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Unlocking the Potential of Nano Husk Ash in Enhancing Trichoderma's Efficacy against Fusarium spp. Causal Twisted Disease: An In Vitro Investigation

Taufiq Hidayat*, Rangga Dony Santoso, Khoirudin, Siti Nur Aisyah, Mulyono.

Abstract: *Shallots, a vulnerable vegetable crop, are often affected by twisted disease caused by Fusarium spp., Enhancing plant resistance is crucial, and one approach is the utilization of biological agents like Trichoderma sp. combined with rice husk ash nanoparticles. This study aimed to assess the efficacy of rice husk ash nanoparticles and Trichoderma sp. in controlling Fusarium spp. causing twisted disease in shallots under in vitro conditions. The dual culture method was employed, where Fusarium spp. and Trichoderma sp. were co-cultured on Potato Dextrose Agar (PDA) with a 3 cm spacing, while nano husk as combine with the PDA. The experimental design was a completely randomized design with three treatments: Rice husk ash nanoparticles with concentration 0.3%, Trichoderma sp., and combination between rice husk ash nanoparticles and Trichoderma sp.. The variables assessed included the characteristics of Trichoderma sp. and Fusarium spp., as well as the percentage of inhibition. Data were analyzed using analysis of variance (α = 5%). The results demonstrated that the combined application of rice husk ash nanoparticles with concentration 0.3% and Trichoderma sp. exhibited the highest resistance percentage, reaching up to 90%. The inhibition mechanism involved competition and antibiosis exerted by the Trichoderma sp. against Fusarium spp.. This finding suggests a promising approach for controlling Fusarium-induced twisted disease in shallots.*

Keywords: *Biological control, Inhibition mechanism, Rice Husk Ash Nano Particles, Twisted disease*

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Introduction

Shallots are a vegetable commodity belonging to the Allium group, commonly used as a seasoning in cooking and valued for its potential in traditional medicine (Pangestuti et al., 2023; Sun et al., 2019; Vuković et al., 2023). This crop holds significant economic importance as a source of income for farmers and contributes to foreign exchange earnings for the country. National production data from 2006 to 2015 reveals fluctuations in shallot production. The production increased from 794,714 tonnes in 2006 to 1,048,927 tonnes in 2010. However, in 2011, shallot production decreased to 893,114 tonnes, followed by an increase to the highest production of 1,233,598 tonnes in 2014. Subsequently, Indonesian shallot production slightly declined by 0.36% in 2015, amounting to 1,229,189 tonnes (BPS, 2017). The decrease in shallot productivity can be attributed to various factors, including pathogen attacks.

Twisted disease, also known as fusarium wilt, is a common affliction in shallot plants caused by the fungus *Fusarium oxysporum* (Aisyah et al., 2022; Herlina et al., 2021). Fusarium wilt can result in yield reductions of up to 50% and even complete crop failure (Cahyaningrum et al., 2023). Utilizing biological agents, such as the fungus *Trichoderma sp.,* through application methods, can be a potential solution. *Trichoderma sp.* is a fungal genus with the capability to act as a biological control agent against plant pathogens. The antagonistic mechanisms employed by Trichoderma sp. include competition, parasitism, antibiosis, and lysis (Mikrobiologi et al., 2009)). Research conducted by (Kurniawan et al., 2006) demonstrated that the addition of Trichoderma sp. inhibited the growth of F. oxysporum Schelect. f. sp. zingeberi Trijillo in kencur plants, with a reduction ranging from 7.9% to 56.3%. Purnomo (2006) stated that Trichoderma sp. exhibited the ability to control fusarium wilt in ginger within five days after infection. Astuti et al. (2021) stated that *Trichoderma*

harzianum is effective as an antagonist against various pathogenic fungi, which typically include *Fusarium* species.

The effectiveness of *Trichoderma sp.* when applied to plants in field conditions is influenced by various environmental factors, including temperature, pH, humidity, and soil characteristics (Gupta et al., 2014). Therefore, it is necessary to incorporate a carrier material that can enhance the potency of Trichoderma sp. as a biocontrol agent. Silica is one potential material that can serve as a carrier. According to Hersanti et al. (2020), the biocontrol delivery system (BDS) relies on a carrier, and silica sourced from rice husk ash has been identified as a suitable carrier for maintaining the survival of antagonistic fungi in a formulated product. In both in vitro and in planta conditions, the application of silica in the form of silicon or silicate at a dose of 6 g/L effectively controls black root rot disease in strawberries (Abd-El-Kareem et al., 2019). Moreover, the addition of rice husk ash can help neutralize soil acidity and strengthen plant tissues, consequently enhancing the development of more pathogen-resistant plants. Furthermore, Silica directly weakens fungal cell walls by interacting with chitin, inducing oxidative stress, and interfering with nutrient uptake, thereby rendering the pathogen more vulnerable to degradation by plant-produced chitinase and β-(1-3) glucanase and also primes defense responses that upregulate these enzymes (Anitha & Rabeeth, 2020; Bakhat et al., 2023).

Rice husk ash (RHA) can be significantly improved through nanoparticle technology by breaking it down into nano-sized silica particles (SiO_2 NPs) that drastically enhance the surface area-to-volume ratio (Cruz-Luna et al., 2021; A. Kumari et al., 2023; Nguyen et al., 2024). This physical improvement enables further invasion into Fusarium hyphae, disrupting cell integrity and spore germination, as well as providing enhanced dispersion in agar media for controlled release of antifungal silica ions that disrupt fungal metabolism (e.g., ergosterol biosynthesis) (Ahamad et al., 2023; Dutta et al., 2023; Mansoor et al., 2021). Furthermore, the infected Fusarium hyphae are also susceptible to the antagonistic activities of *Trichoderma sp.,* such as the release of lytic enzymes (e.g., chitinases), thereby increasing the activity of the biocontrol agent. Importantly, the nanoparticles can also act as sources of micronutrients, which can stimulate the growth of *Trichoderma,* creating a synergistic strategy of sustainable disease management(Ahmad et al., 2024; Atanasova et al., 2021; Tomah et al., 2023). Interestingly, Hersanti et al. (2020) research findings indicate that nano-silica application does not inhibit Trichoderma sp. growth, supporting its compatibility as a synergistic agent.

. This study aims to investigate the effects of rice husk ash nanoparticles, the application of Trichoderma sp., and their combined application on inhibiting the growth of Fusarium spp., the causal agent of wilt disease in shallot plants.

Methods

This research was conducted at the Agrobiotechnology Laboratory, Faculty of Agriculture, Yogyakarta Muhammadiyah University. The study was carried out over a period of two months, from September 2021 to October 2021. Various tools were utilized in this study, including a Bunsen lamp, autoclave, ose needles, scalpel handles, pipettes, test tubes, petri dishes, Erlenmeyer flasks, measuring cups, microscopes, paper covers, fatty cotton, sterile gauze, rubber bands, plastic wrap, an analytical balance, pH sticks, microscope slides, and a ruler.

The materials employed in this study consisted of Fusarium spp. isolates from lowland and midland areas – lowland from Kretek (15 masl) and midland from Imogiri (350 masl), Bantul, Yogyakarta-, Trichoderma sp., Rice Husk Ash Nanoparticle, Potato Dextrose Agar (PDA), disinfectant, Methanol 70%, Ethanol 96% and 70%, 0.1% chloramphenicol, lactophenol cotton blue, and distilled water.

The experiment was laid out in a factorial arrangement in a Completely Randomized Design (CRD) with five replicates per treatment. The source of Fusarium isolates, i.e., lowland (Fusarium R) and medium-land (Fusarium M) isolates, was the first factor. The second variable was the control treatment, comprising three treatments: rice husk ash nanoparticles (0.3 g/L), Trichoderma sp., and a mix of rice husk ash nanoparticles (0.3 g/L) and Trichoderma sp.

Rice husks were transformed into rice husk ash through direct combustion at 350°C. The resulting white ash was separated from black ash using an 80-mesh sieve before undergoing nanofication. This process involved placing 25% white ash in a ball mill, adding 62.5% small iron balls and 12.5% water, and milling for five hours. Post-milling, the water was evaporated by heating at 300°C. Scanning Electron Microscopy (SEM) analysis revealed an average particle size of 62.326 nm, with 87% of particles measuring below 100 nm. Energy Dispersive X-ray Spectroscopy (EDX) indicated the particles comprised 40.36% silica, 46.86% oxygen, 11.93% carbon, and 0.85% potassium (Kokkwilai et al., 2021).

Dual culture technique using an inoculating needle on PDA medium was used for in vitro antagonism assay. On either side of a Petri dish, 3 cm apart, inoculation of Fusarium spp. and Trichoderma sp. isolates was carried out. The toxicity of rice husk ash nanoparticles particles

on Fusarium spp. was also determined by the poisonous method. 0.3 grams of rice husk ash nanoparticles particles mixed in 10 ml of PDA in a Petri dish were homogenized by figure-eight movement prior to solidification. Fungal isolates were later added as done in the first treatment and were incubated for seven days at room temperature. This research focuses on assessing antagonistic activities by Trichoderma sp. and rice husk ash nanoparticles particles on Fusarium spp. by determining the diameter of the zone of growth inhibition.

The collected observational data were analyzed using analysis of variance at a significance level of 5%. If significant differences were found among the treatments, the DMRT test was performed at a significance level of 5%. The obtained data were presented in tabular form, and some results were illustrated through photographs or drawings.

Results

Based on the conducted identification of the characteristics of the Trichoderma sp using (Watanabe, 2010). various microscopic and macroscopic features were observed, as summarized in Table 1 and Figure 1.

Table 1 *Morphological characteristic of Trichoderma sp*

No.	Morphological Characteristic	Macroscopis	Microscopis
1	The Color of Mycelium	Greenish white	
2	Direction of Mycelium growth	Sideways	
3	The Form of Mycelium	Concentric	
4	Hyphae		Ramose
5	Conidiophore		Ramose
6	Conidia form		Obyoid
7	Conidia Color		Pale green

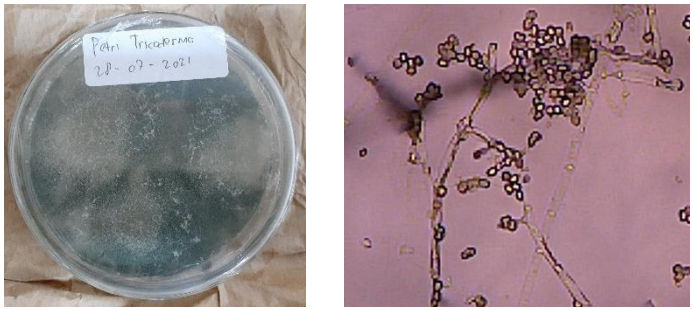

Figure 1
Trichoderma isolate (A) Macroscopic in PDA (B) Microscopic under microscope 40 x 10

In this test, the pathogens or fusarium used consisted of two types, namely Fusarium R (originating from the lowlands) and Fusarium M (originating from the midlands). The macroscopic and microscopic identification of pathogens Fusarium spp. Could be seen in table 2 and figure 2.

Based on the results of variance in Tables 3 showed that there was a significantly different effect of each treatment on the inhibition test on the pathogen Fusarium spp. This can be interpreted that Trichoderma sp. and 0.3 g/L rice husk ash nanoparticles can inhibit and suppress the growth of both Low Fusarium and Medium Fusarium mushroom cultivars in shallot plants well, which is above 50%. The percentage of inhibition in each treatment exhibited a consistent increase over the observation period. In the first treatment involving rice husk ash nanoparticles, the percentage of inhibition progressively rose. For the Low Fusarium experiment, the initial observation on day 2 indicated an inhibition percentage of only 26.67%, which subsequently increased to the highest percentage of 36.75% on day 7. Similarly, in the Intermediate Fusarium experiment, the inhibition percentage started at 29.33% on day 2 and steadily increased to reach the highest value of 40.28% on day 7.

In the second treatment involving Trichoderma sp., the percentage of inhibition also displayed an upward trend over the 7-day observation period. In the Low Fusarium experiment, the inhibition percentage increased from 45.00% on the second day to a remarkable 81.17% on the seventh day. Similarly, in the Intermediate Fusarium experiment, the inhibition percentage rose from 44.33% to 81.59% on the second day and continued to increase throughout the observation period. Furthermore, the combination treatment of rice husk ash nanoparticles and Trichoderma sp. also exhibited a continuous increase in the percentage of inhibition. In the Low Fusarium trial, the second day recorded a notably high value of 75.00%, which further rose to 90.58%. In the Intermediate Fusarium experiment, the second day showed a higher value of 77.33%, and the inhibition percentage continued to increase until it reached 90.81% on the seventh day of observation.

It can be observed in Figures 3a and 3c that *Trichoderma* is capable of controlling *Fusarium* by forming a clear inhibition zone around it. In contrast, when combined with rice husk ash nanoparticles (Figures 3b and 3d), not only is a clear inhibition zone present, but *Trichoderma* growth also tends to be more dominant, covering *Fusarium*

Discussion

Microscopic and cultural observations confirmed that the biological control agent used in this study was *Trichoderma harzianum*. Colonies exhibited rapid growth on PDA, forming circular zones that transitioned from white to light or dark green with granular surfaces due to dense conidiation (Table 1, Figure 1), consistent with previous reports (Nagamani et al., 2020; Pudake et al., 2024; Sharma & Singh, 2014).

Table 2. *Morphological characteristic of Fusarium spp*

No.	Morphological Characteristic	Isolate R		Isolate M	
		Macroscopic	Microscopist	Macroscopic	Microscopist
1	The Color of Mycelium	White		White	
2	Direction of Mycelium growth	diseperse		Cumulate	
3	The Form of Mycelium	rough serrated		smooth protruding	
4	Hyphae		Septate		Septate
6	Conidia form		Obyoid		Curved
7	Number of Septate		2-3		3-4

Figure 2
Fusarium isolate (A) Isolate R in PDA (B) Isolate R under microscope 40 x 10 (C) Isolate M in PDA (D) Isolate M under microscope 40 x 10

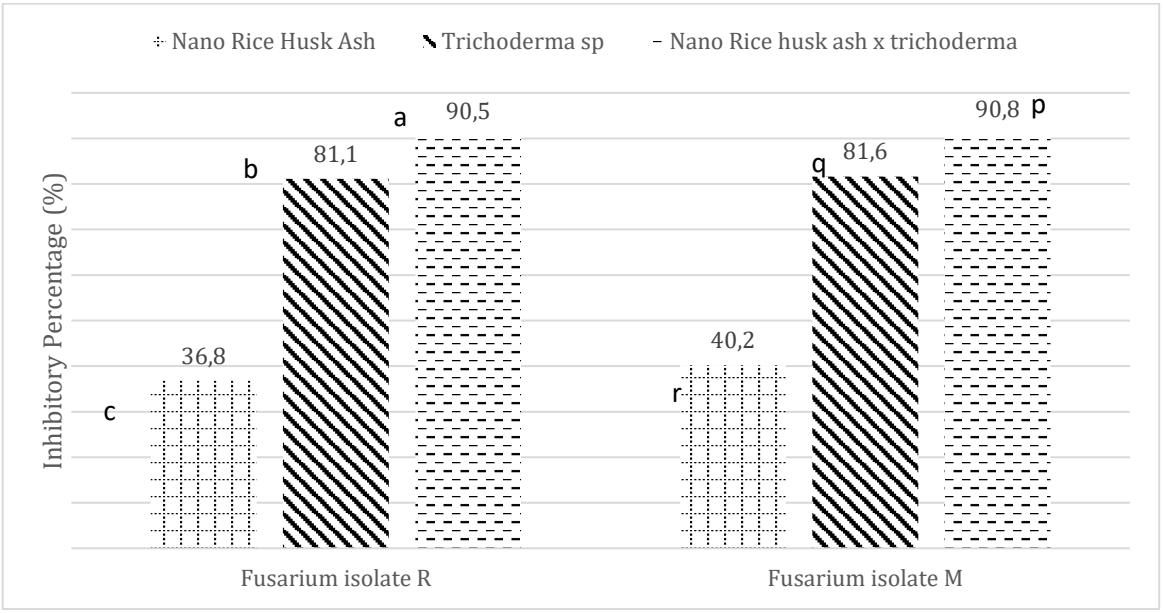

Figure 3.
Inhibitory percentage of Fusarium spp through application of Trichoderma and nano rice husk ash on day 7
* Numbers followed by Same letters show not significantly difference based on the ANOVA (α 5%)

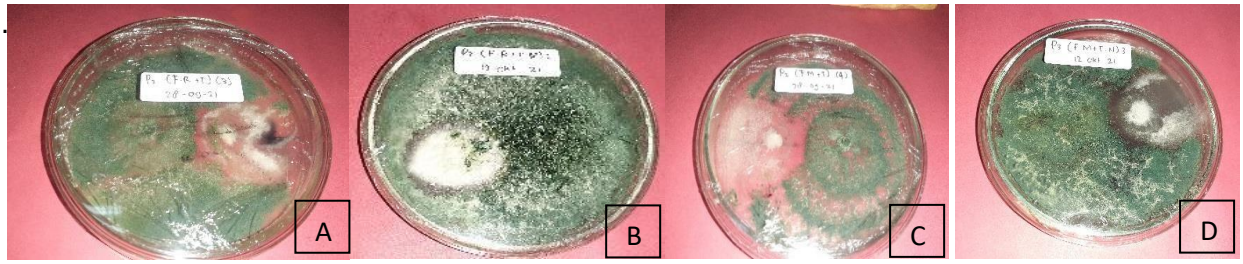

Figure 3
Inhibitory mechanism of Trichoderma and nano husk ash on day 7 (A) Trichoderma against Fusarium R (B) Trichoderma sp. and Rice husk ash nanoparticlesagainst Fusarium R ash , (C) Trichoderma against Fusarium M (D) Trichoderma sp. and Rice husk ash nanoparticlesagainst Fusarium D

The hyphae were hyaline, septate, and branched, with smooth walls measuring 1.5–12 µm in diameter, and branches typically emerging at right angles to the main axis (Shah et al., 2012; Watanabe, 2010). Conidiophores were hyaline, erect, and highly branched, forming divergent or dendritic structures with secondary branches at approximately 90°, bearing one to five ampulliform phialides (5–7 × 3–3.5 µm) arranged in whorls. The conidia were smooth, green, and mostly globose to short obovoid (2.8–3.2 × 2.5–2.8 µm) (Pudake et al., 2024; Shah et al., 2012).

Microscopic and cultural observations confirmed that the causal pathogen in this study was *Fusarium oxysporum*. Both isolates, R and M, showed typical morphological characteristics of the species, though with slight variations in colony and conidial features (Table 2, Figure 2). Isolate R formed white, rough, serrated mycelia with dispersed growth, while Isolate M produced white, smooth, protruding mycelia with cumulate growth patterns. Microscopically, both isolates had septate hyphae and obovoid to curved conidia; however, Isolate R contained 2 to 3 septa, whereas Isolate M had 3 to 4. The colonies appeared cottony to fluffy, ranging from white and creamy pink to dark purple, with orange or reddish-purple pigmentation on the reverse side. Microscopic examination revealed the presence of sickle shaped macroconidia measuring 27 to 35 µm with 1 to 6 septa and oval to obovoid microconidia about 7 by 4 µm, which are diagnostic of *F. oxysporum*. Although isolates R and M differed slightly in appearance, their shared diagnostic features, particularly the characteristic macroconidia and microconidia, confirmed their identity as *Fusarium oxysporum*, consistent with descriptions by (Gupta et al., 2010), Bibi et al. (2024) and (Pešić et al., 2025).

The antagonistic potential of *Trichoderma* spp. against phytopathogenic fungi, particularly *Fusarium oxysporum* isolated from shallot, arises from a combination of interacting mechanisms, including antibiosis, competition for resources, and mycoparasitism. These processes often operate simultaneously and are further strengthened by Rice Husk Ash Nanoparticles, which reshape the physical and biological properties of the environment to enhance *Trichoderma* activity.

Through antibiosis, *Trichoderma harzianum* secretes a diverse arsenal of soluble and volatile metabolites that act on multiple biochemical and structural targets to suppress *Fusarium oxysporum* (Khan et al., 2020; Zhang et al., 2018). Small molecules such as gliotoxin induce oxidative stress and programmed cell death like responses in pathogen hyphae, while peptaibols insert into membranes to form pores that disrupt ionic balance and cause leakage of cytoplasm (Zhang et al., 2018). Volatile compounds including 6-pentyl-2H-pyran-2-one, alcohols, monoterpenes and hydrogen cyanide inhibit spore germination and interfere with hyphal extension and polarity, reducing the pathogen's ability to colonize host tissue(Jin et al., 2020). These metabolites diffuse into the surrounding medium to produce clear inhibition zones, with local concentration determining whether the effect is reversible or lethal; at higher concentrations rapid morphological collapse and loss of viability occur (Morath et al., 2012), and the resulting inhibition zones can be seen in Figure 3a and 3c. Antibiosis also compromises key physiological processes in the pathogen by inhibiting respiratory enzymes and disturbing signal transduction required for growth and virulence, and multiple metabolites often act synergistically so that combined activity exceeds that of individual compounds (Khan et al., 2020). In vitro studies and the present work demonstrate that metabolites from *T. harzianum* produce fast and pronounced antifungal effects against *F. oxysporum* from shallot, supporting antibiosis as a principal mode of suppression (Siswadi et al., 2025).

Through competition for resources, *Trichoderma harzianum* rapidly colonizes substrates and monopolizes space and essential nutrients, causing *Fusarium oxysporum* to be deprived of the resources needed for growth and spread (Harman et al., 2004; Modrzewska et al., 2022). This competitive advantage is evident from the faster growth of *Trichoderma* compared with *Fusarium*, as shown in Figure 3a, 3b, 3c, and 3d. *Trichoderma* produces siderophores that sequester iron and extracellular enzymes such as β-glucanases and proteases that depolymerize complex organic matter into simpler compounds preferentially assimilated by *Trichoderma*, thereby reducing the nutrient pool available to the pathogen (Harman et al., 2004). In addition, secretion of antagonistic secondary metabolites, including alkaloids, paxillin, lolitrem, and steroid tetranone, exerts further pressure on *Fusarium* hyphae, causing shrinkage and loss of vigor (Modrzewska et al., 2022; Sivan, 1989). The combination of rapid substrate colonization, efficient nutrient scavenging, and metabolite production often acts together with mycoparasitic and antibiotic interactions to suppress *F. oxysporum* more effectively than any single mechanism alone (R. Kumari et al., 2025a; Suyanto et al., 2021).

Through mycoparasitism, *Trichoderma harzianum* directly attacks *Fusarium oxysporum* by recognizing, coiling around, and penetrating the pathogen's hyphae, followed by the secretion of cell wall–degrading enzymes such as chitinases, β-glucanases, and proteases that break down structural polymers and cause collapse

of the fungal cell wall (R. Kumari et al., 2025b). Physical contact and hyphal penetration enable *Trichoderma* to absorb nutrients from the weakened pathogen while enzymatic hydrolysis compromises cell integrity, leading to visible disintegration under microscopic observation (Modrzewska et al., 2022). The macroscopic manifestation of this interaction can be observed in Figure 3A–D, where *Trichoderma* grows into and over *Fusarium* colonies, forming inhibition zones and areas of discoloration or halted growth at the contact interface, clear indications of parasitic attachment and tissue degradation. This targeted parasitism is often accompanied by the localized release of antagonistic metabolites that further inhibit pathogen recovery, and the combined effects of mechanical disruption, enzymatic degradation, and chemical antagonism collectively reduce *F. oxysporum* colonization and virulence (R. Kumari et al., 2025a; Suyanto et al., 2021).

Rice husk ash nanoparticles (RHANs) enhance *Trichoderma* activity by modifying the physicochemical environment to favor its antagonistic performance (R. Kumari et al., 2025a). The high surface area and porosity of RHANs improve aeration, moisture retention, and nutrient availability, which collectively promote faster *Trichoderma* growth and colonization compared to *Fusarium* (Suyanto et al., 2021). RHANs also act as microcarriers that facilitate spore adhesion and uniform distribution within the substrate, strengthening *Trichoderma*'s competitiveness for space and resources (Modrzewska et al., 2022). Furthermore, the interaction between RHANs and *Trichoderma* stimulates metabolic activity, leading to elevated production of hydrolytic enzymes such as chitinases and glucanases, as well as antifungal secondary metabolites that enhance both mycoparasitism and antibiosis (R. Kumari et al., 2025a). This synergistic effect accelerates pathogen suppression by promoting enzymatic degradation of *Fusarium* hyphae and reducing pathogen persistence in the rhizosphere.

The synergistic interaction between *Trichoderma* and rice husk ash nanoparticles (RHANs) further demonstrates how nanoscale materials can amplify biological control efficiency. Shams et al. (2023) found that *T. harzianum* and *T. viride* combined with ZnO nanoparticles suppressed *Fusarium solani* and improved plant growth in cherry tomatoes. In this study, the combination of *Trichoderma* sp. with rice husk ash nanoparticles also showed a synergistic effect, achieving 60% inhibition one day after planting compared to four days when *Trichoderma* was applied alone. This faster response is likely due to the pH increase from silica nanoparticles, which creates unfavorable conditions for pathogens and stimulates *Trichoderma* to release more antifungal metabolites. Supporting evidence comes from Ishlah et al. (2022), who found that *T. harzianum* combined with 3000 ppm nano-silica reduced disease intensity and increased shallot yield, and from Raza et al. (2025), who showed that *T. harzianum* with silica-rich rice straw biochar effectively managed chickpea collar rot by enhancing defense enzymes like PPO and PAL. Overall, silica-based materials strengthen both the antagonistic activity of *Trichoderma* and the plant's natural defense system, offering a synergistic approach to sustainable disease control.

Conclusion

The application of rice husk ash nanoparticles has been demonstrated to effectively suppress the growth of low Fusarium by 36% through the competition mechanism, and medium Fusarium by 40% through the antibiosis mechanism. Trichoderma sp. has also been shown to inhibit the growth of Fusarium spp., achieving 81.16% inhibition in low Fusarium through the competition mechanism, and 81.59% inhibition in medium Fusarium through the antibiosis mechanism. The combination of Trichoderma sp. with rice husk ash nanoparticles exhibited enhanced inhibitory effects on Fusarium spp., with the highest percentage of inhibition observed in low Fusarium (90.58%) through the competition mechanism, and in medium Fusarium (90.80%) through the antibiosis inhibition mechanism. However, further research is warranted to investigate the application of Trichoderma sp. in conjunction with rice husk ash nanoparticles for inhibiting moler disease caused by Fusarium spp. in shallot plants in planta.

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