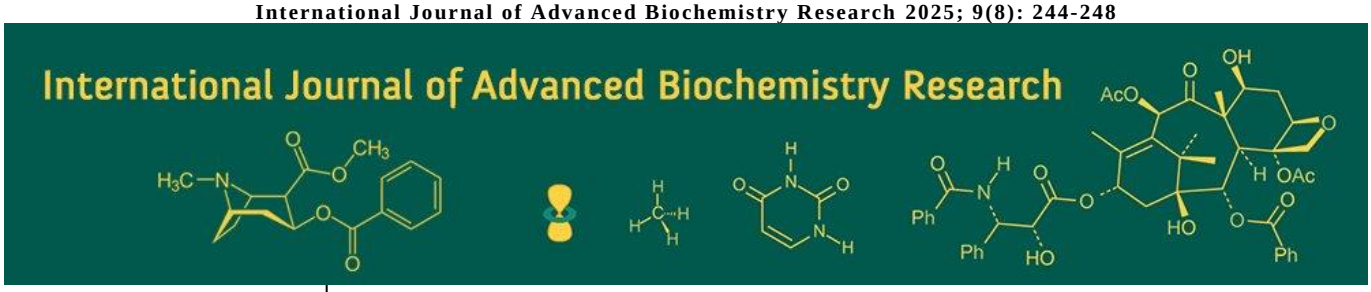

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IN Saiyed
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Dr. NM Gohel
Professor, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
IN Saiyed
Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Harnessing endophytic *Trichoderma* for the suppression of dry root rot disease in mungbean

IN Saiyed and NM Gohel

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Abstract
Mungbean [*Vigna radiata* (L.) Wilczek] is a protein-rich legume crop of significant nutritional and economic importance. However, its productivity is severely hampered by dry root rot, caused by *Macrophomina phaseolina*, a seed and soil borne pathogen prevalent under hot and dry conditions. To explore eco-friendly disease management alternatives, a pot experiment was conducted during *Kharif*, 2024 to assess the bioefficacy of different endophytic *Trichoderma* spp. applied through seed biopriming, soil application and foliar spray. Among the treatments, *Trichoderma asperellum* isolate Tr1 demonstrated the highest effectiveness by significantly improving seed germination, root and shoot growth, seedling vigour and dry weight while recording the lowest disease incidence and highest disease reduction over control. Its performance was statistically at par with *T. asperellum* Tr2 and the fungicide check (carbendazim 12% + mancozeb 63% WP). The study highlights *T. asperellum* Tr1 as a promising biocontrol agent for sustainable dry root rot management in mungbean cultivation.

Keywords: *Trichoderma* spp., *Macrophomina phaseolina*, dry root rot, endophytes

1. Introduction
Mungbean [*Vigna radiata* (L.) Wilczek] is one of the most important and short duration pulse crops that fit well in various cropping systems in India. It is a native Indian crop that is also referred to as green gram or moong. This pulse contains protein, carbohydrates, fiber, oil, vitamins and minerals like potassium, calcium and phosphorus. Mungbean has an average global yield of 721 kg/ha, with approximately 7.3 million hectares cultivated worldwide. In India, it is the third most important pulse crop after chickpea and pigeon pea (Nair and Schreinemachers, 2020) [9]. Mungbean is grown in almost all parts of the country in the Summer and *Kharif* season. In 2024, India's mungbean cultivation was projected to cover 5.18 million hectares, producing 3.10 million tonnes with a productivity of 598 kg/ha. Mungbean alone accounts for 10 per cent of total pulse production and occupies 16 per cent of the pulse cultivation area (Anonymous, 2024) [4].
The productivity of this crop is low due to several biotic and abiotic stresses affecting different crop stages. Several fungal (Pandey *et al.*, 2018) [12] and viral diseases (Nair *et al.*, 2017) [10] pose challenges to mungbean production. One of the major threats is dry root rot, caused by the soil and seed-borne fungus *Macrophomina phaseolina*. This polyphagous pathogen infects over 500 plant species and causes significant yield losses in crops like soybean, sorghum, groundnut and mungbean worldwide (Khan, 2007) [6]. Favourable conditions for DRR include high temperatures (30-35 °C) and low soil moisture. The pathogen survives in the soil for 2-15 years through microsclerotia. In mungbean, it causes sudden wilting, especially after flowering, with dark lesions and necrotic symptoms at the plant base. Traditional chemical control poses environmental risks due to the overuse of synthetic fungicides. As a result, there is growing interest in biological control methods, which offer a sustainable, eco-friendly and cost-effective alternative to managing *M. phaseolina* and other soil-borne pathogens.

2. Materials and Methods
The effectiveness *Trichoderma* isolates in controlling dry root rot of mungbean was assessed under pot conditions using the susceptible cultivar GAM 5. The experiment was conducted in the polyhouse of the Department of Plant Pathology, BACA, AAU, Anand, following a

Completely Randomized Design (CRD) with eight treatments and three replications. *M. phaseolina* sick pots were prepared as per the method described by Nene *et al.* (1981). The inoculum was developed in conical flasks containing sand-maize medium, sterilized at 121 °C and 1.5 kg/cm² for 20 minutes, and inoculated with a 5 mm disc of *M. phaseolina*. These flasks were incubated at 25 ± 2 °C for 15 days. After incubation, the colonized maize grains were mixed with a sterilized soil mixture (vermicompost and sand in a 1:3 ratio). Experimental pots were first washed with tap water and disinfected using a 4% formaldehyde solution before being filled with the inoculated soil. The sick pot technique was then employed to evaluate the efficacy of different *Trichoderma* formulations against dry root rot, with each treatment repeated three times for accuracy and consistency (Abo-Elyousr *et al.*, 2014) [2].

Treatment details

Tr. No.	Treatments
T_1	Seed biopriming + soil application + foliar spray of *T. asperellum* isolate Tr1+ sick soil
T_2	Seed biopriming + soil application + foliar spray of *T. asperellum* isolate Tr2 + sick soil
T_3	Seed biopriming + soil application + foliar spray of *T. harzianum* isolate Tr6+ sick soil
T_4	Seed biopriming + soil application + foliar spray of *T. asperellum* isolate Tr8 + sick soil
T_5	Seed biopriming + soil application + foliar spray *T. asperellum* isolate Tr14+ sick soil
T_6	Seed treatment + soil application of carbendazim 12% + mancozeb 63% WP + sick soil (Fungicide treated check)
T_7	Seed treated with pathogen + sick soil (Negative control)
T_8	Untreated seed + healthy soil (Positive control)

Application of potent endophytic *Trichoderma* spp.

The spore suspension of potent endophytic *Trichoderma* spp. was applied at three different stages: soil application, seed biopriming and foliar sprays.

Soil application and seed biopriming were done prior to sowing, while foliar sprays were done during the seedling stage at 7, 14, 21 and 28 days after germination.

To prepare the fungal cultures, *Trichoderma* spp. was subcultured on a PDA medium and incubated at 25 °C in darkness for two weeks. Spores were harvested by scraping the surface of each culture with a sterile camel-hair brush into a 100 ml glass beaker containing 50 ml of sterile distilled water plus Tween 80 (0.1% v/v). The conidial suspension was stirred, filtered, adjusted to a concentration of $1x10^8$ conidia/ml and used for inoculation.

Before the application of endophytes, seeds were disinfected with 1 per cent NaOCl for three minutes, washed with distilled water three times and dried on sterile blotter paper for 30 minutes. Subsequently, 120 seeds were immersed in 30 ml of conidial suspension ($1x10^8$ conidia/ml) of potent endophytes for four hours while shaking at 180 rpm. The inoculated seeds were then dried in sterile Petri plates for 20 minutes before sowing. The remaining seeds were treated with deionized water containing Tween 80 (0.1% v/v) as a control.

For soil treatment, 5 ml of conidial suspension per pot ($1x10^8$ conidia/ml) was applied to the soil surface before sowing. Control pots were receiving the same volume of sterile deionized water containing Tween 80 (0.1% v/v).

One milliliter of conidial suspension ($1x10^8$ conidia/ml) was sprayed on the first two leaves of plants in each pot. Control plants were sprayed with 1 ml of sterile deionized water containing Tween 80 (0.1% v/v) (Sanchez-Rodriguez *et al.*, 2018; Alves *et al.*, 2021) [13, 3].

Seed treatment and soil application of carbendazim 12% + mancozeb 63% WP were applied @ 3 g/kg seeds and 2.5 g/litre of water, respectively.

Germination count, root and shoot length were measured, after 10 and 20 days of germination, respectively.

The per cent seed germination was calculated as follows:

$$\text{Germination (\%)} = \frac{\text{Numbers of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Vigour index I and Vigour index II were calculated by the formula given by Abdul-Baki and Anderson (1973) [1].

Vigour index I = Seedling length (cm) × Seed germination (%)

Vigour index II = Seedling dry weight (g) × Seed germination (%)

Per cent disease incidence was estimated as follows:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total number of plants}} \times 100$$

The percentage of disease reduction compared to the control was calculated using the following formula (Wheeler, 1969) [15].

$$\text{Disease reduction over control (\%)} = \frac{\text{PDI in control-PDI in treatment}}{\text{PDI in control}}$$

3. Results

During *Kharif* 2024, an experiment was conducted in pot conditions to evaluate the effectiveness of five promising endophytic *Trichoderma* isolates in enhancing plant growth and managing disease. The results, detailed in Table 1 and illustrated in Figure 1, highlight the influence of seed biopriming in combination with soil and foliar application of the selected isolates on key parameters such as seed germination (%), root and shoot length (cm), vigour index I, II, disease incidence (%) and disease reduction over conrol (%) in mungbean plants.

A. Seed germination

The data revealed a significant result across all the treatments compared to the pathogen-treated check. Treatment T_1, T_2 and T_6 (fungicide-treated check) found at par with each other. The germination per cent was in the range of 66.67 to 96.67 per cent, in which the highest germination (96.67%) was recorded in treatment T_1 i.e., seed biopriming + soil application and foliar spray of *T. asperellum* isolate Tr1 + sick soil. Conversely, the lowest germination (66.67%) was observed in treatment T_7 i.e., seed treated with pathogen + sick soil.

B. Root length
Among all the endophytes treated plants, the highest root length of 6.74 cm was recorded in treatment T_1, *i.e*., seed biopriming + soil application + foliar spray of *T. asperellum* isolate Tr1 + sick soil, which was found statistically at par with treatment T_6 (chemical fungicide-treated check) showing a root length of 6.52 cm. In contrast, the shortest root length was observed in treatment T_7 (seed treated with pathogen + sick soil) at 3.93 cm.

C. Shoot length
All the *Trichoderma* inoculated treatments, as well as treatments T_6 and T_8 found to be at par with each other. Treatment T_1, which involved seed biopriming, soil application, and foliar spray of *T. asperellum* isolate Tr1 under sick soil conditions, recorded the highest shoot length of 16.33 cm.

D. Vigour index I
All the treatments showed a significantly higher vigour index as compared to the pathogen-treated check T_7, *i.e*., seed treated with pathogen + sick soil (924.38). Among the endophyte-treated treatments, T_1 (seed biopriming + soil application + foliar spray of *T. asperellum* isolate Tr1 + sick soil) was the most effective, achieving the highest vigour index of 2229.50.

E. Seedling dry weight
Among all the treatments, T_1 (seed biopriming + soil application + foliar spray of *Trichoderma asperellum* isolate Tr1) recorded the highest dry weight (1.22 g) and was statistically superior to all other treatments. Most of the remaining treatments exhibited mediocre performance in terms of dry weight, with the lowest value (0.87 g) observed in T_7 (seed treated with pathogen + sick soil).

F. Vigour index II
The treatment T_1 (Seed biopriming + soil application + foliar spray of *Trichoderma asperellum* isolate Tr1) recorded the highest vigour index (118.22), significantly outperforming all other treatments. Whereas, the pathogen-inoculated control (T_7) recorded the lowest vigour index (59.32).

G. Disease incidence
Among the various treatments, T_1 (seed biopriming + soil application + foliar spray of *Trichoderma asperellum* isolate Tr1 in sick soil) recorded the lowest disease incidence (12.02%) and the highest disease reduction over control (80.28%), indicating its superior efficacy in disease management. In contrast, the highest disease incidence among the endophyte-treated plants was observed in T_3 (seed biopriming + soil application + foliar spray of *T. harzianum* isolate Tr6), recording 33.40% disease incidence and the lowest disease reduction over control (44.98%).

4. Discussion
These findings align with the results of Vinayarani and Prakash (2018) [14], who reported that among several effective endophytic isolates, including *T. harzianum* (Thar DOB-2), *T. atroviride* (Tatro DOB-17), *T. asperellum* (Tasp DOB-19) and *T. harzianum* (Thar DOB-31), the isolate *T. harzianum* Thar DOB-31 was the most effective, recording the lowest per cent disease index (PDI) for rhizome rot (13.8%) and leaf blight (11.6%) in turmeric.
Similar observations were reported by Gonzalez *et al.* (2020) [5] in watermelon plants infected with *M. phaseolina*. The two most effective endophytic isolates, *Trichoderma lentiforme* and *T. harzianum*, showed disease incidence of 0% and 50%, respectively, in the infected plants.
Larran *et al.* (2023) [7] evaluated the effectiveness of eight endophytic *Trichoderma* strains applied as seed coatings to control *M. phaseolina* in soybeans under greenhouse conditions. The treated seeds, sown in pathogen-inoculated soil, showed reduced charcoal rot symptoms and generally improved germination rates.
Manjari (2024) [8] studied the effectiveness of endophytic fungi from medicinal plants in controlling dry root rot in chickpea caused by *M. phaseolina* under pot conditions. The highest disease reduction was achieved with seed biopriming, soil application, and foliar spray of *Chaetomium globosum* (84.61%), followed by *T. asperellum* (76.92%).

Table 1: Effect of potent endophytic *Trichoderma* spp. on growth parameters and DRR incidence in mungbean under sick pot conditions

Tr. No.	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index I	Dry weight (g)	Vigour index II	Disease incidence (%)	Disease reduction over control (%)
T_1	96.67^{a}	6.74^{a}	16.33^{a}	2229.50^{a}	1.22^{a}	118.22^{a}	20.22^{d} (12.02)	80.28
T_2	95.00^{a}	8.18^{ab}	16.04^{a}	2116.58^{a}	1.19^{ab}	113.40^{ab}	21.94^{d} (14.06)	76.76
T_3	86.67^{b}	6.93^{c}	14.89^{a}	1782.20^{b}	1.10^{bc}	95.17^{c}	35.30^{b} (33.40)	44.98
T_4	91.67^{ab}	6.12^{ab}	15.93^{a}	2022.37^{ab}	1.14^{ab}	104.78^{bc}	27.88^{c} (21.88)	64.12
T_5	91.67^{ab}	7.26^{bc}	15.82^{a}	2012.80^{ab}	1.12^{ab}	102.97^{bc}	30.89^{c} (26.36)	56.64
T_6	95.00^{a}	8.11^{ab}	16.27^{a}	2162.48^{a}	1.19^{ab}	113.63^{ab}	21.89^{d} (13.97)	76.94
T_7	68.33^{d}	3.93^{d}	9.61^{c}	924.38^{d}	0.87^{d}	59.32^{e}	51.36^{a} (60.99)	-
T_8	80.00^{c}	5.38^{c}	13.01^{b}	1470.67^{c}	1.01^{c}	80.80^{d}	37.41^{b} (36.90)	39.29
S.Em.±	2.20	0.22	0.59	79.04	0.034	3.97	1.01	-
C.D. at 5%	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	-
C.V. (%)	4.33	6.60	6.98	7.43	5.34	6.98	5.68	-

Note: Figures in parentheses are re-transformed values while those outside of parentheses are transformed values of arcsine
Treatment means with the letter/letters in common are not significant by Duncan's new multiple range test (DNMRT) at a 5% level of significance

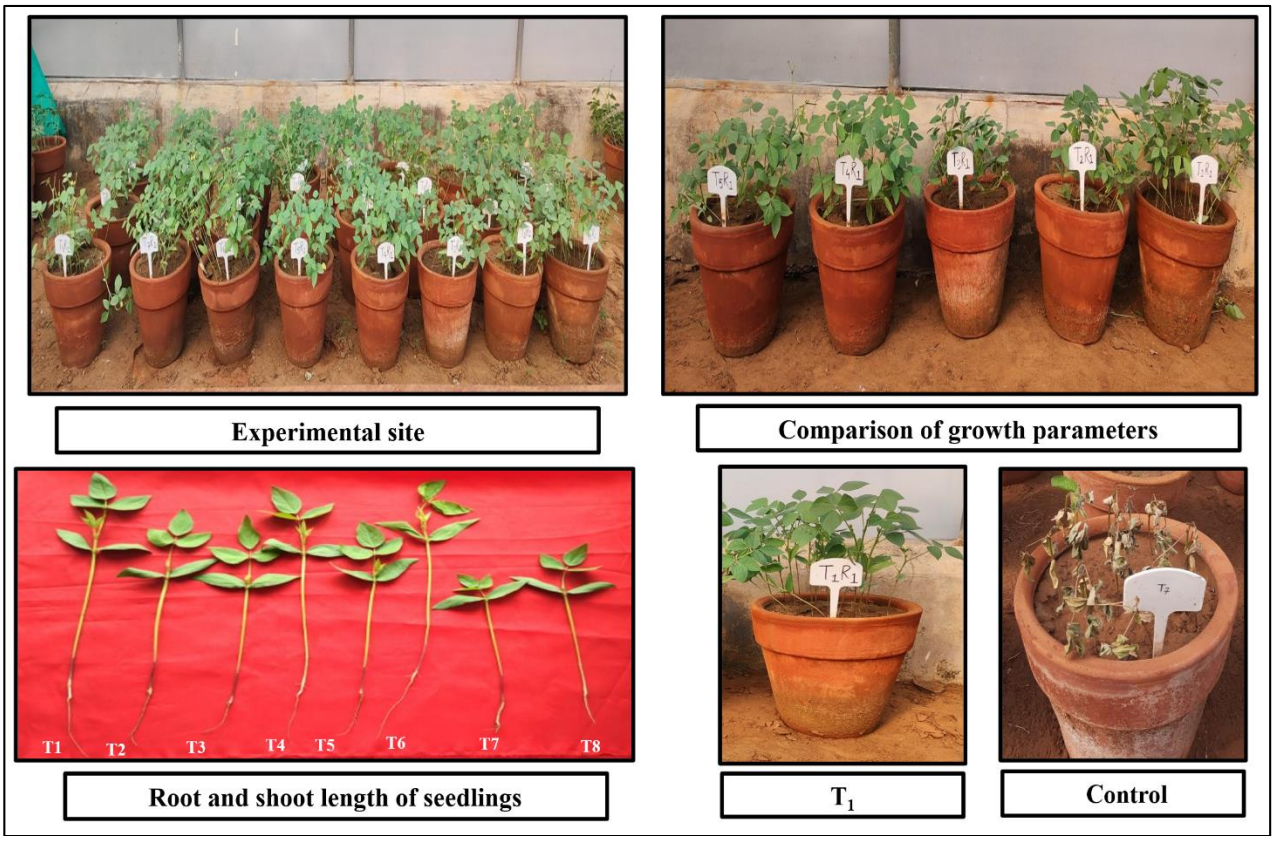

Fig 1: Effect of seed biopriming, soil application and foliar spray of efficient indigenous *Trichoderma* isolates against DRR in mungbean under sick pot conditions

Conclusion

In a nutshell, among all the treatments, T_1 (seed biopriming + soil application + foliar spray of *Trichoderma asperellum* isolate Tr1) consistently performed best across all measured parameters. It recorded the highest seed germination (96.67%), root length (6.74 cm), shoot length (16.33 cm), dry weight (1.22 g), vigour index I (2229.5), vigour index II (118.217), and the lowest disease incidence (12.02%) with the highest disease reduction over control (80.28%). Treatments T_2 (*T. asperellum* isolate Tr2) and T_6 (carbendazim 12% + mancozeb 63% WP) also performed well and were statistically comparable in several parameters. Conversely, T_7 (pathogen-inoculated check) recorded the poorest results across all observations. These findings underscore the efficacy of *T. asperellum* Tr1 as a promising biocontrol agent and a sustainable alternative to chemical fungicides for dry root rot management in mungbean.

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