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Characterization of the antagonistic and mycoparasitic activity of *Nigrospora* spp. against *Fusarium solani*, the causal agent of Fusarium Wilt

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Abstract

Fusarium wilt, caused by *Fusarium solani*, is a destructive disease that limits chili productivity worldwide. The present study aimed to characterize the antagonistic and mycoparasitic activities of five *Nigrospora* species *Nigrospora gorlenkoana*, *Nigrospora guilinensis*, *Nigrospora musae*, *Nigrospora oryzae*, and *Nigrospora rubi* against *Fusarium solani* under in vitro conditions. Antagonistic potential was evaluated using a dual-culture assay, and mycoparasitic mechanisms were observed by scanning electron microscopy (SEM). The results revealed that all *Nigrospora* isolates inhibited the growth of *F. solani* to varying degrees. Among them, *N. rubi* and *N. oryzae* showed the highest antagonistic activity, with inhibition rates of 56.2% and 50.0%, respectively. Microscopic analysis demonstrated distinct mycoparasitic interactions, including attachment, hyphal coiling, penetration, and host lysis. These progressive stages indicate a necrotrophic mycoparasitic strategy, in which *Nigrospora* spp. degrade and lyse the hyphae of *F. solani* through enzymatic and mechanical actions. The study concludes that *Nigrospora* spp. possess strong potential as biological control agents against *F. solani*, offering an environmentally friendly alternative to chemical fungicides. The morphological evidence of mycoparasitism presented in this research provides a foundation for further molecular and biochemical studies to identify the specific enzymes and metabolites responsible for their antifungal activity.

Keywords – Antagonistic activity – biological control – Fusarium wilt – mycoparasitism – *Nigrospora*

Introduction

Chili is a strategic vegetable commodity in Tuban Regency, contributing significantly to the agricultural economy (Karyani et al. 2020, Winarto et al. 2024). Tuban Regency in East Java is known as one of the chili granaries in the province. In 2024, chili production in Tuban reached 19,771.7 tons, with a planting area of approximately 20,581 hectares (Badan Pusat Statistik 2024). This production not only meets the needs of East Java but also supplies chili as far as Jakarta. The economic importance of chili plants is highlighted by their role in generating farmer income and the high demand in the market. Increasing demand for chili has contributed to its widespread cultivation by both large and small scale farmers (Sundari et al. 2023). However, chili plant

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productivity often faces various challenges, one of which is disease attacks caused by soil pathogens, particularly *F. solani*. These two pathogenic fungi are the main cause of Fusarium wilt, which can significantly reduce crop yields (Ibade et al. 2024, Nurtjahyani et al. 2026).

Fusarium solani has historically been used to refer to members of the *Fusarium solani* species complex (FSSC), a taxonomically diverse and phylogenetically complex group. The generic placement of the FSSC has been subject to different taxonomic interpretations. Multilocus phylogenetic analyses and morphological evidence have supported the treatment of FSSC lineages within *Neocosmospora* (Sandoval-Denis et al. 2019, Sandoval-Denis & Crous 2018). However, an alternative phylogenomic framework has argued for retaining the FSSC within *Fusarium*, based on phylogenetic evidence supporting a monophyletic *Fusarium* that includes the FSSC and on the longstanding use of the genus name in plant pathology and other fields (Geiser et al. 2020, O'Donnell et al. 2020). Therefore, In the present study, the term *Fusarium* is therefore used in the broad sense for consistency with the plant-pathological literature on Fusarium wilt and with the nomenclature used in the studies from which the target pathogen was selected. This usage does not imply rejection of the alternative *Neocosmospora* classification.

Recent studies have documented members of the FSSC as pathogens associated with Fusarium wilt of chili and pepper. Chowdary & Kumar. (2023) reported *F. solani* as a causal agent of Fusarium wilt in chili, whereas Engalycheva et al. (2024) identified *F. solani* among the *Fusarium* species associated with wilted pepper plants and confirmed its pathogenicity. Shabir et al. (2026) isolated *F. solani* from wilted chili plants, confirmed its identity using multilocus molecular analyses, and demonstrated its pathogenicity through inoculation assays. Based on these reports, *F. solani* was selected as the target pathogen in the present study because of its documented association with Fusarium wilt and demonstrated pathogenicity, making it a relevant pathogen for evaluating the antagonistic activity of the tested fungi.

Fusarium wilt disease is widespread throughout Indonesia and affects several banana-producing provinces. Surveys conducted between 2006 and 2009 across 15 provinces showed that the incidence of Fusarium wilt varied widely, ranging from 0.08% to 100%, with an average of about 24% (Hermanto et al. 2011). The incidence of Fusarium wilt in the Banyumas region was reported to reach 50%. Fusarium wilt disease was also reported to attack chili plants, causing production losses of up to 50% (Ekaputri et al. 2023). This pathogen systemically infects plants, causing damage to roots, stems, and leaves, ultimately reducing plant vigor and yield (Serrano-Jamaica et al. 2021).

Fusarium wilt causes root rot, which severely limits chili production by impairing the root system and leading to poor nutrient absorption and plant growth (Edward et al. 2013, da Rocha et al. 2016, Filho et al. 2020, Liu et al. 2023). Fusarium wilt is characterized by wilting leaves, discoloration of the xylem vessels, and eventual plant death (Pastrana et al. 2019, Reddy et al. 2023, Lal et al. 2024). Common control efforts include the use of chemical fungicides. However, excessive use of chemical fungicides can lead to resistance in pathogenic fungi (Maciel Ferraz et al. 2014, Reddy et al. 2023, Yadav et al. 2023), environmental pollution (Preeti et al. 2015, Mir et al. 2023), and health risks for humans (Dong et al. 2023, Guo et al. 2024). Therefore, more environmentally friendly and sustainable control alternatives are needed, such as microbes for biocontrol and antimicrobial peptides, to manage Fusarium wilt disease (Wang et al. 2023, Islam et al. 2024).

One environmentally friendly approach to controlling Fusarium wilt is the use of antagonistic fungi (Maitlo et al. 2019). The use of antagonistic fungi is safer than chemical pesticides because it does not leave harmful residues in the soil or plants (Kurniawan et al. 2019, Das & Sabu, 2023). Additionally, antagonistic fungi can attack *Fusarium* through various mechanisms such as directly destroying *Fusarium* hyphae or spores (Bekkar et al. 2016), producing antimicrobial compounds like lytic enzymes (chitinase, glucanase) or antibiotics that can kill or inhibit Fusarium growth (Podgórska-Kryszczuk et al. 2022, Al-Mutar et al. 2023), and inducing plant resistance to Fusarium attack (Awad-Allah et al. 2022, Boulahouat et al. 2023). Therefore, the identification and selection of antagonistic fungi are necessary to improve the efficiency of Fusarium wilt control.

Although several antagonistic fungal strains have been researched and applied, the search for new strains remains necessary because the effectiveness of existing strains is inconsistent across different environments (Tariq et al. 2020, Müller & Behrendt. 2021). Additionally, there is resistance of Fusarium species to antifungal compounds (Venturini et al. 2016, He et al. 2021). Fusarium wilt also has several stages of infection, including root penetration, tissue colonization, systemic spread, and not all antagonistic fungi are effective at all of these stages (Tian et al. 2024). Therefore, new antagonistic fungal strains need to be sought and identified, with the hope that they can help find species that can target various stages of infection and serve as natural control agents for Fusarium wilt disease. Based on the problems outlined, the purpose of this study is to determine the effectiveness and characterize the antagonistic activity of the fungal species *Nigrospora* spp. against the pathogenic fungus *F. solani*.

One of the mechanisms by which antagonistic fungi suppress pathogenic fungi is mycoparasitism, a direct parasitic interaction in which the antagonist recognizes, attaches to, and penetrates the hyphae of the pathogen, leading to its lysis or degradation (Kim et al. 2001, Singh et al. 2024). This mechanism is considered an effective biological strategy for controlling soil-borne pathogens such as *F. solani*. Previous studies have reported that members of the genus *Nigrospora* exhibit mycoparasitic behavior against several plant pathogens (Mustofa & Hastuti 2024). Therefore, understanding both the antagonistic and mycoparasitic activities of *Nigrospora* spp. is essential to evaluate their potential as biological control agents against *F. solani*, the causal agent of Fusarium wilt.

Materials & Methods

Preparation of Antagonistic Fungi and Pathogenic Fungi

This research was conducted in February 2025 at the Laboratory of PGRI Ronggolawe University, Tuban, Indonesia. The antagonistic fungi used in this study were *Nigrospora gorlenkoana* NGUM-1, *N. guilinensis* NGUM-2, *N. rubi* NGUM-3, *N. musae* NGUM-4, and *N. oryzae* NGUM-5. These isolates were originally obtained as endophytic fungi from the leaves and stems of *Heliotropium indicum* and were previously reported by Mustofa et al. (2024). In the previous study, the isolates were identified based on macroscopic and microscopic morphological characteristics using the fungal identification key of Wang et al. (2017). The pathogenic fungus used in the antagonism assay was *Fusarium solani* f. sp. *capsici* (MT371375), obtained from the Microbiology Laboratory fungal collection, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, Indonesia. Before the antagonism assay, all fungal isolates were maintained on potato dextrose agar (PDA) under appropriate laboratory conditions.

Antagonism Power Testing

The interaction between endophytic fungal species and pathogenic fungi was evaluated using the dual culture technique. Each fungal isolate on a PDA plate was incubated at 25°C for 5×24 hours. Each pathogenic fungal colony and each endophytic fungal colony were aseptically cut with a 5 mm diameter cork borer and placed at two points on opposite sides, then incubated at 25°C for 3×24 hours. The antagonistic ability of endophytic fungi against pathogenic fungi was calculated using the antagonism power formula.

$$\textbf{Antagonism Power: } \frac{(r^1 - r^2)}{(r^1)} \times 100 \quad (1)$$

Explanation:
r1 = radius of the pathogenic fungi colony growing away from the fungi
r2 = radius of the pathogenic fungi colony growing toward the fungi

Results

Antagonistic Activity Assay by the Dual-Culture Method

The antagonism activity test of 5 antagonistic fungal isolates, consisting of the fungal species *Nigrospora gorlenkoana* NGUM-1, *N. guilinensis* NGUM-2, *N. rubi* NGUM-3, *N. musae* NGUM-4, and *N. oryzae* NGUM-5 isolated from *Heliotropium indicum* L. (Mustofa et al. 2024), was conducted against the pathogenic fungus *F. solani* f. sp. Capsici MT371375. The results of the study on the antagonism between endophytic fungi and pathogenic fungi consist of morphological data on antagonism (Fig. 1) and measurements of antagonistic effects (Table 1).

Table 1 Antagonistic power of 5 antagonistic fungal isolates against the pathogenic fungus *F. Solani*

Antagonistic Fungi	Pathogenic Fungi	Antagonistic Activity (%)
N. gorlenkoana	*F. solani*	30.4
N. guilinensis	*F. solani*	9.3
N. musae	*F. solani*	28.2
N. oryzae	*F. solani*	50.0
N. rubi	*F. solani*	56.2

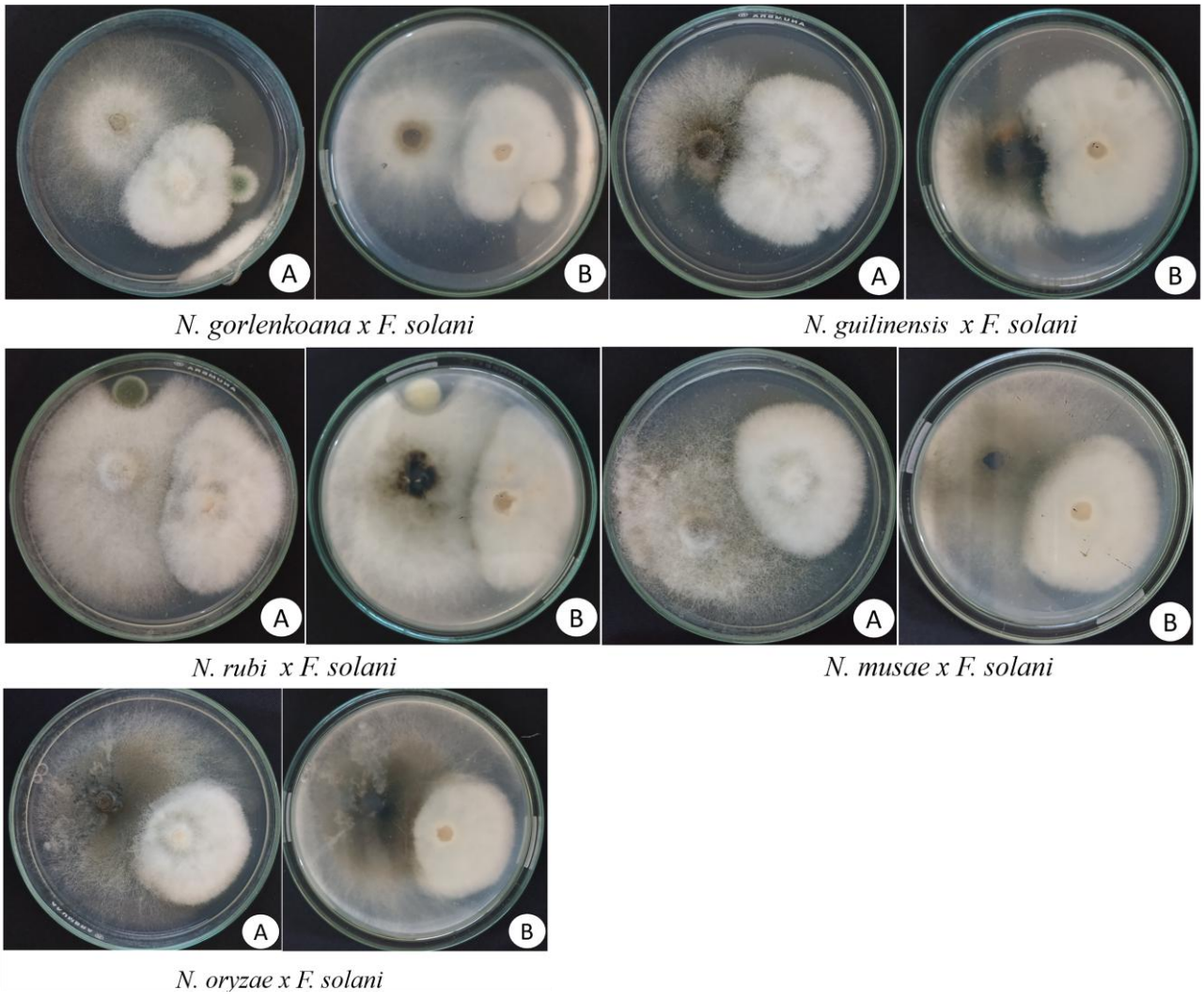

Fig. 1 – Morphology of antagonism between *F. solani* and five antagonistic fungal isolates of *Nigrospora* spp. Code A (top surface view). Code B (bottom surface view).

Table 1 presents the antagonistic activity of five fungal isolates against the pathogenic fungus *F. solani*. The antagonistic activity was expressed as the percentage of inhibition of *F. solani* growth. The observed antagonistic activity varied among the five fungal isolates. *N. rubi* showed the highest antagonistic activity (56.2%), followed by *N. oryzae* (50.0%), *N. gorlenkoana* (30.4%), and *N. musae* (28.2%), whereas *N. guilinensis* showed the lowest activity (9.3%). Because each isolate was evaluated using a single observation without experimental replication, the results are presented descriptively, and no inferential statistical analysis was performed. Therefore, the differences among isolates are interpreted as observed differences in antagonistic activity rather than statistically significant differences.

Analysis of Micoparasitism of *Nigrospora* Species on *F. solani*

The mycoparasitic interaction between several species of *Nigrospora* and *F. solani* hyphae was observed using a scanning electron microscope (SEM) at magnifications of 2500× to 5000× (Fig. 2). These interactions showed different stages of the typical mycoparasitism process, namely attachment, coiling, penetration, and lysis of the host hyphae (Table 2). These patterns demonstrate the ability of *Nigrospora* to recognize, attack, and utilize *F. solani* as a host, which is morphologically consistent with the necrotrophic mycoparasitism mechanism that has been reported in other antagonistic fungi such as Trichoderma and Clonostachys (Atanasova et al. 2013, Viterbo & Horwitz. 2014).

In Fig. A and D, the hyphae of *N. gorlenkoana* (indicated by mark 1) are seen attaching to the hyphae of *F. solani.* This contact is the initial stage of the mycoparasitism process, in which the parasitic hyphae recognize the host cell wall structure, which contains chitin, glucan, and surface proteins as chemical signals. This recognition mechanism is thought to be mediated by adhesin molecules and carbohydrate-binding proteins that play a role in the specific process between hyphae (Zeilinger et al. 2016). Strong adhesion enables further biochemical interactions that trigger the secretion of hydrolytic enzymes at the contact area.

In contrast, Figure B shows the hyphae of *N. guilinensis* (1) coiling around the hyphae of *F. solani* (2). This mechanism demonstrates a more aggressive form of interaction, in which the *Nigrospora* hyphae form tight coils around the host hyphae. This circular structure increases the contact area and allows for mechanical pressure and the secretion of hydrolytic enzymes such as chitinase, β-1,3-glucanase, and protease, which play a role in softening the host cell wall (Inbar & Chet. 1995). This binding mechanism is commonly found in effective mycoparasitic fungi and marks the transition phase from recognition to active penetration. Meanwhile, Figure C illustrates the interaction of *N. musae* hyphae (1) directly penetrating (penetration) into *F. solani* hyphae (2). The structure of the parasitic hyphae appears to form protrusions that penetrate the host cell wall and channel cytoplasmic contents into it. This indicates high enzymatic activity, particularly from cell wall-degrading enzymes such as chitinase and β-glucanase, which break down the polysaccharide cell wall layer of the target hyphae (Harman et al. 2004). This penetration can also be triggered by turgor pressure exerted by *Nigrospora* hyphae, indicating a combination of physical and chemical mechanisms during infection.

The most advanced stage is shown in Fig. E and F, which show the hyphae of *N. oryzae* and *N. rubi* (1) penetrating the hyphae of *F. solani* (2) and causing lysis (damage) to the cell wall structure. The area marked with the number (3) shows the area of degradation, where the wall of the *F. solani* hyphae breaks down and loses its structural integrity. This phenomenon shows that *Nigrospora* not only penetrates but also destroys host hyphae through the secretion of hydrolytic enzymes and antifungal secondary metabolites such as nigrosporin acid and isocumarone (Zhao et al. 2012). The presence of damaged and collapsed hyphal structures indicates host cell death due to lysis.

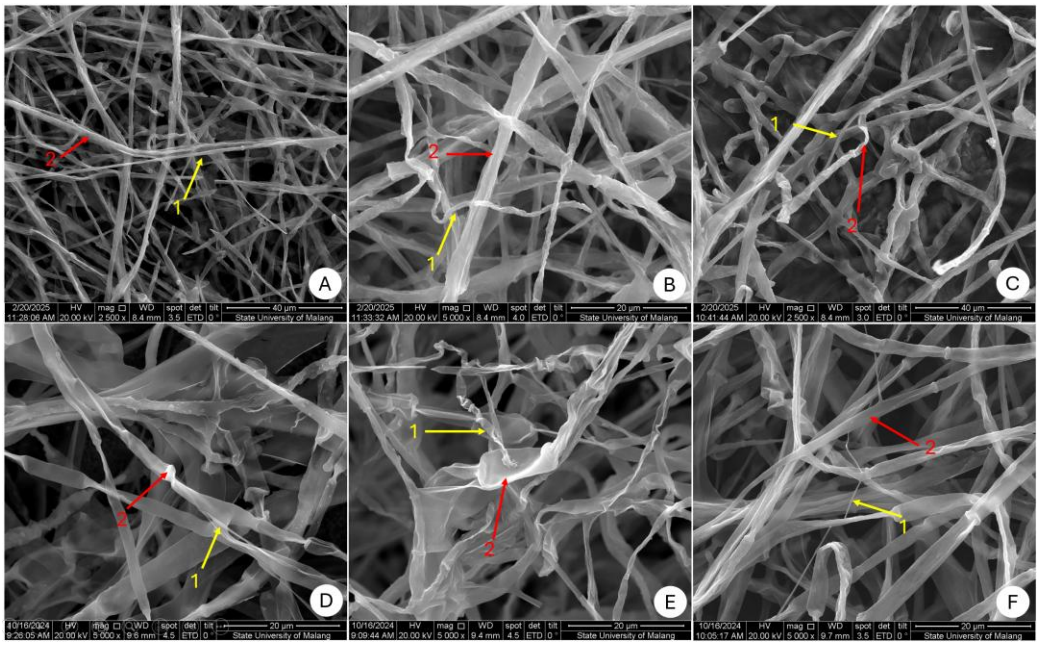

Fig. 2 – Mycorrhizal association of *Nigrospora* spp. with *F. solani*. Fig. A and D Hyphae of *N. gorlenkoana* (1) adhering to hyphae of *F. solani* (2). Fig. B Hyphae of *N. guilinensis* (1) with a mechanism of twining around *F. solani* hyphae (2). Fig. C Hyphae of *N. musae* (1) with a mechanism of penetrating *F. solani* hyphae (2). Fig. E and F Hyphae of *N. oryzae* and *N. rubi* (1) penetrating *F. solani* hyphae (2) and causing lysis. (3) hyphae undergo damage.

Table 2 Observation of Mycoparasitic Interaction Mechanisms of *Nigrospora* spp. Against *F. solani* Under Microscopic Analysis

Panel	***Nigrospora* Species**	**Observed Interaction Mechanism**	**Stage of *F. solani* Hyphal Damage**
A & D	*N. gorlenkoana*	Adhesion to *F. solani* hyphae without clear initial penetration. Surface contact visible with areas that may be tightly adhered.	No penetration yet, no initial lysis; but close contact possible preparation for the next stage.
B	*N. guilinensis*	The coiling mechanism surrounds the hyphae of *F. solani*. Coiling is a complex contact structure that increases the area of interaction and focuses enzymes or physical pressure.	Possibly initial penetration of the membrane or cell wall; no clear lysis yet.
C	*N. musae*	Direct penetration into the hyphae of *F. solani*. The structure of the *N. musae* fungus penetrates the wall/lumen of the host hyphae.	There is internal damage/internal structure damage to the hyphae of *F. solani*: vacuolation, possibly cytoplasmic degradation.
E & F	*N. oryzae* & *N. rubi*	Further penetration and causing lysis; possibly a combination of physical and enzymatic mechanisms. In E & F, penetration is followed by hyphal damage such as wall lysis and loss of structural integrity.	Loss of cellular content, internal compartments ruptured; cell walls may have degraded, causing the host hyphae to become “damaged” – marked as point (3) in image E.

Discussion

This variation in antagonistic power indicates that not all antagonistic fungal isolates have the same effectiveness in controlling *F. solani*. This is supported by the research of Yusnawan et al. (2019), who reported that *Trichoderma* isolates from various rhizospheres showed different levels of inhibition against *F. solani*, with some isolates showing up to 90.8% inhibition. This variation in antagonistic power is influenced by genetic variability and the origin of the isolates, leading to differences in antagonistic activity (Khattak et al. 2018). The origin of the isolate, such as the rhizosphere of specific plants or geographical location, influences the antagonistic properties of fungi. For example, *Pseudomonas* and *Serratia* isolates from different plant rhizospheres and locations exhibit plant-specific and location-specific genotypes, which affect their antagonistic activity against *Verticillium dahliae* (Berg et al. 2006). Additionally, *Trichoderma* spp. isolated from different rhizosphere soils also exhibited varying levels of antagonistic activity against *Aspergillus niger*, which was influenced by their genetic makeup (Haque et al. 2020). Isolates with high antagonistic potential, such as *N. rubi*, have greater potential as biocontrol agents to manage plant diseases caused by *F. solani*. Antagonistic fungi, including species of *Nigrospora*, have been known to have biocontrol potential against various plant pathogens. For example, endophytic fungi from various genera, including *Nigrospora*, exhibit antagonistic effects against *Venturia inaequalis* through the production of antifungal metabolites (Ebrahimi et al. 2022).

Based on the observed antagonistic activity of the five isolates, it was found that the type of antagonism exhibited is competition for space and nutrients. Fungi compete for essential nutrients and space, which can inhibit the growth of other microorganisms (Hiscox et al. 2018, Dullah et al. 2021). For example, *Trichoderma harzianum* competes for nutrients and space, effectively inhibiting plant pathogens (Kordowska-Wiater 2011). Additionally, there is a mechanism of mycoparasitism where it directly attacks and parasitizes other fungi. This involves physical contact, hyphal coiling, and penetration structures to attack and kill the host fungus (Wang et al. 2014, Mustofa & Hastuti. 2024). Additionally, this mechanism also exhibits hyperparasitism, where the antagonistic fungus becomes a parasite of another fungus, causing mycelial malformations and cell wall rupture (Toghueo et al. 2016, Maitlo et al. 2019, Piombo et al. 2022).

Antagonistic fungi can also synthesize secondary metabolites and enzymes such as chitinase, glucanase, and protease, which degrade the cell walls of *F. solani*, causing significant hyphal modifications (Kim & Knudsen. 2013, Erazo et al. 2021, Niu et al. 2024). Antagonistic fungi can also enhance plant defense mechanisms, leading to increased resistance to pathogens. This includes the activation of plant defense enzymes such as catalase and peroxidase, which help reduce the effects of *F. solani* infection (Attia et al. 2022). It should be noted that the identification of *F. solani* in this study was based on classical morphological characteristics and pathogenic relevance, consistent with previous reports on chili Fusarium wilt in Indonesia and Southeast Asia. Moreover, the present work primarily focuses on antagonistic and mycoparasitic interactions between *Nigrospora* spp. and the Fusarium wilt pathogen under in vitro conditions; therefore, detailed multilocus phylogenetic resolution at the genus level (*Fusarium* vs. *Neocosmospora*) was beyond the scope of this study.

The use of biological agents such as *Nigrospora* spp. offers an environmentally friendly alternative to chemical fungicides, making this research expected to reveal the type of antagonism possessed by *Nigrospora* spp., such as antibiosis, competition, or mycoparasitism (Balendres et al. 2025, Dutta et al. 2023). This research is important to support the development of effective and sustainable biological control strategies in chili plant protection and to contribute to agricultural microbiology, particularly in the utilization of antagonistic fungi as biological control agents. Additionally, the results of this study can serve as a basis for developing a biofungicide formulation based on *Nigrospora* spp. for field application, thus helping farmers control Fusarium wilt more economically and environmentally friendly.

Based on the results of mycoparasitism, the sequence of mechanisms from attachment, entanglement, penetration, to lysis shows that *Nigrospora* spp. has complex and adaptive mycoparasitism abilities against *F. solani*. This pattern describes a necrotrophic type of interaction,

in which the parasitic fungus first kills its host and then utilizes the remains of its tissue as a source of nutrition (Vinale et al. 2008, Mustofa et al. 2024). Variations in the interspecies mechanisms of *Nigrospora* indicate differences in the level of aggressiveness and enzymatic effectiveness, which are likely caused by genetic factors, secondary metabolite secretion, and environmental conditions during the infection process.

Ecologically, these results reinforce the potential of *Nigrospora* as a natural biocontrol agent against plant pathogens caused by *F. solani*. Several previous studies have shown that *N. oryzae* and *N. sphaerica* have strong antifungal activity through the production of secondary compounds such as nigrosporolide and isofuranonic acid, which are capable of inhibiting the growth of various pathogenic fungi (Mustofa et al. 2024). Therefore, *Nigrospora* species that exhibit active mycoparasitic mechanisms, as shown in Figures E and F, have the potential to be developed as biocontrol agents for plant diseases caused by Fusarium spp. However, these findings are still morphological in nature and need to be supplemented with molecular and biochemical studies to confirm the enzymatic mechanisms involved. Further research should include analysis of the expression of genes encoding cell wall-degrading enzymes, such as chitinase (chi), β-glucanase, and protease, during the interaction process with *F. solani*. In addition, metabolomic studies are needed to identify antifungal compounds secreted by *Nigrospora* during mycoparasitism. In vivo testing in soil and plant systems is also important to ensure the effectiveness and safety of *Nigrospora* as a biocontrol agent under field conditions. Thus, these SEM images not only provide morphological evidence of *Nigrospora's* mycoparasitism against *F. solani*, but also provide a scientific basis for the development of environmentally friendly and sustainable biological control strategies.

This study revealed that *Nigrospora* species possess significant antagonistic and mycoparasitic activities against *F. solani*, the causal agent of Fusarium wilt. Among the five isolates tested, *N. rubi* and *N. oryzae* exhibited the highest antagonistic potential, inhibiting *F. solani* growth by 56.2% and 50.0%, respectively. Microscopic observations confirmed that *Nigrospora* spp. interact with the pathogen through a series of mycoparasitic stages, including attachment, coiling, penetration, and lysis of host hyphae (Mustofa et al. 2024). These progressive interactions illustrate a necrotrophic mycoparasitic strategy, in which *Nigrospora* spp. destroy the hyphal structures of *F. solani* through both enzymatic degradation and mechanical pressure.

In addition, in vivo experiments under greenhouse and field conditions are necessary to validate the biocontrol efficiency of *Nigrospora* spp. against *F. solani* under natural environmental stresses. Formulation research for developing biofungicide products based on *Nigrospora* spp. should also be pursued to ensure shelf stability, effectiveness, and compatibility with agronomic practices. Integrating these fungi into crop protection systems could offer a sustainable, cost-effective, and environmentally responsible solution to the persistent challenge of Fusarium wilt in horticultural crops.

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