Assessment of liquid, semi-solid, and powder formulations for delivering *Trichoderma* sp. as maize seed coating against *Peronosclerospora maydis*

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ABSTRACT

Downy mildew, caused by *Peronosclerospora* sp., is a major disease impacting maize (*Zea mays* L.) production, resulting in significant yield losses. This study evaluated the effectiveness of three *Trichoderma* sp.-based seed coating formulations (liquid, semi-solid, and powder) in reducing disease incidence and severity in maize. The experiment followed a completely randomized design with four treatments, including a control, replicated five times. The parameters assessed included disease incidence, severity, and control effectiveness over 35 days after inoculation. Results showed significant differences among treatments. The powder formulation (T3) demonstrated superior performance, reducing disease incidence by 42.3% and severity by 44.99% compared to the control (T0), which exhibited the highest disease levels. Control effectiveness of the T3 formulation reached 53.81%, categorized as moderately effective. The enhanced efficacy of the powder formulation is attributed to its ability to maintain high *Trichoderma* sp. populations, enabling sustained antagonistic activity through parasitism, antibiosis, and induced systemic resistance. These findings underline the potential of *Trichoderma* sp.-based seed coatings, particularly in powder form, as an environmentally friendly and practical alternative for managing downy mildew in maize.

Keywords: biological control, downy mildew, maize, seed coating, *Trichoderma* sp.

INTRODUCTION

Maize (*Zea mays L.*) is an essential cereal crop for the Indonesian population, second only to rice (Amzeri, 2017). Its carbohydrate content provides vital dietary fibre, and in certain regions such as Madura, it is often used as a staple carbohydrate substitute. Over time, maize has expanded beyond its role as a food source to be utilised as livestock feed and an industrial raw material. In 2015, East Java became Indonesia's leading maize-producing province, yielding approximately 6.2 tons, with production increasing by 2.4% in 2016 but declining by 0.9% in 2017 (Badan Pusat Statistik (BPS), 2018).

Downey mildew, caused by *Peronosclerospora sp.*, is one of the most destructive diseases impacting maize, leading to yield losses of up to 30% or even total crop failure. Losses as high as 80% have been reported in major maize-growing regions, including East Java, South Sulawesi, and West Kalimantan (Pakki, 2017; Pakki & Talanca, 2016). In Madura, downy mildew caused by *P. maydis* (Khoiri et al., 2021). Farmers often hesitate to replant maize due to insufficient knowledge about disease management. Current control measures, primarily reliant on resistant varieties and synthetic fungicides, remain inadequate and contribute to environmental pollution and pathogen resistance (Djaenuddin et al., 2024; Muis, 2018; Muis et al., 2016).

A sustainable alternative is the use of biological agents such as *Trichoderma sp.*, a non-pathogenic fungus with antagonistic properties that suppress pathogen growth and enhance plant resistance (Khoiri et al., 2023; Syahputra et al., 2017). *Trichoderma sp.* can induce systemic resistance and effectively combat various plant pathogens, making it a promising biocontrol agent (Elfina et al., 2017).

Formulating *Trichoderma sp.* into seed coatings provides a practical and efficient delivery system. Seed coating combines active agents and nutrients, offering protection against pathogens while improving seed germination and plant growth (Rocha et al., 2019). This study focuses on the development formulas and efficacy of *Trichoderma sp*.-based seed coatings in reducing downy mildew infection and enhancing maize productivity.

MATERIALS AND METHODS

Research Location

The research was conducted from September 2021 to April 2022 in the Plant Protection and Environment Laboratory and the Greenhouse of Agrotechnology, Faculty of Agriculture, Universitas Trunojoyo Madura.

Materials

The equipment used in this research included petri dishes, test tubes, beaker glasses, a LAF (Laminar Air Flow) cabinet, inoculation needles, spray bottles, a Bunsen burner, matches, an autoclave, an analytical balance, a hotplate, polybags, rulers, stationery, glass slides, a binocular light microscope, a pH meter, a camera, knives, sterile plastic, tweezers, cotton, cover slips, pipette pumps, dropper pipettes, stirring rods, measuring cylinders, a refrigerator, a gas stove, and other items. The materials used included the endophytic

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strain of *Trichoderma* sp. (Madura strain) obtained from the Plant Protection and Environment Laboratory, 70% alcohol, polyethylene plastic, sterile water, distilled water, glycerol, CMC, molasses, kaolin powder, NPK fertiliser, sterilised topsoil, components for PDA medium (agar, potatoes, distilled water, dextrose), aluminium foil, plastic wrap, tissue rolls, potato dextrose broth (PDB), and Madura 3 maize seeds.

Experimental Design

The research was designed using a Completely Randomised Design (CRD) with a non-factorial approach. The experiment consisted of four seed coating treatments: three types of seed coating formulations and one control, each replicated five times, resulting in 20 experimental units. Each unit contained three plant samples, totalling 60 polybags. The treatments were as follows:

T0 = Control
T1 = Liquid formulation
T2 = Semi-solid formulation
T3 = Powder formulation

The layout obtained after randomisation is presented in Figure 1.

Preparation and Multiplication of *Trichoderma* sp. Isolate

The *Trichoderma sp.* isolate was multiplied by preparing potato dextrose agar medium and was sterilised using an autoclave. The sterilised PDA was poured into petri dishes at a volume of 10 ml per dish. Once the medium solidified, the *Trichoderma sp.* isolate was cultured and incubated for 14 days. For purification, the isolate was transferred to a fresh PDA medium. Using a sterile inoculation needle, a section of the isolate (0.5–1 cm) was excised and placed onto the new medium. The culture was incubated for another 14 days to ensure purity.

Source of Downey Mildew Inoculum

The downy mildew inoculum was obtained from maize leaves infected with *Peronosclerospora maydis* in the field. The leaves were collected in the evening and stored in plastic clip bags. They were washed with clean water to remove dirt and damaged spores and then air-dried. The leaf bases were soaked in a 2% sugar solution to a depth of 3 cm and covered with plastic bags to maintain humidity. The leaves were left overnight in a dark, open area. At 4:00 a.m. (Jakarta time or GMT +7), the leaves were washed under running water and squeezed gently between the thumb and forefinger. Inoculation was performed early in the morning (5:00–6:00 a.m. Jakarta time or GMT +7) by spraying 10 mL of *P. maydis* suspension onto each maize plant's foliage and growth points.

Preparation of Inoculated-Potato Dextrose Broth (PDB)

The preparation of PDB involved extracting a 4 cm^2 disc of *Trichoderma* sp. from a 14-day-old PDA culture using a sterile borer. The isolate was inoculated into PDB and supplemented with 0.9 grams of KH_2PO_4 to maintain the pH at 6–7. The suspension was incubated at room temperature with continuous aeration, illumination, and agitation on a rotary shaker for 48 hours. This process ensured the culture achieved a spore density of approximately 10^8 spores mL^{-1}.

Seed Coating Process

Before coating, maize seeds were disinfected by soaking in 70% alcohol for three minutes, rinsed three times with sterile water, and air-dried in a laminar airflow cabinet for 60 minutes. Three seed coating methods were tested:

1. Liquid Formulation

A liquid formulation was prepared by suspending three petri dishes of *Trichoderma* sp. cultured for seven days in 1 litre of sterile water and 250 grams of dextrose. For every 100 seeds, a suspension from a 14-day-old *Trichoderma* sp. culture (10^8 spores mL^{-1}) was mixed with 75 mL of sterile water and 25 grams of dextrose. The seeds were soaked for 20 minutes and then air-dried for 24 hours.

2. Semi-Solid Formulation

A semi-solid formulation was prepared by mixing 15 mL of potato broth (10^8 spores mL^{-1}) with 6% glycerol, 5% carboxymethyl cellulose (CMC), and 72.9 ml of sterile water. Each 100 seeds were coated with 10 grams of the formulation and 20 mL of sterile water, mixed thoroughly for 20 minutes, and air-dried for 24 hours in a laminar airflow cabinet.

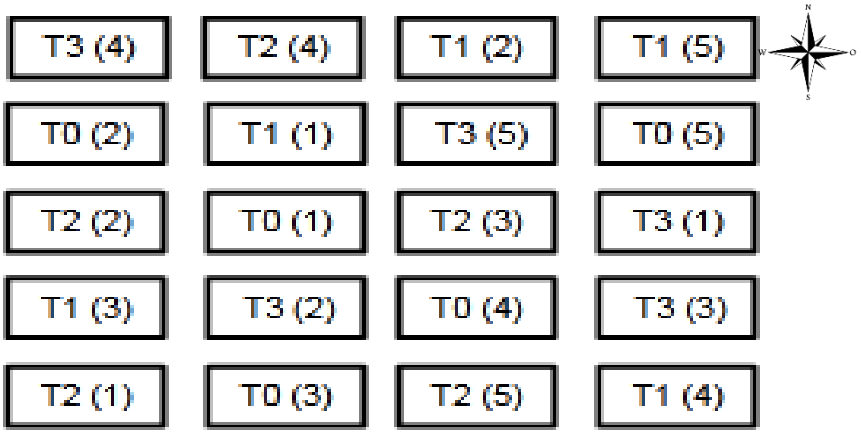

Figure 1. The research layout

3. Powder Formulation

The powder formulation involved mixing 15 mL of potato broth (10^8 spores mL^{-1}) with 1.5 grams of molasses, 5 grams of CMC, 7.1 mL of glycerol, 65 grams of kaolin powder, and 6.4 mL of sterile water. The mixture was oven-dried at 37°C. Maize seeds were soaked in water for two hours before being mixed with the powder formulation to ensure even coating.

Application of Formulated Seed Coatings

The coated maize seeds were stored in polyethylene plastic containers (62 grams per pack) with silica gel in jars to maintain dryness, kept at room temperature (±27°C) for 14 days before assessing seed viability and the *Trichoderma sp.* population. For planting, sterilised topsoil was filled into 15 cm x 15 cm polybags arranged in a greenhouse according to the experimental layout, and the coated seeds were planted 2 cm deep in the prepared soil. Downy mildew inoculation was conducted early in the morning (2:00–4:00 a.m. Jakarta time, GMT +7) by spraying 10 ml of *P. maydis* suspension onto the whorls and leaves of each maize plant seven days after sowing. Plant maintenance involved regular watering every morning and evening (or once daily if moisture levels were sufficient), fertilisation with 0.4 grams of NPK as a basal application before planting, and manual weed removal to minimise competition.

Disease incidence

Observations were conducted weekly after inoculation until 35 days after planting. The number of infected and non-infected plants in each treatment was recorded and calculated using the following formula (Giofanny et al., 2014):

Disease Incidence (%) = ((number of infected plants) / (total number of plants observed)) × 100%

The disease incidence in plants can serve as an indicator for determining plant resistance to pathogen attack (Daryono et al., 2018).

Disease Severity

Observations were also conducted weekly after inoculation until 35 days after planting. Disease severity was calculated by assessing the symptoms on each leaf of the plant, with severity rated according to the scale in Table 2, and the following formula was used (Ulhaq & Masnilah, 2019):

$$C = \frac{\sum(ni\ x\ vi)}{V.Z}\ x\ 100\%$$

Where C is disease severity, ni is the number of leaves in each severity category, vi is the scale value (0-4) for each severity category, V is the value of the highest severity scale, and Z is the total number of leaves observed.

Effectiveness of Control

The effectiveness of the disease control was calculated using the following formula for evaluating of disease control. The effectiveness value was determined by comparing the disease severity of each treatment with the control (Gusnawaty, 2011):

EC = (I Control - I Treatment) × 100%

Where EP is the effectiveness of control with antagonists (%), I Control is the percentage of disease severity in the control, and I Treatment is the percentage of disease severity in the treatment

Data Analysis

The data obtained during the study were analyzed using Analysis of Variance (ANOVA) at a significance level of 5%. If significant differences were found among treatments, further analysis using Duncan's Multiple Range Test at 5% was performed to identify the optimal treatment. According to Elfina et al. (2017), the effectiveness of biological agents can be classified as follows: 0 = ineffective, 1-20% = very ineffective, >20-40% = ineffective, >40-60% = moderately effective, >80% = very effective.

Table 1. Disease resistance categories for downy mildew

Disease incidence (%)	Resistance category
0	Highly resistant
0 < x ≤ 25	Resistant
26 < x ≤ 50	Moderately resistant
51 < x ≤ 75	Susceptible
x > 75	Highly susceptible

Table 2. Downey mildew disease severity scale

Severity score	Descriptions
0	No symptoms
1	Symptoms covering 1-25% of the leaf
2	Symptoms covering 26-50% of the leaf
3	Symptoms covering 51-75% of the leaf
4	Symptoms covering 75-100% of the leaf

RESULT AND DISCUSSION

Based on the results of the Analysis of Variance (ANOVA), significant differences in the incidence of downy mildew were observed from 21 to 35 days after sowing (DAS), but no significant effect was found at 14 DAS (Table 3). Table 1 shows that by 35 DAS, almost all treatments had plants affected by downy mildew. The highest disease incidence occurred in the control treatment (T0) at 94.11%, while the lowest incidence was observed in the powder formulation (T3) at 42.3%. The disease incidence data show that T3 falls under the 'moderately resistant' category, while T1 and T2 are classified as 'susceptible,' and T0 is 'highly susceptible' to downy mildew infection (Table 4). Based on the ANOVA results for disease severity, significant differences in the severity of downy mildew were observed from 21 to 35 DAS, but no significant effect was found at 14 DAS. At 35 DAS, there were significant differences among the treatments. The T3 (powder formulation) treatment showed the lowest disease severity, which was significantly different from the control treatment (T0), with 95.42%. T1 (liquid formulation) showed a significant difference from the control with a severity of 60.84%, and T2 (semi-solid formulation) had a severity value of 77.56% (Table 5).

Control effectiveness is defined as the ability of the biological agent used in seed coating to reduce pathogen attack. The effectiveness was calculated by comparing the disease severity in each treatment with the severity in the control. Based on the calculation from 14 to 35 DAS, no significant differences were observed among the treatments. At 35 DAS, the highest effectiveness was observed in T3 (powder formulation) with a value of 53.81%, while the lowest was seen in T2 (semi-solid formulation). The effectiveness results are presented in Table 4.4, showing that T3 is classified as 'moderately effective' in controlling downy mildew, while T1 is 'ineffective' and T2 is 'very ineffective' (Table 6 and Table 7). This study evaluated the effectiveness of several seed coating formulations containing *Trichoderma* sp. in reducing the incidence and severity of downy mildew caused by *Peronosclerospora* sp. in maize plants. Seed coating treatments using liquid, semi-solid, and powder formulations showed significant differences in disease incidence and severity, while no significant differences were observed for control effectiveness. The seed coating treatment with the powder formulation (T3) had the lowest disease incidence at 43.3%, indicating that *Trichoderma* sp. in powder form is more effective in controlling downy mildew incidence compared to other formulations

Table 3. Average disease incidence (%) observed on different days after sowing (DAS)

Treatment	Average disease incidence (%) on different days			
	14	21	28	35
T0	7.8	54.21 c	73.99 c	94.11 c
T1	0	21 a	46.96 a	59.36 b
T2	0	29.44 ab	54.9 ab	77.14 bc
T3	0	4.98 a	34.3 a	42.3 a

Note: Values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Table 4. Disease resistance categories for downy mildew

Treatment	Average of disease incidence (%)	Resistance category
T0	94.11	Highly susceptible
T1	59.36	Susceptible
T2	77.14	Susceptible
T3	42.3	Moderately resistant

Table 5. Disease severity (%) observed on different days after sowing (DAS)

Treatment	Average of disease severity (%) on different days			
	14 DAS	21 DAS	28 DAS	35 DAS
T0	8.68	56.72 c	76.22 c	95.42 c
T1	0	22.36 a	48.91 a	60.84 b
T2	0	30.92 ab	57.26 ab	77.56 bc
T3	0	5.46 a	35.34 a	44.99 a

Note: Values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Table 6. Control Effectiveness (%) Observed on Different Days After Sowing (DAS)

Treatment	Average of control effectiveness (%) on different days			
	14 DAS	21 DAS	28 DAS	35 DAS
T1	100	60.56	35.82 a	36.23 a
T2	100	45.47	24.86 a	18.7 a
T3	100	90.37	53.62 a	53.81 a

Note: Values followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Table 7. Control Effectiveness Categories for Downey Mildew

Treatment	Control effectiveness (%)	Category
T1	36.23	Ineffective
T2	18.7	Very ineffective
T3	53.81	Moderately effective

Turkan et al. (2023) reported that seed coating containing *T. viridae* increased plant resistance against plant pathogenic fungi. *Trichoderma* sp. inhibits pathogen growth through mycoparasitism and antibiosis, producing enzymes and toxins that are toxic to pathogens, such as viridin, glotoxin, and paraceltin, which can destroy pathogens. Other studies have shown that powder formulations of *Trichoderma* sp. can significantly reduce disease incidence in tomato plants (Awad-Allah et al., 2022; Sallam et al., 2019).

The results suggest that *Trichoderma* sp. applied as a powder formulation is more effective in controlling downy mildew disease incidence. The disease severity showed that T3 (powder) resulted in the lowest severity at 44.99%, while T0 (control) had the highest at 95.42%. The number of *Trichoderma* sp. spores in the powder formulation was likely higher than in the other formulations, which contributed to its greater effectiveness in reducing both incidence and severity. Furthermore, the study highlights that the carrier materials used in seed coatings play a significant role in supporting the viability of *Trichoderma* sp. and its effectiveness in controlling plant diseases.

CONCLUSIONS

Based on the research conducted, it can be concluded that the application of seed coating with a powder formulation containing *Trichoderma* sp. at 35 days after sowing in maize plants effectively reduced the disease incidence by 42.3%, disease severity by 44.99%, and control effectiveness by 53.81%. This performance falls into the category of moderately effective in controlling downy mildew disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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