

RESEARCH PAPER

Characterization antagonism of *Nigrospora* spp. against *Fusarium solani* causing Fusarium wilt in *Capsicum frutescens* L. plants

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ABSTRACT

Fusarium wilt is a major disease affecting horticultural crops in Indonesia, including chili, where it causes root rot, disrupts nutrient uptake, and reduces plant growth. This study aimed to evaluate the antagonistic activity of five *Nigrospora* spp. isolates against *Fusarium solani*, the causal agent of Fusarium wilt in chili plants (*Capsicum frutescens* L.). The tested isolates included *Nigrospora gorklenkoana*, *N. guilinensis*, *N. musae*, *N. oryzae*, and *N. rubii*. Antagonistic activity was assessed using the dual culture method on potato dextrose agar (PDA) at 25 °C for 72 hours. The results showed that all isolates inhibited the growth of *F. solani*, with *N. rubii* exhibiting the highest inhibition (56.2%), followed by *N. oryzae* (50.0%), *N. gorklenkoana* (30.4%), and *N. musae* (28.2%), while *N. guilinensis* showed the lowest inhibition (9.3%). The antagonistic mechanisms involved competition for nutrients and space, as well as mycoparasitism. These findings indicate that *N. rubii* and *N. oryzae* are promising candidates for biological control of Fusarium wilt in chili, offering environmentally friendly alternatives to chemical fungicides. This study provides comparative evidence of species-specific antagonistic activity and highlights the potential of *Nigrospora* spp. for sustainable disease management.

Keywords: Antagonistic fungi, biological control, biofungicides, *F. solani*, mycoparasitism

INTRODUCTION

Chili (*Capsicum frutescens* L.) is an economically important horticultural crop widely cultivated in Indonesia due to its high market demand and significant contribution to farmers' income (Karyani et al., 2020; Winarto et al., 2024). However, its productivity is often constrained by soil-borne pathogens, particularly *Fusarium solani*, the causal agent of Fusarium wilt, which can lead to substantial yield losses. Understanding the biology and impact of this pathogen is therefore essential for developing effective and sustainable disease management strategies.

Fusarium wilt has been widely reported across various regions in Indonesia and affects multiple crops, including chili. Previous studies have documented considerable variation in disease incidence, ranging from low to severe levels depending on environmental

conditions and cropping systems. For instance, disease incidence in certain regions has been reported to reach up to 50%, resulting in significant economic losses (Ekaputri et al., 2023). The pathogen infects plants systemically, causing root rot, vascular discoloration, leaf wilting, and eventually plant death, thereby severely reducing crop productivity (Rocha et al., 2016; Filho et al., 2020; Liu et al., 2023; Serrano-Jamaica et al., 2021).

The management of Fusarium wilt traditionally relies on chemical fungicides; however, their excessive and prolonged use can lead to the development of resistant pathogen populations, environmental contamination, and potential risks to human health (Reddy et al., 2023; Yadav et al., 2023). Consequently, there is an increasing need to develop environmentally friendly alternatives, such as biological control agents, to ensure sustainable crop protection (Islam et al., 2024; Wang et al., 2023). Previous studies by Yusnawan (2013) have demonstrated that antagonistic fungi, including *Trichoderma* spp., exhibit variable inhibitory effects against *F. solani*, depending on isolate characteristics and environmental conditions. In addition, Soesanto et al. (2020) highlighted the important role of natural and biological control agents in suppressing plant pathogens through multiple mechanisms, including antibiosis and mycoparasitism.

Among these alternatives, antagonistic fungi

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have attracted considerable attention due to their ability to suppress plant pathogens through multiple mechanisms. These include mycoparasitism, competition for nutrients and space, production of antifungal metabolites and lytic enzymes (e.g., chitinases and glucanases), and induction of host plant resistance (Bekkar et al., 2016; Al-Mutar et al., 2023; Podgórska-Kryszczuk et al., 2022; Awad-Allah et al., 2022; Boulahouat et al., 2023). Compared to chemical control, the use of antagonistic fungi is considered safer and more sustainable, as it minimizes harmful residues in the environment (Malik et al., 2024; Kurniawan et al., 2019). Therefore, the identification and characterization of effective antagonistic fungi are crucial steps toward improving the biological control of Fusarium wilt.

Despite the availability of several antagonistic fungal strains, their effectiveness often varies under different environmental conditions, limiting their practical application (Müller & Behrendt, 2021; Tariq et al., 2020). In addition, *Fusarium* have shown the ability to develop resistance to certain antifungal compounds (He et al., 2021; Venturini et al., 2016). The infection process of *Fusarium* involves multiple stages, including root penetration, tissue colonization, and systemic spread, and not all antagonistic fungi are equally effective at each stage (Tian et al., 2024). These challenges highlight the need to explore and identify new antagonistic fungal species with consistent and broad-spectrum activity.

In this context, *Nigrospora* spp., a group of endophytic fungi, have emerged as promising candidates for biological control due to their ability to colonize plant tissues and produce bioactive compounds. However, comparative studies on the antagonistic potential and underlying mechanisms of different *Nigrospora* species against *F. solani* remain limited. This study provides a comparative evaluation of five *Nigrospora* species and their mycoparasitic interactions with *F. solani*, supported by morphological and ultrastructural observations.

Therefore, this study aims to evaluate the antagonistic activity and characterize the interaction mechanisms of *Nigrospora* spp. against *F. solani*. It is hypothesized that *Nigrospora* spp. exhibit significant antagonistic activity and mycoparasitic behavior, enabling them to inhibit the growth of *F. solani*.

MATERIALS AND METHODS

Research Site. This study was conducted in February 2025 at the Biology Laboratory, Universitas PGRI

Ronggolawe, Indonesia.

Fungal Materials. The pathogenic fungus used in the antagonism assay was *F. solani* f. sp. *capsici*. The antagonistic fungi consisted of five *Nigrospora* species: *N. gorlenkoana*, *N. gulinensi*, *N. rubii*, *N. musae*, and *N. oryzae*, obtained from the culture collection of the Microbiology Laboratory, Universitas Negeri Malang, Indonesia.

Antagonism Assay. The interaction between endophytic fungal isolates and the pathogenic fungus was evaluated using the dual culture method following Maitlo et al. (2019) with slight modifications. Each fungal isolate was cultured on potato dextrose agar (PDA) and incubated at 25 °C for 5 days. Mycelial plugs (5 mm in diameter) of both the pathogenic fungus and the antagonistic isolate were aseptically cut using a cork borer and placed on opposite sides of a PDA plate.

The plates were incubated at 25 °C for 72 hours. Antagonistic activity was determined by measuring the radial growth of the pathogen in the direction toward (r_2) and away from (r_1) the antagonist. The percentage inhibition of radial growth (PIRG) was calculated according to Pukalski et al. (2024) using the formula:

$$\text{PIRG (\%)} = \left[\frac{(r_1 - r_2)}{r_1} \right] \times 100$$

PIRG = Percentage inhibition of radial growth;

r_1 = Radius of the pathogen colony growing away from the antagonist;

r_2 = Radius of the pathogen colony growing toward the antagonist.

Morphological Analysis using SEM. The morphological interaction between *Nigrospora* spp. and *F. solani* examined using a scanning electron microscope (SEM) (State University of Malang, Indonesia). Observations were conducted at an accelerating voltage of 20 kV with an ETD detector, at magnifications ranging from 2500 × to 5000×.

Sample preparation followed a standard SEM protocol, including fixation, graded ethanol dehydration, and critical point drying, as described by Yuan et al. (2024), followed by mounting and gold coating. SEM observations were used to identify structural indicators of antagonism, such as hyphal deformation, shrinkage, perforation, and lysis, as well as direct interactions including hyphal coiling and penetration by *Nigrospora* spp.

The resulting micrographs were analyzed descriptively to support quantitative inhibition data and

to provide microscopic evidence of the antagonistic mechanisms.

RESULTS AND DISCUSSION

Antagonism Assay. The antagonistic activity of five endophytic fungal isolates—*N. gorlenkoana*, *N. guilinensis*, *N. musae*, *N. oryzae* and *N. rubii*—against the pathogenic fungus *F. solani* was evaluated based on dual culture assays and morphological interactions (Figure 1; Table 1). The results demonstrated clear variation in inhibitory capacity among the tested isolates.

All *Nigrospora* isolates suppressed the growth of *F. solani*, although with markedly different inhibition levels. *N. rubii* exhibited the highest inhibition (56.2%), followed by *N. oryzae* (50.0%), *N. gorlenkoana* (30.4%), and *N. musae* (28.2%), whereas *N. guilinensis* showed minimal inhibition (9.3%). Based on the classification of antagonistic strength proposed by Pal & Gardener (2006) and Ivayani et al. (2018), inhibition values of $\geq 50\%$ are categorized as strong, 25–50% as moderate, and $< 25\%$ as weak antagonism. Accordingly, *N. rubii* (56.2%) and *N. oryzae* (50.0%) are classified as strong antagonists, *N. gorlenkoana* (30.4%) and *N. musae* (28.2%) as

Table 1. Inhibition of *F. solani* by five *Nigrospora* isolates in a dual culture assay

Isolate	Inhibition (% \pm SD)
<i>N. rubii</i>	56.2 \pm 2.1 a
<i>N. oryzae</i>	50.0 \pm 2.5 a
<i>N. gorlenkoana</i>	30.4 \pm 1.8 b
<i>N. musae</i>	28.2 \pm 1.6 b
<i>N. guilinensis</i>	9.3 \pm 1.2 c

Values are presented as means \pm standard deviation (SD) of three replicates ($n = 3$). Data were analyzed using one-way analysis of variance (ANOVA), and means were separated by Duncan's Multiple Range Test (DMRT) at $p < 0.05$. Values followed by different superscript letters within the same column indicate statistically significant differences.

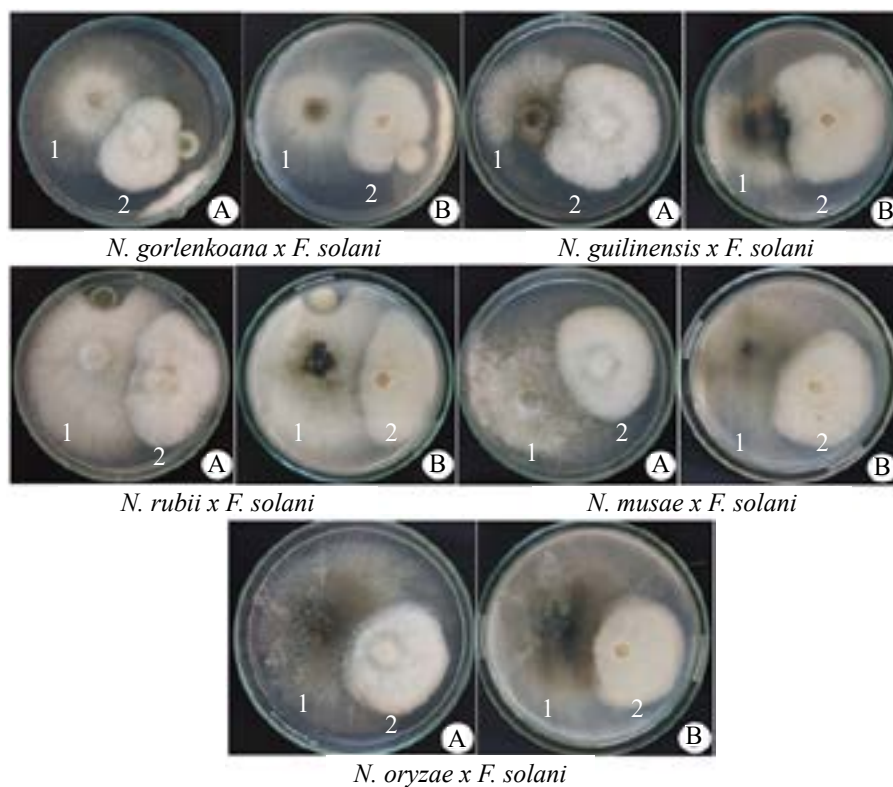


Figure 1. Morphological interactions between *F. solani* and five *Nigrospora* isolates in a dual culture assay. A. Top view of colony interactions; B. Bottom view of colony interactions. Code 1: *Nigrospora*, Code 2: *Fusarium*.

moderate antagonists, while *N. guilinensis* (9.3%) is categorized as a weak antagonist. The data show that the antagonism of the five *Nigrospora* isolates to *Fusarium solani* differs significantly based on one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at $p < 0.05$, as indicated by the different superscript letters in Table 1. This variation suggests that antagonistic efficiency in *Nigrospora* spp. is not only species-dependent but may also reflect functional specialization in ecological adaptation and metabolite production.

The observed differences in antagonistic activity are likely associated with variations in metabolic capacity and enzymatic activity among species. Fungal antagonists are known to produce a wide array of secondary metabolites and hydrolytic enzymes that contribute to pathogen suppression. The higher inhibition observed in *N. rubii* and *N. oryzae* may reflect a more efficient production of antifungal metabolites and cell wall-degrading enzymes such as chitinases and β -1,3-glucanase, which are involved in the degradation of fungal cell walls (Kumar et al., 2024; Tong et al., 2022; Xu et al., 2022). These findings are consistent with previous reports indicating that endophytic fungi exhibit species-dependent antagonistic mechanisms. Similarly, Mustafa et al. (2024) reported that *N. musae* exhibits antagonistic activity mediated by secondary metabolites.

Variability in antagonistic effectiveness is a common phenomenon among fungal biocontrol agents and may arise from differences in genetic background, ecological adaptation, and isolate origin. In addition to antibiosis, antagonistic fungi suppress pathogens through multiple mechanisms, including mycoparasitism, competition for nutrients and space, and induction of host resistance (Islam et al., 2023; Kurchenko et al., 2023). Environmental factors such as temperature, pH, and nutrient availability may further influence the expression of these mechanisms, resulting in variability in inhibition outcomes (Hiscox et al., 2018). Moreover, discrepancies between *in vitro* and *in vivo* performance highlight the need for further validation under field conditions (Yusnawan et al., 2019). Genetic variability and isolate origin also contribute to differences in antagonistic activity (Khattak et al., 2018). These factors collectively explain the differential antagonistic responses observed among the tested *Nigrospora* isolates.

Among the tested isolates, *N. rubii* demonstrated the greatest potential as a biocontrol agent against *F. solani*. Its superior inhibitory activity may be attributed to its enhanced ability to produce bioactive compounds

and adapt to competitive microenvironments. Previous studies have shown that *Nigrospora* species possess diverse biosynthetic capabilities, enabling the production of antifungal metabolites (Lee et al., 2023; Wang et al., 2017). In addition, compounds such as phenazine derivatives have been reported to exhibit strong antifungal activity (Thanabalasingam et al., 2015), although the specific metabolite profile of *N. rubii* requires further investigation. Endophytic fungi, including *Nigrospora*, have also been reported to suppress plant pathogens such as *Venturia inaequalis* through metabolite-mediated mechanisms (Ebrahimi et al., 2022).

Morphological Analysis using SEM. Morphological observations revealed that mycoparasitism was the dominant antagonistic mechanism exhibited by the tested isolates (Figure 2). This mechanism involves direct interaction between antagonist and pathogen, including hyphal adhesion, coiling, penetration, and subsequent degradation of the pathogen's hyphae. In addition to mycoparasitism, competition for nutrients and space may also contribute to pathogen suppression (Dullah et al., 2021; Hiscox et al., 2018). Similar mechanisms have been reported in other fungal biocontrol agents such as *Trichoderma harzianum* (Awad-Allah et al., 2022). The present findings are consistent with previous studies demonstrating that *Nigrospora* species can parasitize *Fusarium* spp. through direct hyphal interaction (Mustofa & Hastuti, 2024; Mustofa et al. 2024). Furthermore, this interaction may involve hyperparasitism, leading to hyphal deformation and cell wall disruption (Maitlo et al., 2019; Piombo et al., 2022; Toghueo et al., 2016). In the present study, the interaction patterns observed suggest that *Nigrospora* spp. employ comparable strategies to inhibit *F. solani*.

These structural alterations are indicative of enzymatic degradation processes mediated by hydrolytic enzymes. Antagonistic fungi can synthesize secondary metabolites and hydrolytic enzymes such as chitinases, glucanases, and proteases, which degrade the cell wall of *F. solani*, resulting in significant hyphal modifications (Erazo et al., 2021; Niu et al., 2024). This mechanism likely contributes to the higher inhibition observed in *N. rubii*, which also exhibited more pronounced hyphal damage under SEM. The higher inhibition percentages observed in *N. rubii* and *N. oryzae* are likely associated with their greater capacity to secrete cell wall-degrading enzymes (chitinase, β -1,3-glucanase, and protease) and to produce bioactive secondary metabolites such

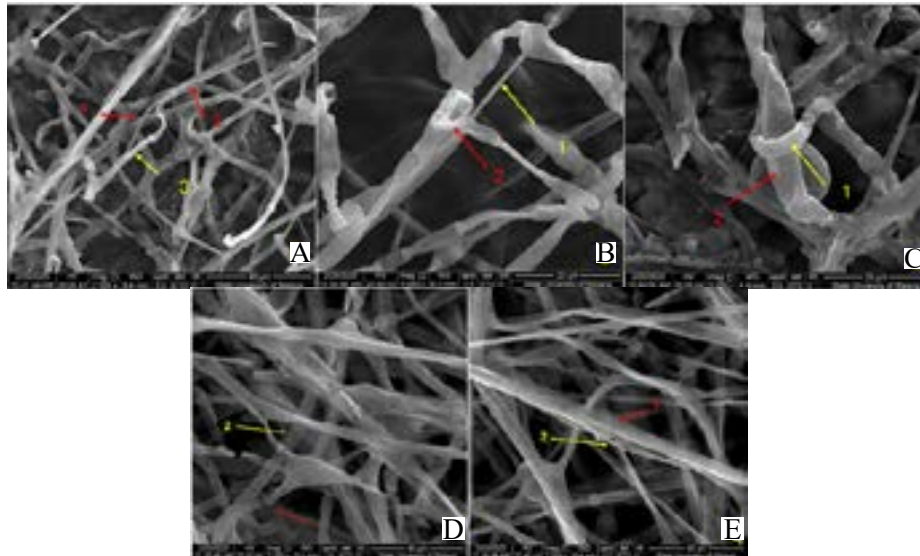


Figure 2. Mechanisms of mycoparasitism of *Nigrospora* spp. against *F. solani*. A. *N. gorlenkoana*; B. *N. guilinensis*; C. *N. oryzae*; D. *N. musae*; E. *N. rubii*. Description: A. Red arrows indicate antagonistic hyphae (2) and their attachment to pathogenic hyphae (1), while yellow arrows indicate damaged pathogenic hyphae (3); B. Penetration of antagonistic hyphae into pathogenic hyphae; C. Coiling of antagonistic hyphae around pathogenic hyphae; D. Distortion of the pathogenic cell wall caused by antagonistic hyphae; E. Lysis and collapse of *F. solani* hyphae.

as phenazine-1-carboxamide, which act synergistically to disrupt the cell wall integrity of *F. solani* and facilitate the mycoparasitic process (Prasetyo et al., 2024; Thanabalasingam et al., 2015). This integrated mechanism, in which enzymatic degradation, metabolite production, and direct mycoparasitic behavior occur sequentially, supports the higher inhibition values recorded for the strong antagonist group (*N. rubii*, *N. oryzae*) compared with the moderate (*N. gorlenkoana*, *N. musae*) and weak (*N. guilinensis*) groups.

Scanning electron microscopy (SEM) analysis provided detailed insights into the ultrastructural interactions underlying mycoparasitism. The sequential stages observed included hyphal recognition and adhesion (Figure 2A), penetration of antagonistic hyphae into the pathogen (Figure 2B), coiling around *F. solani* hyphae (Figure 2C), deformation of the pathogen's cell wall (Figure 2D), and eventual hyphal lysis (Figure 2E). These structural alterations are indicative of enzymatic degradation processes mediated by hydrolytic enzymes such as chitinases, glucanases, and proteases (Mukherjee et al., 2022; Singh et al., 2024). Notably, isolates exhibiting higher inhibition percentages, particularly *N. rubii*, also displayed more pronounced hyphal damage and penetration under SEM, suggesting a direct relationship between quantitative inhibition and mycoparasitic aggressiveness.

In addition to direct antagonism, *Nigrospora*

spp. may contribute to plant defense through indirect mechanisms. Endophytic colonization enables these fungi to enhance host resistance by activating defense-related pathways, including those regulated by jasmonic acid (JA) and ethylene (ET) (Aprile et al., 2022; Pozo et al., 2009). Furthermore, the induction of defense-related enzymes such as catalase and peroxidase may further strengthen plant resistance against *F. solani* infection (Attia et al., 2022). As endophytes, *Nigrospora* spp. can colonize plant tissues without causing harm, thereby promoting plant growth and increasing resistance to pathogens (Dutta et al., 2023).

Overall, the findings of this study demonstrate that *Nigrospora* spp. possess diverse and species-specific antagonistic capabilities against *F. solani*, involving both direct (mycoparasitism and antibiosis) and indirect (host resistance induction) mechanisms. This multifaceted mode of action supports their potential application as environmentally friendly and sustainable alternatives to chemical fungicides in the management of Fusarium wilt.

CONCLUSION

The findings of this study demonstrate that the five *Nigrospora* spp. isolates exhibited varying levels of antagonistic activity against *F. solani*, the causal agent of *Fusarium* wilt in chili plants. These

differences indicate that the effectiveness of *Nigrospora* as a biocontrol agent is influenced by genetic and ecological variability among isolates. All isolates exhibited clear characteristics of mycoparasitism, including hyphal recognition, adhesion, penetration, coiling, and subsequent degradation of the pathogen's cell wall, highlighting their capacity to suppress fungal pathogens. Among the tested isolates, *N. rubii* and *N. oryzae* showed the highest potential as biocontrol agents, suggesting their suitability for sustainable disease management as environmentally friendly alternatives to chemical fungicides. However, further studies under greenhouse and field conditions are required to validate their consistency, efficacy, and practical applicability in agricultural systems.

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AUTHORS' CONTRIBUTIONS

SDN and AM conceptualized and designed the study. AM and SDN performed fungal isolation and antagonism assays, including the maintenance of *Nigrospora* spp. cultures. RD conducted the dual culture experiments. AM collected the antagonism data, while RD performed data analysis and interpretation. AM prepared the manuscript. All authors contributed to reviewing, editing, and approving the final version of the manuscript.

COMPETING INTEREST

The authors declare that they have no competing interests.

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