lead to several negative impacts. Pesticides can contaminate the environment and non-target organisms, including beneficial soil microorganisms, and their residues may pollute soil and water resources (Aktar et al., 2009). Excessive pesticide application may also cause residue accumulation in agricultural products, posing risks to human health (Kurniasih et al., 2013). Furthermore, studies by Widiantini et al. (2017) indicated the emergence of resistance to metalaxyl fungicides in *Peronosclerospora* spp. Therefore, environmentally friendly alternative control strategies are required to reduce the negative impacts of synthetic pesticides.

One alternative control strategy is the use of biological control agents (BCAs). BCAs are living organisms that can suppress plant pests and diseases by predation or parasitism (Muley & Chavan, 2023). One of the potential BCA is *Trichoderma* spp., a saprophytic fungus commonly found in soil, air, and plant surfaces (Gusnawaty et al., 2017; Wang et al., 2022; Yao et al., 2023). *Trichoderma* spp. suppress plant diseases through several mechanisms, including antibiosis, competition for space and nutrients, and parasitism (mycoparasitism or hyperparasitism) (Gusnawaty et al., 2014; Prasad et al., 2023). In addition, *Trichoderma* spp. can indirectly induce plant resistance and promote plant growth (Nakkeeran et al., 2018).

Previous studies reported that *Trichoderma* spp. effectively controlled several plant pathogens, such as clubroot in pak choi, white root rot in rubber seedlings, and vascular streak dieback (VSD) in cocoa seedlings (Amaria & Wardiana, 2014; Harni et al., 2017; Yudha et al., 2016*). Trichoderma* spp. have also been reported as biological control agents against insect pests (Poveda, 2021). Isolate *T. harzianum* T019 increased ergosterol production, which induced defence-related genes in common bean against *Rhizoctonia solani* infection (Mayo et al., 2015). The combination of *T. asperellum* and betel leaf extract was reported to suppress downy mildew incidence in maize (Prasetyo et al., 2021).

The physiological quality of *Trichoderma* spp. inoculum, such as conidial density and viability, which can influence characteristics and the propagation media, is considered an important factor influencing its potential performance as a biological control agent. *Trichoderma* spp. require carbohydrates, proteins, and other nutrients to support their

growth (Jaya et al., 2023; Novianti, 2018). To date, maize and rice are commonly used as substrates for mass propagation of *Trichoderma* spp. However, these substrates are relatively expensive, necessitating the use of alternative, more affordable, and readily available propagation media. Several studies reported that rice, maize, rice bran, rice husk, sawdust, sweet potato, cassava, bran, potato, tofu waste, and millet can be used as substrates for mass propagation of *Trichoderma* spp. (Gusnawaty et al., 2017; Kansrini, 2015; Novianti, 2018; Safitri et al., 2023; Suharni et al., 2023; Utami et al., 2023). Therefore, this study evaluated two *Trichoderma* spp. isolates propagated on different media for their effectiveness in reducing downy mildew (*Peronosclerospora* spp.) incidence in maize.

MATERIALS AND METHODS

This study was conducted using an experimental method at two locations: the Biological Control Agent Laboratory of the West Java Plantation Protection Agency and a field site in Pasirbiru Village, Rancakalong District, Sumedang Regency, at an altitude of 850 metres above sea level. The experiment was conducted from April to May 2025.

Laboratory equipment included a digital balance, beakers, cooking pots, stirrers, stove, Petri dishes, inoculating needles, Bunsen burner, steamer, wooden spatula, trays, electric fan, glass jars, cork borer, test tubes, micropipettes, glass funnels, test tube racks, Laminar Air Flow cabinet (LAF), autoclave, Erlenmeyer flasks, vortex mixer, hemocytometer, hand counter, syringe, microscope slides, cover slips, scalpel, and microscope. Field equipment included 10 kg polybags, stakes, and hoes.

Materials used in the Laboratory included pure cultures of *Trichoderma* spp. obtained from the Indonesian Plant Protection Forecasting Center (BBPOPT) Karawang and the Center for Seed and Plantation Crop Protection (BBPPTP) Surabaya, sterile distilled water, Potato Dextrose Agar (PDA), 70% alcohol, spiritus, toothpicks, cotton, tissue paper, and propagation media consisting of cracked maize and rice bran. Field materials included maize seeds of the Bisma variety, planting media (rice husk, manure, and soil), and maize plants infected with downy mildew.

Experimental Design

Laboratory Experiment

The laboratory experiment employed a Completely Randomised Design Factorial pattern with two factors. The first factor was the *Trichoderma* spp. isolate, consisting of BBPOPT isolate (t_1) and BBPPTP isolate (t_2). The second factor was the propagation medium, consisting of cracked maize (m_1) and rice bran (m_2). Each treatment was replicated five times, with two Petri dishes per replication, resulting in 40 experimental units. Observations included fungal growth parameters (incubation period and medium weight loss before and after incubation) and conidial quality parameters (conidial density and viability).

Field Experiment

The field experiment was arranged in a Randomised Block Design (RBD). Treatments consisted of combinations of two *Trichoderma* spp. isolates propagated on different media that had previously been tested under laboratory conditions to evaluate their effectiveness against downy mildew incidence. Five treatments were applied: A = control, B = t_1m_1 (BBPOPT isolate on cracked maize), C = t_1m_2 (BBPOPT isolate on rice bran), D = t_2m_1 (BBPPTP isolate on cracked maize), and E = t_2m_2 (BBPPTP isolate on rice bran). Each treatment was replicated five times. Each replication consisted of 16 plants grown in two polybags, resulting in a total of 400 observed plants. Observations included downy mildew incidence and the Area Under the Disease Progress Curve (AUDPC).

Propagation of **Trichoderma** *spp. Isolates on PDA Medium*

The *Trichoderma* spp. isolates utilised in this experiment were first propagated on Potato Dextrose Agar (PDA) medium. PDA is a commonly used medium for culturing various fungi and supports mycelial growth effectively (Devi et al., 2018). The PDA medium used was instant PDA, prepared by dissolving 42 g of PDA powder in 1 L of distilled water in a beaker. The solution was heated using the double-boiling method until boiling. The boiled medium was subsequently transferred to an Erlenmeyer flask and sterilised in an autoclave at 121 °C for 45 minutes under 1 atm. Following sterilisation, approximately 10 mL of the medium was aseptically dispensed into Petri dishes.

After solidification, the PDA medium was inoculated with *Trichoderma* spp. within a Laminar Air Flow (LAF) cabinet that had been sterilised using 70% alcohol and ultraviolet light for 15 minutes. Inoculation was performed by transferring an agar plug containing mycelia and spores of *Trichoderma* spp. from an actively growing culture to a fresh PDA medium using an inoculating needle. The inoculated cultures were incubated for approximately 7-14 days, or until the fungal mycelium evenly covered the medium surface.

Propagation of Trichoderma spp. on Cracked Maize and Rice Bran Medium

The preparation of cracked maize medium for *Trichoderma* spp. propagation followed the method commonly used by BPP. The maize grains were washed, steamed until partially cooked, and then air-dried using a fan. Meanwhile, the rice bran medium was prepared according to the method described by Day et al. (2022), with modifications. Rice bran was mixed with water at a 1:2 ratio until the moisture content reached approximately 30%, as indicated by the absence of free water release and the formation of clumps when the medium was compressed by hand (Day et al., 2022). Each medium was weighed at 20 g and placed into a Petri dish (Uruilal et al., 2012). The media were then sterilised in an autoclave at 121 °C for 60 minutes at 1 atm. After cooling, the media were inoculated

with *Trichoderma* spp. by placing one 5-mm agar plug containing the fungal isolate at the centre of the medium surface.

Growth of Trichoderma spp. on Propagation Medium

The observed parameters included fungal growth, assessed using the average incubation period and the difference in medium weight before and after incubation. The average incubation period was determined for all treatment combinations by recording the day on which fungal growth was first observed on the propagation media, calculated from the time of inoculation (Gusnawaty et al., 2017). The difference in medium weight before and after incubation was measured by weighing the medium before inoculation and after incubation, when the entire medium surface had been fully colonised by *Trichoderma* spp., using a digital balance. These measurements were conducted for all treatment combinations.

Conidial Quality Test of Trichoderma spp.

The quality of *Trichoderma* spp. conidia produced on the propagation media were evaluated based on conidial density and viability. Samples were randomly collected from each fungal culture grown on each type of propagation medium. The conidial assessment followed the method established by BBPOPT (2023). For conidial density determination, each sample was homogenised by shaking or stirring. One gram of each sample was weighed and placed into a sterilised 100 mL Erlenmeyer flask. Distilled water was added to reach a volume of 100 mL, followed by the addition of one drop of Tween 80. The flask was covered with aluminium foil, and the conidial suspension was homogenised using a vortex mixer for 15 minutes, resulting in a 10^{-2} dilution. A *Neubauer Improved hemocytometer* was placed on the microscope stage and covered with a cover glass. The counting chamber was located at 100× magnification, and the 0.2 mL aliquot of the 10^{2} diluted suspension was then observed at 400× magnification. A 0.2 mL aliquot of the 10^{-2} diluted suspension was taken using a micropipette and introduced into both the upper and lower channels of the hemocytometer (Figure 1).

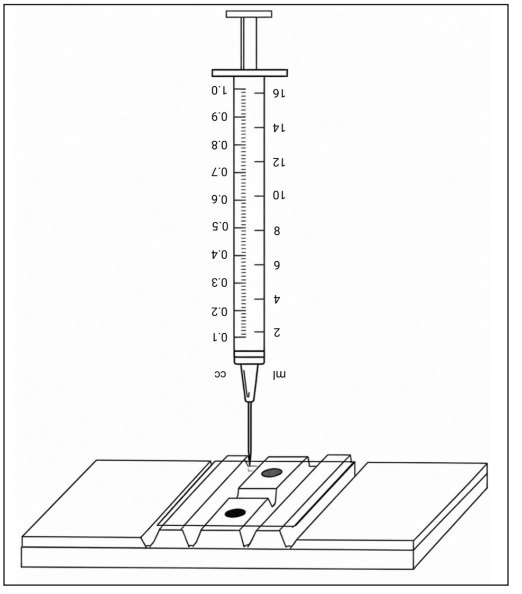

Figure 1. Application of conidial suspension onto the counting chamber
*Source: Center for Forecasting of Plant Pest Organisms (BBPOPT) (2023)

The suspension was allowed to stand for one minute to stabilise the conidia. Conidia were counted diagonally in squares a, b, c, d, and e on both counting chambers using a hand counter. Only conidia within the counting squares and those touching the left and upper boundary lines were counted (Figure 2).

After obtaining the conidial count from both the upper and lower counting chambers, the number of conidia per millilitre was calculated using Equation 1.

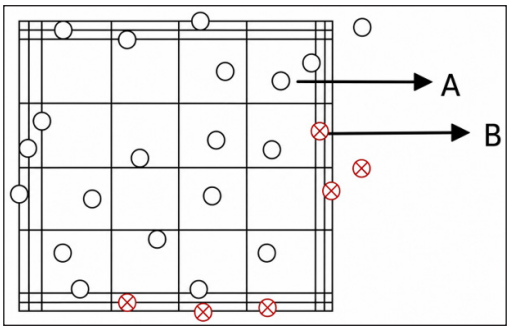

Figure 2. Counting of fungal conidia: (A) conidia included in the count and (B) conidia excluded from the count
*Source: Center for Forecasting of Plant Pest Organisms (BBPOPT) (2023)

$$S = \frac{\bar{X}}{L \times t \times d} \times 10^3 \qquad [1]$$

Where:

S : Conidial density (conidia/mL)
$\bar{X}$: Average number of conidia counted in the upper and lower chambers
L : Counting area 0.04 mm²
T : Chamber depth (0.1 mm)
D : Dilution factor
10^3 : Volume conversion factor (1 ml = 10^3 mm^3)

Conidial viability testing was conducted as a continuation of the conidial density assessment. PDA medium was prepared in Petri dishes and cut into agar discs (0.5 cm diameter) using a cork borer. Three agar discs were placed on a sterile microscope slide as replicates. The 10^{-2} conidial suspension was re-homogenised using a vortex mixer for 3 minutes and subsequently applied to each agar disc using a 1 mL syringe. Each disc received one drop of the suspension and was covered with a cover glass. The prepared slides were placed inside Petri dishes supported by two toothpicks, and two rolls of moist cotton were added to maintain humidity. The Petri dishes were sealed with cling wrap and incubated in the dark. Conidial viability was observed after 16 hours of incubation using a microscope at 400× magnification. Conidia were considered germinated when the germ tube length reached at least half the length of the conidium (Sinclair & Dhingra, 1995). Viability was calculated using Equation 2.

$$\text{Viability} = \frac{\Sigma \text{ Germinated conidia}}{\text{Total observed conidia}} \text{ x } 100\% \qquad [2]$$

Test of Two Trichoderma *spp. Isolates Against Downy Mildew Incidence in Maize*

This test was conducted as a continuation of the conidial quality assessment. Five treatments were applied: control (A), BBPOPT isolate on maize medium (B), BBPOPT isolate on rice bran medium (C), BBPPTP isolate on maize medium (D), and BBPPTP isolate on rice bran medium (E). The maize seeds were prepared by soaking them in water for 24 hours, then draining and incubating in plastic boxes lined with moist tissue paper for another 24 hours. Seed treatment with *Trichoderma* spp. was performed by preparing a 50 mL conidial suspension with a known density and adding 12.5 g of granulated sugar (Turnip, 2015). The conidial suspensions used for seed treatment were prepared based on the measured conidial density from each treatment. Equal suspension volumes were applied to seeds to maintain consistency among treatments. The maize seeds were soaked in the suspension for 30 minutes, while the control seeds were soaked in water containing the same concentration of sugar. Following treatment, the seeds were then drained and placed in plastic boxes according to treatment codes, lined and covered with moist tissue paper to stimulate germination. The seeds were incubated for an additional 48 hours before inoculation with downy mildew.

Downy mildew inoculation was performed using the leaf-insertion method (Adhi et al., 2019). Each treated seed, including the control, was inoculated by inserting maize leaf tissue infected with *Peronosclerospora* spp. into the seed. The inoculated seeds were incubated for 24 hours and subsequently planted at a density of eight seeds per polybag. Observations were conducted from one to five weeks after planting to assess downy mildew incidence. Disease incidence was calculated using Equation 3 (Adhi et al., 2019).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \text{ x } 100\% \qquad [3]$$

The Area Under the Disease Progress Curve (AUDPC) is a single value that describes disease development and is derived from integrating multiple observations of disease intensity, disease incidence, or both (Simko & Piepho, 2012). AUDPC was calculated using Equation 4 (Landeo, 1999, in Kurniawan et al., 2018).

$$AUDPC = \sum_{i=1}^{n}(X_{t+1} + X_t)(D_{t+1} - D_t) \qquad [4]$$

Where:

X_t : Percentage of downy mildew incidence at time t

X_{t+1} : Percentage of downy mildew incidence at the subsequent observation time $t + 1$

$(D_{t+1} - D_t)$: Observation interval between two consecutive assessments

Data were further analysed using Duncan's Multiple Range Test (DMRT) at the 5% significance level to compare treatment means.

RESULTS AND DISCUSSIONS

Growth of Two *Trichoderma* spp. Isolates on Different Propagation Medium

Incubation Period

Both *Trichoderma* spp. isolates showed visible growth on both propagation media at 2 days after inoculation (DAI) (Table 1). Fungal growth was characterised by the emergence of hyphae spreading from the inoculation point toward the surface of the propagation medium. Both isolates completely colonised the surface by 10 DAI; in contrast, on rice bran medium, full colonisation occurred between 10 and 18 DAI (Figure 3).

Table 1
Average incubation period of two Trichoderma *spp. isolates on different propagation Media (Days)*

Treatment	Incubation Period (Days)
t_1m_1	2
t_1m_2	2
t_2m_1	2
t_2m_2	2

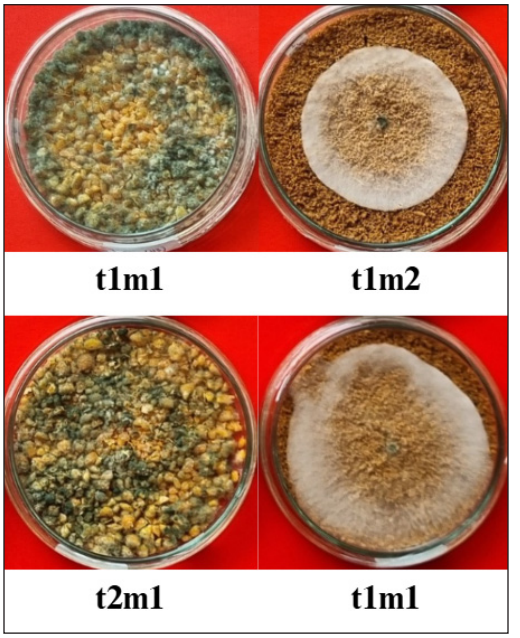

Figure 3. Growth of two *Trichoderma* spp. isolates on different propagation media at 10 Days after Inoculation (DAI)
*Source: Personal documentation (Ida Nurhelawati, 2025)

Based on the average incubation period, both isolates began to grow at 2 DAI, indicating that both propagation media supported *Trichoderma* spp. growth. However, fungal growth was faster on maize medium compared to rice bran medium. Complete colonisation occurred uniformly at 10 DAI on maize medium, whereas colonisation on rice bran medium was more variable. These differences are likely attributable to variations in nutrient composition between the two media. Fungal growth is strongly influenced by the availability of essential nutrients such as carbohydrates and proteins (Basu et al., 2015). Maize contains a higher carbohydrate content, reaching up to 75.64% (Lalujan et al., 2017), whereas rice bran contains approximately 34-62% carbohydrates (Chakraborty & Budhwar, 2018). The higher carbohydrate content in maize likely contributed to the faster mycelial growth of *Trichoderma* spp. observed in this study.

Difference in Medium Weight before and After Inoculation

The difference in medium weight was determined by subtracting the weight before inoculation from the weight after fungal colonisation. Statistical analysis indicated no significant interaction between fungal isolates and propagation media on the difference in medium weight. However, a significant main effect of propagation medium was observed (Table 2). The results indicated that rice bran medium (m_2) exhibited the greatest reduction in medium weight (1.20 g), compared with maize medium (0.88 g).

Table 2
Effect of two Trichoderma *spp. isolates grown on different propagation media on the difference in medium weight before and after inoculation*

Treatment	Weight Before Inoculation (g)	Weight After Inoculation (g)	Weight Difference (g)
Fungal Isolate			
t_1	20	18.75	1.03 a
t_2	20	18.94	1.06 a
Propagation Medium			
m_1	20	19.12	0.88 a
m_2	20	18.80	1.20 b

Note. Different letters indicate a significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% significance level

The greatest reduction in medium weight was observed in the rice bran treatment, with a value of 1.20 g. This finding is consistent with those of Gusnawaty et al. (2017), who also observed the greatest weight reduction in the rice bran medium. The difference in medium weight before and after incubation reflects the metabolic activity of *Trichoderma* spp. The greater weight loss observed in the rice bran medium suggests that its nutrient composition is well suited to the nutritional requirements of *Trichoderma* spp., enabling more rapid substrate decomposition (Gusnawaty et al., 2017).

Rice bran contains major carbohydrate components, including hemicellulose (8.7-11.4%), cellulose (9-12.8%), starch (5-15%), and β-glucan (1%) (Astawan & Febrinda, 2010). *Trichoderma* spp. degrades substrates such as starch, pectin, and cellulose by producing extracellular enzymes, including amylase, pectinase, cellulase, and chitinase, which facilitate nutrient utilisation for growth (Yparraguirre & Galliani-Pinillos, 2020). The high reduction in medium weight therefore further indicates that *Trichoderma* spp. have strong potential as effective organic matter decomposers.

Conidial Quality of Two *Trichoderma* spp. Isolates on Different Propagation Media

Conidial Density

Statistical analysis showed no interaction and no significant main effects of fungal isolates or propagation media on conidial density (Table 3). All treatments produced similar conidial

densities, averaging 10^8 conidia mL^{-1}. The highest conidial density was observed in the BBPPTP isolate (t_2), with 2.55 × 10^8 conidia mL^{-1}. However, this value was not significantly different from t_1 (2.25 × 10^8 conidia mL^{-1}), maize medium (m_1), or rice bran medium (m_2), both of which produced 2.40 × 10^8 conidia mL^{-1}. These results indicate that both propagation media supported the sporulation of *Trichoderma* spp. effectively.

Table 3
Effect of two Trichoderma *spp. isolates grown on different propagation media on conidial density (10^8 conidia mL^{-1})*

Treatment	Conidial Density (10^8 conidia mL^{-1})
Fungal isolate	
t_1	2.25 a
t_2	2.55 a
Propagation medium	
m_1	2.40 a
m_2	2.40 a

Note. Same letters indicate non-significant differences based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

The assessment of conidial density for two *Trichoderma* spp. isolates grown on different propagation media resulted in an average density of 10^8 conidia mL^{-1}. These findings indicate that both isolates produced comparable amounts of conidia across the two types of propagation media tested. Furthermore, the conidial densities obtained in this study exceeded the minimum standard established by the Badan Standardisasi Nasional (2014), which requires ≥10^6 conidia mL^{-1}. High conidial density is closely associated with *Trichoderma* spp.'s ability to suppress pathogen development. This is consistent with the findings of Nabila *et al*. (2021), who reported that a conidial density of 10^7 conidia mL^{-1} was capable of suppressing Fusarium wilt by more than 50%.

Conidial Viability

Conidial viability of *Trichoderma* spp. was observed microscopically after 16 hours of incubation. Conidia were considered germinated when the germ tube length reached at least half the length of the conidium (Sinclair & Dhingra, 1995) (Figure 4).

Statistical analysis revealed a significant interaction between fungal isolates and propagation media on conidial viability (Table 4). The highest conidial viability was observed in the BBPOPT isolate propagated on maize medium (t_1m_1), at 98.61%, and was not significantly different from that

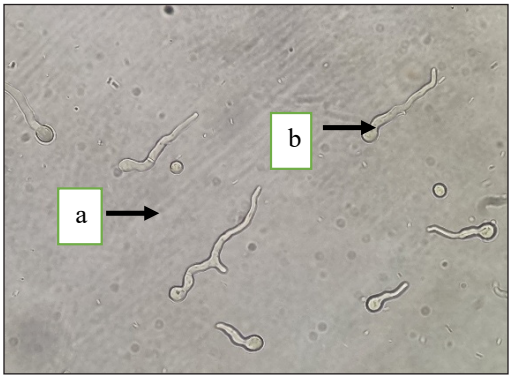

Figure 4. Conidia of *Trichoderma* spp.: (a) Germinated conidia and (b) Non-germinated conidia
*Source: Personal documentation (Ida Nurhelawati, 2025)

Table 4
Effect of two Trichoderma *spp. isolates grown on different propagation media on conidial viability (%)*

Factor t	Factor m	
	m_1	m_2
t_1	98.61 a B	82.70 a A
t_2	96.47 a B	91.46 b A

Note. Different letters indicate significant differences based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

of the BBPPTP isolate on maize medium (t_2m_1), at 96.47%. However, both treatments differed significantly from t_1m_2 and t_2m_2. The lowest conidial viability was recorded for the BBPOPT isolate propagated on rice bran medium (t_1m_2), at 82.70%.

Conidial viability reflects the ability of conidia to germinate and grow normally under optimal conditions (Utami et al., 2023). In this study, an interaction was observed between two *Trichoderma* spp. isolates and different propagation media on fungal conidial viability. The highest viability was recorded in treatments t_1m_1 and t_2m_1, reaching 98.61% and 96.47%, respectively. These values exceed the minimum conidial viability standard for *Trichoderma* spp., set at≥ 60% by the Badan Standardisasi Nasional (2014). The corn-based medium had a significant effect on the conidial viability of both *Trichoderma* spp. isolates. According to Utami et al. (2023), conidial viability is influenced by several factors, including culture age, nutrient content of the growth medium, and environmental factors such as light, temperature, and moisture. Nutrients present in the propagation media can affect fungal survival, sporulation, and antagonistic ability (Mufida et al., 2023). Furthermore, high conidial viability is crucial for supporting fungal growth and development as a biological control agent (Nurbailis & Martinius, 2011).

Effect of Two *Trichoderma* spp. Isolates on Downy Mildew Incidence in Maize

Incidence of Downy Mildew Disease in Maize Plants

Downy mildew disease was first observed one week after planting (WAP). Disease symptoms appeared on newly emerging leaves, characterised by elongated chlorotic streaks parallel to the leaf veins (Figure 5a). In the early morning, white conidial masses were observed on the abaxial surface of infected leaves. Microscopic observations revealed that the conidiophores of the downy mildew pathogen exhibited three levels of branching, were equipped with sterigmata bearing terminal conidia, and had a spherical to sub-spherical shape.

At one week after planting (WAP), the highest percentage of downy mildew incidence was observed in the control treatment (3.93%) and based on statistical analysis, was not

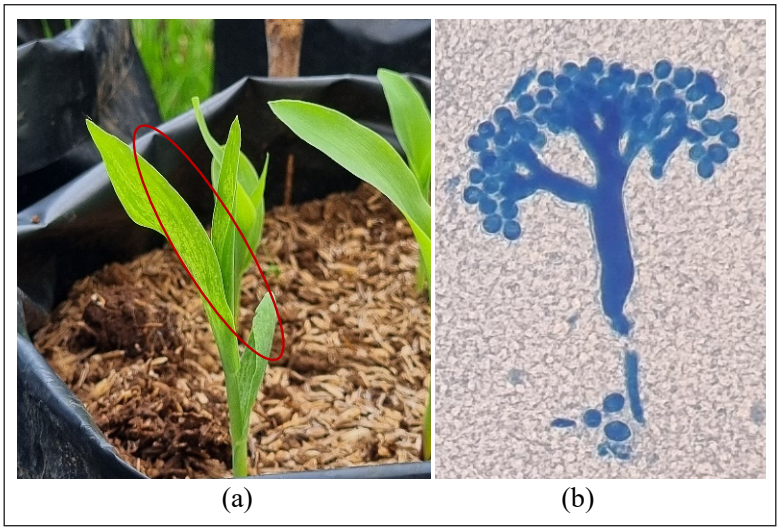

Figure 5. Downy mildew disease: (a) Symptoms of downy mildew at 1 week after planting (WAP) and (b) Conidiophores and conidia of *Peronosclerospora* spp
*Source: Personal documentation (Ida Nurhelawati, 2025)

significantly different from that in treatment t_1m_2 (1.43%) (Table 5). However, disease incidence in the control treatment (3.93%) was significantly higher than in treatments t_1m_1, t_2m_1, and t_2m_2, which showed no symptoms of downy mildew infection (0%). At the second WAP, downy mildew symptoms were detected in all treatments; however, the percentage of disease incidence was significantly lower than in the control. At 3, 4, and 5 WAP, the percentage of disease incidence in the control was not significantly different from that of the other treatments, although the control consistently showed higher disease incidence. At the final observation (5 WAP), downy mildew incidence in the control treatment reached 13.04%, while the lowest disease incidence was recorded in treatment t_2m_1.

Table 5
Effect of two fungal isolates grown on different propagation media on downy mildew incidence (%)

Treatment	Downy Mildew Incidence (%)				
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP
A (control)	3.93 b	6.61 b	7.86 a	9.11 a	13.04 a
B (t_1m_1)	0.00 a	2.68 a	5.28 a	6.51 a	7.76 a
C (t_1m_2)	1.43 ab	2.68 a	5.18 a	7.68 a	10.36 a
D (t_2m_1)	0.00 a	1.22 a	5.00 a	7.50 a	7.50 a
E (t_2m_2)	0.00 a	2.58 a	5.17 a	7.75 a	7.75 a

Note. Values followed by different letters indicate significant differences according to Duncan's Multiple Range Test at the 5% significance level

Based on the experimental results, the lowest percentage of downy mildew incidence was observed at 1 week after planting (WAP), during which disease symptoms were detected only in the control and treatment t_1m_2. At 2 WAP, all treatments exhibited symptoms of downy mildew; however, the percentage of disease incidence in all treated groups remained lower than that in the control. At 3, 4, and 5 WAP, no significant differences in disease incidence were observed among treatments. These results indicate that the two *Trichoderma* spp. isolates propagated on different media suppressed downy mildew incidence only during the early growth stage of maize, specifically within 1-2 WAP. This early suppression may be attributed to *Trichoderma* spp.'s ability to delay the pathogen's incubation period during the host plants' initial growth phase. The relatively high disease incidence observed at 3, 4, and 5 WAP may be influenced by environmental conditions that favour the development and spread of downy mildew. This observation is consistent with the findings of Purwanto et al. (2016), who reported that environmental factors play a critical role in the dissemination of downy mildew disease. In the present study, the temperature during the observation period ranged from 24.5 °C to 29 °C, with relative humidity between 98% and 99%. The optimum temperature for downy mildew development is below 24 °C with relative humidity around 90% (Semangun, 2008). Although the temperature conditions during the experiment were not ideal for downy mildew development, the high relative humidity and continuous availability of susceptible host plants supported pathogen establishment and disease development. Therefore, under environmental conditions conducive to disease development, the treatments applied in this study were insufficient to suppress downy mildew incidence at later growth stages effectively.

Area Under the Disease Progress Curve (AUDPC)

Statistical analysis showed that AUDPC values among all treatments were not significantly different (Table 6). The highest mean AUDPC value was observed in the control treatment (224.35), while the lowest was recorded in treatment t_2m_1 (122.50). The highest downy

Table 6
AUDPC values and percentage of downy mildew suppression

Treatment	AUDPC					Means AUDPC	Inhibition (%)
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP		
A (control)	196.88	224.95	174.93	218.75	306.25	224.35 a	0.00
B (t_1m_1)	0.00	175.00	116.73	175.00	174.93	128.33 a	42.80
C (t_1m_2)	175.00	218.75	131.25	0.00	224.95	149.99 a	33.15
D (t_2m_1)	109.38	109.38	0.00	175.00	218.75	122.50 a	45.40
E (t_2m_2)	109.38	186.66	0.00	163.42	218.75	135.64 a	39.54

Note. Values followed by different letters indicate significant differences according to Duncan's Multiple Range Test at the 5% significance level

mildew suppression was observed in treatment t_2m_1 (45.40%), whereas the control treatment showed no suppression (0%).

The results of the evaluation of two *Trichoderma* spp. isolates propagated on different media indicated no significant differences in AUDPC values compared with the control. The highest AUDPC value was observed in the control treatment (224.35), while the lowest was recorded in treatment t_2m_1 (122.50). A higher AUDPC value indicates greater disease development, whereas a lower AUDPC value indicates reduced disease progression (Suganda, 2001 in Kurniawan et al., 2018). The AUDPC value was inversely related to the percentage of disease suppression; lower AUDPC values were associated with higher levels of disease suppression. The highest suppression of downy mildew was observed in treatment t_2m_1 (45.40%).

CONCLUSION

The propagation medium had a significant independent effect on the reduction in medium weight before and after inoculation with *Trichoderma* spp. Using rice bran media reduces medium weight by up to 1.20 g.

The combination of two *Trichoderma* spp. isolates and different propagation media had no significant effect on conidial density, which ranged from 2.25×10^8 to 2.55×10^8 conidia mL^{-1}. However, it had a significant effect on conidial viability. The highest conidial viability was recorded in *Trichoderma* spp. isolates originating from BBPOPT and BBPPTP, propagated on maize medium, with viability values of 98.61% and 96.47%, respectively.

The BBPOPT and BBPPTP isolates on maize medium showed the lowest numerical AUDPC value (128.33 and 122.50) and the highest numerical disease suppression (42.8% and 45.40%). However, the differences among treatments were not statistically significant. It indicates that the Trichoderma spp. treatments delayed disease development during the early growth stage but did not significantly suppress overall downy mildew progression.

IMPLICATIONS OF THE STUDY

This study demonstrates that using local agricultural by-products, such as cracked maize, for the mass production of *Trichoderma* spp. offers a highly viable, eco-friendly alternative to synthetic fungicides in maize farming. These findings provide a low-cost, sustainable framework that empowers resource-limited farmers to produce effective biocontrol agents, supporting green agriculture initiatives.

The implications of this study are expected to show that *Trichoderma* spp. isolates propagated on corn media, both originating from BBPOPT and BBPPTP, have potential as biological control candidates due to their ability to produce high-quality inoculum. However, further validation through multi-location and multi-season field experiments is required before recommending their practical application for downy mildew management in maize production.

LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

This study demonstrated the potential of *Trichoderma* spp. propagated on different substrates to improve conidial quality and suppress downy mildew development in maize. However, several limitations should be acknowledged. First, the experiment was conducted under a single agroecological condition and within a single planting season, which may limit the broader applicability of the findings across different environmental conditions, cropping systems, and pathogen pressures. The efficacy of biological control agents is strongly influenced by environmental factors, including temperature, humidity, soil physicochemical properties, and indigenous microbial communities, all of which can affect the establishment and antagonistic performance of *Trichoderma* spp. under field conditions. Second, the study evaluated disease suppression primarily through disease incidence and AUDPC parameters without further investigation of physiological, biochemical, or molecular plant responses associated with induced resistance mechanisms. Consequently, specific pathways underlying antagonistic interactions between *Trichoderma* spp. and *Peronosclerospora* spp. remain insufficiently understood. Third, although conidial density and viability were assessed, additional indicators of inoculum quality, including shelf life, rhizosphere colonisation ability, enzymatic activity, secondary metabolite production, and persistence under field conditions, were not examined. Furthermore, the study used only two isolates and two propagation substrates, which may not fully represent the diversity of Trichoderma strains or the locally available organic substrates with potential for large-scale bioformulation development.

Future studies should therefore focus on multi-location, multi-season field evaluations to determine the consistency and stability of *Trichoderma*-based biological control across diverse environmental conditions. Further investigations integrating molecular, transcriptomic, and metabolomic approaches are also required to elucidate the mechanisms underlying induced systemic resistance, pathogen inhibition, and plant-microbe interactions. In addition, future research should explore optimisation of carrier formulations, shelf-life stability, and compatibility with other integrated disease management strategies to enhance field efficacy and commercialisation potential. Screening additional indigenous *Trichoderma* isolates and low-cost agricultural by-products as propagation substrates may also provide opportunities to develop more adaptive, economically feasible, and environmentally sustainable biocontrol technologies for maize production systems.

ACKNOWLEDGEMENT

The authors would like to express sincere gratitude to the co-authors, Ida Nurhelawati, Agus Surya Mulya, and Nunung Sondari, who supported us during the research process. The author also thanks the Plantation Protection Center, Bandung, West Java, Indonesia, for providing the research space and facilities during the research process.

REFERENCES

Adhi, S. R., Widiantini, F., & Yulia, E. (2019). Metode inokulasi buatan untuk pengujian infeksi *Peronosclerospora maydis* penyebab penyakit bulai pada tanaman jagung [Artificial inoculation method for testing *Peronosclerospora maydis* infection causing downy mildew disease in corn plants]. *Jurnal AGRO*, *6(1)*, 77-85. https://doi.org/10.15575/4409

Aktar, W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticide use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology*, *2*(1), 1-12. https://doi.org/10.2478/v10102-009-0001-7

Amaria, W., & Wardiana, E. (2014). Pengaruh waktu aplikasi dan spesies *Trichoderma* terhadap penyakit jamur akar putih pada bibit karet [Effect of application time and *Trichoderma* species on white root fungal disease in rubber seedlings]. *Journal of Industrial and Beverage Crops*, *1(2)*, 79-86. https://doi.org/ https://doi.org/10.21082/jtidp.v1n2.2014.p79-86

Badan Standardisasi Nasional. (2014). *Agens pengendali hayati (APH) - Bagian 3: Trichoderma spp.* [Biological control agent (BCA) - Part 3: Trichoderma spp.] (Standard No. SNI 8027.3:2014). https:// www.scribd.com/document/550086123/SNI-Trichoderma

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M., & Khurana, S. (2015). Evolution of bacterial and fungal growth media. *Bioinformation*, *11*(4), 182-184. https://doi.org/10.6026/97320630011182

Center for Forecasting of Plant Pest Organisms (BBPOPT). (2023). Technical Guidelines of Comparative Testing Between Testing Laboratories in 2023. Directorate General of Food Crops. Ministry of Agriculture.

Chakraborty, M., & Budhwar, S., Vinod, & Pooja. (2018). Nutritional and therapeutic value of rice bran. *International Journal of Green and Herbal Chemistry*, *7*(3), 451-461. https://doi.org/10.24214/ijghc/ gc/7/3/45161

Day, T. M. W., Beja, H. D., & Jeksen, J. (2022). Teknik perbanyakan massal *Trichoderma* sp. pada beberapa media tumbuh sebagai agens pengendali hayati [Mass propagation technique of *Trichoderma* sp. on several growth media as a biological control agent]. *Jurnal Locus Penelitian & Pengabdian*, *1(2)*, 81-89. https://doi.org/https://doi.org/10.58344/locus.v1i2.10

Devi, K. S., Misra, D. K., Saha, J., Devi, P. S., & Sinha, B. (2018). Screening of suitable culture media for growth, cultural and morphological characters of pycnidia forming fungi. *International Journal of Current Microbiology and Applied Sciences*, *7*(8), 4207-4214. https://doi.org/10.20546/ijcmas.2018.708.440

Sinclair, J. B. & Dhingra, O. D. (1995). *Basic Plant Pathology Methods* (2nd ed.). CRC Press. https://doi. org/10.1201/9781315138138

Gusnawaty, H. S., Taufik, M., Triana, L., & Asniah, D. (2014). Karakterisasi morfologis *Trichoderma* spp. indigenus Sulawesi Tenggara [Morphological characterisation of indigenous *Trichoderma* spp. from Southeast Sulawesi]. *Jurnal Agroteknos*, *4(2)*, 88-94. https://doi.org/10.56189/ja.v4i1.204

Gusnawaty, H. S., Taufik, M., Bande, L. O. S., & Asis, A. (2017). Uji efektivitas beberapa media untuk perbanyakan agens hayati *Trichoderma* sp. [Effectiveness of several media for propagation of the biological agent *Trichoderma* sp.]. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *17*(1), 70-76. https:// doi.org/10.23960/j.hptt.11770-76

Harni, R., Amaria, W., Syafaruddin, & Mahsunah, A. H. (2017). Potensi metabolit sekunder *Trichoderma* spp. untuk mengendalikan penyakit vascular streak dieback (VSD) pada bibit kakao [Potential of secondary metabolites of *Trichoderma* spp. to control vascular streak dieback (VSD) disease on cocoa seedlings]. *Jurnal Tanaman Industri dan Penyegar, 4*(2), 57-66. https://doi.org/10.21082/jtidp.v4n2.2017.p57-66

Jaya, K., Ratnawati, Sudewi, S., Jumardin, A, & Zainal, M. (2023). The effect of various carrier media on the growth of *Trichoderma asperellum* TR3 and *Trichoderma* sp. *IOP Conference Series: Earth and Environmental Science*, *1230*, 1-4, Article 012090. https://doi.org/10.1088/1755-1315/1230/1/012090

Kansrini, Y. (2015). Uji berbagai jenis media perbanyakan terhadap perkembangan jamur *Beauveria bassiana* di laboratorium [Evaluation of Various Propagation Media on the Growth of *Beauveria bassiana* Fungus in the Laboratory]. *Agrica Ekstensia Journal*, *9(1)*, 34-39.

Kurniasih, A. S., Setiani, O., & Nugraheni, S. A. (2013). Faktor-faktor yang terkait paparan pestisida dan hubungannya dengan kejadian anemia pada petani hortikultura di Desa Gombong Kecamatan Belik Kabupaten Pemalang Jawa Tengah [Factors related to pesticide exposure and their relationship with the incidence of anemia among horticultural farmers in Gombong Village, Belik District, Pemalang Regency, Central Java]. *Jurnal Kesehatan Lingkungan Indonesia, 12*(2), 132-137. https://ejournal.undip.ac.id/index.php/jkli/article/view/8549

Kurniawan, H., Sulastrini, I., & Suganda, T. (2018). Uji ketahanan klon kentang hasil pesilangan Atlantic × Repita terhadap penyakit hawar daun yang disebabkan oleh *Phytophthora infestans* [Resistance test of potato clones derived from the cross between Atlantic × Repita against late blight disease caused by *Phytophthora infestans*]. *Agrikultura*, *29*(2), 100-104. https://doi.org/10.24198/agrikultura.v29i2.20806

Lalujan, L. E., Djarkasi, G. S. S., Thelma, J. N., Rawung, D., & Sumual, M. F. (2017). Komposisi kimia dan gizi jagung lokal varietas "Manado Kuning" sebagai bahan pangan pengganti beras [Chemical and nutritional composition of local corn var. "Manado Kuning" as rice substitute]. *Jurnal Teknologi Pertanian*, *8*(1), 47-54.

Mayo, S., Gutiérrez, S., Malmierca, M. G., Lorenzana, A., Campelo, M. P., Hermosa, R., & Casquero, P. A. (2015). Influence of *Rhizoctonia solani* and *Trichoderma* spp. in the growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. *Frontiers in Plant Science*, 6, Article 685. https://doi.org/10.3389/fpls.2015.00685

Mufida, N., Suprihadi, A., & Purwantisari, S. (2023). Penggunaan sekam padi dan tongkol jagung sebagai media organik pada perbanyakan miselium jamur *Trichoderma harzianum* [Use of rice husk and corn cobs as organic media for the propagation of *Trichoderma harzianum* mycelium]. *Berkala Bioteknologi*, *6*(1). https://ejournal2.undip.ac.id/index.php/bb/article/view/29119

Muis, A., Nonci, N., & Pabendon, M. B. (2016). Geographical distribution of *Peronosclerospora* spp., the causal organism of maize downy mildew, in Indonesia. *AAB Bioflux, 8*(3), 143-155. https://aab.bioflux.com.ro/docs/2016.143-155.pdf

Nabila, T. T., Nugraheni, I. A., Widiyatmoko, R. S., & Probowati, W. (2021). An in vitro study of the spore densities effect of *Trichoderma* spp. as biocontrol agent for *Fusarium* wilt in cayenne pepper (*Capsicum* sp.). *International Journal of Health Science and Technology*, *3*(1), 117-129. https://doi.org/10.31101/ijhst.v3i1.2238

Nakkeeran, S., Vinodkumar, S., Priyanka, R., & Renukadevi, P. (2018). Mode of action of *Trichoderma* spp. in biological control of plant diseases. In *Biocontrol of Soil-Borne Pathogens and Nematodes* (pp. 81-95). Bioscience Publications.

Nirwanto, H., & Sutikno, S. (2024). Distribusi penyakit bulai jagung *(Peronosclerospora maydis)* pada lahan pertanaman jagung dengan pendekatan geostatistik [Distribution of downy mildew (*Peronosclerospora maydis*) on maize plots based on geospatial approach]. *Journal of Applied Agricultural Research*, *24*(4), 504-516. https://doi.org/10.25181/jppt.v24i4.3431

Novianti, D. (2018). Perbanyakan jamur *Trichoderma* sp. pada beberapa media [Propagation of *Trichoderma* sp. on several media]. *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam*, *15*(1), 35-41. https://doi.org/10.31851/sainmatika.v15i1.1763

Nurbailis & Martinius. (2011). Pemanfaatan bahan organik sebagai pembawa untuk peningkatan kepadatan populasi *Trichoderma viride* pada rizosfir pisang dan pengaruhnya terhadap perkembangan penyakit layu fusarium [The use of organic matter as a carrier to increase *Trichoderma viride* density in the banana rhizosphere and its influence on *Fusarium* wilt disease development]. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *11*(2), 177-184. https://doi.org/10.23960/j.hptt.211177-184

Poveda, J. (2021). *Trichoderma* as biocontrol agent against pests: New uses for a mycoparasite. Biological Control, *159*, Article 104634. https://doi.org/10.1016/j.biocontrol.2021.104634

Prasetyo, J., Ginting, C., Akin, H. M., Suharjo, R., Niswati, A., Afandi, A., Adiwijaya, R., Sudiono, & Nurdin, M. (2021). The effect of biological agents and botanical fungicides on maize downy mildew. *Biodiversitas*, *22*(4), 1652-1657. https://doi.org/10.13057/biodiv/d220409

Purwanto, D. S., Nirwanto, H., & Wiyatiningsih, S. (2016). Model epidemi penyakit tanaman: Hubungan faktor lingkungan terhadap laju infeksi dan pola sebaran penyakit bulai (*Peronosclerospora maydis*) pada tanaman jagung di Kabupaten Jombang [Model plant disease epidemic: Environmental factors related to the rate of infection and distribution patterns downy mildew disease (*Peronosclerospora maydis*) of corn in Jombang Regency]. *Plumula*, *5*(2), 138-152. http://ejournal.upnjatim.ac.id/index.php/plumula/article/view/764

Rashid, Z., Zaidi, P. H., Vinayan, M. T., Sharma, S. S., & Srirama Setty, T. A. (2013). Downy mildew resistance in maize (*Zea mays* L.) across *Peronosclerospora* species in lowland tropical Asia. *Crop Protection, 43*, 183-191. https://doi.org/10.1016/j.cropro.2012.08.007

Safitri, N., Erfandari, O., & Nurmayanti, S. (2023). Effectiveness test of several media for propagation of biological agent *Trichoderma* sp. *Jurnal Agrinika: Jurnal Agroteknologi dan Agribisnis*, *7*(2), 90-96. https://doi.org/10.30737/agrinika.v7i2.4927

Semangun, H. (2008). *Penyakit-penyakit tanaman pangan di Indonesia* [Diseases of food crops in Indonesia] (2nd ed.). Gadjah Mada University Press.

Simko, I., & Piepho, H. -P. (2012). The area under the disease progress stairs: Calculation, advantage, and application. *Analytical and Theoretical Plant Pathology, 102*(4), 381-389. https://doi.org/10.1094/PHYTO-07-11-0216

Suharni, Y., Hakim, L., & Susanna, S. (2023). Pengaruh beberapa media terhadap pertumbuhan *Trichoderma harzianum* isolat lokal asal pala [Effect of several media on the growth of local isolates of *Trichoderma harzianum* from nutmeg]. *Jurnal Ilmiah Mahasiswa Pertanian, 8*(2), 513-522.

Turnip, A. E. J. P. (2015). Pengaruh perlakuan benih dengan *Trichoderma viride* dan *Pseudomonas fluorescens* terhadap keterjadian penyakit bulai (*Peronosclerospora maydis*) pada berbagai varietas jagung (*Zea mays* L.) [Effect of seed treatment with *Trichoderma viride* and *Pseudomonas fluorescens* on the incidence of downy mildew disease (*Peronosclerospora maydis*) in various maize (*Zea mays* L.) varieties]. *Jurnal Agrotek Tropika*, *3*(2), 216-219. https://doi.org/10.23960/jat.v3i2.2000

Uruilal, U., Kalay, A. M., Kaya, E., & Siregar, A. (2012). Pemanfaatan kompos ela sagu, sekam dan dedak sebagai media perbanyakan agens hayati *Trichoderma Harzianum* rifai [Utilisation of sago waste compost, husk and bran as propagation media for biological agent *Trichoderma harzianum* Rifai]. *Agrologia*, *1*(1), 21-30.

Utami, W. P., Syam, N., & HS, S. (2023). Perbanyakan jamur *Trichoderma* sp. pada beberapa jenis media tumbuh dengan metode terbuka dan tertutup [Propagation of *Trichoderma* sp. in several types of growing media with open and closed methods]. *Jurnal AGrotekMAS, 4*(1), 111-118. https://doi.org/10.33096/agrotekmas.v4i1.318

Wang, R., Liu, C., Jiang, X., Tan, Z., Li, H., Xu, S., Zhang, S., Shang, Q., Deising, H. B., Behrens, S. -E., & Wu, B. (2022). The newly identified *Trichoderma harzianum* partitivirus (ThPV2) does not diminish spore production and biocontrol activity of its host. *Viruses*, *14*(7), Article 1532. https://doi.org/10.3390/v14071532

Widiantini, F., Pitaloka, D. J., Nasahi, C., & Yulia, E. (2017). Perkecambahan *Peronosclerospora* spp. asal beberapa daerah di Jawa Barat pada fungisida berbahan aktif metalaksil, dimetomorf, dan fenamidon [Germination of *Peronosclerospora* spp. from several areas in West Java on fungicides containing the active ingredients metalaxyl, dimethomorph, and fenamidone]. *Jurnal Agrikultura*, *28*(2), 95-102. https://doi.org/10.24198/agrikultura.v28i2.15753

Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J., & Chen, J. (2023). *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology*, *14*, Article 1160551. https://doi.org/10.3389/fmicb.2023.1160551

Yparraguirre, H. C., & Galliani-Pinillos, C. L. (2020). Production of *Trichoderma viride* in local organic substrates of the Ica region, Peru. *Journal of Plant Pathology & Microbiology*, *11*(3), 1-7. https://doi.org/10.35248/2157-7471.20.11.490

Yudha, M., Soesanto, L., & Mugiastuti, E. (2016). Pemanfaatan empat isolat *Trichoderma* sp. untuk mengendalikan penyakit akar gada pada tanaman caisin [The utilisation of four *Trichoderma* sp. isolates for controlling clubroot disease in Chinese cabbage]. *Jurnal Kultivasi*, *15*(3), 143-149. https://doi.org/10.24198/kultivasi.v15i3.11771