frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 23 June 2025
DOI 10.3389/fsufs.2025.1609286

OPEN ACCESS

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RECEIVED 10 April 2025
ACCEPTED 02 June 2025
PUBLISHED 23 June 2025

CITATION
Sulieman AME, Elbadri GM, Alanazi NA, Alazmi M, Alrashidi A and Alothman NS (2025) Mitigation of root-knot nematode infestation in tomato plants by mycorrhizal fungi. *Front. Sustain. Food Syst.* 9:1609286. doi: 10.3389/fsufs.2025.1609286

Mitigation of root-knot nematode infestation in tomato plants by mycorrhizal fungi

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Background and objectives: One of main challenges in tomato farming are root-knot nematodes (*Meloidogyne incognita*). By means of improved plant nutrition, induced systemic resistance, and competitive exclusion, arbuscular mycorrhizal fungus (AMF) helps to control nematodes. An arbuscular mycorrhizal fungus (AMF), *Scutellospora heterogama*'s biocontrol potential is assessed in this work as a non-chemical substitute for synthetic nematicides.

Materials and methods: After 1,000 eggs of *M. incognita* were injected into tomato seedlings (*Solanum lycopersicum*), three doses of *S. heterogama* spores (1,000, 1,250, and 1,500 spores per plant) were treated upon them. Three replicates per treatment and a randomized complete block design were used in greenhouses studies. Evaluated were root colonization, gall index, nematode egg count, and plant biomass. The grid-line intersect approach was used to assess AMF colonization; galling index and egg count helped to measure nematode suppression.

Results: All AMF treatments greatly decreased nematode infestation (from 9.33% in control to 3.78%–4.00%) and increased plant biomass. Optimal 1,250 spore dose would have increased shoot dry weight from 2.14 g to 3.40 g. In treated plants, root colonization came at 89% while in controls it came at 0%. Three sequential experimental replicas carried out under the same controlled greenhouse environment produce the results shown below.

Conclusion: In conclusions *Scutellospora heterogama* reduces *M. incognita* stress in tomato quite dramatically. Its application might improve environmentally friendly nematode control. Recommendations for field testing help to confirm its broad relevance.

KEYWORDS

nematicide, arbuscular mycorrhizal fungi, tomato, synthetic pesticides, pathogen

Introduction

Nematodes are microscopic organisms that parasitize plant roots, affecting trees and crops. They are found in many different locations. Across ecosystems, including deep oceans, arid regions, the Arctic, and geothermal springs. Studies show that nematodes, soil-dwelling microorganisms, have widespread negative consequences (Jones et al., 2013; Palomares-Rius et al., 2021). According to the Commission on Genetic Resources for Food, about 80% of food crops are harmed by parasites, especially nematodes (Allender, 2011).

Nematodes' damage is typically overlooked; thus, it is minimized. Root-knot sedentary endoparasitic nematodes induce the formation of large feeding cells in plant roots (Baldacci-Cresp et al., 2015). Singh et al. (2015) and Poveda et al. (2020) estimate a 12.3% global yield loss caused by plant-parasitic nematodes, totaling ~$157 billion. Bradshaw et al. (2016) estimate that invasive insects produce US$70 billion in harm, but the loss is much worse for nematodes.

Parasitic nematodes modify plant form and metabolism, cause root for king, nutritional deficiencies, and abnormal plant growth (Moens et al., 2009; Tileubayeva et al., 2021). Annual crop losses due to plant-parasitic nematodes are estimated at 14.6% in tropical and subtropical regions (Nicol et al., 2011). Elling projected global agricultural losses caused by these nematodes to reach $157 billion, including $13 billion in the United States alone (Elling, 2013). These figures may be underestimated, as nematode-induced damage often goes undetected or is misattributed to other causes. High-value and root-based crops are particularly vulnerable.

Global use of nematicides, a cornerstone of conventional chemical management, has steadily declined in recent decades. Regulatory bodies have tightened controls in response to health and safety concerns, limiting or banning usage. The environmental impacts of synthetic pesticides have driven interest in natural alternatives (Kim et al., 2005). Nematicides are costly, potentially carcinogenic, environmentally hazardous, and ineffective under poor environmental conditions (Desaeger et al., 2020; Pulavarty et al., 2021).

Numerous pests and diseases, including plant-parasitic nematodes, affect tomato (*Solanum lycopersicum* L.) quality and productivity. These pests present a significant challenge to food security, particularly in vulnerable agricultural systems. Meloidogyne species are recognized as major root-knot nematodes impacting tomato crops (Mandal et al., 2021; Mukhtar and Kayani, 2020). Historically, root-knot disease in tomatoes has been documented since the late 19th century. Through several channels, arbuscular mycorrhizal fungus (AMF) has become rather successful biological agents for controlling root-knot nematodes. These include improvement of host plant nutrition and vigor, especially by higher phosphorus absorption, which so indirectly increases the plant's resistance against nematode invasion (Wang, 2017). Root shape and architecture are changed by AMF colonization, hence forming a physical barrier preventing nematode access (Schouteden et al., 2015). Furthermore, in plants, AMF can cause systemic resistance (ISR), thereby activating defense-related genes and increasing the synthesis of phytoalexins and defense enzymes (Vos et al., 2013). Additionally seen is competitive exclusion, whereby AMF occupy cortical root cells that might otherwise be targeted by nematodes, therefore limiting the locations for nematode infection (Azcón-Aguilar and Barea, 1997). These synergistic effects place AMF as interesting biocontrol agents in environmentally friendly nematode control plans.

This study investigates the potential of arbuscular mycorrhizal fungi (AMF) as a biological control agent to suppress populations of root-knot nematodes and promote the growth of tomato plants under nematode pressure.

Materials and methods

Study area

The research was carried out in Ha'il City (Figure 1), in the northwestern area of the Kingdom of Saudi Arabia (KSA) (27° 31′ 0″ N, 41° 41′ 0″ E). Ha'il is an agrarian and pastoral region distinguished by abundant water supplies, arable land, and temperate temperatures. Due to its strategic location, Ha'il has historically played a vital role in the Arabian Peninsula. The region benefits from water availability, fertile soil, and a favorable climate, supporting the cultivation of fruits, vegetables, cereals, barley (Hordeum vulgare), and livestock farming.

Nematode sample collection

Root samples showing root-knot nematode symptoms were collected from infected tomato plants. From every 10 symptomatic plants, five root samples were taken, totaling fifty samples. These were transported in labeled polythene bags to the laboratory and stored at 4°C to preserve nematode egg viability for subsequent analysis.

Nematode identification and egg extraction

Eggs were extracted from heavily galled tomato roots using the method (Hussey and Boerma, 1981). Roots were rinsed, chopped into 1–2 cm segments, and blended for 15–20 s. The resulting suspension was mixed with 1.25% sodium hypochlorite (NaOCl) and gently swirled for 3 min to release eggs. The mixture was passed through a 200 to 25 μm sieve sequence, and the retained eggs on the 25 μm sieve were rinsed and collected in distilled water.

Preparation of tomato seedlings

Seeds of tomato cultivar Beto-86 were surface-sterilized using 1.25% NaOCl for 20 min, rinsed thoroughly, and germinated in peat-based media. At the 4–5 leaf stage, seedlings were transplanted into pots containing sterilized 2:1 clay-to-sand mix. Soil sterilization was conducted at 121°C and 15 psi for 30 to 60 min.

Nematode inoculation

Nematode suspensions were prepared by adjusting egg concentrations to ~200 eggs/mL. Using 5 mL of suspension (~1,000 eggs), each plant was inoculated by creating a small hole near the stem and covering it with soil. Controls received 5 mL of sterile distilled water.

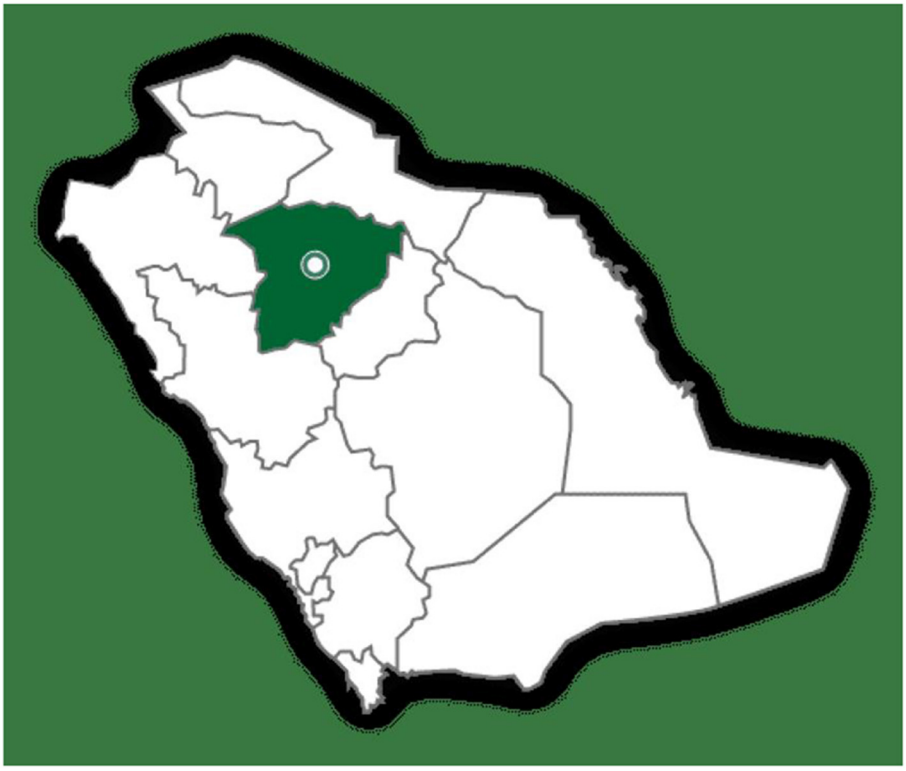

FIGURE 1
Map of Hai'l city, the northwestern district of Saudi Arabia.

Greenhouse conditions

Plants were maintained under controlled conditions (25°C−30°C, 60%−70% RH) with a 12 h light/dark cycle. Soil moisture was monitored using sensors, and watering was done with deionized water as needed. Artificial lighting supplemented natural light to ensure uniform growth.

Arbuscular mycorrhizal fungi (AMF) inoculation

Spores of *Scutellospora heterogama* were isolated from the rhizosphere of Bermuda grass using wet sieving (250, 180, 63, and 45 μm sieves) and decanting. Viability was confirmed via trypan blue exclusion. AMF suspensions were standardized to 250 spores/mL. Each seedling received 4, 5, or 6 mL of the suspension (1,000, 1,250, or 1,500 spores) applied 5 cm below the roots at transplanting. Controls received equal volumes of sterile distilled water.

Experimental design and treatments

A randomized complete block design (RCBD) with five plants per treatment was used. The experiment included:

- Control (no AMF, no nematode)
- Nematode only
- AMF only
- AMF + Nematode (1,000, 1,250, and 1,500 spores per plant)

Each treatment had three replicated experiments conducted under identical greenhouse conditions. Plants were randomized and repositioned weekly.

Evaluation of root colonization and plant growth

Shoot and root lengths, fresh and dry biomass were recorded at harvest. Root colonization by AMF was quantified using the grid-line intersect method (Giovannetti and Mosse, 1980). Roots were stained with 0.05% ink-acetic acid solution and evaluated microscopically.

Gall and egg mass indexing

Galls and egg masses were stained with 0.15 g/L Phloxine B and evaluated under a stereomicroscope using a 0–5 scale. The same scale was used to assess nematode infection severity.

Measurement of parameters

Root galling was graded on a 0–5 scale; egg masses were counted under a stereomicroscope; the grid-line intersect method was used to estimate mycorrhizal colonization. Harvest saw fresh/dry shoot and root weights recorded (Giovannetti and Mosse, 1980).

Evaluations of root colonization and plant growth

Measuring shoot and root lengths, as well as fresh and dry biomass, allowed one to assess plant growth at the end of the experiment. To check how much arbuscular mycorrhizal fungi had grown on the roots, we used the grid-line intersect method described by Giovannetti and Mosse (1980). Root samples were cleaned, stained with 0.05% ink in acetic acid, and dried for 48 h at 62°C. Colonization was quantified via microscopic inspection (Giovannetti and Mosse, 1980).

The percentage of root colonization was calculated using the following formula:

Root Colonization = Number of inter*sections* with mycorrhizal structures × 100.

Total Number of intersections observed.

Statistical analysis

One-way ANOVA was used to compare treatment effects, and Duncan's Multiple Range Test (DMRT) was applied for mean separation at $p < 0.05$. Assumptions of normality and variance homogeneity were validated using Shapiro-Wilk and Levene's tests, respectively (Shapiro and Wilk, 1965; Chialva et al., 2023; Duncan, 1955). Effect sizes were also calculated to assess treatment impact.

Results

Effect of mycorrhizal fungi on plant growth and nematode suppression

Dose-response trend

Increasing AMF spore concentrations from 1,000 to 1,250 enhanced plant growth metrics; 1,500 demonstrated a plateau, implying an ideal threshold.

In tomato (*Solanum lycopersicum* L.), plants infected with *Meloidogyne incognita* and *Scutellospora heterogamy* spores improved growth indices and worm control. Mycorrhizal treatments at 1,000, 1,250, and 1,500 spores per plant considerably improved fresh and dry biomass relative to the control group (Table 1).

However, raising the spore concentration above 1,250 did not increase shoot or root biomass, implying a saturation point in mycorrhizal advantage. Furthermore, a notable decrease in nematode-related indicators, including gall formation and egg mass count, was observed in the treated plants (Table 2).

The nematode infection percentage fell from 9.33% in the control to 3.78%−4.00% in treated plants. Likewise, in treated groups, the count of galls and eggs per plant dropped significantly. In injected plants, mycorrhizal colonization rose to 89%, while it was absent in controls.

Dry shoot weight rose from 2.14 g to 3.40 g; fresh shoot weight rose from 18.00 g (control) to 21.10 g (at 1,250 spores). Nematode control reflected root dry weight dropped from 2.41 g in controls to 1.49–1.67 g in treated plants (Tables 2–4; Figure 2).

TABLE 1 Influence of *Scutellospora heterogama* on tomato growth characteristics.

Mycorrhizal spore concentration	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
1,000 spores	20.34 ± 0.5	3.07 ± 0.2	6.29 ± 0.3	1.55 ± 0.1
1,250 spores	21.10 ± 0.6	3.40 ± 0.3	6.27 ± 0.4	1.49 ± 0.1
1,500 spores	20.73 ± 0.4	2.76 ± 0.2	6.36 ± 0.3	1.67 ± 0.2
Control	18.00 ± 0.5	2.14 ± 0.2	7.56 ± 0.4	2.41 ± 0.3

All mycorrhizal treatments significantly improved shoot and root biomass compared to the control ($p < 0.05$).

TABLE 2 Infection rate and growth parameters of tomato plants inoculated with *Meloidogyne incognita* and treated with *Scutellospora heterogamy*.

Mycorrhizal treatment (mL)	Infection (%)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
1 mL (1,000 spores)	3.89^{a}	20.34^{a}	3.07^{a}	6.29^{b}	1.55^{b}
2 mL (1,250 spores)	4.00^{b}	21.10^{a}	3.40^{a}	6.27^{ab}	1.49^{b}
3 mL (1,500 spores)	3.78^{b}	20.73^{a}	2.76^{ab}	6.37^{b}	1.67^{b}
Control (no AMF)	9.33^{a}	18.00^{b}	2.14^{b}	7.56^{a}	2.41^{a}
Cv%	21.28	6.15	24.99	16.90	26.15
S.E ±	1.25	1.52	0.50	1.28	0.22

According to Tukey's multiple range test, values in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 3 Infection % of shoots and root fresh and dry weights of tomato (*Solanum lycopersicum* L.) plants infected with root-knot nematodes, *Melodogyne* spp. infected with three concentrations of mycorrhizae.

Concentration of mycorrhizae	Infection percentage	Fresh of weight shoots	Dry weight of shoots	Fresh of weight roots	Dry weight of roots
1 mL	3.889^{a}	20.34^{a}	3.067^{a}	6.289^{b}	1.546^{b}
2 mL	4.000^{b}	21.10^{a}	3.400^{a}	6.267^{ab}	1.493^{b}
3 mL	3.778^{b}	20.73^{a}	2.756^{ab}	6.367^{b}	1.667^{b}
control	9.333^{a}	18.00^{b}	2.144^{b}	7.556^{a}	2.411^{a}
Cv%	21.28	6.15	24.99	16.90	26.15
S.E±	1.248	1.518	0.504	1.280	0.217

Values in the same column followed by different letters are significantly different according to 237 Turkey's multiple range tests ($p < 0.05$).

TABLE 4 The legend shows data from the second growing season, including several mycorrhizal colonies, which indicate the presence and abundance of mycorrhizal fungal structures in plant roots, and the percentage of root-knot nematodes, which is the proportion of *Meloidogyne* spp. in the soil or plant roots to the total nematode population.

Concentration of mycorrhizae	Colonization of mycorrhizae	Nematode percentage	Mycorrhizae e root length	Plant root length	Number of galls/ plant	Number of eggs/plant
1 mL	69.26^{c}	38.32^{b}	10.63^{a}	15.99^{a}	590.8^{b}	299.9^{b}
2 mL	90.9^{3b}	38.60^{b}	12.87^{a}	19.04^{a}	600.7^{b}	280.6^{b}
3 mL	75.96^{b}	39.11^{b}	12.44^{a}	17.20^{a}	580.5^{b}	295.0^{b}
control	0.000^{a}	63.20^{a}	0.222^{b}	3.00^{b}	650.8^{a}	390.9^{a}
CV%	15.66	8.14	25.98	28.31	29.5	30.5
S.E±	61.415	13.362	5.514	13.67	3.465	3.627

The total root system length is measured in centimeters by root length and plant root length. Galls are abnormal swellings on roots caused by root-knot nematodes, while eggs per root are the number of eggs in each plant root system.
Values in the same column followed by different letters were significantly different according to Turkey's multiple range tests ($p < 0.05$).

The several concentrations of nematodes investigated produced diverse degrees of plant reaction. Application of mycorrhizal fungus significantly reduced gall development and inhibited nematode population density in inoculated plants. At 4, 5, and 6 mL (equivalent to 1,000, 1,250, and 1,500 spores, respectively), mycorrhizal treatments reduced nematode infection levels. Mycorrhiza-treated plants demonstrated statistically significant improvements in all evaluated parameters—including root galling, egg mass count, nematode population, and plant growth measures—as shown in Figure 2 against untreated controls.

For instance, the total nematode count per root system in the control group was almost 9,000; in the treated group, it dropped to almost 4,000. In plants injected with mycorrhizal fungus, the galls, and egg masses count was likewise much reduced.

Regarding plant development, fresh shoot weight increased from 16.94 g in the control group to 19.23 g in the mycorrhizal treatment group. This suggests that mycorrhizal inoculation increased biomass production. Additional evidence supports this effect, including the increase in shoot dry weight, which rose from 2.00 g in the control plants to 2.86 g in the treated plants (Figure 3).

Unlike 6.6–2 g, the treatment caused fresh and dry roots to weigh less from 5.7 g to 1.1 g (Table 1). Concerning the control zero, the mycorrhizae colonization of 89% in treated plants increased. With a nematode percentage of 62%, mycorrhizae-treated plants were much less than the proportion of 34% of the control plants. Of the treated plants, 34 to 43 percent had roots with mycorrhizae spanning 10 cm when compared to the control group. Whereas, the control plant measured eight centimeters, the treatment plant measured sixteen. Whereas, the number of eggs generated by the control plant rose to 734.7a, the number of eggs generated by the treatment plant dropped to 590 per plant. Furthermore, the number of galls generated by the treated plant dropped to 275 per plant, whereas the number of galls generated by the control plant rose to 328.5 per plant (Table 2).

Seasonal consistency

Reliability of the results was strengthened by similar trends in nematode suppression and growth augmentation reported throughout repetitions.

The treatment resulted in fresh and dry roots weighing less from 5.7 g to 1.1 g, unlike 6.6–2 g (Table 1). Regarding the control zero, the mycorrhizae colonization of 89% in treated plants got higher. Plants treated with a nematode percentage of 62% were far less than the proportion of 34% of the control plants. Comparatively, 34 to 43 percent of the treated plants showed roots with mycorrhizae extending 10 cm compared to the control group. The treatment plant measured 16 m, while the control plant measured eight. The number of eggs produced by the treatment plant was reduced to 590 per plant, while the number of eggs

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FIGURE 2
Segments of galled tomato (*Solanum lycopersicum* L.) in a plastic Petri dish with a five by 5 mm grid.

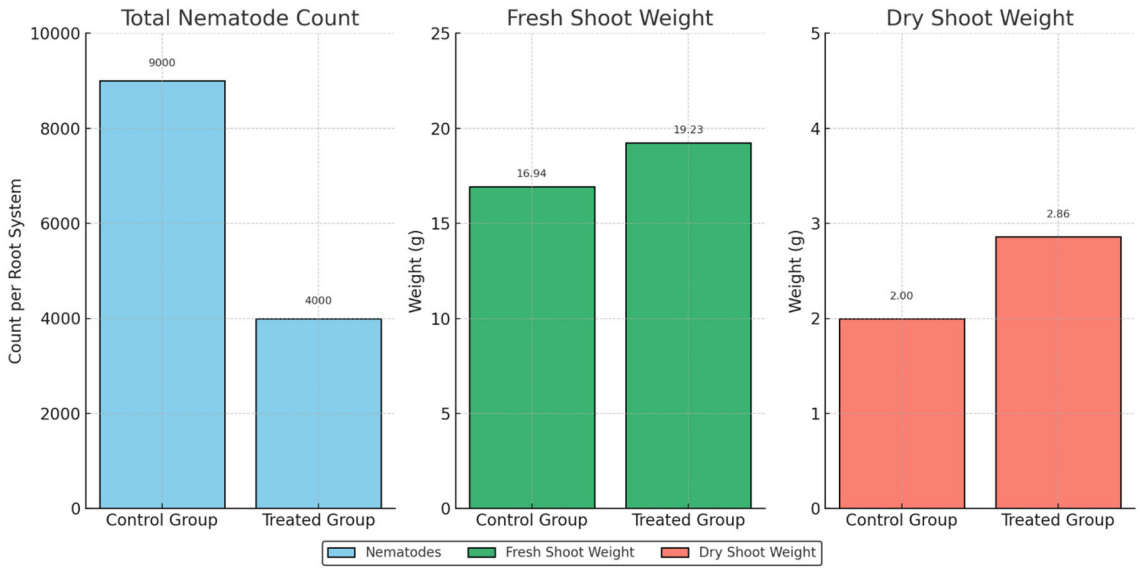

FIGURE 3
Mycorrhizal fungi mitigate nematode stress and improve tomato growth parameters.

produced by the control plant grew to 734.7a. Moreover, whereas the number of galls produced by the control plant climbed to 328.5 per plant, the number of galls produced by the treated plant reduced to 275 per plant (Table 2).

Discussion

Because of its culinary adaptability, nutritional worth, and economic relevance, tomato (*Solanum lycopersicum* L.) is among

the most grown vegetable crops worldwide. Root-knot nematodes (*Meloidogyne* spp.) compromise root structure, limit nutrient absorption, and function as persistent soilborne diseases (Phani et al., 2021; Ma et al., 2023), greatly restricting its productivity. Conventional management methods may raise environmental and health-related issues, from chemical nematicides to resistant cultivars. Therefore, biological solutions like arbuscular mycorrhizal fungus (AMF) become increasingly important.

Treatment comparison

Among treatments, 1,250 spores per plant showed the best combination of reduced nematode infection and increased biomass. In this work, tomato plants pre-inoculated with *Scutellospora heterogama* showed notably increased resistance to *Meloidogyne incognita*. AMF-treated plants displayed lower gall development, decreased nematode infection rates, and better plant growth than untreated controls. These results fit earlier studies by Calvet et al. (2001) and Talavera et al. (2001), which found that early AMF inoculation promoted symbiosis growth and provided defense against nematodes.

Akbar et al. (2023) also noted that AMF-treated plants showed higher shoot and root biomass, most likely because of improved phosphorus absorption and general nutritional efficiency. These results show AMF's ability to stimulate plant vigor and reduce stress caused by nematodes. Furthermore, supporting AMF's function in limiting nematode growth is the decrease in gall numbers and egg mass generation per root system. The present results confirm findings from earlier research involving various crops and AMF species (Liu et al., 2020; da Silva Campos et al., 2017; Cofcewicz et al., 2001).

AMF's suppressive mechanisms likely include physical barrier creation via improved root architecture, competition for root colonization sites, and induction of systemic resistance (ISR) pathways. Newsham et al. (1995) and Wang (2017) proposed that AMF may prime the plant immune system, enabling faster and stronger defenses against nematode attack.

Field limitations

Results derived from sterilized soil under greenhouse conditions must be validated in field trials to confirm effectiveness under real-world scenarios.

Although the outcomes in a greenhouse environment show promise, using AMF in field conditions presents challenges such as soil variability, microbial competition, and cost of AMF production. Sterilized soil may have artificially boosted AMF colonization, and real soil conditions must be tested through field studies.

The variation in outcomes associated with different concentrations of mycorrhizal inocula is a key factor that warrants further exploration. This study found that inoculation levels (1,000, 1,250, and 1,500 spores per plant) reduced the impact of root-knot nematodes in a dose-dependent manner. Environmental factors such as soil type, moisture, and temperature also influence AMF effectiveness.

According to Detrey et al. (2022), tomato plants had fewer galls when inoculated with AMF. Similarly, Schouteden et al. (2015) showed increased resistance to both *M. javanica* and *M. incognita*. This confirms that AMF plays a dual role in growth promotion and biocontrol.

The timing of AMF inoculation is critical. Nematodes invade roots quickly, but AMF colonization takes time. Pre-inoculation offers superior protection, as supported by Rausch et al. (2009) and Sikora et al. (1990).

Using only one AMF species in this work limits the findings. Indigenous or mixed AMF species could provide broader resistance and growth benefits under varied environments. Ultimately, although AMF lowered worm infection and stimulated development, the study did not assess long-term resistance under repeated nematode infestation or secondary pathogen attack. Future studies should explore this interaction in more depth. The findings support integrating AMF into sustainable pest management strategies for tomato crops.

Strengths and weaknesses

This work has great merit in its controlled design, unambiguous dose-response pattern, and exhaustive quantification of plant growth and nematode reduction. Limitations include dependency on greenhouse settings with sterilized soil and absence of long-term studies, nevertheless. Next field research should fill in these voids.

Our findings reaffirm AMF's potential in integrated pest management (IPM) frameworks and warrant further evaluation in diverse agroecological zones.

Conclusions

In conclusion, our work supports the possible biocontrol capacity of *Scutellospora heterogamy* against *Meloidogyne incognita* in tomatoes. Important next steps for converting these discoveries into useful agricultural uses, meanwhile, are improving inoculation time, investigating several AMF species, and confirming efficacy in field settings.

Mycorrhizal fungus considerably lowers root-knot nematode infestation, increasing tomato plant development. Improving plant defenses and nitrogen absorption is a natural and sustainable method that reduces the stress in agriculture by nematodes. Mycorrhiza can be combined with organic additions, less tillage, and crop rotation techniques for pest control. Still, good implementation calls for farmer education and economic viability analysis. Future research should consider natural field trials, dose-response models, temporal investigations, and knowledge of species interactions for best colonization.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AS: Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. GE: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. NAA: Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. MA: Funding acquisition, Software, Supervision, Writing – original draft, Writing – review & editing. AA: Methodology, Resources, Writing – original draft, Writing – review & editing. NSA: Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. We express our gratitude to the Ha'il University for their financing support for this project (R.G. 23/194).

Acknowledgments

The University of Hail Project (RG-23/194) financed this study. We are grateful to the University of Hail KSA for the financial support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

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