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Dose optimization of antagonistic fungal epiphyte for management of walnut anthracnose

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Abstract

Optimization of inoculum density is essential for the successful development of biological control agents, as it determines effective establishment and disease suppression. The present study was conducted during 2025 to optimize the inoculum dose of *Trichoderma pleuroticola* (NK-8), a highly antagonistic walnut epiphytic fungus, against walnut anthracnose under field and greenhouse conditions. Eight inoculum densities ranging from 5×10^2 to 1×10^6 spores ml^{-1} were evaluated along with an untreated control. The results revealed a positive dose-response relationship, with increase in inoculum density the disease suppression also increased. The highest disease suppression under field conditions (68.49%) was recorded at 1×10^6 spores ml^{-1} , followed by 61.16% at 5×10^5 spores ml^{-1} . Similarly, greenhouse evaluation showed maximum disease suppression of 80.30% and 72.81% at 1×10^6 and 5×10^5 spores ml^{-1} respectively. However, the two highest inoculum densities did not differ significantly ($p \leq 0.05$), indicating that 5×10^5 spores ml^{-1} provided disease control comparable to 1×10^6 spores ml^{-1} while requiring a lower inoculum load. Therefore, 5×10^5 spores ml^{-1} was identified as the optimum inoculum density for practical application of *T. pleuroticola* against walnut anthracnose.

Keywords: *Trichoderma pleuroticola*, walnut anthracnose, biological control

Introduction

Trichoderma spp. have emerged as successful and extensively studied fungal antagonists owing to their broad-spectrum activity against numerous plant pathogens. *Trichoderma* spp. naturally colonize the phyllosphere and other ecological niches, where they effectively compete with phytopathogens^[1]. Their success as biological control agents is attributed to multiple mechanisms, including mycoparasitism, competition for nutrients and space, production of antifungal metabolites, secretion of cell wall-degrading enzymes and induction of systemic resistance in host plants^[2]. Initial inoculum dose plays a critical role in determining successful colonization, persistence and subsequent suppression of the pathogen. An inadequate inoculum dose may fail to establish sufficient propagule density, whereas excessively high concentrations may not provide proportional improvement and may increase production costs. Therefore, optimization of inoculum dose is a prerequisite for the development of an effective and economically viable biocontrol strategy. In order to develop such a strategy against walnut anthracnose (*Marssonina juglandis*), this investigation was undertaken with the objective to optimize the inoculum dose of fungal epiphyte namely *Trichoderma pleuroticola*, a predetermined highly antagonistic walnut epiphyte against *Marssonina juglandis*.

Material and methods

The study was conducted during 2025 cropping season in the Division of Plant Pathology, Faculty of Agriculture, Wadura, (SKUAST-K). The promising fungal epiphyte NK-8(*Trichoderma pleuroticola*) was grown on Potato Dextrose Agar (PDA) medium at $25 \pm 1^\circ\text{C}$. Spore mass was harvested in sterile distilled water to prepare a stock of spore suspension. Spore load of the stock suspension was determined by using haemocytometer and working suspension were prepared in sterile distilled water after applying formula $n_1v_1 = n_2v_2$, where n_1 = initial stock concentration, v_1 =

volume of stock solution, n_2 =final concentration after dilution, v_2 = final volume of diluted solution.

As such eight levels of the inoculum dose viz., 5×10^2 , 1×10^3 , 5×10^3 , 1×10^4 , 5×10^4 , 1×10^5 , 5×10^5 , and 1×10^6 were prepared for the evaluation besides one untreated control. Each treatment consisted of spraying the designated inoculum load of NK-8 spore suspension uniformly on the foliage of tagged branches. Following a 12 hour interval to facilitate establishment of the antagonist, the treated branches were challenge inoculated with a conidial suspension of *Marssonina juglandis* adjusted to 1×10^6 conidia ml^{-1} using a haemocytometer [3]. Disease severity was assessed 14 days after pathogen inoculation using 0-9 disease rating scale and the disease intensity (%) was calculated by formula $[\sum(n \times v)/N \times G] \times 100$, where n = number of diseased leaves in each category, v = numerical value of the category N = total number of leaves examined, and G = highest grade value. The experiment was conducted under open field and greenhouse conditions. The treatment allocation was done on tagged branches in RBD and CRD in field and greenhouse environments respectively. The data were statistically analyzed using analysis of variance (ANOVA), and treatments means were separated by least significant difference (LSD) using R software R 4.6.1.

Results

Trichoderma pleuroticola (NK-8) produced a rapidly growing colony on Potato Dextrose Agar (PDA), covering the Petri plate within 3-4 days of incubation at $25 \pm 1^\circ\text{C}$. The colony was initially light green and powdery with circular margins, gradually turning olive green to dark green due to profuse conidial production.

Distinct concentric zonation was also evident on the colony surface. Microscopic examination revealed that the isolate NK-8 produced hyaline spores that were globose, sub-globose to ellipsoidal in shape with $2.8-4.0 \times 2.2-3.0 \mu\text{m}$ in size (Fig 1). These morphological characteristics were consistent with those described for *Trichoderma pleuroticola*.

The *Trichoderma pleuroticola*, irrespective of inoculum dose, suppressed walnut anthracnose under both field and controlled conditions (Table 1, Fig: 2). The data revealed a positive dose response with increased disease suppression at higher test doses. Lower inoculum densities (5×10^2 and 1×10^3 spores ml^{-1}) resulted in comparatively high periodic disease increase of 17.22 per cent and 16.48 per cent and provided only 18.89 per cent and 19.15 per cent disease suppression over the control respectively. The application of 5×10^3 and 1×10^4 spores ml^{-1} reduced periodic disease increase to 15.11 and 12.25 per cent with disease suppression of 22.31 and 34.35 per cent respectively. Further increase in inoculum density of 5×10^4 and 1×10^5 spores ml^{-1} further reduced periodic disease increase to 11.34 and 9.96 with disease suppression of 40.70 and 46.84 per cent respectively. The highest inoculum density (1×10^6) was more effective in reducing periodic disease increase to 5.61 per cent with disease suppression of 68.49 per cent over untreated control. However, two densities 5×10^5 and 1×10^6 showed non-significant ($p \leq 0.05$) effects. In greenhouse condition, the similar trend was also observed. So, the dose density of 5×10^5 was considered as optimum dose for providing comparable efficacy with higher dose, while requiring lower inoculum density.

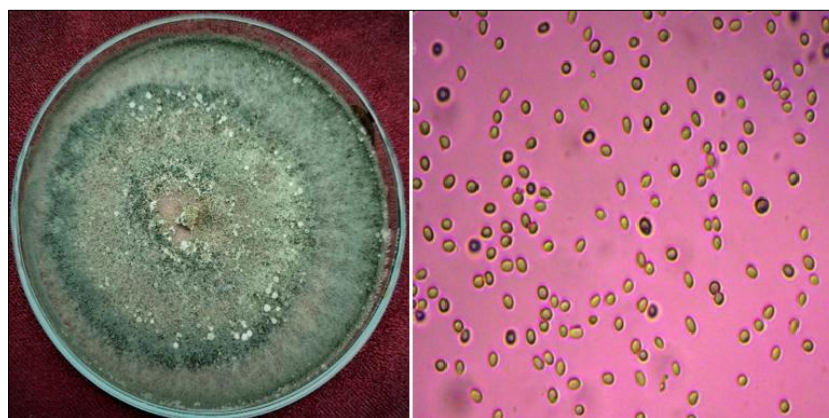


Fig 1: a) Pure colony of *Trichoderma pleuroticola* b) Conidia of *Trichoderma pleuroticola* at 400x

Table 1: Effect of inoculum load on bio-efficacy of *Trichoderma pleuroticola* (NK-8) against walnut anthracnose

Treatments (Spores ml^{-1})	Disease Intensity (%)					
	Field				Greenhouse*	
	Before spray	After spray	Periodic increase	Disease suppression over control (%)	Disease intensity (%)	Disease suppression over control (%)
T1: 5×10^2	2.00 ^a	19.23 ^{ab}	17.22 ^{ab}	18.89	11.95 ^b	26.49
T2: 1×10^3	2.69 ^a	19.17 ^{ab}	16.48 ^{bc}	19.15	11.36 ^b	30.12
T3: 5×10^3	3.31 ^a	18.42 ^{abc}	15.11 ^{bcd}	22.31	10.53 ^b	35.25
T4: 1×10^4	3.32 ^a	15.57 ^{bc}	12.25 ^{cde}	34.35	9.60 ^b	40.95
T5: 5×10^4	2.72 ^a	14.06 ^{bcd}	11.34 ^{de}	40.70	8.48 ^{bc}	47.84
T6: 1×10^5	2.64 ^a	12.60 ^{cde}	9.96 ^{ef}	46.84	5.87 ^{cd}	63.89
T7: 5×10^5	3.13 ^a	9.21 ^{de}	6.08 ^f	61.16	4.42 ^d	72.81
T8: 1×10^6	1.86 ^a	7.47 ^e	5.61 ^f	68.49	3.2 ^d	80.30
T9: Control (pathogen only)	2.05 ^a	23.71 ^a	21.66 ^a		16.26 ^a	
SEm $\pm$	0.54	2.00	1.63		1.16	
LSD (p value ≤ 0.05)	1.63	6.01	4.89		3.47	

*Initial disease score was zero

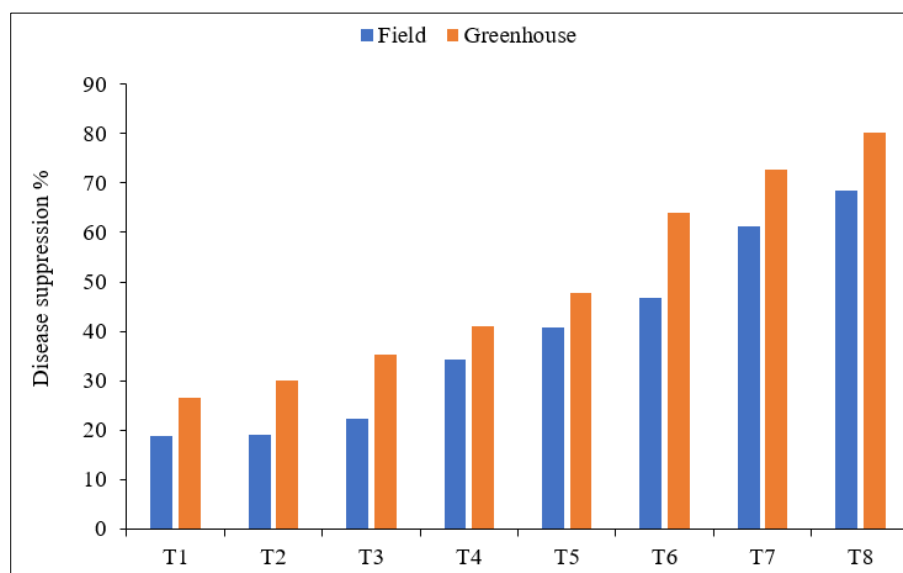


Fig 2: Suppression of walnut anthracnose with variable doses of *Trichoderma pleuroticola* (NK-8) in field and greenhouse environments

Discussion

This study was conducted to assess, for the first time, the efficacy of *Trichoderma pleuroticola* at different inoculum density against *Marssonina juglandis* on walnut. Our results clearly demonstrated that, upon increasing the inoculum density, the disease suppression was significantly enhanced, indicating that the efficacy of the antagonist was closely associated with its population density on the walnut phylloplane. Higher inoculum levels likely facilitated rapid establishment and colonization of the leaf surface, thereby limiting disease development through competition for nutrients and space, mycoparasitism, and the production of antifungal metabolites [4]. Although the highest disease suppression was recorded at 1×10^6 spores ml^{-1} , this treatment was statistically at par with 5×10^5 spores ml^{-1} , indicating that further increasing the inoculum concentration beyond 5×10^5 spores ml^{-1} did not provide a significant additional reduction in disease intensity. Therefore, 5×10^5 spores ml^{-1} may be considered the optimum inoculum density for practical application, as it offers comparable disease control while requiring a lower quantity of inoculum, making it more economical and suitable for large-scale use. The improved disease suppression observed at higher inoculum densities may also be attributed to the greater production of antimicrobial compounds and lytic enzymes by *Trichoderma*, including volatile and non-volatile metabolites, hydrolytic enzymes, ammonia, hydrogen cyanide, and other secondary metabolites that inhibit pathogen growth and infection [5]. These mechanisms collectively contribute to effective biological control once the antagonist has established a sufficient population on the host surface. The present findings are consistent with earlier reports demonstrating the positive relationship between antagonist population density and disease suppression. Padder *et al.* reported that increasing the concentration of endophytic microorganisms significantly reduced the incidence and severity of apple scab [6]. Similarly, Bonaterra *et al.* and Francés *et al.* observed that higher populations of *Pantoea agglomerans* EPS125 effectively suppressed powdery mildew and reduced disease incidence in stone fruits [7, 8]. Comparable observations were also reported by Navaneetha *et al.* found that increasing the conidial concentration of *Trichoderma* spp. enhanced the management of *Alternaria* leaf blight of sunflower and gray mold of castor, with higher inoculum levels providing superior disease control [9].

References

1. Sariah M, Choo CW, Zakaria H, Norihan MS. Quantification and characterization of *Trichoderma* spp. from different ecosystems. *Mycopathologia*. 2005;159(1):113-117.
2. Thambugala KM, Daranagama DA, Phillips AJL, Kannangara SD, Promputtha I. Fungi vs. fungi in biocontrol: An overview of fungal antagonists applied against fungal plant pathogens. *Frontiers in Cellular and Infection Microbiology*. 2020;10:604923.
3. Booth C. Introduction to general methods. In: Booth C, editor. *Methods in Microbiology*. Vol. 4. London: Academic Press; 1971. p. 1-49.
4. Delgado-Ramírez CS, Hernández-Martínez R, Sepúlveda E. Rhizobacteria associated with a native Solanaceae promote plant growth and decrease the effects of *Fusarium oxysporum* in tomato. *Agronomy*. 2021;11(3):579.
5. Bailey BA, Bae H, Strem MD, Crozier J, Thomas SE, Samuels GJ, *et al.* Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biological Control*. 2008;46(1):24-35.
6. Padder SA, Mansoor S, Bhat SA, Baba TR, Rather RA, Wani SM, *et al.* Bacterial endophyte community dynamics in apple (*Malus domestica*) germplasm and their evaluation for scab management strategies. *Journal of Fungi*. 2021;7(11):923.
7. Bonaterra A, Mari M, Casalini L, Montesinos E. Biological control of *Monilinia laxa* and *Rhizopus stolonifer* in postharvest stone fruit by *Pantoea agglomerans* EPS125 and putative mechanisms of antagonism. *International Journal of Food Microbiology*. 2003;84(1):93-104.
8. Francés J, Bonaterra A, Moreno MC, Cabrefiga J, Badosa E, Montesinos E. Pathogen aggressiveness and postharvest biocontrol efficiency in *Pantoea agglomerans*. *Postharvest Biology and Technology*. 2006;39(3):299-307.
9. Navaneetha T, Prasad RD, Venkateswara L. Liquid formulation of *Trichoderma* species for management of gray mold in castor (*Ricinus communis*) and *Alternaria* leaf blight in sunflower (*Helianthus annuus*). *Journal of Biofertilizers & Biopesticides*. 2015;6(1):149.