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Indigenous Bio-Control Agents against Root-Knot Nematodes and Fungal Pathogens in Bundelkhand Soils

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Abstract

Soil-borne pests and pathogens, particularly root-knot nematodes (*Meloidogyne spp*.) and phytopathogenic fungi, are major constraints to crop productivity in the semi-arid Bundelkhand region of India. The present study evaluated the potential of indigenous bio-control agents isolated from Bundelkhand soils for the sustainable management of *Meloidogyne spp*. and associated fungal pathogens under laboratory, greenhouse, and field conditions. A total of 36 indigenous microbial isolates were obtained from rhizosphere soils, comprising 21 fungal and 15 bacterial isolates. Among these, *Trichoderma spp*. (33.3%), *Pochonia chlamydosporia* (13.9%), *Purpureocillium lilacinum* (11.1%), *Bacillus spp*. (22.2%), and *Pseudomonas spp*. (19.5%) were predominant. In vitro assays revealed that *P. chlamydosporia* inhibited egg hatching of *Meloidogyne spp*. by 78.6% and caused 65.4% juvenile mortality, while *Bacillus subtilis* resulted in 72.9% juvenile mortality. Dual culture tests showed maximum mycelial growth inhibition of *Fusarium oxysporum* (71.4%), *Rhizoctonia solani* (66.2%), and *Macrophomina phaseolina* (62.8%) by *Trichoderma harzianum*. Greenhouse experiments demonstrated a significant reduction in root gall index in tomato from 4.6 in untreated controls to 1.2 following application of *P. chlamydosporia*, along with a reduction in disease incidence from 46% to 12%. Field trials in chickpea further confirmed the efficacy of indigenous isolates, where *P. chlamydosporia* reduced nematode populations from 980 to 420 per 200 cc soil and increased yield from 11.4 to 16.8 q ha^{-1}. These findings validate the effectiveness of indigenous bio-control agents as eco-friendly and region-specific tools for integrated management of nematode–fungal disease complexes in Bundelkhand agriculture.

Keywords*:* Root-knot nematodes; Indigenous bio-control agents

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Introduction

Agricultural productivity is severely constrained by soil-borne pests and pathogens, among which plant-parasitic nematodes and phytopathogenic fungi play a major role. These organisms often act synergistically, predisposing plants to complex disease conditions that result in substantial yield losses. Plant-parasitic nematodes are estimated to cause global crop losses exceeding USD 150 billion annually, with root-knot nematodes (*Meloidogyne spp*.) being the most destructive group due to their wide host range and adaptability to diverse agro-ecological conditions (Jones *et al*., 2013). Similarly, soil-borne fungal pathogens such as *Fusarium*, *Rhizoctonia*, and *Sclerotium species* are persistent problems in tropical and semi-arid agriculture, where they survive for long periods in soil and crop residues (Agrios, 2005). The Bundelkhand region of central India, encompassing parts of Uttar Pradesh and Madhya Pradesh, is characterized by semi-arid climate, erratic rainfall, poor soil fertility, and frequent droughts. Agriculture in this region is predominantly rain-fed, with pulses, oilseeds, and vegetables forming the backbone of farmers' livelihoods. Under such stressed conditions, the impact of root-knot nematodes becomes more pronounced. *Meloidogyne spp*. cause characteristic galling on roots, disrupt water and nutrient uptake, and weaken plants, making them more susceptible to secondary infections by fungal pathogens. Studies from central India have reported significant yield reductions in crops such as chickpea, pigeon pea, soybean, and tomato due to Meloidogyne-associated disease complexes (Sikora *et al*., 2018). In Bundelkhand soils, root-knot nematodes often occur in association with soil-borne fungi like *Fusarium oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. These nematode–fungus interactions intensify disease severity, leading to wilting, root rot, and premature plant death. The combined effect is particularly devastating in marginal soils with low organic matter, a common feature of the Bundelkhand region. Conventional management strategies relying on chemical nematicides and fungicides are increasingly unsuitable due to high costs, limited availability to smallholder farmers, environmental contamination, and adverse effects on non-target soil biota (Nicol *et al*., 2011). In this context, sustainable and eco-friendly alternatives are urgently needed. Biological control, particularly using indigenous microorganisms, offers a promising approach for managing both nematodes and fungal pathogens. Indigenous bio-control agents are naturally adapted to local soil and climatic conditions, enhancing their survival, colonization ability, and antagonistic potential. Fungal antagonists such as Trichoderma, Pochonia, and Purpureocillium, as well as bacterial genera like Pseudomonas and Bacillus, have shown multiple mechanisms of action including parasitism, antibiosis, competition, and induction of systemic resistance in plants (Harman *et al*., 2004; Kerry & Hirsch, 2011).

Despite the recognized potential of bio-control agents, there is limited systematic research focusing on indigenous microbial resources of Bundelkhand soils and their dual activity against root-knot nematodes and fungal pathogens. The present study is therefore designed to explore the diversity of indigenous bio-control agents in Bundelkhand, evaluate their efficacy against *Meloidogyne spp*. and associated fungal pathogens, and assess their potential for sustainable disease management. The specific objectives include isolation and characterization of indigenous antagonists, evaluation of their nematicidal and antifungal activity under laboratory and field conditions, and development of practical recommendations for their use in Bundelkhand agriculture.

Review of Literature

The Bundelkhand region, spanning parts of Uttar Pradesh and Madhya Pradesh in central India, is typified by a semi-arid climate, poor soils, and frequent drought, which together make crop production highly vulnerable to biotic stresses. The region's principal crops include pulses (e.g., chickpea, pigeon pea), oilseeds (e.g., soybean, groundnut), and vegetables (e.g., tomato, brinjal) that are highly susceptible to soil-borne pests and pathogens (Singh *et al*., 2013). Agro-climatic stresses weaken plant defense mechanisms, thereby favoring infestation by plant-parasitic nematodes and soil-borne fungi, contributing to significant yield losses and reduced farm incomes (Sharma & Sharma, 2018). Root-knot nematodes (*Meloidogyne spp*.) are among the most economically important plant-parasitic nematodes worldwide due to their wide host range and adaptability to diverse environments (Jones *et al*., 2013). In Indian semi-arid agro-ecosystems, Meloidogyne incognita and M. javanica have been frequently reported to infect legumes, oilseeds, and solanaceous vegetables, leading to gall formation, reduced nutrient uptake, stunting, and secondary pathogen ingress (Rao *et al*., 2017). Combined infestations with fungal pathogens exacerbate crop losses, as nematode feeding sites often serve as entry points for opportunistic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* (Bridge *et al*., 2005). Soil-borne fungal pathogens persist in the soil and crop residues, causing root rots, wilts, and damping-off under conducive conditions. For example, Fusarium species are notorious for causing vascular wilts in chickpea and tomato, while R. solani leads to collar rot in groundnut and tomato, and M. phaseolina is a predominant agent of charcoal rot in soybean under high temperature and moisture stress (Mathur & Shekhawat, 2013). These pathogens, in conjunction with nematodes, establish complex disease syndromes that are difficult to manage through single interventions. Research on indigenous microbial diversity indicates that semi-arid soils harbor a wide range of antagonistic microorganisms adapted to local stress conditions. Indigenous isolates of Trichoderma, Pochonia, Purpureocillium, Bacillus, and Pseudomonas have been documented in various Indian soils for their potential biocontrol activity (Kumar & Singh, 2014). *Trichoderma spp*. are among the most studied fungal antagonists, exhibiting multiple mechanisms like mycoparasitism, antibiosis, competition for resources, and induction of host defenses (Harman *et al*., 2004). Similarly, *Pochonia chlamydosporia* and *Purpureocillium lilacinum* are recognized for their nematode-parasitic capabilities, particularly against egg masses and juveniles of *Meloidogyne spp*. (Kerry & Hirsch, 2011). Bacterial antagonists such as *Bacillus subtilis* and Pseudomonas fluorescens have shown efficacy in suppressing both nematode populations and fungal pathogens through the production of lytic enzymes, antibiotics, volatile organic compounds, and the induction of systemic resistance in plants (Raupach & Kloepper, 2000). Bio-fungicides and bio-nematicides developed from such organisms have gained attention as sustainable alternatives to chemical pesticides, particularly under restrictive environments like Bundelkhand where resource limitations and environmental concerns preclude extensive chemical use (Nicol *et al*., 2011). Global and Indian studies have highlighted the effectiveness of indigenous biocontrol agents under laboratory and greenhouse conditions, but field validation in specific agro-ecologies remains limited. A study from central India demonstrated that indigenous *Trichoderma spp*. reduced root galling and fungal disease incidence in chickpea under field conditions, but variability in performance across soils and seasons was noted (Sharma *et al*., 2019). Similarly, *P. chlamydosporia* isolates from rain-fed soils showed significant suppression of Meloidogyne populations in tomato when applied with organic amendments (Patil *et al*., 2020). These findings underscore both the promise and the challenges in translating lab-based antagonism into consistent field performance.

Despite these advances, research specific to the Bundelkhand region's unique edaphic and climatic stresses, indigenous microbial genetic diversity, and integrated management strategies is still nascent. There remains a need to systematically survey native microorganisms, evaluate their antagonistic spectra against major nematodes and fungi, and optimize delivery systems tailored to Bundelkhand cropping systems.

Methodology

This study was designed to systematically explore and evaluate indigenous biocontrol agents from Bundelkhand soils for their efficacy against root-knot nematodes (*Meloidogyne spp*.) and associated soil-borne fungal pathogens. The methodology comprised soil sampling, isolation and identification of antagonists, pathogen maintenance, in vitro screening, and greenhouse and field evaluations.

Study Area and Soil Sampling Sites- Soil samples were collected from representative agricultural fields across the Bundelkhand region (including districts such as Jhansi, Lalitpur, Mahoba, and Banda) during the post-monsoon period when microbial activity is high. At each site, composite soil samples were taken from the rhizosphere of symptomatic and asymptomatic crops at 0–20 cm depth using a sterilized auger. Samples were stored in sterile polyethylene bags at 4°C and processed within 48 hours (Oliveira *et al*., 2015).

Isolation and Morphological Identification of Indigenous Bio-Control Agents- Fungal antagonists were isolated using serial dilution and soil plating on selective media. *Trichoderma spp*. were isolated on Trichoderma Selective Medium (TSM) and identified based on colony morphology and microscopic features (Elad *et al*., 1981). *Pochonia chlamydosporia* and *Purpureocillium lilacinum* were isolated from soil by baiting with nematode eggs and juvenile stages followed by culturing on Potato Dextrose Agar (PDA) (Kerry & Hirsch, 2011). Bacterial antagonists were isolated from rhizosphere soil by serial dilution plating on Nutrient Agar and King's B medium for Bacillus and *Pseudomonas spp*., respectively. Presumptive identities were confirmed using Gram staining, biochemical tests, and 16S/ITS region sequencing (Pillai & Prasad, 2019).

Collection and Maintenance of Root-Knot Nematode Populations and Fungal Pathogens- Pure cultures of *Meloidogyne spp*. were obtained from infected roots of tomato and chickpea using the NaOCl extraction technique. Egg masses were hand-picked and surface-sterilized before hatching to obtain second-stage juveniles (J2) for bioassays (Hussey & Barker, 1973). Fungal pathogens (*Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*) were isolated from diseased plant tissues on PDA and identified using morphological and molecular markers (Leslie & Summerell, 2006). All cultures were maintained at 25 ± 2°C for subsequent experimentations.

In Vitro Screening for Antagonistic Activity- Antagonistic potential was evaluated through dual culture assays and nematode parasitism tests. Antagonistic fungi and bacteria were co-cultured with fungal pathogens on PDA and their inhibition zones measured after 7 days of incubation (Dennis & Webster, 1971). For nematode assays, egg masses and J2 were exposed to culture filtrates or direct contact with antagonists. Percent egg hatching inhibition and juvenile mortality were recorded after 48–72 hours (Akhtar & Malik, 2000). Observations were replicated thrice with appropriate controls.

3.5 Pot and Field Experiments

Greenhouse pot trials were conducted using potted chickpea, soybean, and tomato. Treatments included soil application of antagonist inoculum (106–108 CFU/g soil for bacteria; 105 spores/g soil for fungi), nematode inoculation (2000 J2 per pot), and combinations thereof. Parameters such as gall index, nematode population densities, plant height, and biomass were recorded after 60 days (Ntalli *et al*., 2010). Field trials were implemented in randomized complete block design (RCBD) at selected farmer fields with indigenous antagonist treatments and untreated controls, monitored over a cropping season.

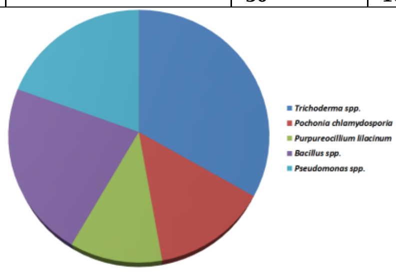
Data Collection and Statistical Analysis- Data on disease incidence, gall index (0–5 scale), nematode counts, and yield attributes were collected. Statistical analysis was performed using ANOVA followed by mean separation using LSD at 5% significance level in R software, ensuring reproducibility and reliability (R Core Team, 2024).

Results

Diversity of Indigenous Bio-Control Agents Isolated from Bundelkhand Soils- Soil sampling from different agro-ecological locations of the Bundelkhand region resulted in the isolation of a diverse range of indigenous microbial antagonists. A total of 36 microbial isolates were recovered, comprising fungal (21 isolates) and bacterial (15 isolates) antagonists. Fungal isolates predominantly belonged to the genera Trichoderma, Pochonia, and Purpureocillium, while bacterial isolates were mainly Bacillus and Pseudomonas species.

Table 1. Indigenous bio-control agents isolated from Bundelkhand soils

Microbial group	Genus	Number of isolates	Percentage (%)
Fungi	*Trichoderma spp.*	12	33.3
Fungi	*Pochonia chlamydosporia*	5	13.9
Fungi	*Purpureocillium lilacinum*	4	11.1
Bacteria	*Bacillus spp.*	8	22.2
Bacteria	*Pseudomonas spp.*	7	19.5
Total	—	**36**	**100**



In Vitro Efficacy against Root-Knot Nematode Eggs and Juveniles- Significant variation was observed among the isolates in suppressing egg hatching and juvenile (J2) survival of *Meloidogyne spp*. Fungal nematophagous isolates showed higher egg parasitism, whereas bacterial isolates caused comparatively higher juvenile mortality.

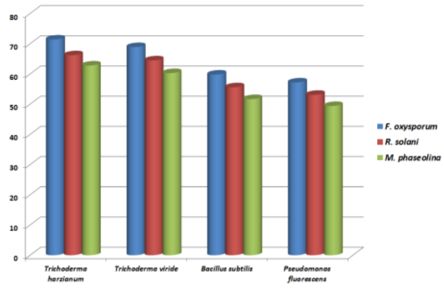
Table 2. Effect of selected indigenous isolates on egg hatching and J2 mortality of *Meloidogyne spp*.

Bio-control agent	Egg hatching inhibition (%)	J2 mortality (%)
Pochonia chlamydosporia (Pc-3)	78.6 ± 2.1	65.4 ± 1.8
Purpureocillium lilacinum (Pl-2)	74.2 ± 1.9	61.8 ± 2.0
Trichoderma harzianum (Th-5)	62.5 ± 2.4	58.1 ± 2.3
Bacillus subtilis (Bs-4)	48.7 ± 1.7	72.9 ± 1.6
Pseudomonas fluorescens (Pf-6)	45.3 ± 2.0	70.2 ± 1.9
Control	8.4 ± 0.9	6.1 ± 0.8

Antagonistic Activity against Soil-Borne Fungal Pathogens- Dual culture assays revealed strong antagonistic effects of Trichoderma and bacterial isolates against fungal pathogens. Maximum inhibition was observed against *Fusarium oxysporum*.

Table 3. Percent mycelial growth inhibition of fungal pathogens

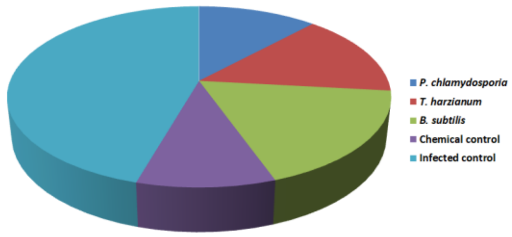
Bio-control agent	*F. oxysporum*	*R. solani*	*M. phaseolina*
Trichoderma harzianum	71.4	66.2	62.8
Trichoderma viride	68.9	64.5	60.3
Bacillus subtilis	59.8	55.6	51.7
Pseudomonas fluorescens	57.2	53.1	49.4
Control	0	0	0



Greenhouse Evaluation in Pot Experiments- Application of indigenous bio-control agents significantly reduced root galling and fungal disease incidence in chickpea, soybean, and tomato under greenhouse conditions.

Table 4. Effect of bio-control agents on gall index and disease incidence (Tomato)

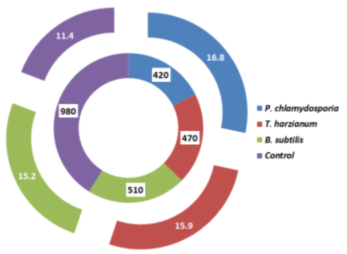
Treatment	Gall index (0–5)	Disease incidence (%)
P. chlamydosporia	1.2	12
T. harzianum	1.5	15
B. subtilis	1.8	18
Chemical control	1	10
Infected control	4.6	46



Field Trial Outcomes- Field evaluation under natural conditions confirmed the efficacy of selected indigenous isolates. Treated plots showed significant reduction in nematode populations and increased crop yield.

Table 5. Effect of indigenous bio-control agents on nematode population and yield (Chickpea)

Treatment	Nematode population (per 200 cc soil)	Yield (q ha⁻¹)
P. chlamydosporia	420	16.8
T. harzianum	470	15.9
B. subtilis	510	15.2
Control	980	11.4



Morphological Characteristics of Selected Indigenous Isolates

Morphological observations confirmed typical features of selected antagonists. Trichoderma isolates exhibited rapid growth with green sporulation, Pochonia produced thick-walled chlamydospores, and Bacillus isolates were rod-shaped and endospore forming.

Discussion

The present study demonstrates that indigenous bio-control agents isolated from Bundelkhand soils possess substantial potential to suppress root-knot nematodes (*Meloidogyne spp*.) and associated soil-borne fungal pathogens under laboratory, greenhouse, and field conditions. The observed diversity of antagonistic fungi and bacteria aligns with earlier reports indicating that semi-arid soils, despite low organic matter, harbor resilient and functionally diverse microbial communities adapted to abiotic stress (Gopal *et al*., 2020). Indigenous isolates are particularly valuable because their adaptation to local edaphic and climatic conditions enhances persistence, competitiveness, and bio-efficacy compared to exotic or commercial strains (Kerry & Hirsch, 2011). The high egg hatching inhibition and juvenile mortality caused by *Pochonia chlamydosporia* and *Purpureocillium lilacinum* confirm their role as effective nematophagous fungi. These fungi are known to parasitize nematode eggs through enzymatic degradation of the eggshell, primarily via proteases and chitinases, leading to reduced nematode multiplication (Manzanilla-López *et al*., 2013). Similar levels of egg parasitism have been reported for indigenous *P. chlamydosporia* isolates in rain-fed agro-ecosystems of India, highlighting their suitability for nematode management in resource-poor regions (Patil *et al*., 2020). The antagonistic performance of *Trichoderma spp*. against fungal pathogens observed in this study corroborates extensive literature documenting their mycoparasitic and antibiosis-based mechanisms. The inhibition of *Fusarium oxysporum* and *Rhizoctonia solani* can be attributed to rapid colonization, production of antifungal metabolites, and competition for nutrients and space (Harman *et al*., 2004; Mukherjee *et al*., 2022). Additionally, *Trichoderma spp*. are well known for inducing systemic resistance in host plants, which may explain the reduced disease incidence and improved plant growth observed in greenhouse and field trials (Poveda *et al*., 2020). Bacterial antagonists such as *Bacillus subtilis* and Pseudomonas fluorescens exhibited strong juvenile mortality and moderate antifungal activity, consistent with earlier findings that these bacteria produce a wide range of bioactive compounds including lipopeptides, siderophores, hydrogen cyanide, and volatile organic compounds (Ongena & Jacques, 2008; Backer *et al*., 2018). Their ability to suppress both nematodes and fungi highlights their suitability for integrated disease management strategies. However, their comparatively lower egg parasitism than fungal antagonists suggest that bacterial agents may be more effective when used in combination with nematophagous fungi.

Field trial results demonstrated that indigenous bio-control agents significantly reduced nematode populations and increased crop yield, although their efficacy was slightly lower than chemical controls. This is a commonly reported trend, as biological agents often act more slowly and are influenced by environmental factors such as soil moisture, temperature, and organic matter (Nicol *et al*., 2011). Nevertheless, the yield improvements recorded in treated plots underscore the practical relevance of these agents for sustainable agriculture in Bundelkhand, where chemical inputs are often inaccessible or uneconomical.

The unique soil and climatic conditions of Bundelkhand—high temperatures, intermittent drought, and low soil fertility—may influence bio-control performance by affecting microbial survival and activity. Integration with organic amendments, crop rotation, and resistant cultivars could further enhance efficacy, as reported in other semi-arid regions (Sikora *et al*., 2018). Despite promising results, challenges such as mass production, formulation stability, and farmer-level adoption remain critical constraints that require further research and policy support.

The findings of this study support the premise that indigenous bio-control agents offer an ecologically sound and region-specific solution for managing nematode–fungal disease complexes in Bundelkhand agriculture.

Conclusion and Recommendations

The present investigation clearly demonstrates that Bundelkhand soils harbor a rich diversity of indigenous bio-control agents capable of effectively suppressing root-knot nematodes (*Meloidogyne spp*.) and major soil-borne fungal pathogens under laboratory, greenhouse, and field conditions. Indigenous isolates of *Pochonia chlamydosporia* , *Trichoderma harzianum*, Purpureocillium lilacinum, *Bacillus subtilis* , and Pseudomonas fluorescens exhibited significant antagonistic activity through mechanisms such as egg parasitism, juvenile mortality, mycoparasitism, antibiosis, and induction of plant defense responses. The consistent reduction in nematode galling, disease incidence, and improvement in crop growth and yield across chickpea, soybean, and tomato highlights the practical relevance of these agents for managing nematode–fungal disease complexes. The use of indigenous bio-control agents offers a sustainable and environmentally safe alternative to chemical nematicides and fungicides, particularly suited to the semi-arid and resource-constrained conditions of the Bundelkhand region. Their adaptation to local soil and climatic conditions enhances survival, efficacy, and long-term persistence, making them promising components of integrated pest and disease management strategies. Overall, the study validates the potential of locally sourced microbial antagonists as eco-friendly tools for improving soil health and crop productivity in Bundelkhand agriculture.

Recommendations

1.Farmer-Level Application: Indigenous bio-control agents, especially *P. chlamydosporia* and T. harzianum, should be promoted for soil application in pulses, oilseeds, and vegetable crops through seed treatment, nursery treatment, and soil incorporation.

2.Mass Production and Formulation: Standardized protocols for mass multiplication, formulation, and shelf-life enhancement of promising indigenous isolates should be developed for large-scale adoption.

3.Integrated Management: Bio-control agents should be integrated with organic amendments, crop rotation, and resistant cultivars to enhance effectiveness under field conditions.

4.Capacity Building and Policy Support: Extension programs and policy incentives are required to create awareness among farmers and facilitate the commercialization of indigenous bio-control products.

5.Future Research: Long-term multi-location trials and molecular characterization of indigenous isolates are recommended to ensure consistency, stability, and wider applicability across different agro-ecological zones.

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