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Biocontrol Efficacy and Plant Growth-Promoting Traits of Indigenous *Trichoderma* Isolates against *Fusarium* Wilt in Chickpea

Varala Krishnaveni[1], Kota Sathish[2], Ashwini Govindrao Patil[1]
Kendre Ashatai Hanumantrao[1], K.D. Navgire[1]

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ABSTRACT

Background: *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* significantly limits chickpea (*Cicer arietinum* L.) productivity in semi-arid regions. Native *Trichoderma* spp. offer potential for sustainable disease management and growth promotion.
Methods: Nineteen *Trichoderma* isolates were obtained from chickpea rhizosphere soils in Marathwada, Maharashtra, during the *rabi* season, 2023-24. Isolates were screened against the pathogen using dual culture assays and eight promising isolates were identified by ITS sequencing. A pot experiment with chickpea cv. BDNGK-798 was conducted using seed and soil applications of *Trichoderma*, followed by pathogen inoculation. Growth parameters, wilt incidence and micronutrient (Zn, Cu, Fe, Mn) uptake were recorded 65 days after sowing.
Result: Eight isolates of *Trichoderma* significantly suppressed *Fusarium* wilt while enhancing germination, seedling vigour, growth, yield and micronutrient content. *T. yunnanense* (CTA2, CTP4) and *T. pleuroticola* (CTB2) were the most effective, demonstrating strong dual potential as biocontrol agents and biofertilizers under controlled conditions, indicating their applicability for improving chickpea productivity in stressed environments.

Key words: Chickpea, *Fusarium* wilt, Micronutrient uptake, Plant growth promotion, *Trichoderma*.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a vital pulse crop, particularly in semi-arid regions, with India accounting for the largest share of global production (ICAR-IIPR, 2022). In addition to being rich in protein and essential nutrients, chickpea contributes to soil fertility through biological nitrogen fixation. In India, it is cultivated over 10.91 million hectares, producing approximately 13.75 million tonnes annually (ICAR-IIPR, 2022). Among the Indian states, Maharashtra contributes about 26% of the national chickpea output; however, productivity in the Marathwada region remains low due to several biotic and abiotic stresses (Kumari and Malik, 2024, Sontakke *et al*., 2020).

Among these, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is considered the most devastating disease, causing yield losses ranging from 10% to 90%, depending on environmental conditions and pathogen virulence (Muche *et al*. 2022). Although the deployment of resistant cultivars and the application of fungicides offer partial control, these strategies often prove inadequate and raise ecological concerns due to their long-term impacts (Singh *et al*., 2022).

Biological control presents a sustainable and eco-friendly alternative, with *Trichoderma* spp. gaining increasing attention for their broad-spectrum antagonistic mechanisms. These include mycoparasitism, antibiosis, competition for nutrients and space and the induction of systemic resistance in host plants (Harman *et al*., 2004; Waghunde *et al*., 2016; Trivedi *et al*., 2020). Additionally, *Trichoderma* species are known to promote plant growth by producing phytohormones and enhancing the uptake of micronutrients, particularly iron, zinc and manganese (Aishwarya *et al*., 2020; Illescas *et al*., 2021; Siddiqui *et al*., 2025).

Despite the rising interest in *Trichoderma* based biocontrol, limited studies have explored the efficacy of native strains adapted to specific agro-ecological zones, such as the chickpea rhizosphere of Marathwada. Locally adapted isolates may offer superior effectiveness due to their compatibility with local soil and climatic conditions (Reddy *et al*., 2024; Kumari *et al*., 2025). Notably, lesser-studied species like *T. yunnanense* and *T. pleuroticola* have demonstrated promising biocontrol potential globally

[1]Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth University, Parbhani-431 402, Maharashtra, India.
[2]Department of Agricultural Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492 102, Chhattisgarh, India.

Corresponding Author: Varala Krishnaveni, Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth University, Parbhani-431 402, Maharashtra, India.
Email: drkrishnaveni1995@gmail.com

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(Yu *et al.* 2007). Pathogenic invasive microbes *Trichoderma pleuroticola* transform bacterial and fungal community diversity in Auricularia cornea crop production system (Ye *et al*. 2023). however, their role in chickpea disease suppression, plant growth promotion and micronutrient dynamics under Indian conditions remains largely unexplored.

Therefore, the present study aimed to (i) isolate and characterize native *Trichoderma* spp. from chickpea-growing soils of Marathwada, (ii) evaluate their antagonistic activity against *F. oxysporum* f. sp. *ciceris* and (iii) assess their influence on chickpea growth, yield and micronutrient uptake under controlled pot culture conditions. It was hypothesized that native *Trichoderma* isolates would exhibit strong antagonistic activity and enhance chickpea growth and micronutrient uptake more effectively due to their adaptation to local edaphic and climatic conditions.

MATERIALS AND METHODS

The experiment was conducted during the *rabi* season of 2023-24, at the Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra.

Collection and Isolation of *Trichoderma* spp.

Rhizosphere soil samples were collected from healthy chickpea plants across three agro-climatic zones of the Marathwada region. Serial dilutions (10^{-4} to 10^{-7}) of the soil samples were prepared and plated on *Trichoderma* Selective Medium (TSM) as described by Elad *et al*. (1981). Plates were incubated at 28±2°C for 5-7 days. Colonies exhibiting typical *Trichoderma* morphology were purified using the hyphal tip method (Rifai, 1969; Bisset, 1991) and maintained on potato dextrose agar (PDA) at 4°C for further use.

Antagonistic Activity *In Vitro*

The antagonistic potential of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *ciceris* (Foc) was evaluated using the dual culture technique (Dhingra and Sinclair, 1985). PDA plates were inoculated with 5 mm mycelial discs of both the pathogen and the *Trichoderma* isolate placed opposite each other. Control plates were inoculated with the pathogen alone. After 7 days of incubation at 28 ± 2°C, the percentage inhibition of pathogen growth was calculated using Vincent's formula (Vincent, 1947):

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where
C = Radial growth of the pathogen in control (mm).
T = Radial growth of the pathogen in dual culture (mm).

Molecular characterization

Genomic DNA was extracted from selected *Trichoderma* isolates and the internal transcribed spacer (ITS) region was amplified using primers ITS1 and ITS4 (White *et al.*, 1990). PCR products were sequenced and species identification was confirmed through BLAST analysis. Gel electrophoresis images and culture plate photographs were documented with high resolution and appropriate captions.

Development of *Trichoderma* formulations

For mass multiplication, selected *Trichoderma* isolates were cultured in 1 L of potato dextrose broth (PDB), each inoculated with five 5 mm mycelial discs. Cultures were incubated at 28±2°C for 14 days on a rotary shaker at 100 rpm. The biomass was harvested and mixed with sterilized talc powder in a 1:2 ratio, along with 5 g/kg of carboxymethyl cellulose to prepare talc-based formulations containing approximately 10^6 cfu/g. Shelf-life and viability were monitored over a 90-day storage period, showing stable colony-forming unit (10^6 cfu/g) counts.

Pot experiment

A pot culture experiment was carried out under greenhouse conditions during the *rabi* season of 2024 using chickpea cultivar BDNGK-798 (sourced from Agricultural Research Station, Badnapur, Maharashtra). Surface-sterilized seeds were treated with talc-based *Trichoderma* formulations at 10 g/kg seed rate. Additionally, soil in each pot was inoculated with 25 g of the formulation. Foc inoculum was applied to all treatments except the healthy control at 2% w/w, standardized based on the weight of colonized substrate (Sand maize media) mixed with soil.

The experiment was laid out in a completely randomized design (CRD) with three replications. Micronutrient content (Zn, Mn, Cu, Fe) in 30-day-old seedlings was analyzed using atomic absorption spectrophotometry following wet digestion (Rashid, 1986). At 65 days after sowing, plant growth parameters, yield attributes, nodulation and wilt incidence were recorded. Disease incidence (DI) was calculated as:

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total plants}} \times 100$$

Statistical analysis

Data were statistically analyzed using R software under a completely randomized design. Percentage data were arc-sine transformed and treatment means were compared using standard error of the mean (SEm) and critical difference (CD) at P = 0.01 (Panse and Sukhatme, 1978).

RESULTS AND DISCUSSION

Isolation and Identification of *Trichoderma* spp.

Nineteen *Trichoderma* isolates were obtained from chickpea rhizosphere soils across eight districts of Marathwada and identified based on morphological and cultural characteristics using Potato Dextrose Agar (PDA) medium, as described by Gams and Bissett (2002). The isolates were designated by location: Parbhani (CTP1-CTP4), Nanded (CTN1-CTN3), Jalna (CTJ1-CTJ2), Aurangabad (CTA1-CTA2), Latur (CTL1-CTL2), Osmanabad (CTO1-CTO2), Beed (CTB1-CTB2)

and Hingoli (CTH1-CTH2) (Fig 1 and 2). A high isolation frequency (86.36%) indicates the widespread occurrence and diversity of *Trichoderma* spp. in chickpea-growing soils, consistent with findings from Harman *et al*. (2004), who reported the dominance of *Trichoderma* in agricultural rhizospheres. This highlights their potential for further use in biological control and plant growth-promotion (Reddy *et al*., 2024; Kumari *et al*., 2025).

Biocontrol efficacy

Dual culture assays showed that all nineteen *Trichoderma* isolates inhibited *Fusarium oxysporum* f. sp. *ciceri* mycelial growth, with inhibition ranging from 39.64 to 86.30%. Eight isolates demonstrated significant inhibition (71.90-86.30%) (Fig 3), with *T. yunnanense* (CTA2) showing the highest (86.30%), followed by CTP4 (84.81%) and CTJ1 (84.08%). Other isolates such as CTN2, CTB2 and CTB1 also showed

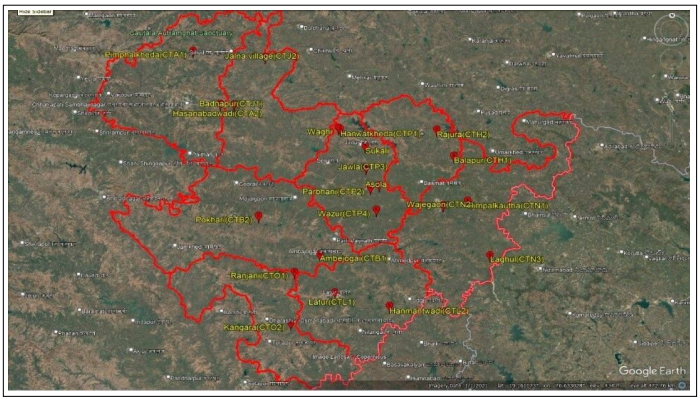

Fig 1: Geospatial mapping of chickpea rhizosphere soil and *Trichoderma* isolates across Marathwada's diverse agroclimatic zones.

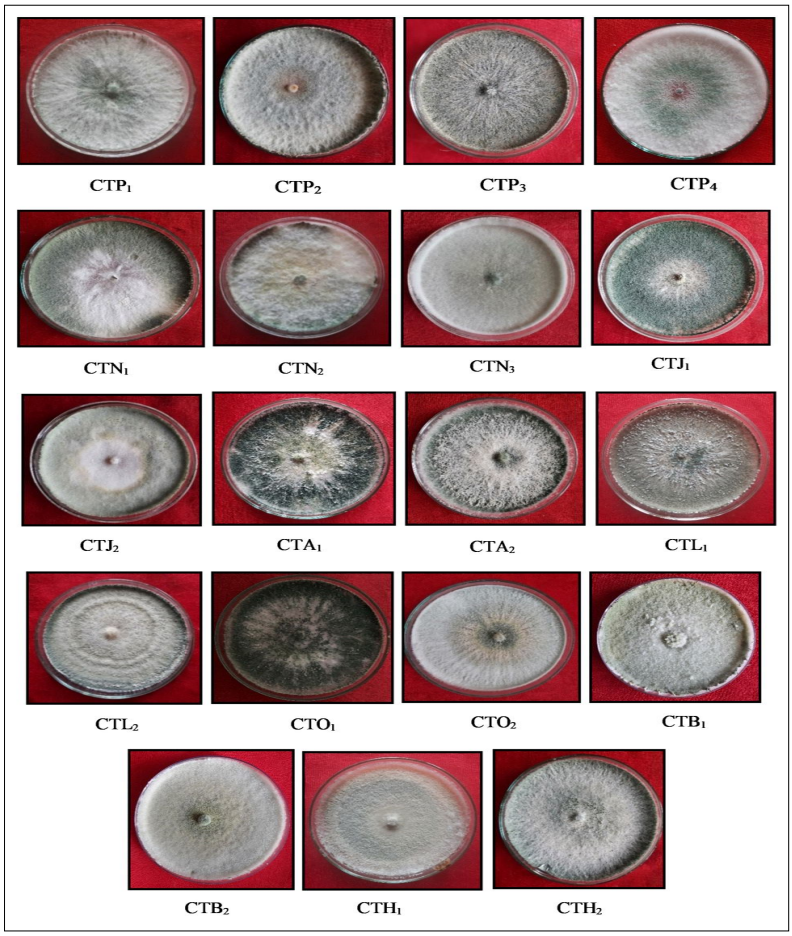

Fig 2: Nineteen *Trichoderma* isolates on potato dextrose agar medium.

strong activity. These results confirm the strong biocontrol potential of *Trichoderma* spp., especially *T. yunnanense* and *T. pleuroticola*, consistent with recent studies highlighting their efficacy in chickpea wilt management (Joshi and Sunkad 2025; Parmar and Gohel, 2024). Variability in inhibition may be due to differences in antibiotic, siderophore and enzyme production (Syed *et al*., 2023; Reddy *et al*., 2024).

Molecular identification

Eight selected isolates were molecularly confirmed using ITS1 and ITS4 primer sequencing (Fig 4). PCR amplification and ITS sequencing (~650 bp) validated species-level identification as *T. yunnanense* (CTP4, CTN2, CTJ1, CTA2, CTO2), *T. rifai* (CTL2), *T. simmonsii* (CTB1) and *T. pleuroticola* (CTB2), showing 97.93-99.83% similarity. Sequences were deposited in NCBI GenBank (OP781938-OP781945). Combined morphological and ITS analyses revealed significant diversity among isolates, highlighting their prevalence in chickpea cropping systems and potential as valuable microbial resources (Surma *et al*., 2025).

Micronutrient uptake enhancement

Data tabulated in Table 1, revealed that seed treatment and soil application of *Trichoderma* isolates significantly enhanced micronutrient uptake in chickpea at 30 DAS. *T. yunnanense* isolate CTA2 exhibited significantly highest uptake of zinc (26.57 ppm), copper (8.27 ppm), iron (46.03 ppm) and manganese (16.50 ppm), compared to control and pathogen control. Other effective isolates included *T. yunnanense* (CTP4, CTJ1, CTN2) and *T. pleuroticola* (CTB2), which also significantly improved micronutrient content. These increases are attributed to siderophore and organic acid production by *Trichoderma*, enhancing micronutrient solubility and plant availability, thereby improving soil fertility and chickpea nutrition. Similar findings have been reported by Aishwarya *et al*., 2020; Ali *et al*., 2022; Syed *et al*., 2023; Chalie-U Rokozeno *et al*., 2025, who observed improved nutrient uptake in chickpea, respectively, following *Trichoderma* application.

Plant growth promoting attributes and wilt incidence

Data on growth promoting characters of chickpea (Table 1) revealed that *Trichoderma*-treated plants showed improved germination (73.67-86.33%), vigour index (2800-4241), number of branches (3.77-5.33), nodules (7-12), pods (10.33-16.33), biomass and yield per plant (4.00-7.17 g), with CTA2 producing the maximum. *Trichoderma yunnanense* (CTA2) recorded the highest germination% (86.33%), vigour

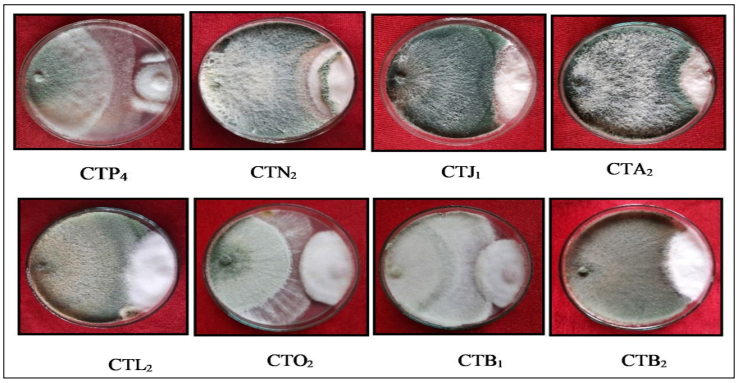

Fig 3: Antagonistic efficacy of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *ciceri* using dual culture assay.

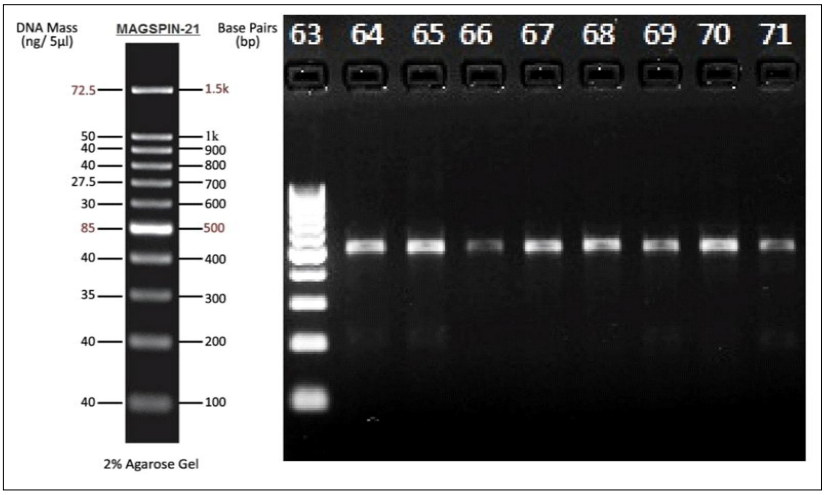

Fig 4: ITS region amplification in eight *Trichoderma* isolates (T1-T8).

Table 1: Effect of *Trichoderma* isolates on germination, micronutrient uptake, wilt incidence and plant growth-promoting traits of chickpea (*Cicer arietinum* L.).

Treatment no.	Germination (%)	Micronutrient uptake				Wilt Incidence (%)	Plant vigour Index	No. of branches/Plant	Shoot fresh Wt (g)	Shoot dry Wt (g)	Root fresh Wt (g)	Root dry Wt (g)	Nodules /plant	Pods/ plant	Yield/ plant (g)
		Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)										
T1: *T. yunnanense* (CTP_4)	83.33 (65.93)	24.40	8.17	45.58	16.33	24.17 (29.43)	3813.89	4.67	4.46	2.42	1.41	0.91	10.67	14.67	6.52
T2: *T. yunnanense* (CTN_2)	80.00 (63.41)	20.33	7.33	40.75	15.47	24.33 (29.54)	3448.00	4.00	4.33	2.27	1.27	0.80	9.33	13.67	5.13
T3: *T. yunnanense* (CTJ_1)	80.00 (63.41)	20.97	8.07	43.43	15.67	22.50 (28.31)	3501.33	4.33	4.37	2.33	1.30	0.83	9.67	14.00	5.67
T4: *T. yunnanense* (CTA_2)	86.33 (68.33)	26.57	8.27	46.03	16.50	16.50 (23.95)	4241.84	5.33	4.57	2.50	1.42	0.92	12.00	16.33	7.17
T5: *T. rifai* (CTL_2)	75.00 (59.99)	15.89	5.95	35.64	13.83	32.50 (34.74)	2913.00	3.77	4.00	1.97	0.98	0.53	7.00	10.67	4.20
T6: *T. yunnanense* (CTO_2)	73.67 (59.11)	16.63	4.39	32.66	12.67	39.67 (39.02)	2800.07	3.77	3.97	1.90	0.99	0.54	7.33	10.33	4.00
T7: *T. simmonsii* (CTB_1)	79.00 (62.70)	17.79	6.32	37.53	14.33	29.33 (32.77)	3143.41	4.00	4.20	2.13	1.07	0.63	7.67	12.33	4.93
T8: *T. pleuroticola* (CTB_2)	84.00 (66.48)	19.67	8.17	38.42	16.17	22.50 (28.29)	3554.04	4.33	4.27	2.27	1.26	0.78	9.00	13.33	5.03
T9: Pathogen control	61.33 (51.54)	15.42	4.05	31.63	11.67	61.50 (51.63)	1899.29	1.87	2.93	0.90	0.69	0.23	4.00	4.00	1.67
T10: Control	70.33 (56.98)	14.97	3.48	29.16	11.00	0	2597.64	2.83	3.77	1.70	0.90	0.46	6.00	8.33	3.23
SE(m)±	0.90	0.22	0.07	0.33	0.33	0.55	70.92	0.07	0.06	0.05	0.03	0.03	0.30	0.39	0.06
CD (P = 0.01)	2.67	0.67	0.20	0.97	0.98	1.64	210.69	0.22	0.18	0.14	0.09	0.07	0.89	1.17	0.18

Data are mean of three replications; values in parentheses represent angular-transformed data.

index (4241), number of branches per plant (5.33), nodules per plant (12.00), pods per plant (16.33) and yield per plant (7.17 g), significantly exceeding both the control and pathogen control. These values were statistically at par with treatments involving *T. yunnanense* (CTP4, CTJ1, CTN2) and *T. pleuroticola* (CTB2). Similarly, fresh and dry weights of shoots and roots were significantly enhanced under *T. yunnanense* (CTA2) and *T. yunnanense* (CTP4). Observations on wilt incidence 65 days after sowing, showed *T. yunnanense* (CTA2) had the lowest wilt incidence (16.39%), followed by *T. yunnanense* (CTJ1) at 22.50% and *T. pleuroticola* (CTB2) at 24.02%, indicating superior disease suppression by these isolates.

These findings are consistent with reports by Chohan *et al*. (2024) and Parmar and Gohel (2024), who demonstrated the plant growth-promoting and biocontrol potential of *Trichoderma* spp. in legumes. Additionally, similar improvements in growth and yield were observed in legume crops treated with *Trichoderma* isolates, as reported by Chalie-U Rokozeno *et al*., 2025; Syed *et al*., 2023. These dual benefits reinforce the role of *Trichoderma* as both biocontrol agents and biofertilizers (Venkataramanamma *et al*., 2022; Haque *et al*., 2025).

Implications and future directions

The superior performance of *T. yunnanense* (CTA2, CTP4) and the promising results from *T. pleuroticola* (CTB2) show strong potential as dual-purpose bio-inoculants for managing Fusarium wilt and enhancing chickpea yield. While these results are encouraging under controlled conditions, extensive field validation across diverse environments is essential to confirm their efficacy and consistency. Moreover, multi-gene phylogenetic analyses and detailed biochemical studies will provide deeper insights into their mechanisms and genetic stability. Further research on formulation stability and scalability will be crucial to support their commercialization and practical use. This integrated approach will facilitate the development of robust, scalable bioformulations to aid sustainable intensification of chickpea production.

CONCLUSION

This study successfully isolated and characterized indigenous *Trichoderma* spp. from chickpea rhizosphere soils of the Marathwada region, identifying *T. yunnanense* (CTA2, CTP4) and *T. pleuroticola* (CTB2) as the most effective isolates in suppressing *Fusarium* wilt and promoting chickpea growth under controlled conditions. These isolates significantly enhanced seed germination, seedling vigour, yield attributes and micronutrient uptake, demonstrating their dual potential as biocontrol agents and biofertilizers. The findings underscore the value of native *Trichoderma* strains adapted to local agroclimatic conditions for sustainable disease management and yield improvement in chickpea cultivation.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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