Biological control of Root-knot nematodes by the fungus *Fusarium solani*

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Abstract. Plant-parasitic nematodes belonging to the genus *Meloidogyne* are known as root-knot nematodes. In regions with hot weather or brief winters, they are found in the soil. This nematode can be infected by microbial parasites like fungi. Therefore, this study was performed to identify the fungal parasite that infect nematode *Meloidogyne* spp. *Fusarium solani* has been isolated from nematode in the rhizosphere of a Okra plant infected with root-knot nematodes. The morphological and molecular identifications appeared to be identical by 100%. The pathogenicity test indicated that *F. solani* did not infect the radish seeds. 96% of the seeds germinated in the distilled water. However, *F. solani* was responsible for 93% of the seeds germinating. After 24 hours, the hatchability rate was 18.52%; after 72 hours, it was 28.6%. At the 25% concentration, the hatchability was 18.26%; at the 100% concentration, it was 28.39%. The mortality% on the larvae of root-knot nematodes between the three concentrations, 25%, 50%, and 100%, were 42.81, 56.49, and 64.65%, respectively. Additionally, the mortality% of larvae has also been significantly impacted by time. The difference between 72 and 24 hrs and 58.32 and 51.09 was statistically quite significant, respectively. While the interaction effect was not significant.

Key words: Eggs hatchability. *Fusarium solani*, larval mortality, *Meloidogyne* spp., Molecular identification

1. Introduction

Nematodes are invertebrate creatures of living things. Their morphologies elongate, thread like, or circular, or eelworms, and they can be either free-living or parasitic (endoparasites and exoparasites of other species). Nematodes are among the most successful and versatile animal groups, second only to insects, in terms of species or ecological range [1]. There are many fungi are harmful to some nematode species, their interaction with soil worms goes beyond parasitism and into the realm of predation. Approximately 200 taxonomically diverse fungal species are capable of attacking active nematodes, which are useful creatures that range in length from 0.1 to 1.0 mm [2]. These fungi were divided into three major categories by [3]: endophytic fungi, saprophagous fungi, and nematophagous fungi. However, some fungal species, such as *Arthrobotrys oligospora*, are able to consume nematodes and their eggs. These fungi are known as nematophagous fungi [4]. Therefore, nematophagous fungus use a variety of strategies to target nematodes, such as predatory fungi like *Drechslerella* spp. and

Arthrobotrys oligospora, which create extensive hyphal networks and constricting rings to capture nematodes. Endoparasitic fungus, like *Drechmeria coniospora*, are nematode parasites that adhere directly to nematodes in order to kill them. Sedentary nematode stages, including worm eggs, cysts, and adult females, are targeted by facultative parasitic fungus such *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, and *Pochonia rubescens* [5, 6]. Our investigation therefore sought to isolate and identify the fungus responsible for the nematode-mortality state root-knot nematodes under Basra- Iraq conditions.

2. Materials and Methods

2.1. Isolation of nematode-parasitic fungi

These experiments were conducted in the laboratories of the College of Agriculture, University of Basra. The soil samples were collected from the rhizosphere of infected plants Okra by the root-knot nematode *Meloidogyne spp*. This area is considered a rich environment in pathogenic fungi and predators of nematodes. Then, the nematode-parasitic fungi were isolated from the infected nematode fungi [7] **Figure 1**. The infected nematodes were extracted from the soil using flotation and filtration methods [8], and then cultured on Potato Dextrose Agar (PDA) for the growth of parasitic fungi [9]. After 3 days of incubation at 25°C, fungal colonies appeared [10].

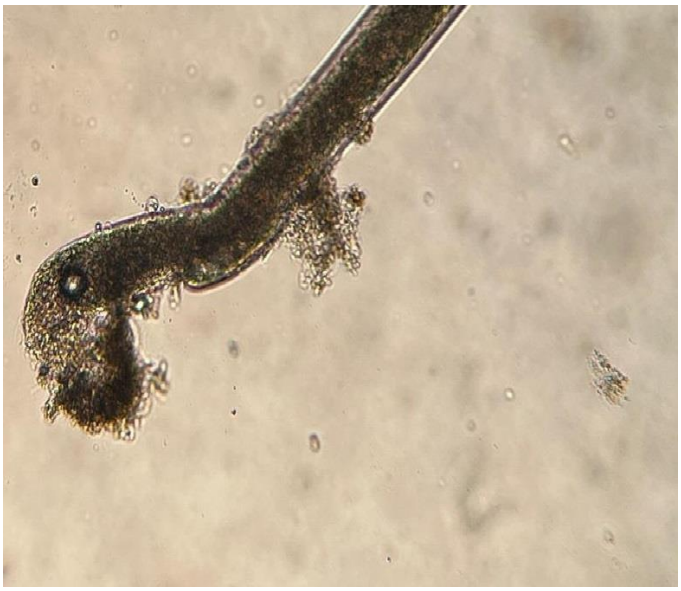

Figure 1: nematode infected with fungus

2.2. Fungal Identification

2.2.1. Morphological identification

The morphological identification has been accomplished after 7-day fungal growth in the plate by Dr. Yehya A. Salih. This identification depends on the featured characteristic of fungi [11].

2.2.2. Molecular identification

The molecular identification has been achieved using the Polymerase Chain Reaction (PCR), targeting Internal Transcribed Spacer (ITS) [12, 13]. The gene was excreted and amplified according to [12]. The amplified gene was compared to the genes that are already excised and internationally registered in the National Center for Biotechnology Information (NCBI); in addition, the evolutionary analyses (phylogenic tree) were conducted in MEGA7 [14].

2.2.3. Inoculate fungal preparation

The fungal filtrate was prepared by culturing isolated fungi in a liquid nutrient medium (Potato Dextrose Broth (PDB)) under sterile conditions. The medium was added to a sterile glass beaker and maintained in an incubator at an appropriate temperature (usually 25 ± 2 °C) with continuous stirring or shaking to ensure proper fungal growth [15]. After an incubation period of 5 to 7 days, the fungi were separated from the liquid medium using sterile filtration to obtain a filtrate free of fungal cells.

2.2.4. In vitro evaluation of fungal impact on seed germination

This test was conducted to show the effect of the fungus isolated from the nematode on the plant. The Radish (*Raphanus sativus*) seeds were surface-sterilized using a 1%–2% sodium hypochlorite solution and then rinsed with sterile distilled water. Radish seeds were chosen because they grow quickly in the laboratory and are easy to obtain and handle. The prepared Potato Dextrose Agar (PDA) medium was put into sterile Petri dishes. The plates were inoculated with the investigated fungal isolate, while uninoculated plates served as controls. Each treatment was administered three times. All plates were incubated for five to seven days at 25 to 28 °C after the sterilized radish seeds were placed on the PDA surface. The rate of germination was monitored daily throughout the incubation period [16].

2.2.5. Preparation of Root-Knot Nematode Eggs and larvae

The eggs of the root-knot nematode *Meloidogyne spp*. were prepared from the infected Okra plants with their entire root systems. The roots were then thoroughly washed to remove any adhering soil. The roots are then cut into small pieces and treated with a dilute 0.5% to 1% sodium hypochlorite (NaOCl) solution for 3–4 minutes to facilitate the separation of the eggs from the plant tissue [8] . A sequential sieve through two fine sieves (usually 250 μm and then 25–30 μm) is used to separate the eggs from the wash solution. After that, the eggs are collected in a clean container, and the eggs can be incubated under suitable conditions (temperature 25-28°C) to obtain second-stage larvae (J2) suitable for use in laboratory experiments [18].

2.2.6. Effects of a fungal filtrate on the eggs and larvae of root-knot nematodes

The effect of fungal inoculate on the eggs and larvae of root-knot nematodes *Meloidogyne* spp. was evaluated in the laboratory using three different inoculate concentrations: 25%, 50%, and 100%. Three duplicates of each concentration of the fungal inoculum were administered to 1 milliliter of each egg and larva in order to ensure the accuracy of the data [17].

3. Results and Discussion

3.1. Isolation and morphological identification

The *F. solani* is phenotypically characterised by its cotton colonies on the PDA medium, which range in colour from white to grey. It is also characterised by the production of large, multi-barrier crescent conidia (3–5 barriers), and small, single-celled ones, in addition to solid bodies (chlamydospores) that form inside or at the ends of the hyphae, and are a distinctive feature of the fungus [11].

3.2 Molecular identification of fungus species

According to the molecular identification, the Table 1 describes how BLAST was used to compare the sequence similarities between the strain PV840062.1 and other isolates in the NCBI GenBank database. With significant genetic similarity (≥99.8%) to isolates from several geographic locations, the BLAST analysis strongly suggested that the fungal isolate PV840062.1 is *Fusarium solani*. The PV840062.1 isolate has 100% query coverage, which

indicates that every strand of the sequence corresponds to the GenBank entries. With the exception of one sequence from the Netherlands (MH865998.1), which displays 99.80% identity, indicating a nearly perfect match, the majority of matches display 100% sequence identity. As a result, these sequences come from a variety of nations, such as China, Spain, Iraq, and the Netherlands, demonstrating the widespread distribution of *Fusarium solani* and the high level of conservation of the identifying gene.

Table 1: Synopsis of all similarities between the Basra isolate and the NCBI- BLAST results

Fungal species	GenBank accession number*	Query coverage	Percent of sequence identity	Country
Fusarium solani	PV840062.1	100	100	Iraq
Fusarium solani	KY484924.1	100	100	Spain
Fusarium solani	OK323244.1	100	100	China
Fusarium solani	LN828134.1	100	100	Spain
Fusarium solani	MH865998.1	100	99.80	Netherlands
Fusarium solani	LN828115.1	100	100	Spain

*In NCBI: https://www.ncbi.nlm.nih.gov/genbank

While the Figure 2 revealed that the evolutionary history was inferred using the Neighbor-Joining method. With a branch length sum of 0.00727276, the ideal tree is shown. The Poisson correction method was used to calculate the evolutionary distances, which are expressed in terms of the number of amino acid substitutions per site. Analysis was done on six amino acid sequences. The coding data was translated under the assumption of a typical genetic code table. All positions that had gaps or insufficient information were eliminated. A total of 138 locations were included in the final dataset.

[19] detected *F. solani* f. sp. *glycines* using primers EF-728F/EF-986R and NMS1/NMS2. Additionally, they attested that the use of those two distinct subspecies facilitates identification and encourages us to develop additional primer designs in order to identify the subspecies isolate.

Figure 2: Evolutionary relationships of fungal isolates.

3.3 Evaluation *Fusarium solani* in vitro

As seen in Figure 3, there was no discernible difference in the radish seed germination between the fungal filtrate and distilled water control groups ($p > 0.05$). The seed germination was registered at 96% in the distilled water compared to the fungal filtrate, which scored 93%. Our findings demonstrates that the fungus, or the present strain isolated from nematodes, did not produce any pathogenicity in radish seeds in the laboratory, despite the fact that *F. solani* is recognized to be a plant and seed pathogen. Design a new primer, perhaps, and we might be able to identify the subspecies. According to a study by [20], *F. graminearum* had the highest rate of reduction, achieving 50% in laboratory seed germination.

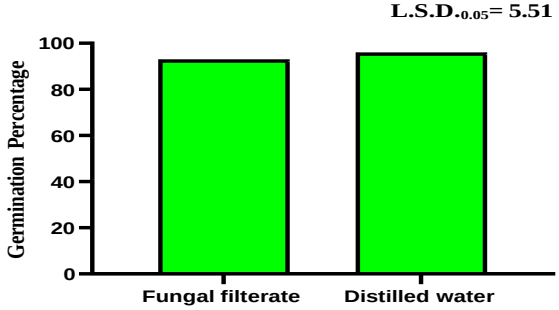

Figure 3: Effect of *Fusarium solani* filtrate on the radish seed germination %

3.4 Effect of *Fusarium solani* filtrate on the eggs hatching

Table 2 illustrates the effect of filtrate concentration. The mean hatching % sharply decreased as the *F. solani* filtrate concentration rose. The differences between the three concentrations were statistically significant, suggesting that raising the filtrate concentration significantly reduced egg hatching.

Nonetheless, we can see how the hatching% is impacted by exposure duration. The average inhibition of hatching increased over time. It was discovered that the inhibition of egg hatching increased significantly between 24 and 72 hours, as well as between 48 and 72 hours, but not between 24 and 48 hours.

However, several treatment combinations showed greater differences, indicating that concentration and exposure duration have significant interacting effects. As a result, the inhibition of egg hatching was significantly boosted by both longer exposure times and higher concentrations.

Our results confirm that the filtrates of *F. solani* were toxic to the root-knot nematode. This agrees with the study of [21, 22]. Whom showed that the mushroom is poisonous as well to juveniles of *M. incognita*, inhibiting hatching and/or suppressing eggs. The production of poisonous filtrates by *F. solani* is the cause [23].

Table 2. Inhibitory Effect of *Fusarium solani* filtrates on the hatching % of Root-Knot nematode eggs

Time (hours)	25%	50%	100%	Mean of time effect
24	13.56	18.37	23.63	18.52
48	17.62	21.78	27.84	22.41
72	23.62	28.64	33.71	28.65
Mean of concentration effect	18.26	22.93	28.39	23.19

LSD at 0.01: For concentration = **3.38,** for time = **4.01,** for interaction (time × concentration) = **6.84.**

3.5 Effect of the fungal filtrate on the larval mortality%

Table 3 shows the data at three exposure intervals: 24, 48, and 72 hours; the mortality% of larvae root-knot nematodes *Meloidogyne* spp. was assessed in relation to varying concentrations (25%, 50%, and 100%) of *F. solani* filtrate.

The results clearly demonstrate that there is a considerable increase in larvae mortality with increasing filtrate concentration and exposure period. The differences in mortality% between the three concentrations, 25%, 50%, and 100%, at all exposure times were clearly statistically significant, indicating the concentration influence. At 100% concentration, the highest mortality was consistently recorded, followed by 50% and then 25%.

But the mortality% has also been significantly impacted by time. The difference between 72 and 24 hrs, 58.32 and 51.09, was statistically significant, respectively.

The difference between 48 and 24 hrs was 3.45, and between 72 and 48 hrs, it scored 3.78; however, it was not statistically significant. Similarly, the interaction effect, or the relationship between time and concentration, also showed both significant and non-significant changes. The mortality difference between 100% concentration at 72 hrs (68.42%) and 25% at 24 hrs (38.73%) was 29.69%, indicating a substantial interaction effect. As long as *F. solani* is able to degrade lignocelluloses [24, 25]. It might be capable of root-knot nematode parasitism.

Table 3. Effect of the fungal filtrate of *Fusarium solani* on the mortality % of root-knot nematode larvae.

Time (hours)	25%	50%	100%	Mean of time effect
24	38.73	53.83	60.72	51.09
48	42.88	55.92	64.82	54.54
72	46.82	59.72	68.42	58.32
Mean of concentration effect	42.81	56.49	64.65	54.65

LSD at 0.01: For concentration = **4.19,** for time = **4.37,** for interaction (time × concentration) = **7.84.**

Conclusion

The purpose of this study was to evaluate the parasitic potential of *F. solani*, which was isolated from the root-knot nematode. The results of this The results of this study demonstrate that *F. solani* can affect both larvae and egg hatchability of *Meloidogyne* spp.

References

[1] Pokharel, R. R., Larsen, H., Hammon, B., Gourd, T., & Bartolo, M. (2009). Plant parasitic nematodes, soil and root health in Colorado onion fields. *Western Colorado Research Center, Colorado State University. Annual report. Ft. Collins: Colorado State University*. 39-44.

[2] Karakaş, M. (2020). Nematode-destroying fungi: Infection structures, interaction mechanisms and biocontrol. *Communications Faculty of Sciences University of Ankara Series C Biology*, *29*(1), 176-201.

[3] Moosavi, M. R., & Zare, R. (2020). Fungi as biological control agents of plant-parasitic nematodes. In *Plant defence: biological control* (pp. 333-384). Cham: Springer International Publishing.

[4] Su, H., Zhao, Y., Zhou, J., Feng, H., Jiang, D., Zhang, K. Q., & Yang, J. (2017). Trapping devices of nematode-trapping fungi: formation, evolution, and genomic perspectives. *Biological Reviews*, *92*(1), 357-368.

[5] Al-Ani, L. K. T., de Freitas Soares, F. E., Sharma, A., de los Santos Villalobos, S., Valdivia-Padilla, A. V., & Aguilar-Marcelino, L. (2022). Strategy of nematophagous fungi in determining the activity of plant parasitic nematodes and their prospective role in sustainable agriculture. *Front Fungal Biol 3: 863198*.

[6] Kishore, K., Mukesh, C., and Meghvansi, K. (2022). Sustainable Management of Nematodes in agriculture: Organic management. Available at: https://link.springer. com/10.1007/978-3-031-09943-4

[7] Khan, M. R., et al. (2021). Isolation and characterization of nematophagous fungi from rhizosphere soil of infected plants. Journal of Applied Microbiology, 130(3), 785-795.

[8] Hussey, R. S., & Barker, K. R. (1973). A comparison of methods of collecting inoculaof *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 57, 1025-1028

[9] Li, X., et al. (2022). Biocontrol potential of *Purpureocillium lilacinum* against root-knot nematodes in tomato plants. Biological Control, 165, 104854.

[10] Zhang, Y., et al. (2023). Efficacy of *Trichoderma* spp. in managing *Meloidogyne incognita* populations in greenhouse conditions. Crop Protection, 159, 106120.

[11] Abd-Elsalam, K. A., & Yassin, M. A. (2023). Morphological and molecular identification of *Fusarium* species associated with root rot diseases. Journal of Fungal Research, 27(2), 115–124.

[12] Salgado-Salazar, C., et al. (2022). Molecular Identification and Antifungal Susceptibility of *Fusarium* Clinical Isolates. Mycoses, 65(1), 45-56.

[13] O'Donnell, K., Kistler, H. C., Cigelnik, E., &Ploetz, R. C. (2014). Molecular characterization of *Fusarium solani* isolates using IGS-RFLP analysis. Phytopathology, 104(6), 627-634

[14] Jurado, M., Salgado-Salazar, C., &Chaverri, P. (2022). Molecular Identification of *Fusarium* Isolates from Plant Hosts Using Multilocus Sequence Typing. Journal of Fungi, 8(3), 231.

[15] Kumar, A., Sharma, S., & Singh, R. (2021). Preparation and characterization of fungal culture filtrates for biocontrol applications. Journal of Applied Microbiology, 130(4), 1234-1245

[16] Junges, E., Muniz, M. F. B., de Oliveira Bastos, B., Oruoski, P., &Michelon, C. J. (2017). Techniques microbiolization seed forage radish with *Trichoderma* spp. and *Bacillus subtilis*. RevistaBrasileira de CiênciasAgrárias, 12(2), 135-141.

[17] Bokhari, F. M. (2005). Study of the effect of fungal filtrates on *Meloidogyne javanica* eggs and juveniles. King Abdulaziz University Research, Saudi Arabia.

[18] Godinez-Vidal, D., Edwards, S. M., &Groen, S. C. (2024). Root-knot nematode egg extraction protocol. Protocols.io. DOI: 10.17504/protocols.io.eq2lyj5nqlx9/v1

[19] Li, S., & Hartman, G. L. (2003). Molecular detection of *Fusarium solani* f. sp. glycines in soybean roots and soil. Plant pathology, 52(1), 74-83.

[20] Abdullah, A. A., Al-Tarjuman, J. K., & Al-Mamary, H. A. S. (2024). Detection of Seed–Borne Fungal Pathogens Associated with Barley (*Hordeum vulgare*) Seeds and Their Bio-Control Under Laboratory Conditions. In IOP Conference Series: Earth and Environmental Science (Vol. 1371, No. 3, p. 032042). IOP Publishing.

[21] Costa, M.J.N., Campos, V.P., Pfenning, L.H. & Oliveira, D.F. (2001). Fungus Žltrates toxicity to *Meloidogyne incognita*. Fitopatologia Brasileira 26, 749-755.

[22] Randhawa, N., Singh, P., Sandhu, K.S. & Bhatia, A. (2001). Effect of culture Žltrates of soil fungi on hatching of *Meloidogyne incognita*. Plant Disease Research 16, 280-282.

[23] Chen, S.Y., Dickson, D.W. & Mitchell, D.J. (2000). Viability of *Heterodera glycines* exposed to fungal Žltrates. Journal of Nematology 32, 190-197.

[24] Wang, G., Wang, X., Yang, Z., Wang, S., Li, W., Shang, S., ... & Wang, L. (2024). Effects of *Fusarium solani* on the Growth and Development of *Anoplophora glabripennis* Larvae. Microbial Ecology, 87(1), 23.
[25] Abed, A. Z., Fayyadh, M. A., & Heydari, R. (2023). Morphology Root-Knot Nematode Meloidogyna incognita on Cucumber Plant in Southern Iraq. In IOP Conference Series: Earth and Environmental Science (Vol. 1158, No. 7, p. 072012). IOP Publishing.