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Soil application of different species of *Trichoderma* for the management of charcoal rot of faba bean caused by *Macrophomina phaseolina*

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Abstract
An experiment was conducted to determine the effect of soil application of *Trichoderma harzianum, T. hamatum, T. viride, T. virens, T. atroviride* and *T. koningii* against charcoal rot of faba bean (*Vicia faba* L.) caused by *Macrophomina phaseolina* under pot condition. Inoculation with *M. phaseolina* resulted in a significant decline in the plant growth (18-23%), biomass production (15-25%), and root nodulation (15-20%) over control. Treatments with *Trichoderma* spp. however reduced root rot severity (36-43%) and subsequently improved the plant growth (7-13%), seed germination (10-15%) and nodulation (20-25%) of infected faba bean plant. *T. harzianum* and *T. hamatum* proved to be most efficient biocontrol agents in reducing the disease severity (40-43%), and subsequent improving the plant growth (9-63%) and root nodulation (85 & 90%).

Keywords: disease management, *vicia faba, macrophomina phaseolina*, *trichoderma* spp

Introduction
Faba bean or broad bean (*Vicia faba* L.) is among the oldest legume crops in the world. It is major source of dietary proteins of high biological value, energy, vitamins and mineral (Joshi *et al*., 2014) [4]. In India, Faba bean is cultivated in the Northern states during winter (Rabi) in plains and during rainy season (Kharif) in hilly and mountains region. Faba bean grown as sole crop and as intercropped or mixed crops with variety of combination even as guard or border crop in eastern India (Singh *et al*., 2012) [12]. However, productivity of the crop is quite lower than the global average in India. Constraints that contribute to low productivity of faba bean include lack of good quality seeds and improper cultural practices and also various abiotic and biotic factors (Singh *et al*., 2009) [2]. Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid has been considered as one of the most devastating diseases of faba bean (Singh *et al*., 2007) [13]. Its prevalence is favoured by hot, dry weather or when unfavourable environmental conditions stress the plant (Wrather *et al*., 2001) [15]. Faba bean is susceptible to *M. Phaseolina* at different growth stages and the fungus attack all plant parts (Agrios, 2005) [1]. Pin head size dark coloured pycnidia appear on the epicotyls and hypocotyls followed by seedling death due to obstruction of xylem vessels. Economic losses due to dry root-rot on different crops may reach up to an extent of 80% (Sen, 2000) [11].
Seed and soil borne nature of the *M. phaseolina* creates difficulties for an effective disease management. Since the fungus survive in soil for longer periods, its management through biocontrol agent may prove much effective. *Trichoderma* species have been mainly registered as effective antagonists of soilborne pathogens, such as *Macrophomina phaseolina* (Khan and Gupta, 1998) [7], *Sclerotinia minor* (Ibarra-Medina *et al*., 2010) [3], *S. sclerotiorum* (Mansour *et al*., 2008), *Fusarium* spp. (Khan *et al*., 2011) [5], *Rhizoctonia solani* (Khan *et al*.2014) [5], *Phytophtora* spp. (Mpika *et al*., 2009), *Pythium* spp. (Mishra, 2010), etc. In addition to their antagonistic activity, *Trichoderma* strains have also demonstrated growth promoting properties enhancing seed germination, shoot and root length and vigour indexes of various plant seeds (Khan and Anwer, 2011) [6]. However, the effectiveness of *Trichoderma* spp. against *M. phaseolina* infecting faba bean has been rarely tested. Hence, the present study was aimed to study the efficacy of six *Trichoderma* spp. through soil application on charcoal rot of faba bean under pot condition.

Materials and Methods
Isolation, Identification and mass culture of *Macrophomina phaseolina* and *Trichoderma* spp.
Pure cultures of *Machrophomina phaseolina* and six species of *Trichoderma* viz., *T. harzianum* (Rifai), *T. viride* (Pers), *T. koningii* (Oudem), *T. hamatum* (Bonord) Bainier, *T.*

atroviride (Bissett), and *T. virens* (Rehner and Samuels) were procured from I.A.R.I, New Delhi. Mass cultures of *Trichoderma* spp. and *M. phaseolina* were prepared on potato dextrose broth (PDB) in 500 ml conical flasks. After incubation for 7 days at 27±2ºC, the mycelial mats were collected from the flasks, and homogenized in the distilled water. The 5ml fungus suspension containing 1gm mycelium of *M. phaseolina* or *Trichoderma* spp. (10^{4-5} conidia/ ml), was applied to 25 cm diameter pots filled with 1.5 kg autoclaved soil compost mixture (3:1).

Treatment and plant culture

The suspension of *Trichoderma* spp. was applied a day after the pathogen inoculation, and the seed sowing was done a day later. The seeds of *V. faba* cv. Pusa Sumeet treated with commercial *Rhizobium* were sown @ 10 seeds/ pot. In total 14 treatments, seven without pathogen (uninoculated) and seven with pathogen (inoculated) were maintained with 5 replicates/ treatment (Table 1). The pots were arranged in a CRBD on the roof receiving uniform sunlight. The pots were watered daily. The experiment was terminated three months after sowing. During this period, pots were watered on alternate days. The Seed germination was recorded two weeks after sowing. Thereafter, the seedlings were thinned to one seedling/ pot. At harvest, plants were gently uprooted for maximum root recovery and disease severity, root nodulation, plant growth (length of root and shoot) and biomas production (fresh and dry weight) were determined. The Disease severity was measured on the basis of 0-5 scale, where 0=No rotting and the plant without any visible symptoms; 1=1-10% rotting; 2=11-25% rotting; 3=26-60% rotting; 4=61-80% rotting; 5=80-100% rotting.

Population of *M. phaseolina* in soil

Soil population was determine at planting 1 month, 2 month, 3 month, 3.5 month in terms of CFU using the dilution plate method (waksman) 10 gram of solution was collected from the rhizosphere of most of each pot and process separately to determine population of pathogen. To transfer the soil in a conical flask first we have shade dried and sieved the soil and then 100 ml of water was added the suspension was shaken for 10 min and serially diluted to 10^{-5}. 0.1 ml suspension was spread over solidified PDA from final solution under laminar flow. 5 plates were maintained from each treatment then incubated them 25±2ºC for 6 days and colonies were counted under colony counter (American optical Darkfield Quebec Colony Counter Model 3330).

Statistical analysis

The observations taken from five replicate pots on seed germination, plant growth and biomass production, and root nodulation were analysed by two factor analysis of variance (ANOVA) and least significance difference (LSD) was calculated p≤0.05 using MINITAB-T on Windows. Percent variation over control was also calculated. The means of observations are presented in tabular and figure forms.

Result and Discussions

Plant growth

Faba bean plants cv. Pusa Sumeet grown in the soil inoculated *M. phaseolina* became stunted in growth, and progressive wilting, premature dying, and loss of vigour were observed. Inoculation with *M. phaseolina* resulted in the root rot of faba bean severity on 0-5 scale (Table 1).

Table 1: Effect of biogent on plant growth and biomass production of faba bean grown in soil inoculated with *M. phaseolina*.

Treatment	*M. phaseolina*	Shoot length (cm)	Root length (cm)	Shoot fresh wt. (gm)	Root fresh wt.(gm)	Shoot dry wt. (gm)	Root dry wt. (gm)
Control	Not inoculated	39.5	12.6	16.6	3.2	9.6	1.3
T. viride	Not inoculated	42.3	14.5	24.1	4.2	12.8	1.88
T. hamatum	Not inoculated	43.9	16.5	27.1	4.9	14.3	2.01
T. harzianum	Not inoculated	42.9	15.6	25.5	4.5	13.9	1.91
T. virens	Not inoculated	41.4	14.2	23.5	4.1	11.6	1.76
T. koningii	Not inoculated	40.5	13.9	22.1	3.9	10.9	1.62
T. atroviride	Not inoculated	40.1	13.5	20.6	3.8	10.5	1.5
Control	Inoculated	30.6	10.3	12.5	2.4	7.9	1.1
T. viride	Inoculated	32.7	12.8	20.3	3.8	9.1	1.4
T. hamatum	Inoculated	32	12.2	19.4	3.6	8.8	1.38
T. harzianum	Inoculated	32.9	13.3	20.5	4	9.4	1.43
T. virens	Inoculated	31.8	11.8	19.1	3.4	8.6	1.34
T. koningii	Inoculated	31.2	11.4	18.6	3.1	8.4	1.31
T. atroviride	Inoculated	30.9	10.9	18.2	3.0	8.1	1.29

Each value is mean of 5 replicates.

Plant biomass production

Application of *T. hamatum* in the pots without *M. phaseolina* (non-infested soil) significantly increased the length of shoot (11%) and root (30%) of faba bean. Application of *T. harzianum* and *T. virens* also promoted the shoot and root length by 8 and 23%, and 15 and 12% respectively over control. Inoculation with *M. phaseolina* caused 22 and 18% suppression in the length of shoot and root, respectively over control. Application of *Trichoderma* spp. checked the suppressive effect of *M. phaseolina* leading to significant enhancement in the plant growth variable. Highest improvement in the length of shoot (7%) and root (29%) was observed with the treatment of *T. hamatum* followed by *T. harzainum* with an increase of 6% (shoot length) and 24% (root length) over control. Significant effect of *T. viride, T. virens, T. koningii* and *T. atroviride* was recorded only on root length (5-18%) over control, (Table. 1). T. *hamatum was* found to improve the fresh and dry weight of shoot (63% and 48%) and root (53% and 54%), respectively over control in non-infested soil (Table. 1). With rest of the *Trichoderma* spp. the enhancements in fresh and dry weight of shoot and root were in the order of *T. harzianum* (50 & 44% and 40 & 46%)>*T. viride* (41&45% and 20&33%)>*T. virens* (25 & 31% and 35 & 45%)>*T. koningii* (24 &31% and 9 & 14%)>*T. atroviride* (18 and 21% and 15 & 25%) respectively over control.

The bean plants grown in the soil infested with *M. phaseolina* exihibited significant reduction in the fresh weight of shoot and root and dry weight of shoot and root [(24%, 25%, 17% and 15%) respectively over control (Table. 1). The treatments with of *T. harzianum* and *T. hamatum* improved above biomass variables by 18-66% and 15-58% respectively in the soil infested with the root rot fungus. Other *Trichoderma* spp. evaluated in the study also significantly enhanced the biomass of faba bean plants grown in the pathogen infested soil (Table. 1)

Root nodulation

Application of *Trichoderma* spp. promoted the root nodulation that varied with *Trichoderma* spp. In non-infested soil significantly greater nodulation was recorded due to application of *T. hamatum* (57%) followed by *T. harzianum* (48%) and *T. viride* (34%), respectively over control (Figure 1).

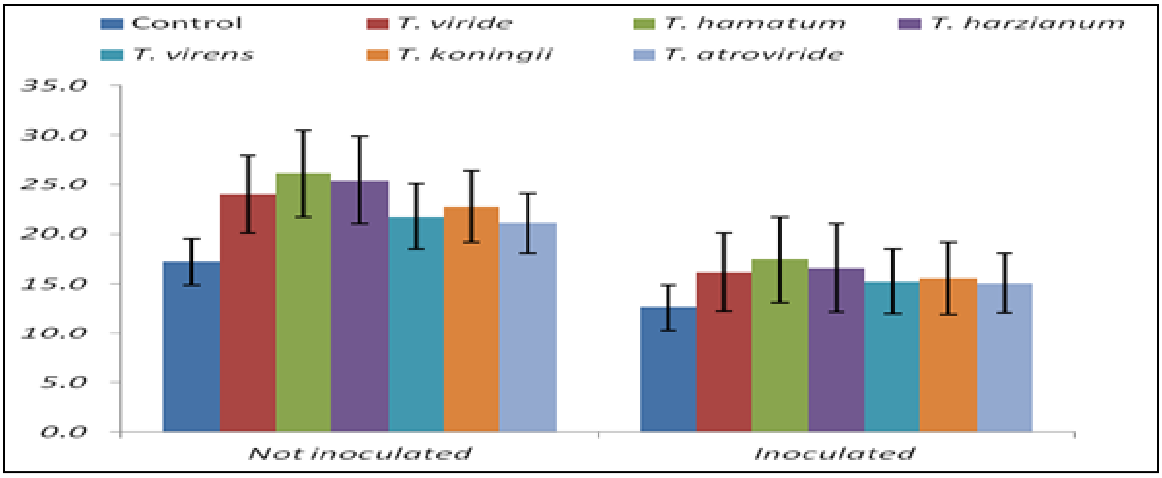

Fig 1: Effect of application of *Trichoderma* spp. on total number of nodule/ root system

Infection with *M. phaseolina* caused 26% reduction in the number of root nodule/ root system over control (Fig 1). However application of *T. hamatum* and *T. harzianum* increased root nodulation by 38% and 31%. Next in effectiveness was *T. viride* (28%) followed by *T. virens* (23%), *T. koningii* (20%) and *T. atroviride* (19%) respectively over control.

Yield and yield attributes

Table 2: Effect of biocontrol on yield and yield attributes of faba bean in soil inoculated with *M. phaseolina*

Treatments	*M. phaseolina* (1g/kg soil)	No. of Pods/ Plant	No. of seeds/ Plant	Yield/ Plant (g)
Control	Not inoculated	50.5	5.2	20.3
T. harzianum	Not inoculated	60.3 (19.4%)	6.8 (30.7%)	23.0 (13.3%)
T. hamatum	Not inoculated	59.1 (17.0%)	6.7 (28.8%)	23.0 (13.3%)
T. viride	Not inoculated	58.9 (16.6%)	6.6 (26.9%)	22.5 (10.8%)
T. koningii	Not inoculated	58.5 (15.8%)	6.6 (26.9%)	21.4 (5.4%)
T. virens	Not inoculated	58.1 (15.0%)	6.4 (23.0%)	21.1 (3.9%)
T. atroviride	Not inoculated	58.0 (14.8%)	6.1 (17.3%)	21.1 (3.4%)
Control	Inoculated	40.6 (24.3%)	3.3 (57.5%)	12.3 (65%)
T. harzianum	Inoculated	51.1 (25.8%)	4.2 (27.2%)	13.9 (13.0%)
T. hamatum	Inoculated	51.0 (25.6%)	4.0 (21.2%)	13.6 (10.5%)
T. viride	Inoculated	49.9 (22.9%)	3.8 (15.1%)	13.6 (10.5%)
T. koningii	Inoculated	48.5 (19.4%)	3.8 (15.1%)	13.3 (8.1%)
T. virens	Inoculated	47.3 (16.5%)	3.7 (12.1%)	13.1 (6.5%)
T. atroviride	Inoculated	45.9 (13.0%)	3.5 (6.0%)	12.7 (3.2%)

Each value is mean of 5 replicates.

Uninoculated plant show enhancement in yield. When treated with biocontrol agent, maximum enhancement was recorded by soil application of *T. harzianum* (19.4%) in number of pods per plant, (30.7%) in number of seeds per plant and (13-3%) in yield followed by *T. hamatum*, *T. viride*. *T. koningii*, *T. virens*, and *T. atroviride* (14.8-17%) in number of pods per plant, (17.3-28.8%) in number of seeds per plant and (3.4-13.3%) in yield respectively over control in uninoculated plant. Infection by root rot fungi in legumes significantly suppresses root nodulation (Khan *et al.*, 2002) [7]. *Trichoderma* spp. enhanced the nodulation by suppressing *M. phaseolina* (Dubey *et al.*, 2010) as well as synergism with *Rhizobium* spp. (Khan *et al.*, 2014) [4]. However plant grown in inoculated soil showed significant reduction in number of pods/ plant, number of seeds/ plant and in yield by 24.3% 57.5% and 65% respectively over control. Treatment with *T. harzianum* in inoculated soil found to be very effective in enhancing the number of pods per plant (25.8%), number of seeds/ plant (27.2%) and yield (13%). Next in effectiveness was *T. hamatum*, *T. viride*, *T. koningii*, *T. virens* and *T. atroviride* (13-25.6%) in number of pods per plant, (8-21.2%) in number of seeds per plant and (3.2-10.5%) in yield respectively over control. *T. virens and T. atroviride* found to be least effective.

Conclusion

The investigation has indicated that the selected *Trichoderma* species, especially *T. hamatum* and *T. harzianum* could be potentially managing charcoal rot on faba bean. The BCA, besides suppressing the pathogen also acted as a growth promoter and significantly improved the growth and biomass of faba bean plants grown in the soil infested or not infested with *M. phaseolina*. The study has highlighted the significance of using *T. hamatum* and *T. harzianum*, in integration with *Rhizobium* in pulse cultivation.

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