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RESEARCH ARTICLE

IN VITRO SCREENING OF *TRICHODERMA* SPP AGAINST COLLAR ROT PATHOGEN

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ABSTRACT

The success of establishing new plantations of a perennial crop like coffee mainly depends on the use of healthy seedlings with good vigour for planting. For this purpose, it is most essential to protect the seedlings in the primary and secondary nursery beds from attacks of pest and diseases. Coffee seedlings at the nursery stage are susceptible to fungal diseases. In the persent study four *Trichoderma* spp were tested for their ability to inhibit soil borne pathogen *Rhizoctonia solani* Kuhn. causing collar rot in coffee seedlings. Dual culture technique revealed that *T. hamatum* (75.68%) highly inhibited mycelial growth of pathogen followed by *T.koningii* (65.92), *T.viride* (57.90%) and *T.harzianum* (52.71%) respectively. This preliminary results are valuable for further studies.

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INTRODUCTION

Coffee is the second largest traded commodity in the world, next to petroleum products and hence aptly described as 'Brown Gold'. It is grown in about 80 countries across the globe, of which about fifty are considered to be the major producers. Collar rot or damping off disease occurs on 1 to 4 month old tender coffee seedlings in the coffee nursery. Sometimes, the collar rot pathogen attacks coffee seeds sown on the seed beds and prevent its germination. However, the pathogen fails to establish on coffee seedlings when the stem at the collar region turn brown and become harder. *R.solani* Kuhn. is a soil inhabiting fungus with a wide host range including coffee. The pathogen is capable of surviving in the soil for many months in the form of sclerotia on fallen debris. Under favorable conditions, sclerotia germinate into mycelia and infect coffee seedlings. Two phases of this disease are described as under : 1) Pre – emergence stage: The pathogen invades embryo and endosperm of the nursery seed beds; consequently seeds start rotting, disintegrate and fail to germinate. 2) Post – emergence stage: Seedlings show brown to black discolouration at the collar region of the stem leading to rotting of the tissues. Growing tip of the seedlings wilts, collapses and dies (Sudhakar and Bhat 2016). Biological control of plant diseases is considered as one of the viable alternative methods to manage plant diseases (Barakat and Al-Masri 2005, Pal and Gardener 2006). Application of fungicides is not economical in the long term because they pollute the environment, leave harmful residues and can lead to the development of resistant strains of the pathogen with repeated use (Vinale *et al*., 2008). However, use of biological control is safe, nonhazardous for human, farm animals and avoids environmental pollution (Abdel-Kadir *et al*., 2002). The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries (Janisiewicz *et al*., 2000).

The bio control exercised by *Trichoderma* can occur by several antagonistic mechanisms such as nutrient competition, antibiotic production and mycoparasitism. Mycoparasitism has been reported as the major antagonistic mechanism displayed by *Trichoderma* spp. After host recognition, *Trichoderma* spp attaches to the host hyphae via coiling and penetrate the cell wall by secreting cell wall degrading enzymes which allow them to bore holes into the fungal host and extracts nutrients

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for their own growth. Most phytopathogenic fungi have cell wall that contain chitin as a structural backbone arranged in an regularly ordered layers and β, 1-3 glucan as a filling material arranged in an amorphic manner. Chitinases and β, 1-3 glucanases have been directly involved the mycoparasitism interaction between *Trichoderma* spp and its host (Kubickek *et al*., 2001). The present study was aimed to evaluate the antagonistic activity of fungal biocontrol agents against collar rot pathogen in laboratory conditions.

MATERIALS AND METHODS

Isolation of pathogen

Collar rot infected coffee seedlings were collected from coffee nursery at Perumparai, Dindigul (Dt). Infected seedlings were washed under tap water for about 5 minutes to remove soil particles. Infected stem parts (1 to 2 mm) were cut into small pieces by sterilized blade then surface sterilized with 70 % acohol for 1 min. The pieces were then washed thrice with sterilized distilled water and dried by sterilized blotting paper. These pieces were placed in Petri dishes (90-mm diameter) containing 20 mL potato dextrose agar (Peeled potato –200 g, Dextrose –20 g, Agar– 15 g and distilled water – 1000 ml, pH – 5.6) medium amended with streptomycin (250 mg/L). and incubated at 28 ± 2°C for 7 days. These isolated pathogen was purified separately by transferring the tip of the mycelia into PDA slants and maintained as stock cultures for further studies.

Fungal biocontrol agents

The genus *Trichoderma,* species such as *T.hamatum, T.harzianum, T.koningii* and *T.viride* were isolated from rhizosphere soil of coffee field of Perumparai, Dindigul Dt. The isolated and identified four *Trichoderma* spp were selected for antagonistic activity based on their biocontrol potential .

Dual culture technique

Selected four *Trichoderma* spp evaluated against *R. solani* Kuhn. by the dual culture technique (Morton and Strouble 1955). Mycelial discs 5 mm in diameter were excised from the edge of an actively growing antagonist and the pathogen was cultured on opposite ends of a petri dish equidistant from the periphery. A completely randomized experimental design was used with nine replicates for each isolate. In control petridish, in place of antagonist, a sterile agar disc was inoculated on the side opposite to pathogen. Inoculated plates were incubated at 25 ± 1^0 C for 5- 7 days. After the incubation period, radial growth of pathogens was masured and the percent inhibition of average radial growth was calculated relative to the controls as follows:

$$L = [(C – T) / C] \times 100$$

Where L is the percentage inhibition of radial mycelial growth, C is radial growth of the pathogen in the control ; T is radial growth of the pathogen in the presence of *Trichoderma* spp (Edington *et al*., 1971).

The degree of antagonism between each of the *Trichodema* species and test pathogens in dual culture was scored on scale of R1 - R5 that is, R1=*Trichoderma* completely overgrew pathogens (100 % over growth); R2= *Trichoderma* overgrew at least two-third pathogens (75 % over growth); R3= *Trichoderma* colonizes on one half of the pathogens (50 % over growth); R4= *Trichoderma* and the pathogens contact point after inoculation and R5= Pathogens overgrow bioagent - *Trichoderma* (Bell *et al*., 1982).

Statistical analysis

Statistical analysis was performed following a completely randomized design (CRD) with nine replicates in each treatment. The data was subjected to analysis of variance (ANOVA) using statistical software SPSS 10.0,1999 and significance of various treatments were evaluated by Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

In this preliminary investigation, an attempt was made to screen the antagonistic potencial of coffee rhizosphere native bioagents against *R. solani* Kuhn. the causal organism of collar rot disease in coffee seedlings. The isolated pathogen was identified as *R. solani* Kuhn. based on their colony morphology by using standard fungal manuals. The present study revealed that *in vitro* dual culture technique, the isolated and identified selected four biocontrol fungal agents are *T.hamatum, T.harzianum, T.koningii* and *T.viride* were tested the efficiency of inhibiting the mycelial growth of *R. solani* Kuhn.. Among the four *Trichoderma* spp, the highest antagonistic effect against the mycelial growth inhibition of pathogen was found with *T. hamatum* (75.68 ± 1.27%), followed by *T.koningii* (65.92 ± 3.50%), *T.viride* (57.90 ± 4.73 %) and *T.harzianum* (52.71 ± 2.96 %) respectively (Table 1and Plate 1). Similar findings on the interaction of *Trichoderma* species (*T.hamatum* T614, *T. hamatum* T612, *T. harzianum* T447, *T. harzianum* T969, *T.virens* T523 and *Trichoderma* sp.T) were recorded by Hajieghrari *et al*., (2008) for *Fusarium graminearum*, *R. solani* and *Macrophomina phaseoli*. Shalini and Kotasthane (2007) studied seventeen *Trichoderma* strains were screened against *R. solani in vitro*, all strains including *T. harzianum, T. viride* and *T. aureoviride,* inhibited the growth of *R. solani.* The earlier studies also revealed that antimicrobial metabolites produced by *Trichoderma* is effective against a wide range of fungal phytopathogens eg., *Fusarium oxysporum, R.solani, Curvularia lunata, Bipolaris sorokiniana* and *Colletotrichum lagenarium,C.acutatum, C.gloeosporioides* Svetlana *et al*. (2010).

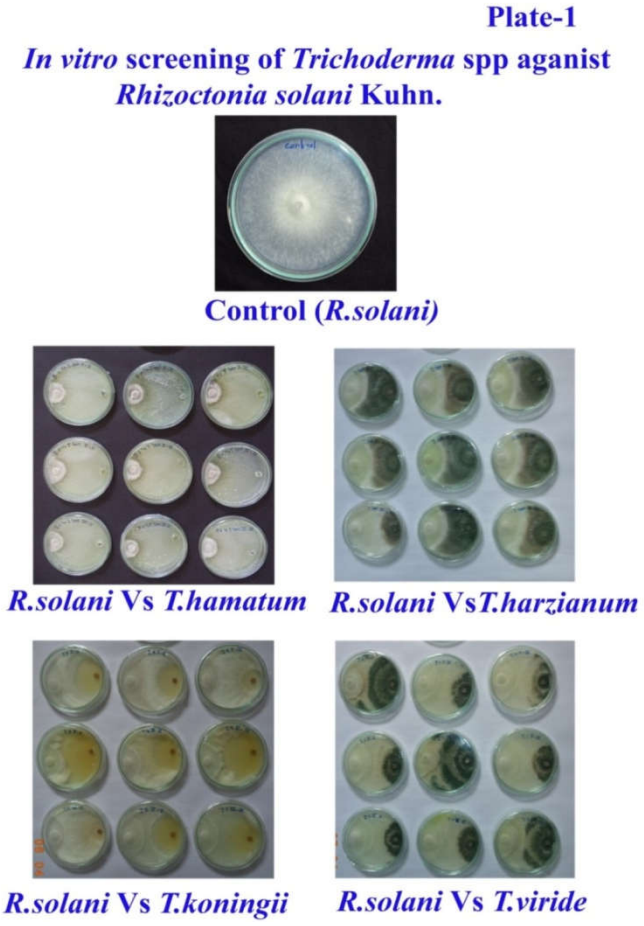
The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi Rajkonda *et al*. (2011). Although, Wilson *et al*. (2008) found the genus *Trichoderma*, species such as *T. hamatum*, *T. harzianum, T. reesei*, *T. virens*, and *T. viride* have demonstrated excellent antagonistic activity against *R. solani* on potato pot and or field tests. Shafiquzzaman *et al*. (2009) reported that *Trichoderma harzianum* (Th), *T. viride* (Tv) and *T. koningii* (Tk) upon evaluation via dual culture technique resulted in suppression of

soil borne pathogens of different vegetables viz. *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiarum* under *in vitro* conditions. Similarly, *T. viride* (Tv-1), *T. viride* (Tv-2), *T. harzianum* (Th-1) inhibited the growth of *R. solani* Radwan *et al*. (2007).

Table 1. *In vitro* screening of *Trichoderma* spp against *R. solani* Kuhn

Trichoderma spp	Mycelial growth inhibition (%) Mean ± SD	Bell's scale*(R)
T.hamatum	75.68 ± 1.27 [a]	R2
T.harzianum	52.71 ± 2.96 [d]	R3
T.koningii	65.92 ± 3.50 [b]	R3
T.viride	57.90 ± 4.73 [c]	R3

Mean followed by the same letter do not differ significant at 0.05 according to DMRT.



Trichoderma virens T523 and *Trichoderma* sp. were evaluated against five isolates of soil borne phytopathogenic fungi via dual culture techniques resulted in suppression of *Fusarium graminearum, R. solani (AG4 and AG5), Macrophomina phaseoli* and *Phytophtora cacturum* Behzad *et al*., (2008).

Conclusion

This study concluded that *in vitro* dual culture technique of *T. hamatum* was found to be most effective inhibition aganist the mycelial growth of pathogen. The use of fungal biocontrol agents is not only safe for farmers and consumers but also good for the environment.

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