

The Role of *Trichoderma* spp. as a Biocontrol Agent of Damping Off Disease and Soybean Biostimulant

Peran *Trichoderma* spp. sebagai Agen Biokontrol pada Penyakit Rebah Semai dan Biostimulan Kedelai

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ABSTRACT

Domestic demand for soybeans in Indonesia continues to increase each year, yet the average production has declined. To meet national needs, the government must even import soybeans. One of the causes of low soybean production is damage from plant pests and diseases, particularly damping-off caused by *Sclerotium rolfii*, which can lead to total plant death under severe infection. One promising, environmentally friendly, and cost-effective approach to disease management is the use of biological control agents such as *Trichoderma* spp., which function not only as biocontrol agents but also as biostimulants. This research was conducted at the Plant Pest and Disease Laboratory, Faculty of Agriculture Brawijaya University and Central Laboratory of BALITKABI for *in vitro* experiments and *in vivo* study located at a greenhouse of Agriculture Experimental Land Brawijaya University, Jatimulyo, Lowokwaru, Malang. A completely randomized design was used with five treatments and five replications. Applications of *Trichoderma harzianum* and *T. asperellum*, either individually or in combination, successfully reduced the incidence and severity of damping-off disease by 14% to 26.6% under field conditions. These treatments also increased the total phenolic content of the plants, indicating enhanced resistance, thus supporting the role of *Trichoderma* as an effective biocontrol agent. In addition, the combined application of *Trichoderma* species significantly increased the number of soybean leaves, indicating a biostimulant effect.

Keywords: biocontrol, biostimulant, soybean, total phenolic compounds, *Trichoderma*

ABSTRAK

Permintaan dalam negeri akan kedelai dari tahun ke tahun terus meningkat, akan tetapi rerata produksi mengalami penurunan. Bahkan untuk memenuhi kebutuhan nasional, pemerintah harus melakukan impor kedelai. Rendahnya produksi kedelai di Indonesia di antaranya disebabkan adanya gangguan OPT seperti penyakit rebah semai (*Sclerotium rolfii*) yang pada tingkat serangan berat menyebabkan kematian. Salah satu pendekatan pengendalian yang menjanjikan karena bersifat ramah lingkungan, efektif, dan efisien adalah dengan memanfaatkan agens hayati seperti jamur *Trichoderma* spp.. Penelitian ini dilaksanakan secara *in vitro* di Laboratorium Penyakit Tumbuhan, Departemen HPT Fakultas Pertanian Universitas Brawijaya (FB-UB) dan di Laboratorium Sentral BALITKABI serta secara *in vivo* di rumah kaca lahan percobaan FP-UB dan secara *in vitro* menggunakan rancangan acak lengkap dengan 5 perlakuan dan 5 ulangan. Aplikasi *T. harzianum* maupun *T. asperellum* secara tunggal dan kombinasi mampu menurunkan persentase insiden maupun intensitas penyakit busuk pangkal batang yang disebabkan oleh patogen *S. rolfii* antara 14% hingga 26.6% di lapangan dan meningkatkan

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senyawa total fenolik tanaman sebagai indikator tingkat ketahanan tanaman sehingga dikatakan sebagai agen biokontrol yang efektif. Sedangkan kombinasi antara spesies *Trichoderma* memengaruhi peningkatan jumlah daun tanaman kedelai.

Kata kunci: biokontrol, biostimulan, kedelai, senyawa total fenolik, *Trichoderma*

INTRODUCTION

Soybean (*Glycine max*) is a vital legume crop with strategic importance in global food security due to its high protein content and diverse utilization in food, feed, and industrial products. In Indonesia, soybean constitutes a primary protein source in daily consumption. Despite growing domestic and global demand, national soybean production has exhibited a declining trend, resulting in a widening gap between supply and demand. According to Setjen Pertanian (2020), soybean production in Indonesia decreased by over 30% between 2018 and 2019 from 650 000 tons year⁻¹ to 424 189 tons year⁻¹, and by 2020, domestic supply fulfilled only approximately 20% of national needs (Setjen Pertanian 2021). Consequently, Indonesia remains dependent on imports to meet its soybean consumption demands.

One of the critical challenges limiting soybean productivity is the prevalence of plant diseases, particularly damping-off disease caused by *Sclerotium rolfsii* Sacc. (Kusumaningrum *et al.* 2007). *S. rolfsii* (teleomorph: *Athelia rolfsii*) was very detrimental for soybean production. This soil-borne fungal pathogen is known for its broad host range (Semangun 2004) and persistence in the soil through resilient sclerotia structures. Infected plants typically exhibit basal stem rot, wilting, and eventual death, leading to severe yield losses (Sukamto and Wahyuno 2013).

Although the use of chemical fungicides remains a common method for managing damping-off, the long-term application of these chemicals poses serious environmental and agronomic concerns. These concerns include the accumulation of chemical residues, harm to non-target organisms, a reduction in soil microbial diversity, and the development of fungicide-resistant pathogen strains (Kusumaningrum *et al.* 2007). These drawbacks have encouraged the exploration

of environmentally sustainable alternatives, particularly biological control strategies

Trichoderma spp. have received significant attention among various biocontrol agents due to their proven antagonistic activity against numerous soil-borne pathogens (Harman *et al.* 2004). These fungi exhibit multiple modes of action, including mycoparasitism, competition for nutrients and space, and production of antifungal metabolites. *Trichoderma* spp. are also known to enhance plant defense mechanisms by inducing systemic resistance and promoting plant growth through improved nutrient uptake and phytohormone modulation.

Recent studies have highlighted their role in inducing systemic resistance in host plants. For example, *Trichoderma* spp. were shown to reduce the severity of downy mildew in maize plants by up to 88.89% by stimulating the production of reactive oxygen species (Asmawati 2016). Additionally, *Trichoderma* has demonstrated plant growth-promoting effects, such as increased stem diameter and leaf number in soybeans (Rizal and Susanti 2018).

Many studies have assessed the effectiveness of individual *Trichoderma* isolates. However, combining compatible strains is often recommended to enhance efficacy and broaden their functional range (Cook 1993; Sangeetha *et al.* 2009). Mixed applications have been reported to provide greater biocontrol efficiency than single-isolate treatments (Andrews 1982; Sangeetha *et al.* 2009). Based on this background, the present study aimed to evaluate the potential of *T. harzianum* and *T. asperellum*, both individually and in combination, as biocontrol agents against *S. rolfsii* and as biostimulants to enhance soybean growth. The outcomes are expected to support the development of environmentally sound technologies for improving the health and productivity of soybean crops.

MATERIALS AND METHODS

This research was conducted from May to August 2022 at multiple locations, including the Plant Disease Laboratory, Faculty of Agriculture, Brawijaya University, the Central Laboratory of BALITKABI and the greenhouse of UB's Agricultural Experimental Field located in Jatimulyo, Malang, East Java.

The study consisted of two separate experiments: The first objective is to evaluate the biocontrol activity of *Trichoderma* spp. against *S. rolfii*. The second objective is to assess their biostimulant potential on soybean vegetative growth. Both experiments were arranged in a completely randomized design with five treatments and five replications per treatment. Each experimental unit contained five soybean plants, for a total of 125 plants per experiment. The *Trichoderma harzianum* (TD2) and *Trichoderma asperellum* (Abx) used in this study have been deposited in the culture collection of Brawijaya University.

The treatment for the biocontrol experiment is as follows: The negative control (T0) did not contain fungal or pathogen application. The positive control (T1) was inoculated with *S. rolfii*. The *T. harzianum* + *S. rolfii* combination was T2. The *T. asperellum* + *S. rolfii* combination was T3. The *T. harzianum* + *T. asperellum* + *S. rolfii* combination was T4. In the biostimulant experiment, treatments T2, T3, and T4 were applied without *S. rolfii*.

Pathogenicity Assay of *Sclerotium rolfii*

The pathogenicity of *S. rolfii* was confirmed by inoculating soybean seedlings of the Anjasmoro variety with sclerotia at the base of the stem. Symptoms, including stem rot, wilting, and plant death (Figure 1), were monitored to validate the effectiveness of the inoculation procedure.

Compatibility Test of *Trichoderma harzianum* and *Trichoderma asperellum*

The compatibility of *T. harzianum* and *T. asperellum* was tested using dual culture on potato dextrose agar (PDA) medium. The absence of inhibition zones and mutual growth after seven days indicated their compatibility for combined application.

Planting Medium Preparation

The planting medium was a mixture of sterilized soil, organic manure, and wet rice husks at a ratio of 2:1:1. The soil mixture was sterilized by spraying with 4% formalin, sealed in plastic bags, and incubated for seven days. Then, the medium was then air-dried for seven days before use.

Preparation and Application of *Trichoderma* sp. Suspension

T. harzianum and *T. asperellum* were cultured in potato dextrose broth medium. For application, 100 mL of fungal culture was diluted into 1000 mL of sterile water.

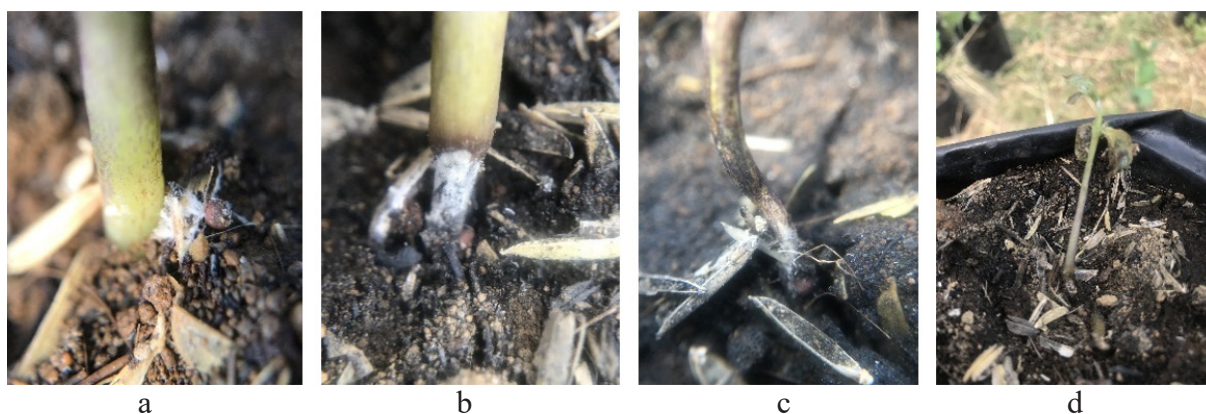


Figure 1 Damping off disease in soybean plants. a, Pathogen penetration process; b, Infectious and colonization process; c, Infection spreads; and d, Plants wilt and death.

A 60 mL aliquot of the suspension was applied to each planting hole immediately after soybean seeds were sown. The application was carried out once at the beginning of the experiment. The suspension *T. harzianum* contained of 5.65×10^6 conidia mL⁻¹ and *T. asperellum* at 2.75×10^6 conidia mL⁻¹.

Inoculation of *Sclerotium rolfsii* to Soybean Seedlings

Sclerotia were harvested from cultures of *S. rolfsii* and applied to 5-days-old (DAP) seedlings. In accordance with the treatment, three sclerotia were placed at the base of each seedling (Purwanto *et al.* 2016).

Observation

Several parameters were observed to evaluate *Trichoderma* spp.'s effectiveness as a biocontrol agent and plant growth promoter. To assess disease occurrence, observations were conducted starting 10 days after planting (DAP) and continued weekly for four weeks. Disease incidence was determined by calculating the percentage of infected plants relative to the total number of plants, using the following formula (Silaban *et al.* 2015).

$$DI = \frac{n}{N} \times 100\%, \text{ with}$$

DI, percentage of disease incidence; n, number of infected plants and N, the total plant population.

Disease severity was evaluated using a visual scoring scale ranging from 0 to 3, based on symptom severity (Table 1) as defined by Chairudin *et al.* (2018). The level of infection in each treatment was then determined by

Table 1 Symptom scores of collar rot disease

Score	Description attack
0	No visible symptoms
1	Mild symptoms, slight discoloration at the base of the stem, no wilting
2	Moderate symptoms, spotting with stem wilting, some plants remain viable
3	Severe symptoms, complete wilting or plant death

calculating the percentage of disease severity using this formula.

$$DS (\%) = \frac{\sum_{i=0}^Z (n_i \times v_i)}{N \times V} \times 100\%, \text{ with}$$

DS, disease severity; n, total infected plants; v, scale value damage; Z, the highest scale (v = 3); and N, quantity observed plants.

To assess plant resistance, total phenolic compounds were analyzed as biochemical indicators. Leaf samples were collected at 32 DAP from the second fully expanded leaf from the top of each plant. A composite sample of 1 g was extracted using 80% ethanol and centrifuged at 5000 rpm for 45 minutes. Using a UV-Vis spectrophotometer, the resulting supernatant was analyzed using the Folin–Ciocalteu method (Singleton *et al.* 1999) with absorbance measured at 765 nm.

Two parameters were observed for evaluating vegetative growth: plant height and number of leaves. Measurements were taken weekly from 1 to 4 weeks after planting (WAP). Plant height was measured from the soil surface to the shoot apex. The number of leaves was determined by counting fully expanded leaves that were not damaged by more than 50%. These parameters were recorded to assess the biostimulant potential of *Trichoderma* spp. on soybean vegetative development.

Data Analysis

The experimental data were analyzed using analysis of variance (ANOVA) at a 5% significance level and regression. If there is a significant difference among treatments, then further test using Tukey honest significant difference test α 0.05. All statistical analyses were performed using Microsoft Excel and SPSS software.

RESULT

Compatibility of *T. harzianum* and *T. asperellum* on PDA

The compatibility test between *T. harzianum* and *T. asperellum* showed that after 10 days of incubation, both isolates grew simultaneously on the same medium without

forming inhibition zones. This indicates that the two species are compatible for combined application (Figure 2). This compatibility supports their potential for synergistic use as a biocontrol agent and biostimulant.

Disease Incidence

The application of *Trichoderma* spp significantly decreased the incidence of damping-off disease in soybean plant treatments. Compared to the positive control (T1), treatments T2 (*T. harzianum* + *S. rolfsii*),

T3 (*T. asperellum* + *S. rolfsii*), and T4 (*T. harzianum* and *T. asperellum* + *S. rolfsii*) were effective in reducing disease incidence. Among the treatments, T3 showed the highest reduction, lowering the incidence by approximately 17%, followed by T4 (15%) and T2 (14%) (Figure 3). These results demonstrate the potential of *Trichoderma* spp. in suppressing collar rot incidence under greenhouse conditions.

Disease Severity

Disease severity was assessed based on a scoring scale and analyzed over a four-week observation period. Results showed that all treatments involving *Trichoderma* spp. significantly reduced disease severity compared to the pathogen-only control (T1). The combination treatment (T4) demonstrated the most significant reduction in disease severity, with values ranging from 21.3% to 26.7% across the four weeks of observation (Table 2). These findings confirm the effectiveness of both individual and combined applications of *T. harzianum* and *T. asperellum* in limiting disease progression.

Total Phenolic Compound

The total phenolic content in soybean leaves was analyzed using a standard gallic acid curve using *Folin Ciocalteu reagent* (Table 4).

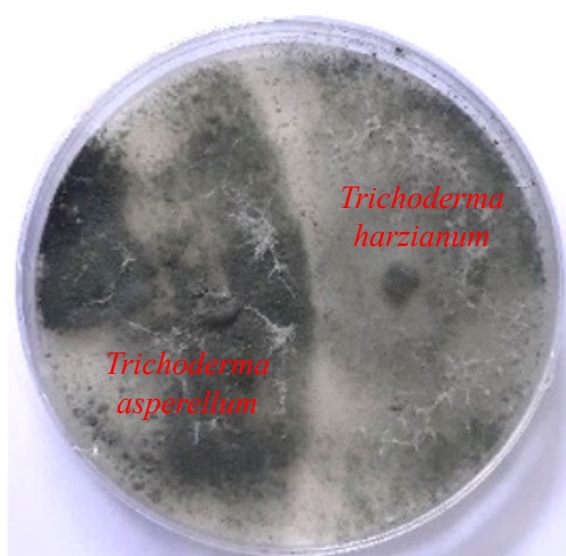


Figure 2 Compatibility between *Trichoderma harzianum* and *Trichoderma asperellum* on potato dextrose agar medium.

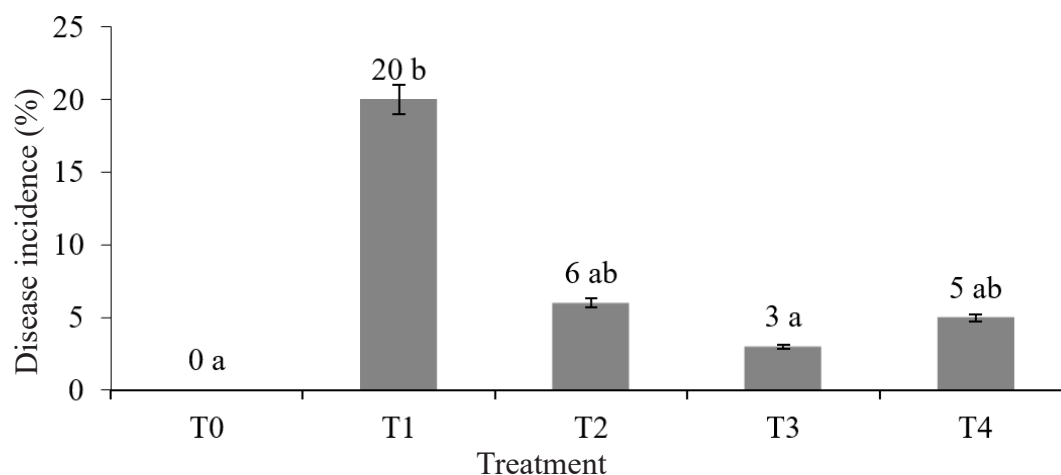


Figure 3 Disease incidence of collar rot disease in soybean plants. T0, Negative control (without fungi applications); T1, Positive control (*S. rolfsii* only); T2, *T. harzianum* + *S. rolfsii*; T3, *T. asperellum* + *S. rolfsii*; T4, *T. harzianum* + *T. asperellum* + *S. rolfsii*. Means followed by the same letter are not significantly different by Tukey test α 0.05.

The results revealed an increase in phenolic compounds in treatments involving *Trichoderma* spp. The highest phenolic content was observed in T2 (*T. harzianum* + *S. rolfesii*), reaching 626.9 GAE mL L⁻¹, followed by T3 (*T. asperellum* + *S. rolfesii*) with 578.4 GAE mL L⁻¹. These values were substantially higher than those in the pathogen control (T1, 473.4 GAE mL L⁻¹) and were similar to or greater than the negative control (T0) (Table 4). These results suggest that *Trichoderma* spp. may contribute to the induction of plant defense mechanisms by accumulating phenolic compounds.

Relationship Between Total Phenolic Content and Disease Suppression

Regression analysis indicated a negative linear correlation between total phenolic content and disease incidence and severity. An increase in total phenolic levels by 5% was associated with a reduction in disease incidence by approximately 4.56%, and a reduction in disease severity by 2.29%. The coefficients of determination (R²) were 0.3862 for disease incidence and 0.2554 for disease severity, suggesting that phenolic compounds

played a moderate role in disease suppression (Figure 4).

The Plant Height Average of Soybean

The application of *Trichoderma* spp. had no statistically significant effect on plant height at any stage of observation (1–4 WAP). Although slight differences in mean values were observed, particularly in T4, the variation was not significant when compared to the control groups (Table 5). This finding indicates that the primary effect of *Trichoderma* spp. in this study was related more to disease resistance than to vegetative elongation.

The Average of The Number of Soybean Leaves

Only the number of leaves significantly responded to the treatments among the vegetative growth parameters. At 4 WAP, the combination treatment (T4) resulted in the highest average number of leaves (4.48), significantly greater than the other treatments (Table 6). This finding indicates the biostimulant potential of *Trichoderma* spp., particularly when applied in combination.

Table 3 Disease severity of collar rot disease in soybean plants

Treatment ^a	Disease severity at...week after planting ^b (%)			
	1	2	3	4
T0	0.0 a	0.0 a	0.0 a	0.0 a
T1	10.7 bc	30.7 c	44.0 c	49.3 c
T2	4.0 ab	6.7 ab	20.0 ab	22.7 ab
T3	9.3 ab	14.7 abc	22.7 abc	28.0 ab
T4	20.0 c	21.3 bc	26.7 bc	26.7 ab

^aT0, Plant negative control (without fungi applications); T1, Plant positive control (applications of *S. rolfesii*); T2, *T. harzianum* + *S. rolfesii*; T3, *T. asperellum* + *S. rolfesii*; and T4, *T. harzianum* + *T. asperellum* + *S. rolfesii*.

^bMeans followed by the same letter are not significantly different by Tukey test α 0.05.

Table 4 Phenolic compound content in each treatment

Treatment ^a	Phenolic compound (GAE mL L ⁻¹)
T0 (Control)	539.9
T1 (<i>S. rolfesii</i>)	473.4
T2 (<i>T. harzianum</i> + <i>S. rolfesii</i>)	626.9
T3 (<i>T. asperellum</i> + <i>S. rolfesii</i>)	578.4
T4 (<i>T. harzianum</i> + <i>T. asperellum</i> + <i>S. rolfesii</i>)	537.1

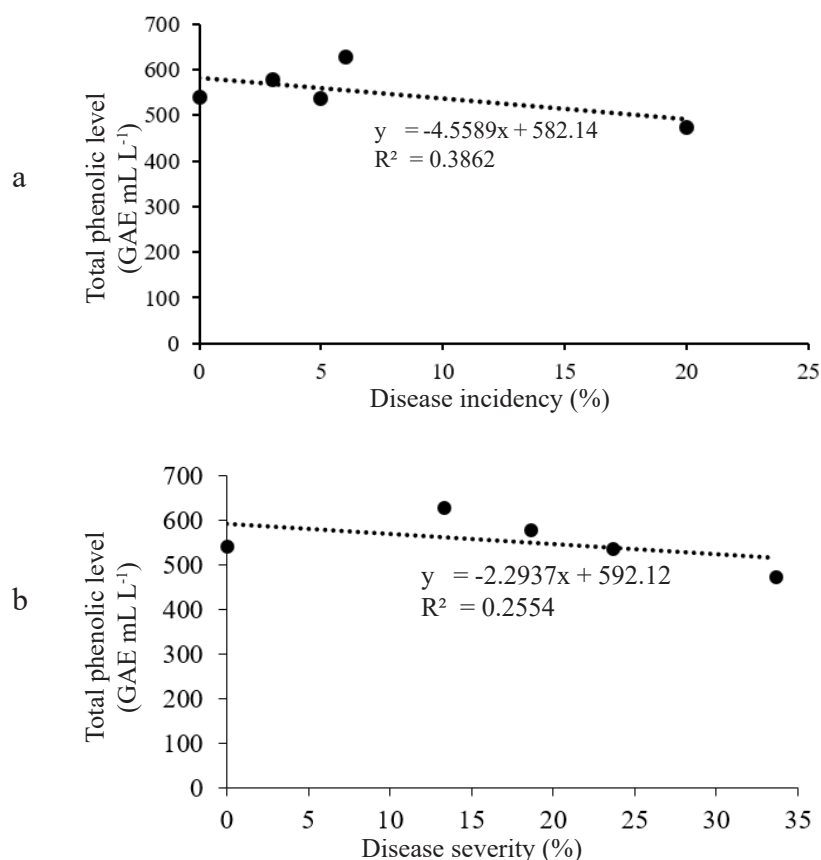


Figure 4 Correlation between total phenolic compound levels and disease incidence (a) and disease severity (b) of basal stem rot disease in soybean plants.

Table 5 Height of soybean plants treated with *Trichoderma* spp.

Treatment ^a	Plant height at ... week after planting (cm)			
	1	2	3	4
T0	5.55	9.14	13.26	16.61
T1	4.85	6.83	9.49	12.35
T2	5.68	7.90	11.30	14.23
T3	5.75	8.61	13.09	16.11
T4	5.97	9.52	13.59	16.78

^aT0, Plant negative control (without fungi applications); T1, Plant positive control (applications of *S. rolfsii*); T2, *T. harzianum* (10⁶ conidia mL⁻¹); T3, *T. asperellum* (10⁶ conidia mL⁻¹); and T4, *T. harzianum* combination *T. asperellum*.

Table 6 Number of leaves of soybean plants treated with *Trichoderma* spp.

Treatment ^a	Number of leaves after ... week after planting ^b			
	1	2	3	4
T0	2.52	2.56	2.84	3.72 a
T1	2.28	2.20	2.24	2.92 ab
T2	2.76	2.88	2.84	3.52 ab
T3	2.52	3.08	2.96	3.84 ab
T4	2.96	3.48	3.68	4.48 b

^aT0, Plant negative control (without fungi applications); T1, Plant positive control (applications of *S. rolfsii*); T2, *T. harzianum* (10⁶ conidia mL⁻¹); T3, *T. asperellum* (10⁶ conidia mL⁻¹); and T4, *T. harzianum* combination *T. asperellum*.

^bMeans followed by the same letter are not significantly different by Tukey test α 0.05

DISCUSSION

The application of *Trichoderma* spp., both individually and in combination, was proven effective in suppressing collar rot disease caused by *Sclerotium rolfsii* in soybean plants. This result aligns with previous findings, which demonstrated the ability of *Trichoderma* species to control plant pathogens through competition, antibiosis, mycoparasitism, and induction of systemic resistance (Howell 2003). The observed reduction in disease incidence and severity indicates that *T. harzianum* and *T. asperellum* possess high biocontrol potential.

The total phenolic content in plants treated with *Trichoderma* spp. increased. Suggests that these fungi contribute to the activation of plant defense responses. Phenolic compounds function as antimicrobial agents and strengthen cell walls, thus limiting pathogen development (Vagiri *et al.* 2017). In this study, regression analysis confirmed that higher phenolic levels were negatively correlated with disease severity, indicating the contribution of induced resistance mechanisms. This is supported by Temaja *et al.* (2018), who noted that phenolic compounds and enzymes such as peroxidase and chitinase are part of the plant's defense arsenal against pathogen infection. *Trichoderma harzianum* treatment resulted in the highest phenolic accumulation, possibly due to its production of antifungal metabolites such as harzianic acid and harzianopyridone (Contreras-Cornejo *et al.* 2016). On the other hand, *T. asperellum* plays a role in modulating plant gene expression related to defense, such as lipoxygenase and phenylalanine ammonia-lyase (PAL), leading to the synthesis of phenolic compounds and enhanced resistance (Contreras-Cornejo *et al.* 2016).

In terms of plant growth, while *Trichoderma* spp. did not significantly affect plant height during the early vegetative phase, a notable increase in leaf number was observed at 4 weeks after planting (WAP) in the combination treatment. This result supports previous findings that *Trichoderma*

can function as a biostimulant, improving plant vigor by enhancing nutrient availability or stimulating phytohormone production (Sjam *et al.* 2014; Rizal and Susanti 2018). The limited effect on plant height may be attributed to the growth stage of the soybean plants during observation. Simanjuntak (2005) states that significant morphological changes are not always observable in the early phenological stages, especially in short-duration experiments. Additionally, Ousley *et al.* (1994) emphasized that the effect of *Trichoderma* on plant growth is influenced by plant genotype and environmental conditions.

Compatibility testing between *T. harzianum* and *T. asperellum* confirmed their ability to coexist without mutual inhibition, supporting the feasibility of their combined application. Similar results were reported by Hanudin *et al.* (2012) and Nawfetrias *et al.* (2016), who found that compatible strains of *Trichoderma* can be mixed to expand the spectrum of biocontrol activity. Combined use of compatible strains has been recommended to enhance the effectiveness of biological control (Sangeetha *et al.* 2009).

In conclusion, this study demonstrated the dual role of *Trichoderma* spp. as biocontrol agents and biostimulants in soybean cultivation. Further research is needed to elucidate the molecular mechanisms underlying induced systemic resistance and evaluate these fungi's long-term effects under field conditions. Additional assessment of their impact on generative growth, yield components, and hormonal analysis will strengthen the understanding of their functional roles in crop improvement.

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