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Research Article

ADVANCE FORMULATION TECHNOLOGIES FOR BIOFUNGICIDE (*TRICHODERMA HARZIANUM*) COFFEE RESIDENTIAL CULTURE IN PULNEY HILLS

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ABSTRACT

Trichoderma harzianum is one of economical importance for the production of industrial enzymes (cellulases and hemicellulases), antibiotics and their action as biocontrol agents against plant pathogens based on various mechanisms. The ecological importance of this genus, particularly of its mycelium, is to take part in the decomposition of plant residues in soil. *T. harzianum* is a potential fungal biocontrol agent against a range of plant pathogens. So there is a need to evaluate the efficacy of the native isolates of *T. harzianum* to promote the growth and yield parameters of coffee, wilt disease under in vitro and in vivo conditions. Hence the study focuses on fifteen native *Trichoderma* antagonists were isolated from healthy coffee rhizosphere soil in different geographical regions. *Rhizoctonia solani* is the most important pathogen involved in seedling disease. In the antagonistic activity isolates of *T. harzianum* was evaluated in vitro, against isolates of *R. solani* in dual culture. There was highly significant interaction was observed between isolates of *Trichoderma* and *R. solani*. All isolates of *T. harzianum* showed varying levels of antagonism against *R. solani*. Similarly, the interaction between *Trichoderma* isolates and *R. solani* was a highly significant source as biocontrol agent and world distribution of various commercially available *T. harzianum* formulations.

Keywords: *Trichoderma harzianum*, Bio-control, Fungal Antagonists, Capsule biocontrol formulation.

INTRODUCTION

*Trichoderma harzianum*is economically important for the production of industrial enzymes (cellulases and hemicellulases), antibiotics and their action as biocontrol agents against plant pathogens based on various mechanisms such as the production of antifungal metabolites, competition for space and nutrients and mycoparasitism (Howell, 2003). The ecological importance of this genus, particularly of its mycelium, is to take part in the decomposition of plant residues in soil. Mycoparasitic *Trichoderma* strains are able to recognize the host hyphae, coil around them, develop haustoria and penetrate the cell wall of the host (Abdullah *et al*., 2007). Characterization of the antagonistic effect of *Trichoderma harzianum* is the first step in utilizing the full potential of *T. harzianum* for specific anti-plant pathogenic applications. Promotion of growth and inducing resistance in plants is a mechanism which has been described for members of this genus (Harman, 2006). Several species of *Trichoderma* were used as biological control agents against soil borne plant pathogenic fungi (Küçük & Kivanç, 2004). The advantage of using *Trichoderma* in managing soil borne plant pathogens are eco-friendly, effective, ease of mass culturing with less cost of production and growth promoting effect. However, commercialization of

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Trichoderma for its utility in field crops could not be achieved successfully. A series of abiotic and biotic parameters have an influence on the biocontrol efficacy of *Trichoderma harzianum*.

Rhizosphere is a dynamic region governed by complex interactions between plants and the organisms that are in close association with the root. Beneficial relationships exit between Rhizosphere organisms and plants, which ultimately affect root function and plant growth. It includes the use of plant growth-promoting organisms and the suppression of plant diseases and weeds using biocontrol agents (Hillel & Hatfield, 2005). Coffee is a brewed drink prepared from roasted coffee beans, the seeds of berries from certain coffee species. The two most commonly grown are Coffee Arabica and Coffee robusta. In Tamil Nadu, Coffee is cultivated in and around lower Pulney hills, of Dindigul district and Yercaud of Salem district.

As world population is increasing, it is insufficient to fulfill the food demand of populations. So to feed the growing population of the world, farmer heavily relies on the use of chemical fertilizer. The chemical fertilizer increases yield in agriculture but are expensive andcauseharmfuleffecttotheenvironment.Theyreducenon-renewableenergyresourceslike coal and natural gas which produce of greenhouse gases contributing to global warming (Bhattacharjee *et al*., 2008). At present, there are approximately 7 billion people living in the world and this number is undoubtedly expected to rise to approximately 8 million around 2020 (Conway, 2012). With the expected rise in worldwide population there is increasing environmental damage as consequence of rapid growth in industrialization and urbanization (Glick, 2012).

Fungal biocontrol agents comprise of fungal inocula either alone or in combination (consortium), exerting direct or indirect benefit on plant growth and crop yield through different mechanism. Selected fungal species are used as biocontrol agents. Mycorrhiza which form mutualistic relationship with plant roots of more than 80% of plant including many important crops and forest tree species, which beneficially act as an effective bio-control agent (Rai *et al*., 2013). In recent years, use mycorrhizal fungi as inoculum has increased its importance due to its multifarous role in plant growth and yield and resistance against biotic and abiotic stresses. Penicillium, Aspergillus and Trichoderma species are fungal biocontrol agents combined with phosphate solubilizing microorganisms, have been used to develop plant growth by enhancing phosphorus absorption in plant further helps in metabolic activity.

MATERIALS AND METHODS

Collection of sample

The soil samples were randomly collected from the rhizosphere region of mature coffee plants in the agricultural fields of different localities in Thandigudi, Dindigul District, Tamil Nadu, India.

Isolation of *Trichoderma harzianum*

One gram of soil sample was randomly taken and it was added to 10 ml of sterilized distilled water to make serial dilution of 10^{-1} to 10^{-6}. 1 ml from 10^{-2} to 10^{-4}dilutions of the sample was inoculated in to the fungal specific medium like Rose bengal agar and PDA agar. Finally the plates were incubated at 30℃ for 3-5 days. After incubation the colonies were isolated and it was transferred to potato dextrose agar slant and stored at 4℃ for further use.

Morphological characterization of *Trichoderma harzianum*

Morphological characterization of *T.harzianum* was carried out for the genus differentiation, culture colour and structure of conidiophores/conidia (Latha & Mukherjee, 2002).

Testing of biocontrol ability: Antagonistic test

T. harzianum was originally isolated from the soil of coffee plants grown in field soil and collected from different plant growing areas. They were cultured on potato-dextrose agar (PDA, Difco, Detroit, MI,) or Rose bengal agar at 28 °C for five days. After an incubation period, colonies were purified and determined to be *Trichoderma* spp. (Rifai, 1969). *Trichoderma* isolates were grown in 50-ml potato dextrose broth (PDB, Difco), for 10 ± 2 days at room temperature. Mycelium was harvested by filtration through a piece of filter paper and washed with distilled water. Further the mycelium was used for antagonistic activity.

Plate confirmation test

Plate confrontation assays of *T.harzianum* isolated from coffee soil region were evaluated in vitro as antagonists against isolates of *R. solani* (pathogen). Dual cultures were carried out by using one-week-old cultures of *R. solani* and *T. harzianum* on PDA. The agar medium was inoculated with a 5-mm-diameter disc of antagonist positioned diametrically opposite a 5-mm-diameter disc of the pathogen. The distance between discs was approximately 5 cm. The cultures were grown at 28 ± 3 ℃, and measurements were taken after four days. In the control treatment, a sterile agar disc (0.5 mm dia m) was placed in dish instead of *Trichoderma* isolates. There were three replicates for each treatment. At the end of the incubation period, redial growth was measured. The efficiency of *T.harzianum* in suppressing radial growth was calculated as follows: (C–T)/C × 100, Where, C is radial growth measurement of the pathogen in the control and T is radial growth of the pathogen in the presence of *T.harzianum.*

Formulation of fungal biocontrol agent

There is wide variety of formulation types, liquid, solid as well as capsule in biocontrol. The main types, currently used for organism have been classified into dry products

and suspension. Major types of biocontrol formulations are: Liquid formulation of *T. harzianum*, Solid formulation of *T. harzianum*, Capsule formulation of *T. harzianum.*

Liquid formulation of *Trichodrema harzianum*

The strains used for liquid biocontrol formulation was *Trichoderma*. It was cultivated Potato dextrose agar media or Rose Bengal agar media. Then it was incubated in rotary shaker at 30℃ for 24hours. After incubation, we had to check the optimal temperature and pH of the broth for the survival of the microorganism. Then it was directly used as biocontrol (Figure 1). The shelf-life of liquid biocontrol was higher than other bio control agents (up to180days) (Navaneetha *et al*., 2015).

Solid formulation of *Trichoderma harzianum*

The biocontrol agent selected for the study was *T. harzianum*. Commercially used talc based inoculum of *T. harzianum* was used. Vermicompost and farmyard manure based carrier materials were used for mass production of the biocontrol agents. Mass production of the *T.harzianum* was done each of the above selected carrier material (talc or vermicompost or farmary manure). Enrichment of carrier material with *T. harzianum* was done at the rate of 1kg/20 kg of carrier material (Jeyarajan *et al*., 1994).

Capsule formulation of *Trichoderma harzianum*

In capsule biocontrol formation, the Gelatin capsule was used for preparation, because it was Easy to handle, Eco-friendly, Biodegradable, cheaper source and non-toxic to environment. The best quality of talcum was collected and mixed with inoculum and shade dried for few hours. After dried, it was packed in the Gelatin capsule with suitable physical parameters. There are further sizes of capsules such as 250 mg, 350 mg and 1000 mg were used for capsule biocontrol formulation.

RESULT AND DISCUSSION

One of the biggest threats to coffee growers world-wide are emerging fungal wilt diseases, in particular tracheomycosis caused by *Gibberella xylarioides* (anamorph *Fusarium xylarioides*) (Geiser *et al*., 2006). In Ethiopia incidences of tracheomycosis are reported to concern 60% of total crops, and are accompanied by significant yield losses due to severe damage and ultimate death of millions of coffee bushes (Girma *et al*., 2004). Integrated Disease Management programs would be of a great potential to combat Fusarium pests in Ethiopia. The fungal genus Trichoderma (teleomorph Hypocrea, Ascomycota, Dikarya) contains some of the most potent biocontrol agents used today (Harman, 2000). In addition, it has been shown that several of its taxa occur as endophytes particularly in the tropical arboreous plants, and that the strains often exhibit high antagonistic activity against pathogens of these plants (Samuels, 1996; Zhuge *et al*., 2005). However, the importance of Trichoderma or other endophytic fungi as a part of endogenous microbial diversity and their application in the biological control of tracheomycosis on the coffee plants has not been investigated yet

In the present work, three colonies were isolated from the PDA agar plates that were subjected to antagonistic characterization and subjected to test its shelf-life ability by three formulation viz., solid, liquid and capsule formulation. The Rhizosphere soil has highest number of isolate compare to non- rhizospheric soil reported by Reyes Castro, (2006). In the present study the role of bio control activity have been reported. Species identification and differentiation in this genus is only possible with the study of morphological features viz., culture colour and structure of conidiophores/ conidia, etc., (Latha & Mukherjee, 2002). Figure 2 shows the growth of T. *harzianum* in PDA agar plate and slant.

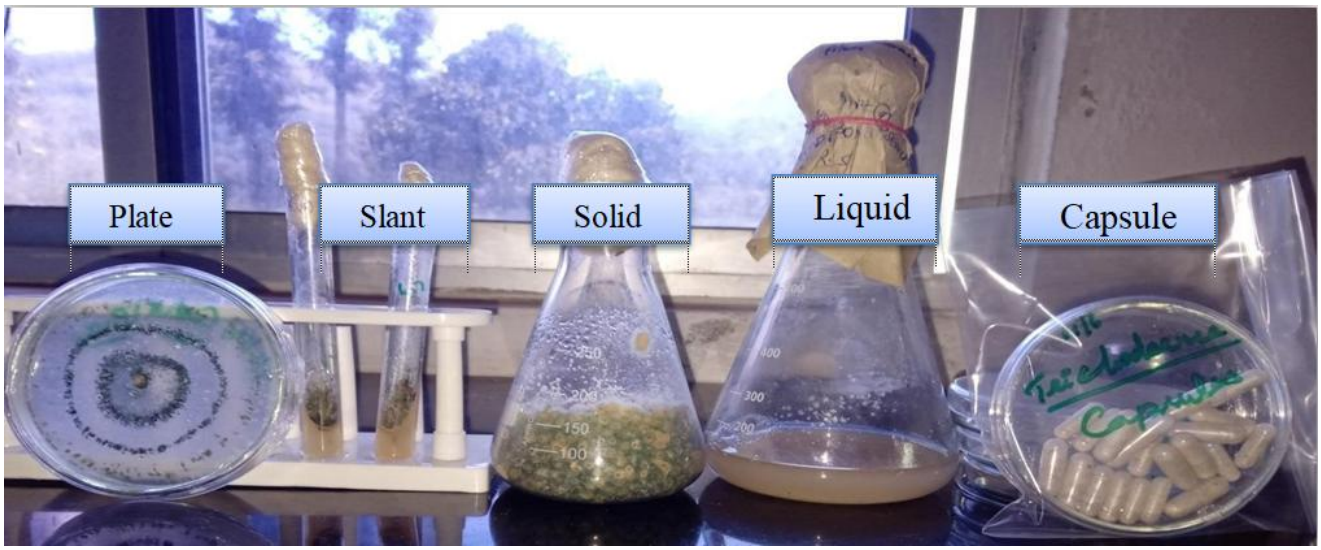

Figure 2. Formulation of biocontrol agent.

Figure 2. Isolation of T.*harzianum* in PDA plate ans slant.

Trichoderma species are economically important for their production of industrial enzymes (cellulases and hemicellulases), antibiotics and their action as biocontrol agents against plant pathogens based on various mechanisms such as the production of antifungal metabolites, competition for space and nutrients and mycoparasitism (Howell, 2003). The ecological importance of this genus, particularly of its mycelium, is to take part in the decomposition of plant residues in soil. Mycoparasitic Trichoderma strains are able to recognize the host hyphae, to coil around them, develop haustoria and penetrate the cell wall of the host (Abdullah *et al*., 2007). Characterization of the antagonistic effect of Trichoderma species is the first step in utilizing the full potential of Trichoderma species for specific anti-plant pathogenic applications (Figure 3).

A microbial biological control agent may express different mechanisms against pathogens during their antagonistic activity; it weakening or destroying the pathogen by parasitize the pathogen directly, by producing antimicrobial compounds, by compete for space and nutrients, by producing enzymes that attack the cell components of the pathogens (Agrios, 2005) Antibiosis, production of antibiotic compounds and inhibition of other microbes, is the most important mechanism expressed by the antagonistic bacteria. In this study, the antagonistic effect expressed by the Trichoderma spp. in dual culture method might be due to the one or combination of all the above mechanisms (Figure 4). However, the results of all other three assays for Trichoderma spp. and assay for Bacillus spp. mainly depended on the ability of producing antimicrobial compounds and degradative enzymes by the tested antagonistic organisms. It has been already reported that Bacillus spp. has ability to produce a large number of antifungal metabolites such as bacitracin, gramicidin S, polymyxin, tyrotricidin, bacilysin, chlotetaine, iturin A, mycobacillin, bacilomycin, mycosubtilin, fungistatin and subsporin *B. anthracis, B. circulans and B. polymyxa* and *B. sphaericus* had inhibitory effect on the mycelial growth of tested fungi. This shows that above bacteria are potent antagonists of *P. aphanidermatum*. Similar results were reported by some other researchers, where different strains of soil isolates of *Bacillus* sp. revealed antagonistic effect against *P. aphanidermatum*. In the present study among the tested nine Bacillus spp. Five failed to show any inhibition zone. This may be due to the lack of ability to produce antimicrobial compounds which are inhibitive to *P. aphanidermatum* or may be inadequate production of antimicrobial compounds.

Based on the antagonistic activity it is observed to be higher with 55% of inhibition for *R. solani*, followed by the minimum inhibition with 32 for *P.fluorescence* (Table 1).

Figure 3. Determination of antagonistic activity.

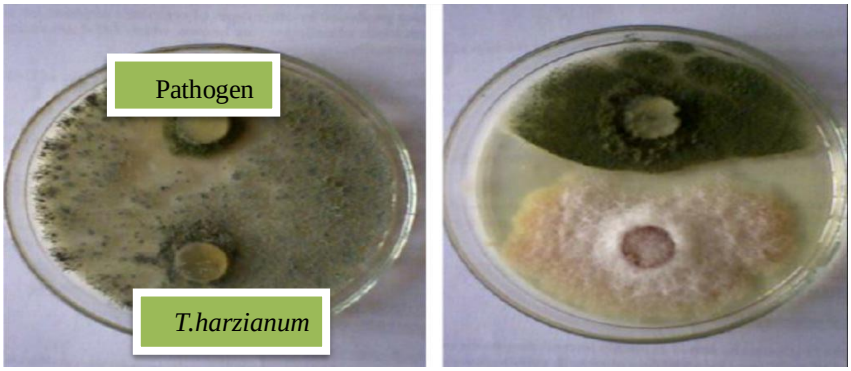

Figure 4. Antagonistic activity.

Table 1. Antagonist test.

Treatment	Percentage of Inhibition
T.harzianum	55%
P.fluorescence	32%
B.subtilis	49%
R.solani	55%

CONCLUSION

The Rhizosphere soil has highest number of isolate compare to non- rhizospheric soil reported by authours. In the present study the role of bio control activity have been reported. The isolation of Species identification and differentiation in this genus is only possible with the study of morphological features viz., culture colour and structure of conidiophores/ conidia, etc. In these work, three colonies were isolated from the PDA agar plates that were subjected to antagonistic characterization shown the result.

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