Coffee Husk Extract as a Growth Medium for *Trichoderma brevicompactum* and Its Antagonistic Activity Against *Fusarium* sp. and Stimulation of Chili Seed Germination

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Abstract

Coffee husk waste, rich in nutrients and bioactive compounds, was evaluated as an alternative medium for Trichoderma brevicompactum and as a biocontrol agent against Fusarium sp. The study tested its effectiveness in inhibiting Fusarium sp. in vitro and improving the germination of infected chili seeds. This experiment was conducted from April to July 2025 using an experimental method using a completely randomized design (CRD). Parameters observed included the physicochemical properties of the medium, spore density, mycelial biomass weight, inhibition against Fusarium *sp., germination percentage, and vigor index of chili seeds. Data analysis was performed descriptively for the physical properties of the medium, using a t-test for spore density, and analysis of variance (ANOVA) followed by Honestly Significant Difference (HSD) test at the 5% level for seed growth parameters. The results showed that coffee husk extract was able to support the growth of T. brevicompactum with a spore density of 2.0 × 10^9 spores mL^{-1} and was not significantly different from Potato Dextrose Broth medium, indicating its potential as a more economical alternative liquid medium. The filtrate of the coffee husk extract–based formulation showed an inhibition rate against* Fusarium *sp. of 31.31%. The formulation treatment was also able to maintain chili seed viability with germination percentages of 93.3–96.7% and vigor index in the good category. Coffee husk extract has the potential to be developed as an alternative growth medium for T. brevicompactum.*

Keywords: *Biological control agent, chili, coffee husk extract, Fusarium sp., T. brevicompactum.*

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of the important horticultural commodities with high economic value and a high level of consumption in Indonesia, including in Gorontalo Province. Chili is an essential food ingredient in the daily diet of the community. Along with population growth and increasing public demand, chili consumption continues to increase (Kadir et al., 2025). Gorontalo Province is known as one of the centers of cayenne pepper production; however, data from the Central Bureau of Statistics indicate that cayenne pepper productivity has declined in recent years. Productivity during the 2018–2019 period reached 93.43–90.38 quintals ha^{-1}, then decreased in the 2020–2021 period to 73.90–60.84 quintals ha^{-1}, and declined again in the 2022–2023 period to 55.05–63.15 quintals ha^{-1} (BPS of Gorontalo Province, 2023). This decline in productivity indicates that there are obstacles in the chili farming system, one of which is caused by attacks by plant pathogens.

Fusarium wilt caused by Fusarium oxysporum is one of the major diseases in chili plants that can lead to plant death and significant yield losses. This disease can spread rapidly through soil and water flow, especially in areas with steep topography (Ihkwanisa et al., 2023). Infection by Fusarium sp. has been reported to cause yield losses of up to 50% and may even result in crop failure (Abdila &

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Maduratna, 2021). The high intensity of this disease is one of the main factors contributing to the decline in chili productivity in various regions (Ibrahim et al., 2022).

Control of Fusarium wilt in chili plants is still predominantly based on the use of synthetic pesticides. However, excessive and continuous use of pesticides not only increases production costs but also has the potential to cause environmental pollution and trigger the emergence of pathogen resistance, which in turn can threaten the sustainability of agricultural systems (Suyanto et al., 2024). Therefore, alternative and more environmentally friendly and sustainable control strategies are needed, one of which is through the use of biological control agents.

Biological control agents are known to be effective in suppressing the development of soil-borne pathogens and are relatively safe for the environment (Probowati et al., 2021). Several studies have reported that biological control agents are able to effectively suppress Fusarium sp. infection in chili plants (Firlandiana et al., 2024). One biological control agent with strong potential for development is the fungus Trichoderma sp., which has antagonistic properties against various pathogens, is saprophytic, and plays a role in maintaining the balance of soil microorganisms (Nurjannah, 2020). In addition, Trichoderma sp. is known as a good biodecomposer and is capable of producing secondary metabolites, such as organic acids, that support plant growth (Iswati et al., 2024).

Propagation of Trichoderma sp. can be carried out using either solid or liquid media. Liquid media are more widely used because they allow faster nutrient absorption and support optimal fungal biomass formation through agitation during the fermentation process (Fadlillah et al., 2023). The use of liquid media is also considered more efficient and economical than solid media for in vitro propagation of microorganisms (Mbiyu, 2012). The development of liquid biopesticides with Trichoderma sp. as the active ingredient is considered more practical for field application and allows for more stable control of fungal biomass.

One organic material with potential for use as a fermentation medium for Trichoderma sp. is coffee husk waste. This waste is generated in large quantities during coffee bean processing; however, its utilization remains limited (Maysixteen & Haryadi, 2022). In Gorontalo Province, coffee production reaches an average of 126.76 tons per year with a plantation area of 1,294.42 ha, resulting in abundant coffee husk waste (BPS of Gorontalo Province, 2023). To date, coffee husk waste is generally only piled up or burned, which can potentially pollute the environment and degrade ecosystem quality (Purnamasari & Wahyuni, 2021). Therefore, the utilization of coffee husk waste as a medium for propagating Trichoderma brevicompactum is expected to be an economical and ecological solution in the development of biological control of plant diseases.

Based on the above description, this study aimed to evaluate the potential of coffee husk extract as a growth medium for T. brevicompactum, the effectiveness of T. brevicompactum formulations based on coffee husk extract in suppressing the in vitro growth of Fusarium sp., and their effects on the germination of chili seeds infected with Fusarium sp.

MATERIALS AND METHODS

Place and Time of Study

The study was conducted from April to July 2025 at the Biological Control Agents Laboratory of the Agricultural and Horticultural Plant Protection Center of Gorontalo Province, located in Toto Selatan Village, Kabila District, Bone Bolango Regency, Gorontalo, Indonesia.

Materials and Equipment

The materials used included coffee husk waste, isolates of Trichoderma brevicompactum and Fusarium sp. from the collection of the Agricultural Plant Protection Center Laboratory (BPTP of Gorontalo Province), chili seeds of the Juwita 25 F1 variety, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), sterile distilled water, 70% alcohol, sodium hypochlorite (NaOCl), potassium permanganate ($KMnO_4$), chloramphenicol antibiotic, synthetic fungicide Dithane M-45, granulated sugar, Whatman No. 42 filter paper, and germination media in the form of sterile tissue paper.

The equipment used included an autoclave, laminar air flow cabinet, shaker, water bath, hot plate, vortex mixer, aerator, binocular microscope (Olympus CX33), haemocytometer, digital pH meter, analytical balance, micropipette, Petri dishes, Erlenmeyer flasks, beakers, test tubes, inoculating needles, cork borers, 0.22 µm nylon syringe filters, fermentation bottles, and other supporting laboratory equipment.

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Research Method

An experimental method was used to evaluate the potential of coffee husk extract as a growth medium for T. brevicompactum and its potential as a biopesticide to suppress the in vitro growth of the pathogenic fungus Fusarium sp. and as a plant growth promoter.

Rejuvenation and Virulence Test of Isolates

Isolates of T. brevicompactum and Fusarium sp. were rejuvenated on sterile PDA medium and incubated for 7 days at room temperature. A virulence test of Fusarium sp. was conducted using surface-sterilized chili seeds to confirm the pathogen's ability to cause infection symptoms before being used in subsequent experiments.

Preparation of Fusarium sp. Inoculum Suspension

The Fusarium sp. suspension was prepared from 7-day-old cultures grown on PDA by adding sterile distilled water followed by homogenization. Spore density was adjusted to 10^6 conidia mL^{-1} using serial dilution (Sandiase et al., 2023).

Formulation and Propagation of T. *brevicompactum* in Coffee Husk Extract

Coffee husk extract was prepared by boiling coffee husk waste in water for approximately 20 minutes, followed by filtration and the addition of granulated sugar, then sterilized using an autoclave at 121 °C for 15 minutes. After cooling, the extract medium was inoculated with T. brevicompactum and fermented for 9 days in six series (bottles). Observations were conducted from day 4 to day 9 of fermentation, with potato extract medium used as the control (Fadlillah et al., 2023).

Antagonism Test and In Vitro Effectiveness

The antagonistic ability of T. brevicompactum against Fusarium sp. was tested using the dual culture method. Furthermore, the effectiveness of the formulation was evaluated using the poisoned food technique, by growing Fusarium sp. on PDA medium supplemented with the filtrate of the T. brevicompactum formulation. Observations were carried out for 8 days of incubation (Rahmadani et al., 2021). To determine the level of pathogen growth inhibition, the percentage inhibition was measured by determining the diameter of Fusarium sp. colonies in the treatment and control. Percent inhibition was calculated using the following formula:

$$\text{PP} = \frac{D1-D2}{D1} \times 100\%$$

Description:
PP = Inhibition Percentage,
D1 = Average fungal diameter in the control (mm),
D2 = Average fungal diameter in each treatment (mm).
(Chatri et al., 2022).

Formulation Test as a Growth Promoter for Chili Seeds

The test was conducted using a Completely Randomized Design (CRD) with five treatments and three replications. The treatments included soaking seeds in sterile distilled water, soaking in Fusarium sp. suspension, soaking in synthetic fungicide followed by Fusarium sp. suspension, soaking in T. brevicompactum formulation based on coffee husk extract, and soaking in T. brevicompactum formulation based on coffee husk extract followed by Fusarium sp. suspension. Parameters observed included germination percentage and vigor index of chili seeds during 14 days of incubation (Hanif & Susanti, 2019).

Observation Parameters

The parameters observed included the physicochemical properties of the medium (temperature, pH, and color), conidial density, mycelial biomass weight, percentage of inhibition against Fusarium sp., germination percentage, and vigor index of chili seeds (Mirsam et al., 2021).

Data Analysis

Physicochemical property data were analyzed descriptively. Conidial density was analyzed using a t-test. Inhibition percentage, germination percentage, and vigor index data were analyzed using analysis of variance (ANOVA), and when significant differences were found, the test was followed by an Honestly Significant Difference (HSD) test at the 5% significance level.

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RESULTS AND DISCUSSION

Physical and Chemical Properties of *T. brevicompactum* Formulation in Coffee Husk Extract

In vitro testing of coffee husk extract was conducted to evaluate its potential biological activities, such as antibacterial, antioxidant, or cytotoxic activity against specific test cells. The results provide preliminary information regarding its effectiveness. The physicochemical characteristics of coffee husk waste formulation and Potato Dextrose Broth (PDB), observed before fermentation and after 7 days of fermentation, included pH, temperature, and color, as presented in Table 1.

Table 1. Physicochemical Characteristics of Coffee Husk Extract Medium and Potato Dextrose Broth (PDB)

Parameter	Control (PDB)		Coffee Husk Extract	
	Initial	Day 7	Initial	Day 7
Temperature	28.6 ^{0}C	28 ^{0}C	29.8 ^{0}C	25.4 ^{0}C
pH	5.93	5.44	7.68	5.12
Color	Yellowish brown	Light green	Reddish brown	Dark brown

The results in Table 1 indicate changes in pH, temperature, and color in both the control medium and the coffee husk extract medium. Under initial conditions before fermentation, the temperature of the media tended to be higher. This occurred because the media had not yet been colonized by microorganisms, so residual heat from sterilization or initial reactions in the medium had not been utilized by biological activity. After fermentation with the inoculation of T. brevicompactum, the medium temperature decreased. This decrease indicates that T. brevicompactum had passed the active growth phase and entered the stationary phase on the seventh day of fermentation. In this phase, metabolic activity decreases as nutrients become limited, resulting in reduced heat production (Mengesha et al., 2022). This condition is consistent with the findings of Kabir et al. (2025), who reported that fermentation temperature generally decreases when microorganisms are no longer in the active growth phase.

In addition to temperature, pH values also decreased after fermentation in both media. This decrease in pH indicates that during fermentation, T. brevicompactum produced secondary metabolites in the form of organic acids, causing the medium to become more acidic. Harman et al. (2004) explained that Trichoderma sp. is capable of producing metabolic compounds such as gliotoxin and harzianic acid, which play a role in antagonistic mechanisms against pathogenic microorganisms. The decrease in pH in the coffee husk extract medium is presumed to be caused by microbial fermentation activity that produces organic acid compounds, such as acetic acid and lactic acid, during substrate degradation (Sukmawati et al., 2023).

Changes in medium color were also clearly observed in both treatments after fermentation. In the control medium, the color changed from yellowish brown to light green, which is a characteristic of Trichoderma sp. colony growth (Amalia & Elviantari, 2023). Meanwhile, in the coffee husk extract medium, the color changed from reddish brown to dark brown. This color change can be associated with the presence of polyphenol oxidase enzymes contained in coffee husk, which produce brown pigments (Trinafianita & Widyaningsih, 2018).

Overall, the results of this study indicate that the 7-day fermentation process caused changes in the physical and chemical properties of the media, serving as indicators of the biological activity of T. brevicompactum. More pronounced changes in the coffee husk extract medium compared to the control medium suggest that coffee husk extract is a promising alternative medium to support the growth of T. brevicompactum and has the potential to enhance its effectiveness as a biological control agent against plant pathogens.

The comparison of spore density between the control medium and the coffee husk extract–based medium was statistically analyzed using a t-test to determine whether there were significant differences between treatments. The results of observations on the spore density of T. brevicompactum in each treatment are presented in Table 2.

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Table 2. Results of the t-Test on the Effect of Coffee Husk Extract and PDB on Conidia Density

Treatment	Conidia Density (spores / ml)
Control (PDB)	8.2×10^{9ns}
Coffee Husk Extract	2.0×10^{9ns}

Note: p-value > 0.05

Based on Table 2, the spore density of T. brevicompactum in the coffee husk extract treatment was not significantly different from that in the control medium (PDB). This indicates that the use of coffee husk extract as a liquid medium is able to support the sporulation process of T. brevicompactum with effectiveness comparable to that of PDB, which is commonly used as a standard medium. However, it has not yet been able to produce sporulation performance as high as that of PDB. PDB medium is naturally rich in readily available nutrients such as glucose, allowing fungal growth and sporulation to occur optimally (Fatimah et al., 2022). This condition differs from coffee husk extract–based media, which have a more complex nutrient composition.

According to Febriani et al. (2023), coffee husk contains polyphenols, lignocellulose, and complex carbohydrates that can support microbial growth but require prior degradation before being optimally utilized. Therefore, coffee husk extract can be categorized as an economical and promising alternative medium, although quantitatively it produces lower spore density compared to PDB.

Observations of the relative growth rate (RGR) and absolute growth rate (AGR) were conducted to determine the growth dynamics of T. brevicompactum colonies in different media, including coffee husk extract medium and the control (Potato Dextrose Broth/PDB). The results of observations on relative growth rate (RGR) and absolute growth rate (AGR) are presented in Table 3.

Mycelial Biomass

Observations of the relative growth rate (RGR) and absolute growth rate (AGR) were conducted to determine the growth dynamics of *T. brevicompactum* colonies on different media, including coffee pulp extract medium and the control (Potato Dextrose Broth/PDB). The observations of relative growth rate (RGR) and absolute growth rate (AGR) are presented in Table 3.

Table 3. Mycelial Growth of *T. brevicompactum* on the Control Medium and Coffee Husk Extract.

Observation Day	Relative Growth Rate (RGR)		Absolute Growth Rate (AGR)	
	Control (PDB)	Coffee Husk Extract	Control (PDB)	Coffee Husk Extract
4	6.43	7.59	6.43	7.59
5	8.42	7.94	14.39	9.00
6	10.62	8.24	19.43	10.50
7	10.32	5.08	8.77	-10.75
8	8.64	3.81	-1.44	-3.8
9	7.12	2.66	-3.56	-5.37

Based on Table 3, the mycelial growth rate in the control medium (PDB) showed a normal growth pattern during the exponential phase before declining in the stationary phase. Meanwhile, growth in the coffee husk extract medium showed a relatively slower response, especially after entering the late growth phase. This difference in growth rate is presumed to be caused by differences in nutrient composition between the two media. The control medium (PDB) is based on potato and dextrose, which are rich in simple glucose and therefore easily utilized by fungi for energy synthesis and mycelial formation (Basarang et al., 2016). In contrast, the coffee husk extract medium contains complex compounds such as lignocellulose, tannins, and polyphenols (Ramdhoani et al., 2025), which require a longer time to be degraded before they can be utilized by *Trichoderma* sp. as energy sources.

The highest RGR and AGR values in both the control and coffee husk extract treatments were achieved on day 6, indicating that at this phase the mycelium was in the exponential phase of active growth. Under these conditions, nutrient availability was still optimal, supporting physiological processes and primary metabolism for biomass formation. After day 6, RGR and AGR values decreased

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in both treatments, indicating that the mycelium had entered the stationary phase and even progressed toward the decline phase. This is presumably due to nutrient limitation and the accumulation of toxic metabolites, which are less supportive of metabolic processes (Harman et al., 2004).

Overall, mycelial growth in the coffee husk extract medium was still able to follow a growth pattern similar to that of the control up to day 6, indicating that coffee husk extract has potential as an alternative medium for the growth of *T. brevicompactum*. Although growth was slightly slower than in the control medium, this medium has economic and ecological potential because it utilizes abundant agricultural waste and supports the concept of environmentally friendly biological control through the use of organic materials.

Potential of *T. brevicompactum* Formulation in Coffee Husk Extract as a Biological Control Agent

The percentage of inhibition of the *T. brevicompactum* formulation in coffee husk extract against the growth of *Fusarium* sp. indicates the ability of the formulation to act as a biological control agent in suppressing the growth of the pathogen. The inhibition values observed during the observation period are presented in Figure 1.

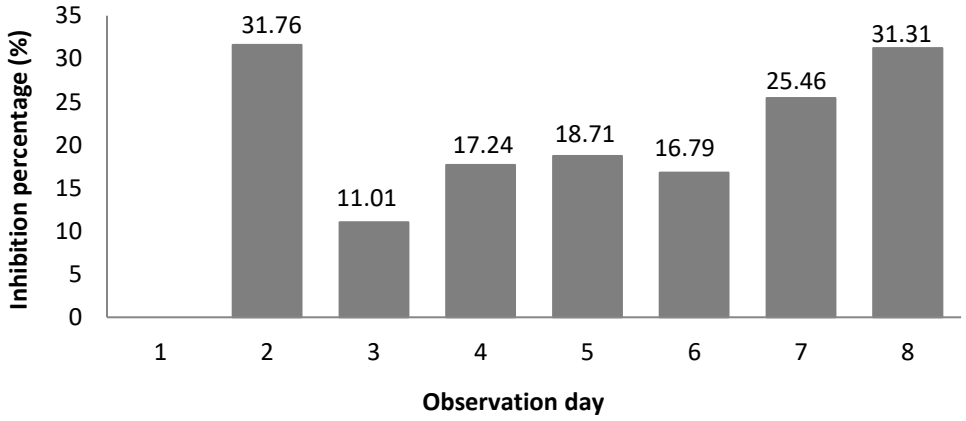


Figure 1. Inhibition Effect of *T. brevicompactum* Formulation in Coffee Husk Extract on the Growth of *Fusarium* sp.

Based on the observations in Figure 1, the filtrate of the *T. brevicompactum* formulation based on coffee husk extract was able to inhibit the growth of *Fusarium* sp. The fluctuating inhibition percentages observed during the observation period reflect the dynamic biological interactions between the antagonistic agent and the pathogen. The effectiveness of the filtrate in inhibiting *Fusarium* sp. indicates that the coffee husk extract formulated with *T. brevicompactum* contains antifungal metabolites. This is consistent with Ikhwanisa et al. (2023), who reported that *Trichoderma* sp. can produce hydrolytic enzymes such as β-1,3-glucanase, chitinase, and cellulase, which degrade the main structural components of the *Fusarium* sp. cell wall, thereby damaging hyphal integrity and increasing inhibitory effectiveness.

On days 2 and 8, the filtrate showed the highest inhibition percentages. On other days, the inhibitory effect was relatively lower, even reaching the lowest value on day 3. This pattern indicates that the effectiveness of the *T. brevicompactum* formulation filtrate based on coffee husk extract is not constant but is strongly influenced by the stability of active compounds and the enzymatic activity present in the fermentation products of *T. brevicompactum* with coffee husk extract as the substrate (Chatri et al., 2022). The decrease in inhibition on day 3 is presumed to be due to the degradation of active metabolites, such as phenolics, flavonoids, and other antibiosis compounds produced by *T. brevicompactum*. These compounds are known to be susceptible to oxidation and chemical structural changes, leading to reduced antifungal effectiveness (Jumadi et al., 2021).

The increase in inhibition on days 7 and 8 is presumed to occur because the *T. brevicompactum* filtrate becomes more stable, allowing its secondary metabolites to act more effectively. This change reflects alterations in fungal metabolite composition according to growth phase, and antimicrobial activity generally increases in more mature culture phases (Khan et al., 2020). This is in line with reports that some fungal metabolites may decrease in stability due to environmental conditions or prolonged incubation (Jayalakshmi et al., 2021).

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In addition to cell wall degradation, *Trichoderma* sp. also produces metabolites such as siderophores that can bind ferric ions (Fe^{3+}) in the culture environment, thereby reducing the availability of essential nutrients for *Fusarium* sp., which strongly depends on iron for respiration processes and enzymatic activity. This nutrient competition limits iron availability to the pathogen and inhibits nutrient uptake, contributing to the suppression of *Fusarium* sp. growth in PDA culture media (Cui et al., 2025).

Potential of *T. brevicompactum* Coffee Husk Extract Formula as a Growth Promoter under Biotic Stress

This study aimed to evaluate the effectiveness of a *T. brevicompactum* formulation based on coffee husk extract on seed germination and vigor index of chili seeds under *in vivo* conditions. The observed germination data are presented in Table 4.

Table 4. Germination Percentage and Vigor Index (%) of Chili Seeds Treated with Coffee Husk Extract Formula

Treatment	Growth Promotion (%)	
	Germination	Vigor Index
Control	96.7b	96.7b
Fusarium sp.	30a	0.00a
Fungicide + *Fusarium* sp.	93.3b	96.7b
Coffee husk extract formula	93.3b	66.67ab
Formula + *Fusarium* sp.	96.7b	70.00ab
HSD 5%	**13.89**	**73.18**

Note: Means followed by the same letter in the same column are not significantly different at the 5% HSD (Honestly Significant Difference) test.

Based on Table 4, the treatment with single inoculation of *Fusarium* sp. (P1) showed a very significant decrease in germination percentage and vigor index compared to the other treatments. This indicates that the pathogen is virulent and capable of severely damaging the physiological systems of the seeds, thereby disrupting germination and early seedling growth (Afriani et al., 2019). In contrast, the control (P0), fungicide (P2), *T. brevicompactum* coffee husk extract formula (P3), and the combination of formula + pathogen (P4) showed no significant differences in germination percentage, indicating that the coffee husk extract formula was effective in maintaining seed viability even under biotic stress. This suggests that *T. brevicompactum* and the bioactive compounds in the coffee husk extract play roles in effectively suppressing pathogen activity from the early stages of growth.

The fungicide treatment (P2) and the control (P0) were not significantly different from each other and were able to maintain seed vigor under optimal conditions. This is consistent with Setiyowati et al. (2023), who reported that broad-spectrum fungicides are effective in inhibiting the development of pathogenic fungi and thus maintaining the physiological integrity of seeds. However, treatments using the coffee husk extract formula without pathogen (P3) and the coffee husk extract formula combined with *Fusarium* sp. (P4) were not significantly different from each other, but were significantly different from the worst condition caused by pathogen infection. This indicates that the coffee husk extract formula was able to provide physiological protection to the seeds, keeping vigor within a good category, although not as high as the control. This ability is supported by the potential of coffee husk bioactive compounds such as polyphenols, caffeine, and chlorogenic acid, which have been reported to possess antifungal properties and suppress the growth of *Fusarium* sp. (Virginia et al., 2024).

The vigor value in P3 tended to be lower than in P4. This difference suggests that the presence of *Fusarium* sp. at controlled levels in P4 may have triggered a biological defense response in *Trichoderma* sp. This condition enhances the accumulation of secondary metabolites and the activity of defense-related enzymes, indicating activation of the phenylpropanoid pathway induced by *Trichoderma* sp. (Iswati et al., 2025). As a result, seeds in P4 had higher vigor than those in P3. The reduction in P3 is presumed to be due to mild phytotoxic effects of phenolic compounds such as chlorogenic acid, caffeine, tannins, and gallic acid, which at high concentrations in coffee husk extract may inhibit seed physiological activity (Azzahra et al., 2024).

Overall, these results indicate that coffee husk extract is effective in maintaining chili seed germination at a level comparable to the control, and although the vigor index is slightly lower than that

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of the fungicide treatment, it remains within a good category. This suggests that the coffee husk extract formula has potential to be used as an environmentally friendly biological control alternative to improve seed viability while simultaneously suppressing *Fusarium* sp. infection.

CONCLUSION

Coffee husk extract has the potential to be used as an economical and environmentally friendly alternative growth medium for *Trichoderma brevicompactum*, with spore density comparable to that of PDB medium. The formulation of *T. brevicompactum* based on coffee husk extract is able to inhibit the growth of *Fusarium* sp. in vitro and maintain chili seed germination and vigor index in the good category, indicating its potential to be developed as a biological control agent for disease management.

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