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Potential of Nematophagous Fungi from Kutalimbaru, North Sumatra, Indonesia, as Biological Control Agents to Manage Root Knot Nematodes, *Meloidogyne incognita*

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Abstract

Root-knot nematodes (RKN), especially *Meloidogyne incognita*, are one of the most damaging and widespread plant-parasitic nematodes (PPNs) in cultivated plants worldwide. The intensive application of chemical nematicides in controlling these pests raises environmental and ecological concerns, prompting the need for sustainable alternatives. This study aimed to isolate and evaluate the predatory potential of indigenous nematode-trapping fungi (NTF) from the rhizosphere soils of healthy tomato plants against *M. incognita* under in vitro conditions. Soil samples were processed using the soil sprinkle technique, and fungal isolates capable of forming traps were screened by exposing them to second-stage juvenile (J2) of *M. incognita*. This study isolated 20 nematophagous fungi that have antagonistic activity against *M. incognita* from the rhizosphere soil of tomato plants. Among these, four isolates were identified as nematode-trapping fungi, with TRK 04 showing the highest predation efficacy, followed by TRK 07, TRK 09, and TRK 16. All four isolates were identified as part of the Arthrobotrys genus. These findings highlight the potential of indigenous nematophagous fungi as sustainable biocontrol agents for managing root-knot nematodes. Their application could support the development of environmentally friendly agricultural practices and reduce reliance on chemical nematicides. Further, in vivo studies are recommended to validate their efficacy under field conditions.

Keywords: *Arthrobotrys thaumasia*, biocontrol, indigenous, nematode-trapping fungi, TRK isolate

Introduction

Plant-parasitic nematodes (PPNs) are major agricultural pests that cause significant yield losses globally, with estimated economic damage reaching up to USD 1500 billion annually, consisting of about 4300 species, which account for about 15% of the entire nematode family (Mesa-Valle et al., 2020; Wu et al., 2023). Among them, root-knot nematodes (RKN) from the *Meloidogyne* spp. are the most destructive, infecting over 5500 plant species and severely impairing root systems. *Meloidogyne incognita*, in particular, is a dominant species with a broad host range and global distribution, and they parasitize more than 5500 plant species, including a large number of cultivated plants (Ahmad et al., 2021; Eken et al., 2024). In

Indonesia, *Meloidogyne* spp. infections have been reported to reduce tomato yields by 20% to 40% (Damayanti et al., 2018), posing a serious threat to horticultural productivity.

Current management strategies for RKN heavily rely on synthetic pesticides, including insecticides with nematicidal activity (Winarto et al., 2019). While effective in the short term, such chemical applications lead to adverse environmental impacts, including accumulating toxic residues, developing pathogens resistance, and disrupting soil biodiversity (Xiao et al., 2016; Hahn et al., 2019). These concerns underscore the urgent need for alternative, sustainable, and eco-friendly solutions to mitigate nematode infestations in crop systems.

Nematophagous fungi (NF) are natural antagonists of nematodes and have gained attention as promising biological control agents. These fungi can directly attack, immobilize, kill, or repel nematodes. Moreover, they can also interfere with nematode host-seeking behavior, outcompete them for essential resources, or exert a combination of these antagonistic effects (Schouten, 2016). According to Li et al. (2015), more than 700 species of nematophagous fungi have been described. These fungi belong to diverse phylogenetic groups, including Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota. Several potentially useful biological control agents of root-knot nematodes (*Meloidogyne* spp.) have also been successfully isolated, including species of Paecilomyces, Pochonia, Trichoderma, Fusarium, Arthrobotrys, Monacrosporium, Aspergillus, and Penicillium (Aminuzzaman et al., 2018).

Among NF, nematode-trapping fungi (NTF) from the *Arthrobotrys* genus are widely reported as effective biocontrol agents. Approximately 100 NTF, including species from Drechslerella, Dactylellina, and Arthrobotrys, have been morphologically described and illustrated (Li et al., 2015). Bioassays of several NTF species, especially Arthrobotrys, against nematodes showed positive results. Research by Monteiro et al. (2019) showed that adding *Arthrobotrys flagrans* per gram of soil can reduce the number of *M. javanica* juveniles by 73%. Other information was also reported by Purba et al. (2022); the results showed that *A. thaumasia* and *A. musiformis* were highly effective in suppressing *M. hapla* populations on tomato plants, achieving reductions of 93% and 97%, respectively. However, the biocontrol potential of local NTF strains—especially those adapted to the rhizosphere soils of healthy tomato plants in Kutalimbaru, North Sumatra, Indonesia (3°24’34.61”N, 98°30’32.07”E)—remains under-explored.

This study aimed to explore and isolate potential indigenous NTF from the rhizosphere of healthy tomato plants in Kutalimbaru District, North Sumatra, Indonesia, and evaluate their in vitro antagonistic activity against *M. incognita*. Identifying and screening effective isolates can contribute to developing sustainable biocontrol solutions and reduce dependence on synthetic nematicides in Indonesian agriculture.

Methods

This research was conducted from October 2024 to February 2025 at the Integrated Research Laboratory, Faculty of Medicine, Universitas Sumatera Utara. Soil samples were collected from a tomato cultivation field located in Kutalimbaru Subdistrict, Deli Serdang Regency, North Sumatera, Indonesia (3°24’34.61”N, 98°30’32.07”E).

Soil Sample Collection

Soil samples were collected following the method described by Duddington (1955) by establishing three plots measuring 50 × 50 cm, each spaced at least 10 meters apart. From each plot, 100 grams of soil were taken at 10–20 cm depth using a sterilized shovel. The collected samples were then thoroughly mixed. Subsequently, 100 grams of the composite soil sample were wrapped in aluminum foil, placed in

a labeled sterile plastic container, and stored at 4 °C for five days before use.

Nematode Extraction

The roots of tomato plants that form root knots due to nematode infection were obtained from the Institut Pertanian Bogor Experimental Garden. Eggs were collected following the procedure described by Hussey and Barker (1973); infected roots were carefully removed from the soil, thoroughly washed with tap water, and cut into segments approximately 1–2 cm in length. The root pieces are then soaked in a solution of 0.05% NaOCl and poured through a set of sieves with mesh sizes of 75 µm and 25 µm. The eggs were retained on the 25 µm sieve and thoroughly rinsed with a gentle stream of tap water for 2–3 minutes to eliminate NaOCl residue. The rinsed eggs were transferred into a clean beaker containing sterile distilled water and incubated at room temperature (25 °C) to obtain *M. incognita* second-stage juveniles (J2). The number of hatched J2 was quantified using a counting chamber, and the juveniles were subsequently used for the following experimental procedures.

Identification of *M. incognita* was based on the morphology of the perineal pattern of the adult female (Taylor and Sasser, 1978). Adult females of *M. incognita* were obtained by dissecting infected tomato roots at galling sites. The neck region of the female nematode was incised, and the body was pressed to release internal contents. The remaining cuticle was treated with 45% lactic acid to remove tissue residues. The posterior region containing the perineal pattern was mounted on a slide, oriented upward, and observed under a compound microscope.

Isolation and Screening of Nematophagous Fungi

Nematophagous fungi were isolated using the soil sprinkle technique and purified using the single spore isolation method. One gram of soil sample was sprinkled into CHF-WA medium and incubated in dark conditions at 22°C–25°C for three days until fungal mycelia had fully grown across the petri dish. The petri dishes containing soil isolates colonized by fungi were then inoculated with approximately 100 second-stage juveniles (J2) of *M. incognita*, which were previously hatched from extracted eggs. The hatched J2s were counted using a counting dish under a stereomicroscope, and 100 individuals were suspended in 200 µL of sterile distilled water and gently dispensed onto the agar surface using a micropipette under aseptic conditions. The plates were incubated again in the dark until dead nematodes were observed. If dead nematodes were found around the fungal mycelia, the fungi surrounding the dead nematodes were then subcultured into a PDA (Potato Dextrose Agar) medium to obtain pure fungal cultures. The pure fungal cultures were then re-cultivated on CHF-WA medium and inoculated with *M. incognita* to observe the presence or absence of mycelial traps formed (trapping structures) under a microscope (Berhanu et al., 2022; Tarigan et al., 2020).

Assessing the In Vitro Predatory Activity of NTF Isolates

The in vitro test of NTF was conducted based on the experimental method (Hastuti et al., 2023) with modifications. Fungi with mycelial traps were selected for in vitro testing and cultured on Corn Meal Agar (CMA) medium, then incubated at 25°C for 3 days or until the fungal mycelia entirely covered the petri dish. The petri dishes containing the fungal mycelia were then inoculated with a suspension of approximately 1000 second-stage juveniles (J2) of *M. incognita*, prepared in sterile distilled water, and applied aseptically. The dishes were kept in a dark room, and nematode populations were observed and counted at 12, 24, 36, 48, and 72 hours after inoculation.

Variables Observed

Morphological characteristics of nemato-phagous fungi

Morphological characteristics of nematophagous fungi obtained from tomato rhizosphere soil, observed macroscopically (colony shape, texture, and color) and microscopically (hyphae, conidia, and conidiophores). Morphological identification was conducted by using a light microscope with magnificent 400× and 100×, and potential fungal isolates to species level was done using Swe et al. (2008), Yu et al. (2014), Winarto et al. (2019).

In vitro activity against *M. incognita*

Population of nematode-trapping structures. The existance of trapping structures (e.g., three-dimensional adhesive networks) were examined under inverted microscope at 40× and 100× magnification. To monitor the temporal dynamics of *M. incognita* populations, the number of active (motile) and immobilized (paralyzed or dead) J2 was recorded at predetermined time intervals—12, 24, 36, 48, and 72 hours after inoculation.

Mortality of *M. incognita*. The mortality of *M. incognita* was calculated by comparing the number of dead juveniles to the total inoculated population, expressed as a percentage. Generally, *M. incognita* mortality calculated using the formula (Ali *et al*., 2024):

$$Mortality\ (\%) = \frac{Number\ of\ dead\ juveniles}{Total\ number\ of\ juveniles} \times 100\% \quad .....(1)$$

Data Analysis

Data regarding morphological characteristics is presented descriptively in tables. The population and mortality of *M. incognita* were subjected to Analysis of Variance (ANOVA) to determine whether significant differences existed among treatments. A Tukey's test ($P < 0.05$) was performed as a post-hoc analysis. Graphical representations of population trends and nematode mortality over time were constructed using GraphPad Prism version 10.

Result

Morphological Characteristics of Nematophagous Fungi

A total of 20 nematophagous fungal isolates that have antagonistic activity against *M. incognita* were obtained from soil samples collected from tomato plantations in Kutalimbaru District, North Sumatra, Indonesia. Among the 20 isolates, only TRK 04, TRK 07, TRK 09, and TRK 16 showed the formation of typical trapping structures, along with conidia and immobilized nematodes, indicating that they belong to Nematode Trapping Fungi (NTF). The remaining 16 isolates did not form visible traps, and their antagonistic mechanisms against nematodes remain to be further investigated (Table 1).

Table 1. Characterization of Nematophagous Fungi

Isolates	Colony Character				Trapping Device
	Form	Texture	Color	Surface	
TRK 01	Filamentous	Cottony	Green	Raised	-
TRK 02	Filamentous	Cottony	Yellowish White	Umbonate	-
TRK 03	Round with Radiating Margin	Velvety	Purplish Red	Flat	-
TRK 04	Round with Raised Margin	Cottony	White	Raised	+
TRK 05	Filamentous	Velvety	Grayish Green	Raised	-
TRK 06	Filamentous	Glanular	White	Raised	-
TRK 07	Filamentous	Cottony	White	Raised	+
TRK 08	Filamentous	Velvety	Yellowish White	Flat	-
TRK 09	Filamentous	Cottony	White	Raised	+
TRK 10	Filamentous	Cottony	White	Umbonate	-
TRK 11	Concentric	Glanular	Green	Raised	-
TRK 12	Filamentous	Cottony	White	Raised	-

Isolates	Colony Character				Trapping Device
	Form	Texture	Color	Surface	
TRK 13	Filamentous	Velvety	Grayish Yellow	Raised	-
TRK 14	Round with Radiating Margin	Cottony	Green	Raised	-
TRK 15	Filamentous	Cottony	Brownish White	Raised	-
TRK 16	Filamentous	Cottony	White	Raised	+
TRK 17	Filamentous	Cottony	White	Flat	-
TRK 18	Round with Radiating Margin	Cottony	Whitish Green	Raised	-
TRK 19	Filamentous	Glanular	Greenish Yellow	Raised	-
TRK 20	Filamentous	Glanular	Yellow	Raised	-

Note: TRK = Tomato Rhizosphere Kutalimbaru

The macroscopic observation showed that there were morphological characteristics in the shape of the colony (filamentous and round with a raised margin). Meanwhile, the colony color was predominantly white, and the mycelial growth appeared cottony in texture (Figure 1). Other isolates did not form trapping structures, which may indicate that they were not NTF but could control nematodes through different mechanisms (Figure 2).

The colony of isolate TRK 04 at 25°C on PDA after seven days had a 6 cm diameter, with a slightly brownish white color on the upper and lower surfaces, hyaline hyphae, septate, branched, and ellipsoidal conidia, forming a three-dimensional adhesive trap. These data were morphologically similar to the fungus *Arthrobotrys thaumasia* Schenck, Kendr, and Pramer (Ascomycota: Orbiliaceae) described by Swe et al. (2008); Yu et al. (2014).

The colony of isolate TRK 07 at 25°C on PDA after seven days had a diameter of 8 cm, whitish. Mycelium hyaline, branched and septate. Conidiophore erect, hyaline. Conidia hyaline, ellipsoidal or obovoidal, 1-3-septate. Capturing nematodes by means of three-dimensional adhesive networks suggests that TRK 07 is identified as *Arthrobotrys sinensis* Scholler, Hagedorn, and Rubner (Ascomycota: Orbiliaceae) based on the same species described by Yu et al. (2014).

TRK 09 formed whitish, rapidly growing on PDA. Mycelium hyaline, septate, and branched. The conidiophores were hyaline, 2–8-septate, erect, and simple. Conidia were globose to turbinate or broadly spindle-shaped, rounded at the distal end, and truncated at the base. Capturing nematodes by means of three-dimensional adhesive networks. These suggest that TRK 09 was identified as *Arthrobotrys eudermata* Scholler, Hagedorn, and Rubner (Ascomycota: Orbiliaceae) based on the species Yu et al. (2014) described.

TRK 16 showed colony characteristics similar to *Arthrobotrys musiformis* (Ascomycota: Orbiliaceae) based on species described by Swe et al. (2008) and Yu et al. (2014). Characterized by white colonies on both the upper and lower sides of the media. Mycelium was spreading, hyaline, septate, and branched. Conidiophores are hyaline, septate, erect, and branched. Conidia were obovoid or ellipsoid in shape, with 3-9 conidia on each conidiophore.

In Vitro Activity Against *M. incognita*

Four potential NTF isolates (TRK 04, TRK 07, TRK 09, and TRK 16) demonstrated the ability to reduce the number of second-stage juveniles (J2) of *M. incognita* after in vitro incubation. Among them, TRK 04 showed the highest efficacy, achieving the greatest reduction in J2 numbers after 72 hours of incubation. This was followed by TRK 07, TRK 09, and TRK 16, while the control

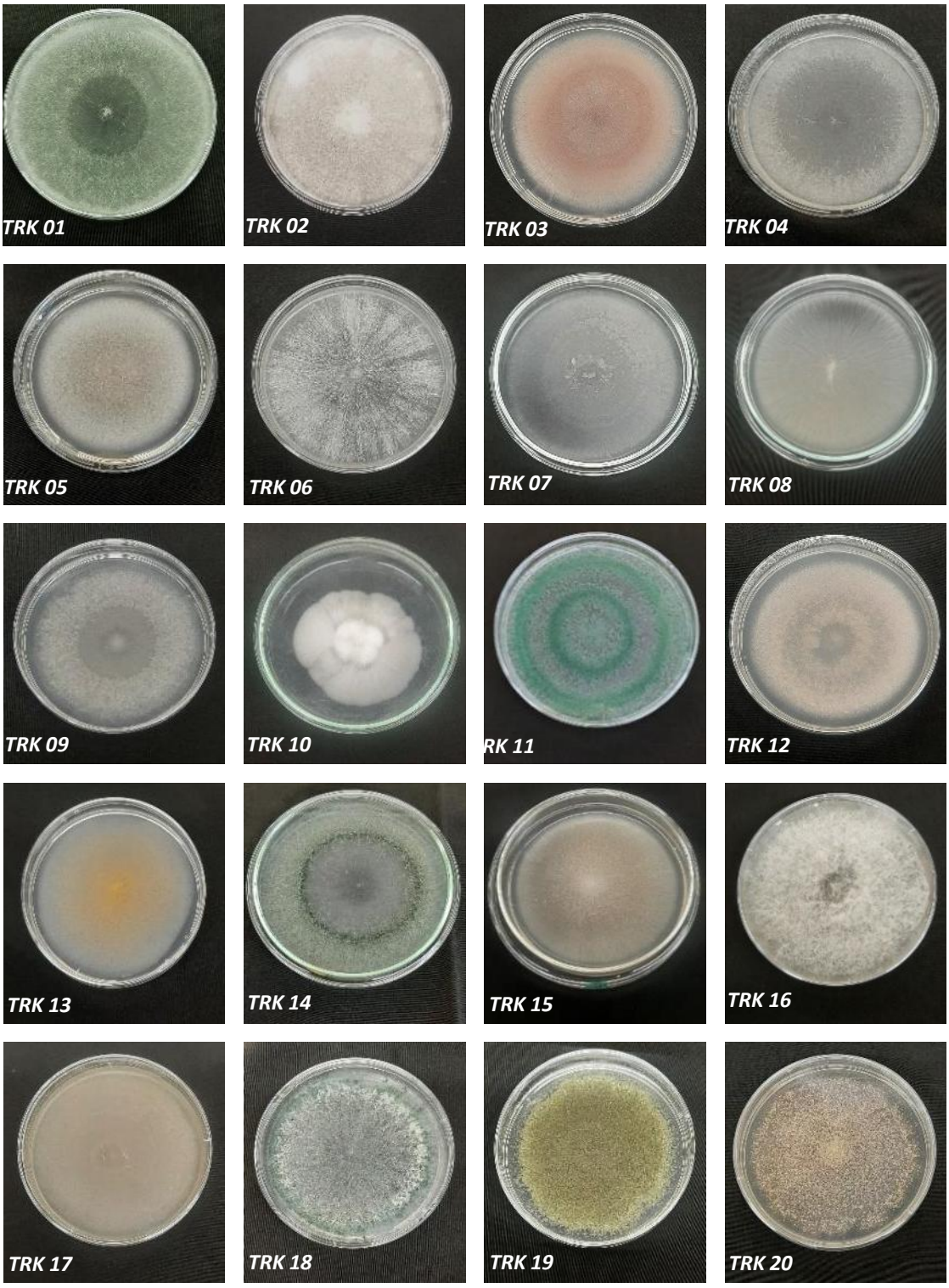

Figure 1. Colonies of nematophagous fungi grown on PDA medium

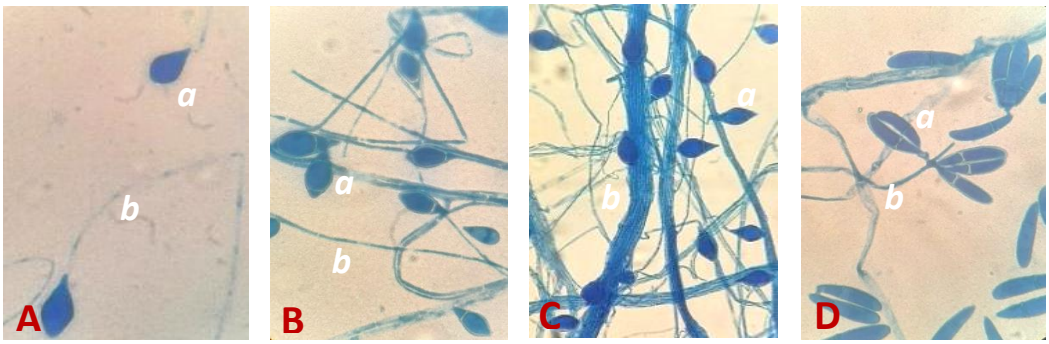

Figure 2. Microscopical feature of potential nematode trapping fungi isolates: (A) TRK 04; (B) TRK 07; (C) TRK 09; and (D) TRK 16 (a. conidia; b. conidiophore) (Magnification: 400x)

treatment exhibited no significant reduction (Figure 3). TRK 04 has the highest effectiveness, with the greatest decrease in the number of J2 at 72 hours, followed by TRK 07, TRK 09, and TRK 16 which also show a reduction effect, although with lower levels of efficacy. Meanwhile, the control showed no significant change.

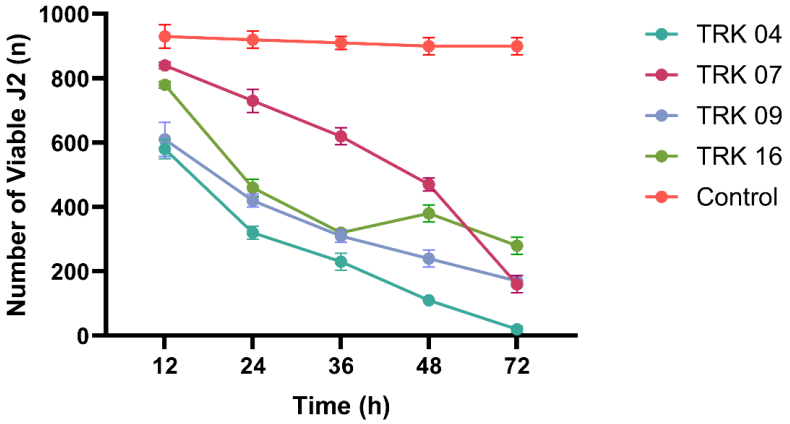

Figure 3. *Meloidogyne incognita* population (n) under co-incubation with Nematode Trapping Fungi (NTF) Isolates at different time observations

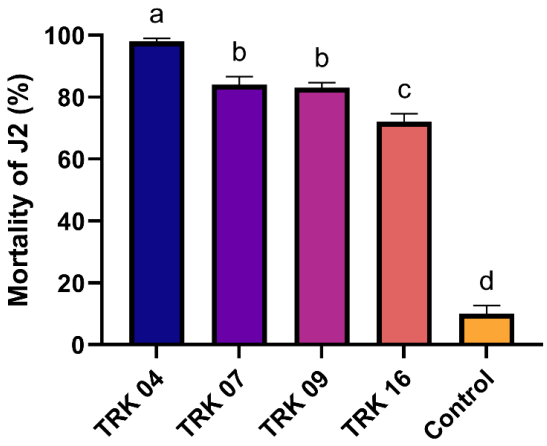

Figure 4. Effect of Nematodes-Trapping Fungi (NTF) Isolates on J2 Mortality of *Meloidogyne Incognita* After 72-h Incubation

The capture and paralysis of nematodes by NTF increased progressively with each additional day of incubation. The graph of the percentage mortality of second-stage juvenile (J2) *M. incognita* after 72 hours of treatment with various isolates (TRK 04, TRK 07, TRK 09, TRK 16) compared to the control is shown in Figure 4. After 72 hours of incubation, isolate TRK 04 showed the highest *M. incognita* mortality rate (98%), followed by TRK 07 (84%), TRK 09 (83%), and TRK 16 (72%). The activity of the Nematode-Trapping Fungi is shown in Figure 5.

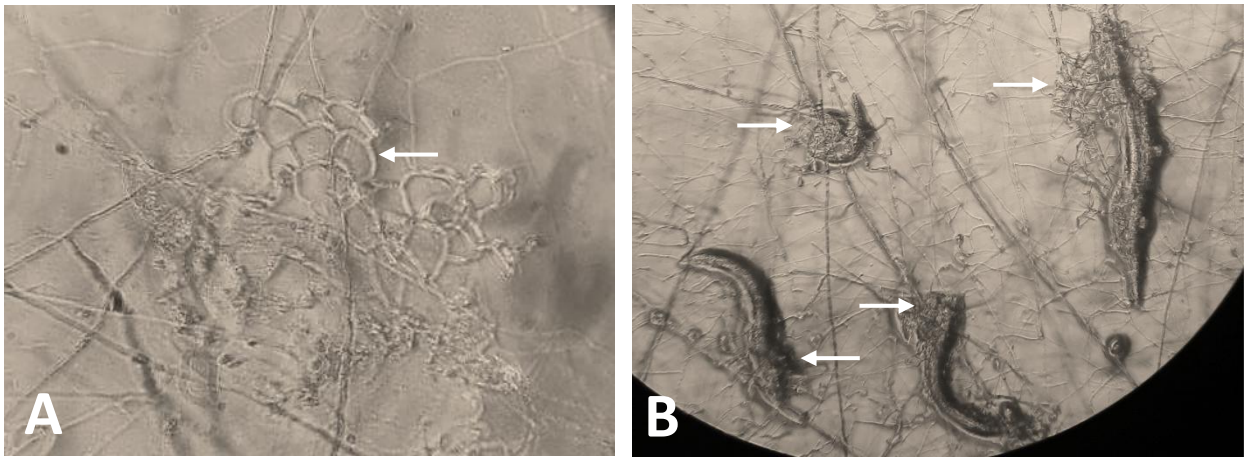

Figure 5. Predatory activity of Nematode Trapping Fungi (TRK 04) on CHF-WA; (A) Trapping device (arrow) (B) Predatory activity against *M. incognita* (arrrow) (Magnification: 100x)

Discussion

Plant-parasitic nematodes are among the most damaging pests in vegetable production systems, with *Meloidogyne* spp. representing the most destructive genus (Gowda et al., 2017). After infecting the roots, *Meloidogyne* spp. triggers the infected cells to multiply, forming root galls, a characteristic of root-knot nematodes (RKN) (Soliman et al., 2021). Because NTFs are natural enemies of nematodes, they represent promising candidates for nematode control (Sun et al., 2024). Therefore, applying NTF is important as an environmentally friendly and cost-effective alternative to controlling RKN (Wernet and Fischer, 2023). According to Hiep et al. (2019), nematophagous fungi consist of more than 200 fungal species with a wide taxonomic diversity, all of which can attack live nematodes (juveniles, adults, and eggs) and utilize them as a source of nutrition. This group includes nematode-trapping fungi, egg-parasitic fungi that can survive saprophytically in the soil, and endoparasites (obligate parasites). Numerous studies have investigated the effectiveness of various types of nematophagous fungi against root-knot nematodes and found that these fungi play an important role in the rhizosphere of agricultural plants.

In vitro screening of nematophagous fungi is an important method to evaluate their potential in controlling plant-parasitic nematodes before being utilized as biological control agents. When observing isolates capable of forming traps on CHF-WA medium under a microscope, the nematodes that came into contact with the traps adhered to the trap bundles and could not move freely (Figure 5). According to Wu et al. (2023), these traps release chemical compounds that induce a comatose state in the nematodes and suppress their motility while enzymes degrade the nematode body walls. The fungal mycelia then invaded and

proliferated inside the nematode's body until they were completely decomposed. This is in line with Wang et al. (2023), who stated that NTFs are capable of detecting nematodes in their environment and utilizing specialized hyphal structures to capture and digest them. While infecting nematodes, NTF produces various extracellular enzymes, including collagenase, serine protease, and chitinase. These enzymes disrupt the nematode's epidermal structure, degrade its protein components, and facilitate the fungus penetration and subsequent colonization of the nematode.

The experimental results demonstrated the biocontrol potential of four NTF isolates against *M. incognita.* During the observation period, the effectiveness of nematode predation increased progressively. In line with the findings of Doolotkeldieva et al. (2021), the predatory fungi began exhibiting active trapping activity within 24 hours when sufficient nematodes were present, and most nematodes were captured within 48 hours. However, the predation efficiency differed among the isolates tested. Isolate TRK 04 exhibited the highest predation activity, followed by TRK 07, TRK 09, and TRK 16. This may be due to differences in the attractiveness between the JPN strains and *M. incognita.* For example, certain fungal strains may not produce sufficient adhesive traps in response to chemical compounds released by nematodes, thereby reducing the probability of nematode entrapment. Based on this factor, it is suspected that the frequency of contact between the TRK 04 isolate and *M. Incognita* was higher than the other three isolates, which increased the likelihood or rate of hyphal specialization into predatory rings, making predation efficiency against *M. incognita* much higher at the same timeframe.

The morphological characteristics observed under macroscopic and microscopic examination confirmed that the four isolates belonged to the genus *Arthrobotrys*. This identification aligns with Zhang et al. (2022), highlighting that Arthrobotrys represent the largest and most complex genus of nematode-trapping fungi within the family Orbiliaceae. Due to their unique survival strategies in capturing nematodes, members of the Arthrobotrys are widely distributed in diverse habitats worldwide. These fungi are predominantly found in soil or sediment from various ecosystems, including agricultural land, forests, mangroves, and freshwater environments. Most Arthrobotrys species exhibit strong saprophytic capabilities and high reproductive potential, enabling rapid colonization of soil environments and making them excellent candidates for developing biocontrol agents against parasitic nematodes. Additionally, due to the high species diversity and distinct morphological characteristics of their conidia and conidiophores, this group also serves as a valuable model for evolutionary studies of nematode-trapping fungi within the genus (Zhang and Hyde, 2014).

In this study, the most potential isolate with the highest predatory ability, TRK 04, identified as *Arthrobotrys thaumasia* based on macroscopic and microscopic observations, exhibits strong potential as a biocontrol agent due to its ability to suppress *Meloidogyne incognita* by 98% after 72 hours of incubation. The nematophagous potential of *A. thaumasia* isolates reported in this study aligns with the findings from previous research regarding the reduction in the number of *M. incognita* infective juveniles. For example, Kassam et al. (2021) reported that *A. thaumasia* was able to reduce the number of *M. incognita* infective juveniles by 82%, while Eken et al. (2024) reported an in vitro reduction exceeding 70% in J2 of *M. incognita* by *A. thaumasia*. Furthermore, in another in vitro study, *A. thaumasia* was reported to reduce *M. hapla* infective juveniles by 89.27% (Hastuti et al., 2023). Another study also revealed that applying *A. thaumasia* as a fungal suspension to tomato plants decreased nematode populations

by 93% and enhanced plant growth (Purba et al., 2022). To further validate these results, additional in vivo studies are needed to investigate the potential and effectiveness of our native isolates in the biological control of root-knot nematodes.

Conclusion

This study isolated 20 nematophagous fungi that have antagonistic activity against *M. incognita* from the rhizosphere soil of tomato plants. Among these, four isolates were identified as nematode-trapping fungi, with TRK 04 showing the highest predation efficacy, followed by TRK 07, TRK 09, and TRK 16. All four isolates were identified as part of the Arthrobotrys genus. These findings highlight the potential of indigenous nematophagous fungi as sustainable biocontrol agents for managing root-knot nematodes. Their application could support the development of environmentally friendly agricultural practices and reduce reliance on chemical nematicides. Further, in vivo studies are recommended to validate their efficacy under field conditions.

Declaration

Author contribution

Wira Risa Lina Simanjuntak contributed to the conceptualization, methodology, investigation, data curation, formal analysis, and writing – original draft. Liana Dwi Sri Hastuti is the corresponding author, and Yurnaliza is the co-author; they are responsible for supervision, resources, validation, and writing – review, and editing. All authors have read and approved the final version of the manuscript.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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