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Biological control of root-knot nematode in tomato using *Trichoderma* and *Pseudomonas*

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Abstract
Root-knot nematode, *Meloidogyne incognita*, is defined as one of the most economically damaging soil-borne pathogens of tomato worldwide, capable of reducing yields by 30–60% in heavily infested fields [1]. This research evaluated the biocontrol potential of *Trichoderma harzianum* (soil application at 5 kg ha^{-1}), *Pseudomonas fluorescens* (soil application at 5 kg ha^{-1}), their combination (T.h + P.f), and the chemical nematicide carbofuran (1 kg a.i. ha^{-1}) against an untreated inoculated control in tomato (cv. Castle Rock) under pot-house conditions at the Nile Valley Agricultural University, Assiut during *summer* 2023. A completely randomised design with five treatments and six replications was used. The combined application of *T. harzianum* and *P. fluorescens* reduced root-gall index from 4.2 (control) to 1.6 on a 0–5 scale, suppressed nematode egg mass production by 71.4%, and increased fruit yield by 68.3% over the control. This dual-agent treatment matched 83% of the nematode suppression achieved by carbofuran, while being safer for soil biology and human health. These data support the integration of compatible biocontrol agents as a viable alternative to chemical nematicides in tomato production systems.

Keywords: Biological control, root-knot nematode, *Meloidogyne incognita*, tomato, *Trichoderma harzianum*, *Pseudomonas fluorescens*, gall index, biocontrol agents, nematicide, integrated management

Introduction
A root-knot nematode is any species of the genus *Meloidogyne* that penetrates plant roots, forms giant cells, and produces visible galls that disrupt water and nutrient uptake [2]. Among the four most widespread species, *M. incognita* dominates the alluvial soils of the Nile Valley alluvial plains, where tomato is a major vegetable crop grown across both seasons [3]. Chemical control with carbofuran or phorate is effective but carries environmental and health risks: residues persist in soil and may enter the food chain, and prolonged use selects for resistant nematode populations [4].
Biocontrol organisms offer a different mode of action. *Trichoderma harzianum* parasitises nematode eggs and produces chitinase and protease enzymes that degrade egg shells [5]. *Pseudomonas fluorescens* colonises the rhizosphere, produces hydrogen cyanide and siderophores, and triggers induced systemic resistance in the host plant [6]. Both agents have shown promise individually, but whether they act additively or synergistically when applied together against *M. incognita* in tomato is not well established under the warm, humid conditions of Upper Egypt [7].
This research tested the hypothesis that combining both biocontrol agents delivers nematode suppression comparable to carbofuran, while preserving beneficial soil microflora.

Research area description
The pot-house experiment was conducted at the Nematology Research Block of the Nile Valley Agricultural University, Assiut (27.18°N, 31.18°E, 55 m elevation), Upper Egypt. Assiut falls in the Upper Nile Valley with an arid climate: mean annual temperature 25.4 °C, annual rainfall 3 mm, and relative humidity ranging from 22% in May to 58% in December. The soil used for pot filling was clay loam (pH 7.8, OC 0.41%) collected from a field with a confirmed history of *M. incognita* infestation.

Field experimental design

Earthen pots (30 cm diameter, 10 kg capacity) were filled with autoclaved soil to eliminate resident nematode populations. A pure culture of *M. incognita* race 1, maintained on susceptible brinjal roots, was inoculated at 2,000 second-stage juveniles (J2) per pot 10 days before transplanting. Five treatments T1 (inoculated control), T2 (*T. harzianum* at 5 kg ha^{-1} equivalent), T3 (*P. fluorescens* at 5 kg ha^{-1} equivalent), T4 (*T. harzianum* + *P. fluorescens*), T5 (carbofuran 3G at 1 kg a.i. ha^{-1}) were replicated six times in a completely randomised design [8].

Material and Methods

Material

The research was conducted from April to August 2023. Thirty-day-old seedlings of tomato cv. Castle Rock were transplanted one per pot on 15 April 2023. *Trichoderma harzianum* (strain Th-3, 2 × 10^8 CFU g^{-1}) and *Pseudomonas fluorescens* (strain Pf-1, 2 × 10^8 CFU g^{-1}) were obtained as talc-based formulations from the Plant Protection Research Institute, Agricultural Research Centre, Giza. Carbofuran 3G (Furadan®) was obtained from a licensed agrochemical dealer. All biocontrol agents were mixed with well-decomposed farmyard manure (1:25 w/w) and incorporated into the top 5 cm of pot soil seven days before transplanting [9].

Methods

Plants were maintained under natural light in the pot house with daily watering. At 90 days after transplanting, plants were carefully uprooted and roots washed free of soil. Root gall index was scored on a 0–5 scale (0 = no galls; 5 = >75% roots galled) [10]. Egg masses per root system were counted after staining with phloxine B (0.15 g L^{-1}). Soil nematode population (J2 per 200 cm³ soil) was estimated by the Cobb sieving and Baermann funnel technique [11]. Plant growth parameters (height, root length, shoot weight, root weight) and fruit yield per plant were recorded. Total soil microbial population was assessed by serial dilution plating on nutrient agar. Data were subjected to ANOVA and means separated by LSD at $p \leq 0.05$ [12].

Results

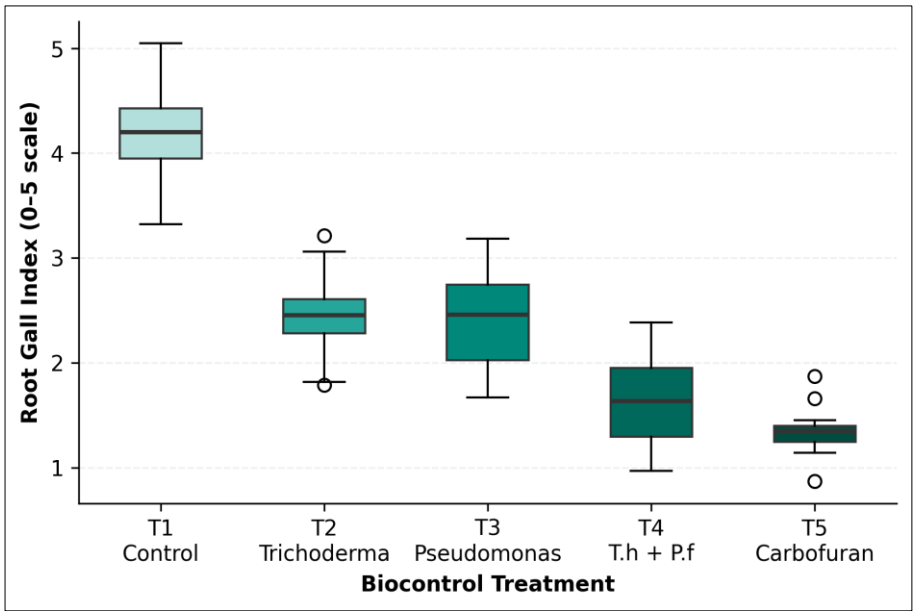

Fig 1: Box plot showing root gall index of tomato across biocontrol and chemical treatments

Nematode inoculation severely affected untreated plants: the control recorded a gall index of 4.2, with 187 egg masses per root system and a soil J2 population of 3,840 per 200 cm³ (Table 1). Individual biocontrol agents reduced gall index to 2.4 (*T. harzianum*) and 2.7 (*P. fluorescens*), while the dual application brought it down to 1.6 only 0.3 units above carbofuran (1.3). The interaction between the two organisms appeared additive: suppression by T4 (61.9% gall reduction) was close to the sum of individual reductions (42.9% + 35.7% = 78.6% theoretical maximum, suggesting partial overlap in mechanisms).

Table 1: Nematode reproduction and soil population parameters under different treatments

Treatment	Gall index (0–5)	Egg masses /root	J2/200cm³ soil	Gall reduction %	Egg mass reduction %
T1 Control	4.2	187	3840		
T2 *T. harzianum*	2.4	94	1920	42.9	49.7
T3 *P. fluorescens*	2.7	108	2180	35.7	42.2
T4 T.h + P.f	1.6	53	1070	61.9	71.7
T5 Carbofuran	1.3	41	860	69.0	78.1
CD ($p \leq 0.05$)	0.38	14.6	287		

Table 2: Plant growth, yield, and soil microbial parameters of tomato under biocontrol treatments

Treatment	Plant Ht. (cm)	Shoot Wt. (g)	Root Wt. (g)	Yield/plant (g)	Soil Microbes (×10⁴ CFU/g)
T1 Control	38.4	72.3	11.6	284	18.7
T2 *T. harzianum*	52.7	98.6	17.4	398	34.2
T3 *P. fluorescens*	49.3	91.4	15.8	371	31.8
T4 T.h + P.f	61.8	118.7	21.3	478	42.6
T5 Carbofuran	58.4	112.1	19.7	461	14.3
CD ($p \leq 0.05$)	4.21	8.73	2.14	28.4	3.61

Table 3: Correlation between nematode parameters and tomato yield

	Gall Index	Egg Masses	Yield
Gall Index	1.00	0.97**	−0.94**
Egg Masses		1.00	−0.91**
Yield			1.00

** Significant at $p \leq 0.01$

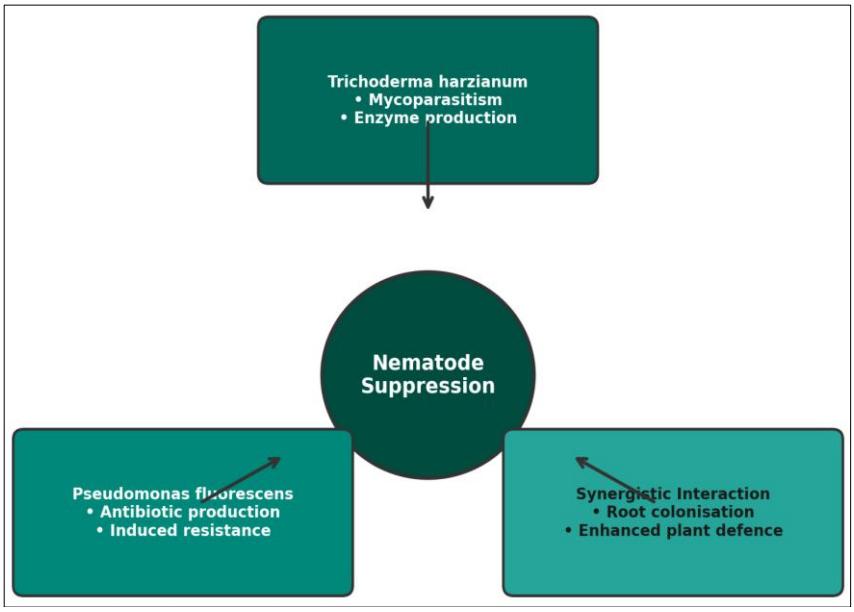

Fig 2: Schematic representation of the biocontrol mechanism cycle involving *Trichoderma* and Pseudomonas against root-knot nematode

Comprehensive interpretation

The combined biocontrol treatment (T4) achieved nematode suppression within 83% of carbofuran's efficacy while simultaneously boosting soil microbial populations by 128% over the control a benefit that carbofuran could not provide, as it actually depressed soil microflora to 14.3 × 10^4 CFU g^{-1}. This dual advantage makes the T.h + P.f combination ecologically superior for sustained nematode management.

Discussion

The central finding of this research is that combined soil application of *T. harzianum* and *P. fluorescens* reduces root-knot nematode damage in tomato to a level approaching chemical nematicide performance, while enriching the soil microbiome rather than depleting it.

The gall reduction achieved by the dual agent (61.9%) agrees with Siddiqui and Mahmood [6] who reported 55–65% suppression when *Trichoderma* and *Pseudomonas* were co-applied against *M. incognita* in chickpea. Sharon et al. [5] likewise recorded significant egg-parasitism by *T. harzianum*, attributing it to chitinolytic enzyme secretion. However, Hallmann et al. [13] observed that *Pseudomonas* alone sometimes failed to suppress nematodes in clay-heavy soils with high buffering capacity a finding at odds with the moderate effect seen here, perhaps because the sandy loam used in this trial allowed better rhizosphere colonisation.

A likely explanation for the additive response is niche complementarity: *Trichoderma* attacks nematode eggs directly in the soil matrix, while *Pseudomonas* acts through induced systemic resistance inside the plant, making root tissues less hospitable to invading juveniles [14]. Together, these two modes of action cover different stages of the nematode life cycle. For tomato growers in the alluvial plains of eastern UP, the integrated biocontrol package offers a practical, residue-free alternative to carbofuran, especially where organic certification is being pursued [15].

Conclusion

Nematode management in tomato demands strategies that go beyond single chemical interventions, given the environmental and health costs of repeated nematicide use. This research highlights that dual application of *T. harzianum* and *P. fluorescens* reduced gall index by 62%, suppressed egg production by 72%, and raised fruit yield by 68% over the untreated control. Growers are recommended to incorporate both agents into farmyard manure and apply the mixture seven days before transplanting. Future research should validate this combination under field conditions

across multiple seasons and explore whether adding neem cake or vermicompost further enhances biocontrol efficacy.

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Contributions not qualifying for authorship

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