Int.J.Curr.Microbiol.App.Sci (2016) *5*(4): 32-38

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 4(2016) pp. 32-38**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.504.006

In-Vivo Evaluation of Competitive Parasitic Ability and Rhizosphere Colonisation of Different *Trichoderma* Isolates

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Keywords

Rhizosphere colonisation, Competitive parasitic ability, *Trichoderma*, *Sclerotinia sclerotiorum*, chickpea (*Cicer arietinum* L.).

Article Info

Accepted:
08 March 2016
Available Online:
10 April 2016

A B S T R A C T

The rhizosphere competence of *Trichoderma* isolates was conducted with chickpea, using natural and sterilized soils of Neemrana, Rajasthan. The rhizosphere population of *Trichoderma* increased at an increasing rate from 15 to 30 DAS and 30 to 45 DAS and thereafter increased but at a decreasing rate and finally declined after 45 DAS, in both soil types. There was appreciable more number of rhizosphere populations of antagonists in rhizosphere soil of test crop observed at 45 DAS than at 15 and 30 DAS. Competitive parasitic ability was conducted in two different soil conditions, *viz*., natural and steam sterilized soil collected from three states. Under natural soil of Uttar Pradesh, the isolate UP:Bam003 and MP:Kha030 appeared most efficient in their competitive parasitic ability against sclerotia of *S. sclerotiorum*, whereas the isolate MS:Mar016 was with intermediate effect and the isolate UP:Kus008 were poor competitive colonizer on sclerotia of *S. sclerotiorum*. Considering the conidial form of inoculum, the isolate UP:Bam003 appeared most efficient colonizer of sclerotia of *S. sclerotiorum*, whereas UP:Kus008 exhibited as least colonizer and isolate MP:Kha030 and MS:Mar016 showed intermediate colonizing ability of sclerotia. While considering the chlamydospores form of inoculum, the isolate UP:Bam003 was best competitive colonizer of sclerotia of test pathogen showing 91.5% colonizing ability.

Introduction

The ability of *Trichoderma* species to colonize and establish themselves in the rhizosphere is of crucial importance to achieve efficient control of phytopathogens around the seeds and the roots (Baker 1991; Papavizas 1985). Some *Trichoderma* strains, described as rhizosphere competent and can cause an asymptomatic infection of roots, behave as endophytes, colonizing the root epidermis and outer cortical layers and release bioactive molecules, responsible for increased plant resistance to various biotic and abiotic stresses through induced or acquired systemic resistance (McLean *et al*., 2005; Shoresh *et al*., 2010). The competitive parasitic ability of *Trichoderma* spp. using

sclerotia as live baits of several pathogens has been studied (Bhagat and Pan, 2009; Roy and Pan, 2005b) but few researches has been done on *Sclerotinia sclerotiorum*. Therefore, the aim of this study is to depict the rhizosphere colonisation and competitive parasitic ability of *Trichoderma* isolates in chickpea rhizosphere using sclerotia of *S. sclerotiorum* as live bait.

Materials and Methods

Competitive Parasitic Ability of *Trichoderma* Isolates

This experiment was conducted in two types of soils *viz*., natural and sterilized soils of Neemrana, Rajasthan to assess the ecological adaptability of *Trichoderma* spp. 200g air dried (natural and sterilized soil) was mixed thoroughly with mycelial, conidial and chlamydospores inocula of test isolates of *Trichoderma* separately, moisture holding capacity of each soil type was adjusted to 50% and fitted into the earthen cups (100 ml).

25 sclerotia of *S. sclerotiorum* were buried 0.5 - 2.0 cm deep, covered with perforated aluminum foil and incubated at 28±1°C for 7 days.

The sclerotia of *S. sclerotiorum* were harvested separately by floatation and sieving method (Rodriguez-Kabana *et al*., 1974) and plated on modified *Trichoderma* selective medium (TSM) after surface sterilization with 1.0% sodium hypochlorite solution for 2-3 min. and air dried. The petridishes seeded with sclerotia of *S. sclerotiorum*, were incubated at 28±1°C for 7 days and the sclerotia with colonization by *Trichoderma* spp. in each treatment were recorded. The percentage colonization of sclerotia of both pathogens by *Trichoderma* spp. was calculated by dividing the number of sclerotia yielding *Trichoderma* by total number of sclerotia seeded in the petridishes.

Rhizosphere Colonization of *Trichoderma* Isolates

The rhizosphere competence of test isolates of *Trichoderma* was conducted with chickpea (*Cicer arietinum* L.), using natural and sterilized soils of Neemrana, Rajasthan. Two kg potting mixture of soil (natural and sterilized soil) and farm yard manure (2:1 v/v) was mixed thoroughly with 20 g of wheat bran + mustard cake (20%) formulation of *Trichoderma* isolates (1 x 10^8 cfu/g of product), MHC of the potting mixture was adjusted to 50% and filled into the earthen pots.

Twenty five seeds of chickpea were sown per pot. The experiment was replicated four times and seeds of test crop were allowed to germinate. The pots were supplied with irrigation water whenever required to maintain the MHC of potting mixture to 50%. The rhizosphere soil was collected by gently uprooting the test crops and brushing the soil adhered to roots after 15 DAS. The observation was repeated at 15 days interval and the data were recorded upto 45 DAS. The rhizosphere soil collected was mixed thoroughly and the rhizosphere population of *Trichoderma* spp. was estimated by soil dilution technique (Dhingra and Sinclair, 1995). Ten mg of soil samples of each isolate-crop combination was suspended in 10 ml sterilized distilled water. The suspension was serially diluted upto 10^8 and 1 ml each of these aliquot was plated on modified TSM with five replications. The inoculated Petridishes were incubated at 28±1°C for 7 days and the number of colony forming units per g of rhizosphere soil for each isolate was counted. The total number of cfu/g of rhizosphere soil was the

rhizosphere population of *Trichoderma* spp.

Results and Discussion

Competitive Parasitic Ability

The data on competitive parasitic ability of four isolates of *Trichoderma* sp. *viz*. UP:Bam003 (*T. asperellum)*, UP:Kus008 (*T. viride)* MP:Kha030 *(T. koningiopsis) and* MS:Mar016 (*T. viride)* against sclerotia of *S. sclerotiorum* have been presented in Figure 1. The experiments were conducted in two different soil conditions, *viz*., natural and steam sterilized soil, collected from Bambawad (UP), Hamirpur (UP), Parbhani (Maharashtra) and Narsingpur (MP).

Under natural soil of Uttar Pradesh, the isolate UP:Bam003 and MP:Kha030 appeared most efficient in their competitive parasitic ability against sclerotia of *S. sclerotiorum,* whereas the isolate MS:Mar016 was with intermediate effect and the isolate UP:Kus008 were poor competitive colonizer on sclerotia of *S. sclerotiorum*. Considering the conidial form of inoculum, the isolate UP:Bam003 appeared most efficient colonizer of sclerotia of *S. sclerotiorum* than other isolates tested under natural soil of UP, whereas UP:Kus008 exhibited as least colonizer and isolate MP:Kha030 and MS:Mar016 showed intermediate colonizing ability of sclerotia.

While considering the chlamydospores form of inoculum, the isolate UP:Bam003 was best competitive colonizer of sclerotia of test pathogen showing 91.5% colonizing ability, whereas MS:Mar016 (58.25%) resulted into least aggressive requiring high inoculum level under natural soil of Maharashtra. The other antagonistic isolates were found with intermediate effect under natural soils of UP and MP.

In steam sterilized soil, the situation changed further regardless of form of inocula or isolates of *Trichoderma* itself, where they required very low level of inocula (either mycelia or conidia or chlamydospores form) than that in the natural soil condition. Surprisingly, the isolate UP:Kus008 showed the maximum competitive colonizing ability in mycelial form of inoculum whereas the isolate MS:Mar016 and UP:Bam003 was with intermediate effect and the isolate MP:Kha033 were poor competitive colonizer on sclerotia of *S. sclerotiorum*. Isolate MS:Mar016 exhibited highest percent colonization conidial form of inoculum and UP:Bam003 showed in chlamydospore form of inoculum. The possible justification of this study could be credited to the fact that the natural soil often does not allow introduced biocontrol agents to perform well due to some abiotic and biotic factors (Knudsen and Bin, 1990 and Bae and Knudsen, 2005). Soil solarisation and sterilization upsets the ecosystem to the extent that it may allow proliferation of *Trichoderma* spp. Present results corroborated with the findings of Lewis and Papavizas (1985) where they suggested that mycelial inoculum was better than conidial inoculum, for soil application and chlamydospres were less sensitive to biotic and abiotic stresses.

Rhizosphere Colonization

The perusal of entire results on rhizosphere colonization of *Trichoderma* spp. from MP, UP and Maharashtra, under natural and sterilized soils indicated that all isolates of *Trichoderma* significantly colonized the rhizosphere of chickpea crop tested compared to control. The rhizosphere population of *Trichoderma* increased at an increasing rate from 15 to 30 DAS and 30 to 45 DAS and thereafter increased but at a

decreasing rate and finally declined after 45 DAS, in both soil types. But there was appreciable more number of rhizosphere populations of antagonists in rhizosphere soil of test crop were observed at 45 DAS than that at 15 and 30 DAS.

Figure.1 Percentage Colonization by *Trichoderma* Isolates in Three Different Inoculums Levels in Natural and Sterilized Soil

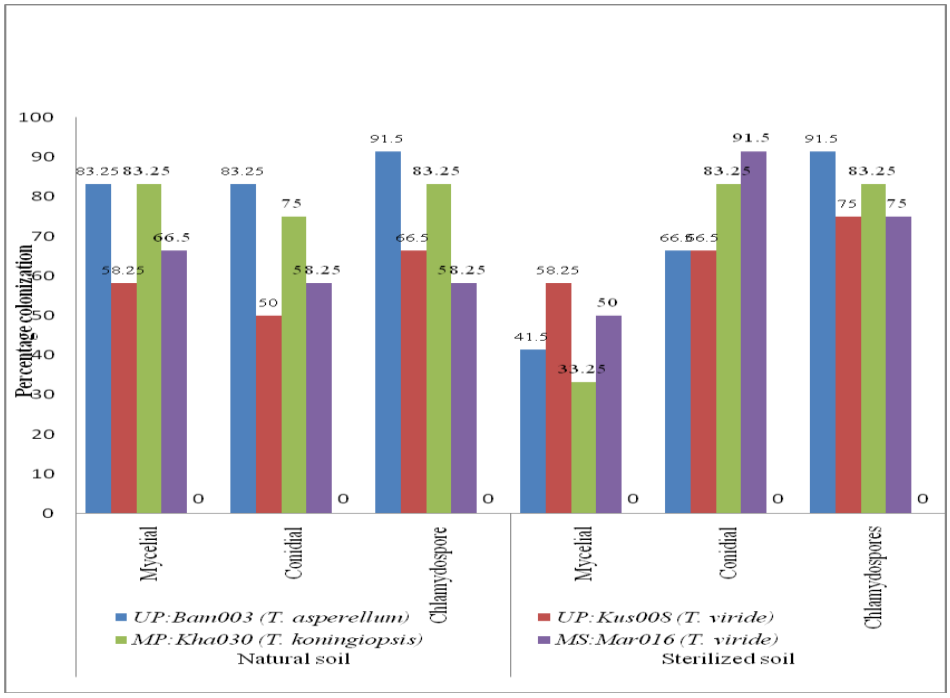


Figure.2 Rhizosphere Colonization by *Trichoderma* Isolates in Terms of their CFU Count (1x 108 cfu/g Soil)

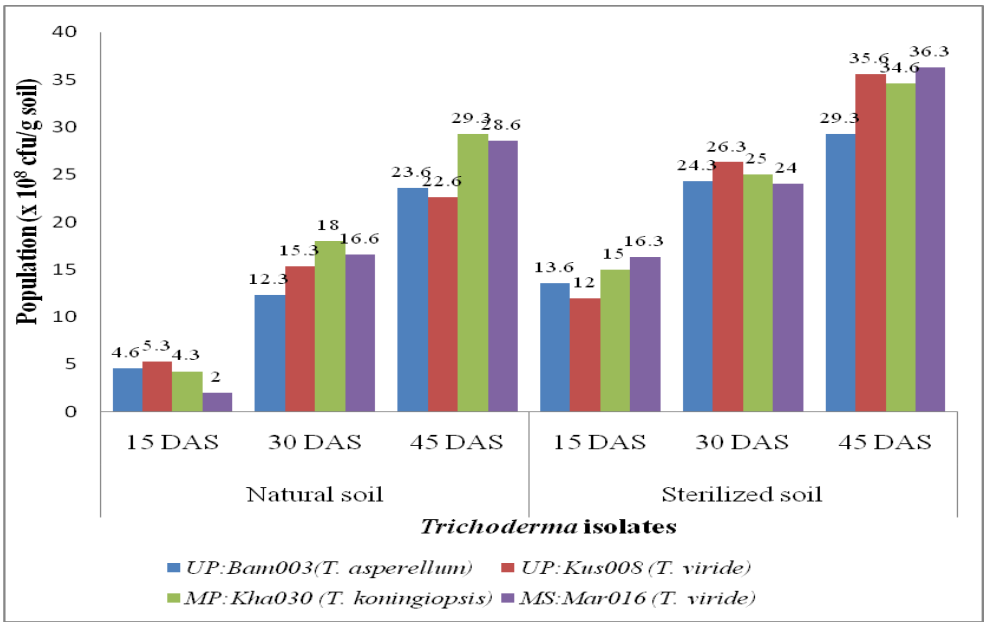
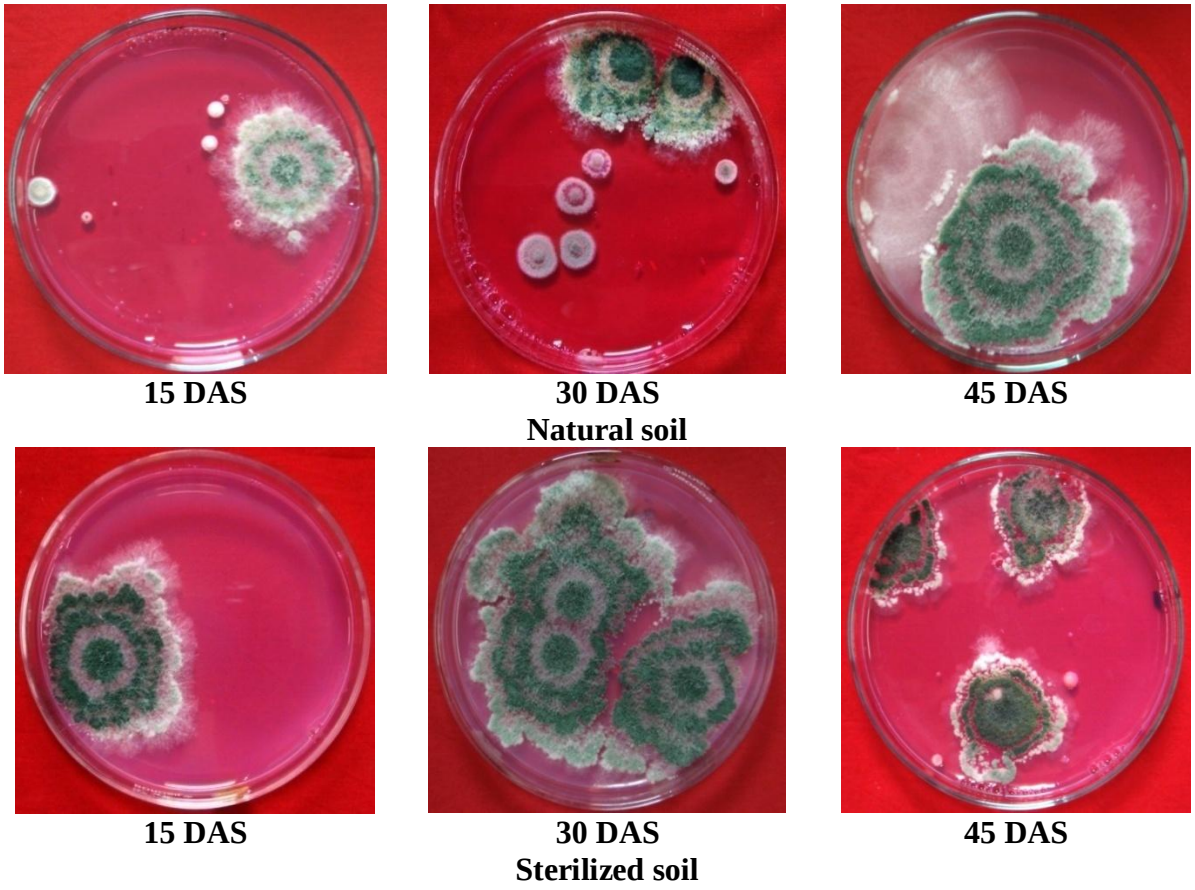


Plate.1 *Trichoderma* Isolated from Rhizosphere Soil of Chickpea to Check the Presence of Root Colonization by *Trichoderma* in Natural and Sterilized Soil after Soil Incorporation at 15, 30 and 45 Days of Sowing (DAS)



In chickpea (Figure 2), highest rhizosphere population of *Trichoderma* was recorded with the isolate MP:Kha030 (18 and 29.3 x 10^8 cfu/g of soil at 30 and 45 DAS), followed by MS:Mar016 (16.6 and 28.6 x 10^8 cfu/g, at 30 and 45 DAS), UP:Kus008 (15.3and 22.6 x 10^8 cfu/g at 30 and 45 DAS) and UP:Bam003 (12.3 and 23.6 x 10^8 cfu/g at 30 and 45 DAS) in natural soil of Rajasthan, whereas the rhizosphere population of 24 and 36.3 x 10^8 cfu/g of soil at 30 and 45 DAS in chickpea was recorded with MS:Mar016 in sterilized soil of Neemrana, Rajasthan which also appeared as most efficient rhizosphere colonizer in chickpea (Plate 15). The rhizosphere populations of antagonist were less in natural soil compared to sterilized soil. The isolate MS:Mar016 were the most efficient colonizer under both natural and sterilised soils of Neemrana. The isolate UP:Kus008 was recorded with least rhizosphere population in chickpea rhizosphere under both natural and sterilized soils and rest isolates were with intermediate category in view of their rhizosphere competence in chickpea.

Sharma *et al.* (2010) reported that high population of *Trichoderma* (2 x 10^6 to 80 x 10^6 cfu/g colony population) between flowering and pre-harvesting was observed in the samples collected from the rhizospheric soil samples. The rhizosphere

competent isolate of *Trichoderma* spp. was found to produce diffusible metabolites in the rhizosphere which actively influenced the growth of *Trichoderma*–colonized plant due to their action as plant growth regulators (auxin and/or auxin-like compound) (Vinale *et al*., 2008a,b).

The effectiveness of *Trichoderma* as seed treatment is probably determined not only by their biocontrol qualities but also by their abilities to multiply in the rhizosphere when applied to soil and their PGPR properties. In present findings there were increase in the rhizosphere population of *Trichoderma* spp. with the advancement of crop age upto 45 DAS and thereafter the population declined marginally. Benitez *et al.* (2004) reported that *Trichoderma* has a strong capacity to mobilize and take up soil nutrients, thus making it more efficient and competitive than many other soil microbes. The present findings suggested that there was less rhizosphere population of *Trichoderma* spp. when they were applied to crops which were grown in natural soil as compared to sterilized soil. The possible explanation of these findings may be due to ecological specificity of *Trichoderma* spp. (Bae and Knudsen, 2005; Papavizas, 1985) and existence of microbial competition which does not allow flourishing the antagonist in that situation (Whipps, 2001).

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How to cite this article:

Monika Singh, O.P. Sharma, Someshwar Bhagat and Neetu Pandey. 2016. *In-Vivo* Evaluation of Competitive Parasitic Ability and Rhizosphere Colonisation of Different *Trichoderma* Isolates. *Int.J.Curr.Microbiol.App.Sci.* 5(4): 32-38.
doi: http://dx.doi.org/10.20546/ijcmas.2016.504.006