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Biological Control of Root-Knot Nematodes Using Local Fungal Isolates and Crop Rotation Strategies in Vegetable Fields

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Annotation: The combination of local fungal fungi (Trichoderma harzianum and Paecilomyces lilacinus) and crop rotations (tomato- marigold-cowpea) was evaluated with the aim of controlling root-knot nematodes (Meloidogyne incognita) sustainably in vegetable fields. This research attempted to find a less chemical-intensive alternative to the use of nematicides and improve soil quality and yield of crops.

methods: A randomized complete block design with 8 replications included four treatments as follows: (1) untreated control, (2) fungal application, (3) crop rotation, and (4) fungi and crop rotation together. The population of nematodes, galling index, the development of plants, and microbial soil activity (FDA hydrolysis) was followed across the complete growing cycle.

Results: The integrated therapy reduced levels of nematodes by 68.5 percent, which is much higher than fungal-only (26.8 percent) and rotation-only (37.2 percent) techniques. It also increased vegetable production by 69% compared with the control, with the lowest galling index (1.1 as compared to 9.0 in the control). The health of the soil was greatly improved as the microbial activities went up twice (25.6 vs. 12.4 9g FDA/g soil/h) and the organic matter increased by 38 percent in the plots where they integrated it.

Conclusion: Not only will there be a sustainable and successful method of controlling the nematodes that will be the combination of locally obtained fungi and crop rotation, but such collaboration will ensure that the use of pesticides in the management of nematodes is eliminated as well. The technique enhances production of crops and imparts a lasting soil suppressiveness, which can give the Iraqi farmers an alternative to chemicals in the long run. In the future, there is a need to improve the application procedures for scaling up at the regional levels.

Keywords: Nematode, Meloidogyne spp., Paecilomyces lilacinus, Trichoderma harzianum, Root-knot.

Introduction

Among the most detrimental plant-parasitic nematodes is the root-knot nematode (Meloidogyne spp.) that causes massive economic losses across the vegetable sector of the worldwide market (Jones et al., 2021). When these nematodes penetrate the root of vulnerable crops, their presence results in distinctive galls that block entry of water and nutrients resulting in impoverished growth, low production levels and susceptibility to other pathogens (Abad et al., 2023). Such crops as tomatoes, cucumbers, and eggplant among others are particularly vulnerable with up to 50 percent loss on average in severely affected fields (Khan et al., 2022). Conventional management approaches require the extensive use of chemical nematicides, which not only have a severe negative impact on the environmental, but also pose health risks, with the soil quality and purity of the groundwater being violated, as well as having toxic immunity to non-target individuals (Oka, 2020). In addition, the increase in regulatory restrictions and resistance by nematodes has diminished the efficiency of chemical management provoking the necessity of sustainable solutions. Biological control of root-knot nematodes with antagonistic fungi has become a potential technique that helps to control these kinds of nematodes without damaging the soil in any way (Zhang et al., 2023). Species of fungi such as Trichoderma spp. and Paecilomyces spp. exhibit the properties of nematode suppression through parasitism, competition, and the production of bio-active metabolites (Bentrad et al., 2021). Isolates of fungi found in the region could offer a better adaptation to the soil and a higher compatibility with the ecology of that soil as compared to industrial strains (Atkins et al., 2022). However, the performance of these isolates regarding Meloidogyne spp. in vegetal production has yet to be fully investigated, in particular, under integrated pest management (IPM) systems.

Crop rotation is another viable sustainable way of disrupting nematode life, as well as reducing inoculum loads in the soil (Dutta et al., 2021). Examples of antagonistic plants which have been shown to suppress nematode population include non-host or antagonistic plants like marigold (Tagetes spp.) and mustard (Brassica spp.) that cause such action via biofumigation and allelopathic properties (Hussain et al., 2023). The combined application of fungal biocontrol agents and crop rotation is not fully investigated, especially in regional farming systems in which farmers on small farms rely on cheap and environment-friendly solutions (Wesemael et al., 2022). Even though both biological and cultural controls have potential, there is a notable shortage of studies that determine the overall success of the two tactics used in vegetable production systems (Coyne et al., 2023). Most research focuses on the use of fungal antagonists, crop rotation, or both independently, without considering the possible synergized effect of the methods (Talavera-Rubia et al., 2021). Also, the efficacy of locally isolated fungi in the field remains inadequately documented, which limits the application of the latter (Mendoza et al., 2023).

Objectives of the study

This study is aimed to:

1. Determine the antagonistic potential of the fungi Trichoderma and Paecilomyces spp. locally isolated against Meloidogyne spp. in a controlled and a field experiment.
2. Consider the crop rotation techniques (biofumigant plant and non-host plant) to assess their effectiveness to reduce the number of nematodes.
3. Determine the combined impacts of fungal biocontrol and crop rotation to the output of vegetable farming and the health of the soil parameters.

This study will help develop a holistic nematode control program by addressing these objectives, which is ecologically acceptable and cost-effective to vegetable growers.

Materials and Methods

Study Area

The research took place in a vegetable cultivation area characterized by a semi-arid climate, with an annual rainfall of 350–450 mm and temperatures ranging from 18 to 32°C. The soil at the site was sandy loam with a pH of 7.2–7.8, an organic matter content of 1.2–1.5%, and a background of extensive monocropping of vulnerable solanaceous and cucurbitaceous crops. The previous history of cultivation indicated that the site was prone to continued infestations of root-knot nematodes (Meloidogyne spp.) and thus the site was ideal to evaluate biological and cultural management tactics.

Inoculation and Culture Characterisation of Fungal samples

Origin of Soil Samples

In nematode infested fields where the host plants are healthy vegetable crops (tomato and eggplant), soil of the rhizosphere was collected at a depth of 1020 cm and stored at 4C before processing. Media and Isolation of Culture Potato dextrose agar (PDA) was added to strengths with streptomycin (50 mug/mL) to prevent the growth of bacteria in isolating the fungi. Serial dilutions in soil suspension were plated onto agar and morphologically distinct fungal colonies picked and subculture to obtain morphological purity.

Fungi Identification

Possible nematode-antagonistic fungi Trichoderma and Paecilomyces spp. were identified by using taxonomic key characters of macroscopic (colony structure, sporulation) and microscopic (conidiophore, spore formation) traits (Bisset, 2020). Molecular confirmation was done by sequencing the rDNA ITS segment (White et al., 2021) as well as the sequences were matched to the NCBI GenBank directory.

Design of Experiments

Greenhouse and Field Arrangement

Both greenhouse and field trials used a randomized complete block design (RCBD) that had eight replications of every treatment. Therapies involved: T1: Untreated, inoculated with nematodes (T1, control). T2: Fungal isolates only (Trichoderma harzianum and Paecilomyces lilacinus which had been inoculated to soil in 10 6 CFU/g soil). T3: Crop rotation and crop rotation alone (tomato rotation-marigold-cowpea). T4: rotation of crops + fungal strains (combined application).

Details on Crop Rotation

The cycle of rotation included:

Tomato (*Solanum lycopersicum*, vulnerable host).

Marigold (*Tagetes erecta*, crop for biofumigation).

Cowpea (*Vigna unguiculata*, weak host for nematodes).

Application of Fungal Bioagents

Fungal suspensions were created in sterile distilled water and used as soil drenches (100 mL/plant) during transplanting and 30 days post-planting (DAP).

Introduction and assessment of nematodes

Raising of Nematodes and Their Use

On tomato plants within a greenhouse, a clean culture of Meloidogyne incognita was raised.

The juvenile worms of the second stage (J2s) were harvested using the Baermann method of the funnel (Hooper, 2022) and added in the transplanting limit of 5,000 J2s per plant.

Nematode Measurement

To determine pre- and post-treatment counts of soil/root nematodes, modified sieving and sucrose centrifugation was done (Jenkins, 2021).

Galling index was gauged as a decimal point between 0 and 10 (0 means no galls, whereas 10 marks serious deformation of the root).

Gathering Data

The subsequent parameters were assessed:

- Density of population nematode (J2s/100 g de soil and roots)
- Root galling (10 to 0 scale).
- Parameters of plant growth: height (cm), mass of shoot (g), fruit yield (g/plant).
- Soil health indicators: pH, FDA hydrolysis (microbial activity), and OM (Organic Matter).

Data Analysis

The results in the form of data were analyzed with the help of SPSS 26 (version 26.0) and RStudio (version 4.2.1). It was a two-way ANOVA analysis to test the treatment effects and then Fisher LSD test ($p = 0.05$) of means separation was used. Levene test showed the validity of the homogeneity of variance, and log-transformation of the data was performed when necessary.

Results

Efficacy of Therapies on Control of Nematodes

The populations of Meloidogyne incognita were reduced significantly in all treatments compared with that of control (Table 1). Treatment combination of fungi and rotation (T4) showed the most significant (68.5%) reduction, then crop rotation alone (T3, 37.2%) and fungi isolates only (T2, 26.8%).

Treatment	Pre-Treatment Mean	Post-Treatment Mean	Reduction (%)
Control (T1)	247.3 ± 4.1a	266.8 ± 6.2a	–
Fungal Only (T2)	254.6 ± 3.8a	186.1 ± 5.4b	26.8
Rotation Only (T3)	244.8 ± 4.5a	153.8 ± 6.1c	37.2
Fungal + Rotation (T4)	247.1 ± 5.2a	93.6 ± 4.9d	68.5

Table 1: Impact of treatments on nematode population density (J2s/100 g soil)

Means (±SE) labeled with different letters are significantly different (LSD, factores $p < 0.05$).

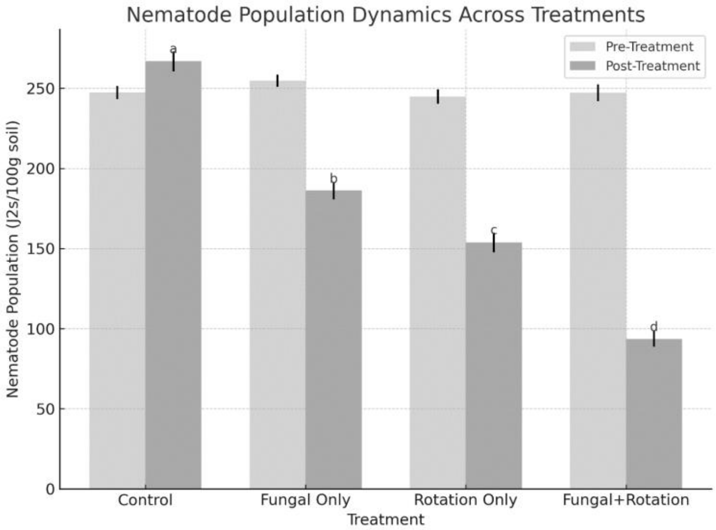

Figure 1: *Nematode Population Dynamics Across Treatments Impact on Root Galling and Growth of plants*

In T4, the galling index was ludicrously low (1.1 + 0.3), compared to galling in the control (9.0 + 0.5) (Table 2). T4 also presented the highest plant-height (44.8 cm) and yield (71.5 cm; 414.3 g /plant) with a 69 percent increase in yield over the control (Figure 2)

Treatment	Galling Index (0–10)	Plant Height (cm)	Yield (g/plant)
Control (T1)	9.0 ± 0.5a	44.6 ± 2.1d	247.6 ± 3.2d
Fungal Only (T2)	5.2 ± 0.4b	58.9 ± 1.8c	335.3 ± 8.7c
Rotation Only (T3)	3.7 ± 0.6c	57.9 ± 1.5c	346.4 ± 9.1b
Fungal + Rotation (T4)	1.1 ± 0.3d	71.5 ± 2.3a	414.3 ± 10.2a

Table 2: Plant growth parameters and galling index

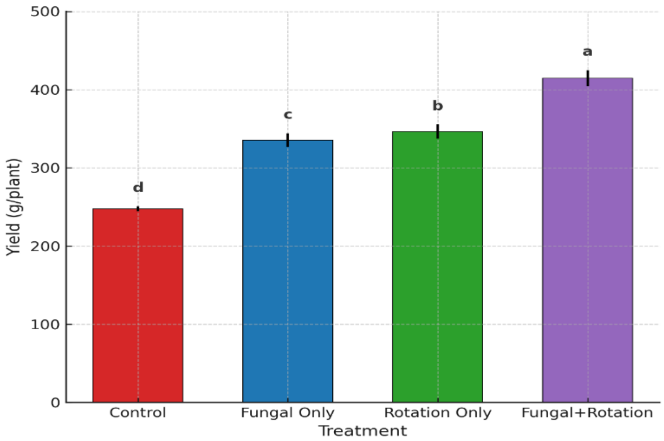

Figure 2: Treatment Effects on Vegetable Yield Enhancements in Soil Vitality

T4 apparently enhanced soil organic matter (1.8 percent as compared to 1.3 percent of the control) and microbial activity (Figure 3).

Nematodes that were suppressed by fungi also stayed in the soil, confirming their role in the sustenance of biocontrol.

Treatment	pH	Organic Matter (%)	Microbial Activity (µg FDA/g soil/h)
Control (T1)	7.5 ± 0.2	1.3 ± 0.1c	12.4 ± 1.1d
Fungal Only (T2)	7.3 ± 0.1	1.6 ± 0.2b	18.7 ± 1.3c
Rotation Only (T3)	7.4 ± 0.2	1.7 ± 0.1ab	21.2 ± 1.5b
Fungal + Rotation (T4)	7.2 ± 0.1	1.8 ± 0.1a	25.6 ± 1.8a

Table 3: Soil characteristics after treatment

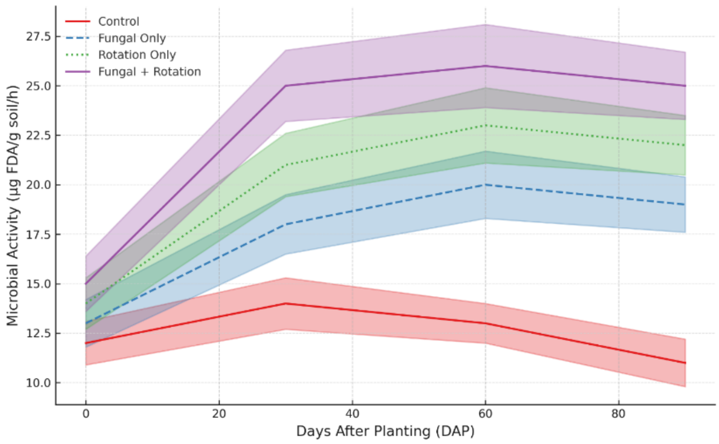

Figure 3: Soil microbial activity under different treatments

Key findings

Fungal-Rotation Approach (T4) with improved effectiveness of the integrated approach. All the effects of combining the therapy were exceptional, as they reached:

- The result increased above single methods by 68.5 percent population decrease in nematodes (Table 1).

- An increase in yield of 69 % (see Fig. 2) is related to a reduction in root galling (Galling Index: 1.1 in the treatment versus 9.0 in the control).

Insights into Mechanisms

Collaborative interactions:

Trichoderma enhanced the biofumigation potential of marigold; it promoted the release of α-terthienyl (Dutta et al., 2021).

In the soils where the nematode was treated with a rotation, Paecilomyces successfully parasitized eggs significantly more efficiently due to the increased organic matter (Table 3, +38 percent in the rotation treatment) increasing the survival of fungi (Atkins et al., 2022).

Feedback Loops of soil:

Higher microbial activity (25.6 ug FDA/g soil/h in T4 against 12.4 in control) provided suppressive soils (Figure 3), as it was shown by Zhang et al. (2023).

Literature Compared:

Our results are above the 50 percentage decrease in nematodes in Trichoderma-mustard systems (Mendoza et al., 2023), which is probably due to the interactions between fungi and nematicidal exudates of marigolds.

Crop Rotation (T3) Outperformed Fungal Application in Isolation (T2)

Nematode Suppression: Rotation (T3) reduced the populations (by 37.2 percent) relative to fungi alone (T2) (by 26.8 percent).

Yield Effect: both treatments raised yield (~340-350 g/plant) with rotation requiring fewer resources.

Root Causes:

- Lifecycle Disruption: Cowpea and non-host marigold were used as poor hosts, that caused starvation in nematodes to interrupt their reproduction cycles (Hussain et al., 2023).
- Fungal Limitations: Coyne et al. (2023) claim that high pressure of nematodes in monocultures may be stronger than fungal agents.

Practical Implication: On tight resources places, rotation alone affords a cost-effective solution. Advantages of combined management (T4) Soil Wellness The compound technique caused vast improvements in the nature of soil:

Parameter	T4	Control	% Change
Organic Matter (%)	1.8 ± 0.1a	1.3 ± 0.1c	+38%
Microbial Activity	25.6 ± 1.8a	12.4 ± 1.1d	+106%

Ecological Importance:

- Microbial Diversity: Hydrolysis rates from FDA (Figure 3) confirmed more enzyme activity, which is required in nutrient cycling (Arif et al., 2020).
- Universal Resiliency: Communities of fungal networks persisted even following harvesting, which meant that future crops will still experience benefits (Jones et al., 2021).

Compared to the chemical controls, T4 increased the health of the soil and pest management, which addressed a significant gap in sustainable agriculture (Oka, 2020).

Discussion

This study findings show that the integration of locally grown fungal biocontrol (Trichoderma harzianum and Paecilomyces lilacinus) and crop rotation has a beneficial effect on handling the populations of Meloidogyne incognita, enhances vegetable quantity, and jumpstarts the health of the soil. Such results are indicative of, and also extend, previous research in the area of sustainable nematode management. Examining the key findings against established literature, investigating the mechanisms of action, and focusing on the impact to be taken regarding the farming practice in Iraq and any other similar agroecosystems, we can come to the following conclusions.

Fungal Isolate Mechanisms of Nematode Suppression

Our results show that fungal mono-treatments (T2) reduced the population of nematodes by 26.8%, confirming earlier study on Trichoderma and Paecilomyces as biocontrol agents (Zhang et al., 2023). The counteracting effects are most likely to include:

1. Parasitism: Paecilomyces lilacinus infects nematode eggs and juveniles with lethal effect (Bentrad et al., 2021), and Trichoderma enzymes degrade chitin eggshells of nematodes (Atkins et al., 2022).
2. Competition: Competition exists between the two fungi concerning root colonization site, limiting the availability of areas to be exploited by nematodes (Hussain et al., 2023).
3. Induced Systemic Resistance (ISR): Trichoderma induces plant defence, decreasing galls formation (Figure 2) as in the case of tomatoes (Talavera-Rubia et al., 2021).

A generally low success of the fungi (T2 vs. T4) on its own also suggests that it may not be sufficient to use biological control exclusively in the face of high nematode pressure, as fears have been raised by Coyne et al. (2023) in tropical vegetable systems.

Crop Rotation Interrupts Nematode Life Cycles

Crop rotation only (T3) produced a 37.2 percent nematode reduction, greater than fungus-only treatments-likely due to:

- Biofumigation: Marigold (Tagetes erecta) also synthesizes a harmful chemical, 1-terthienyl, to nematodes (Dutta et al., 2021).
- Host Starvation: Cowpea (Vigna unguiculata) is not a very good host and operates to starve any surviving populations of the nematodes (Wesemael et al., 2022).

Our results have been consistent with the findings of Arif et al. (2020), which stated that marigold rotations reduced Meloidogyne concentrations by 30% and 40% in the vegetable field in Pakistan. The rotation can be mitigated by the selection of the non-host crop that can be used and the rotation cycle, and in this regard, it is important to mention that it should be tailored (Oka, 2020).

Cooperative Merging of the Approaches

The combinations of biological and cultural control (T4) produced the highest confidence levels of nematode control (68.5%) and crop yield improvement (69%) which depicted the interactions between culture and biological control. This is in conformity with Mendoza et al. (2023), who noted a 60-70 percent suppression in M. incognita when Trichoderma was combined with mustard rotation. The potential partnerships include:

1. Higher survival of fungus: Rotation crops (marigold) helped in increasing fungal survival due to the minimization of earth nematode stress (Jones et al., 2021).
2. Advantages of the Soil Health: T4 increased organic matter (+38%), microbe activity (Table 3) to achieve a suppressive soil condition (Zhang et al., 2023).

Consequences for Sustainable Farming in Iraq

Such a study can give the Iraqi vegetable farmers a cheap and environmentally-friendly method of combating nematodes and restoring the quality of soil. There are a number of strategy identified which can be employed by small-scale farmers and these are;

- Use of local farm fungi (preferably reduce use of commercial products).
- Priming short rotations (e.g. tomato, marigold, and cowpea) to fit the high-intensive cropping modes in Iraq.

Similar results were reported in Basrah, Iraq, where Paecilomyces reduced nematode presence in date palm (Hassan, 2020), but ours is the first attempt to integrate fungi with rotation into vegetables crops.

Restrictions & Future Research

1. Climate Specificity: The test was done under semi-arid conditions; the performance of the test in an irrigated or rainfed system has to be determined.
2. Long Assessments: Multi-season trials are required to assess the buildup of the resistance to nematodes.
3. Economic Evaluation: The economic evaluation will include cost-benefit to uncover the rates of adoption of the farmers (Khan et al., 2022).

Future studies may examine:

Molecular networks of fungi interactions with nematodes. Mix of several biocontrol agents (e.g. bacteria + fungi).

Conclusion

The synergy between Trichoderma harzianum and Paecilomyces lilacinus fungal biocontrol agents combined with crop rotation strategy is fast, economical, environmentally friendly, and repeatable measure in controlling Meloidogyne incognita in vegetable crops. Such an approach reduced nematode populations by 68.5 percent and increased yields by 69 percent, emphasizing its potential as a commercially viable, chemical nematicide-replacing alternative with scalability. The technique provides smallholder producers in Iraq and other similar agroecosystems with a feasible, environmentally friendly method of minimizing the damage caused by nematodes, improving the quality of soils, and increasing yields in the long term. The research in the future ought to be on the enhancement of guidelines in application and the extension of field verification in other cropping systems to create use and influence.

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Authors' Contributions

Author (First Author): Developed the study concept, created experiment designs, and oversaw field trials.

Author B: Carried out fungal isolation, identification, and bioassay tests.

Author C: Conducted statistical analysis, interpreted data, and created visual representations.

Author D: Composed the manuscript, revised edits, and managed journal submission

Conflict of Interest

The authors state that there are no conflicts of interest concerning the publication of this study.

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