J. Mycopathol. Res. ***58(3)*** *: 187-191, 2020;* (ISSN 0971-3719)

Effective management of Collar Rot disease caused by *Rhizoctonia solani* Kuhn. in coffee using native biocontrol isolates

M. SUDHA*, MADHU S. GIRI, SANTOSHREDDY MACHENAHALLI, A.P.RANJINI AND N. SURYA PRAKASH RAO

Division of Plant Pathology, Central Coffee Research Institute, C. R. Station (P.O.) – 577 117, Chikkamagaluru district, Karnataka, India

Received : 24.07.2020 RM Accepted : 30.08.2020 Published : 26.10.2020

Coffee is an important plantation crop in India. *Coffea arabica* and *C. canephora* are two commercially exploited types of coffee and both are susceptible to fungal diseases. Propagation of coffee plants is mainly through seeds in India. *Rhizoctonia solani* is known to cause decay of seeds referred as Collar Rot/Damping-off of coffee seedlings in the nursery beds and can lead to 10 to 25% mortality of seedlings under favourable conditions and is prevailing in all the coffee growing areas of India. For effective management of this disease by biocontrol method the present study was carried out. Sixty rhizosphere soil samples were collected from different coffee growing regions of Chikkamagaluru district, Karnataka at different altitudes having mono and mixed shade pattern. Fifteen *Trichoderma* and ten fluorescent pseudomonad strains were isolated from the coffee rhizosphere soils. The potentiality of *Trichoderma* and fluorescent pseudomonad strains were assessed in terms of per cent inhibition of mycelial growth of *R. solani*. Among the different *Trichoderma* isolates CB-Tr.3 showed maximum mean per cent inhibition (57.78%) of mycelial growth followed by CB-Tr.4 (56.67%). Whereas, among ten fluorescent pseudomonads, isolate CB-FP.10 inhibited (65.18 %) mycelial growth of *R. solani*. Seed germination of 90.52% and high vigour index (1438.06) was observed in *T. harzianum* (CB-Tr.3) treated seeds compared to untreated control (82.13% seed germination and 1178.34 vigour index).

Key words: Bioagents, fluorescent Pseudomonads, coffee, collar rot, *Rhizoctonia solani*, *Trichoderma harzianum*

INTRODUCTION

Collar Rot or Damping-off disease in coffee is caused by the common soil inhabitant *Rhizoctonia solani* Kuhn. which affects coffee seedlings both in the primary seed beds and secondary nursery. Both the commercially exploited cultivars *Coffee arabica* and *Coffea canephora* are susceptible to Collar Rot disease. Excess soil moisture due to poor drainage, watering, overcrowding of seedlings favour the fungus growth and spread of the disease. *Rhizoctonia solani* is soil borne in nature and survives in soil for many months in the form of sclerotia. The infection by this fungus can lead to 10-25% mortality of seedlings under favourable conditions. It infects embryo, endosperm and radical during germination leading to rotting of the seeds and is called as pre-emergence damping-off. Infected seedling in the button stage show brownish discoloration on the stem near the collar region below the cotyledon leading to rotting of the tissue and wilting of growing apex leading to death of seedlings and is termed as post emergence damping-off. To mitigate the problem of Collar Rot, carbendazim 50 WP is used for seed treatment and drenching in the nursery bed. Continuous use of the chemicals in nursery may lead to reduction in the population of soil beneficial microflora. Hence, Biocontrol agents can serve as an alternative to chemical fungicides and is an ecofriendly approach for soil borne disease management. In view of this, a systematic study has been initiated to isolate and identify the potential strains of bio-agents from coffee habitats

*Corresponding author : sudhaccri@gmail.com

and use them for the management of Collar Rot disease.

MATERIALS AND METHODS

Collection of soil samples and isolation of bioagents

Sixty coffee rhizosphere soil samples were collected from different coffee growing regions of Chikkamagaluru district, Karnataka at different altitudes having mono and mixed shade pattern. Representative soil samples were drawn and subjected for isolation of *Trichoderma* and fluorescent pseudomonads by following serial dilution method on *Trichoderma* selective medium ($MgSO_4$: 0.20 g, KH_2PO_4: 0.90 g, NH_4NO_3: 1.0 g, KCl: 0.15 g, Glucose: 3.0 g, PCNB: 20 g, Rose Bengal: 0.15 g, Chloramphenicol: 0.25 g, Agar-agar: 15 g, Metalaxyl: 30 g, Distilled water: 1 L). One ml of (10^{-3} dilution) was poured on to Petri dishes containing *Trichoderma* selective medium for selective isolation of *Trichoderma* species and incubated at 25±2° C. Culture plates were observed at regular intervals for the appearance of colonies of *Trichoderma*. The fungal colonies with white mycelium, which later changed into different shades of green on the culture medium, were selected, examined picked up on the basis of their morphological and microscopic characteristics and purified by hyphal tip isolation technique. Purified *Trichoderma* spp. were transferred to Potato Dextrose Agar (PDA) slants. Bacterial colonies showing the characteristic fluorescence in King's B Medium (KBM) under UV-light were picked up, purified, and maintained on KBM slants. The pure cultures of both *Trichoderma* spp. and fluorescent pseudomonads were maintained and stored at 4°C for further studies.

Isolation of Rhizoctonia solani from collar rot infected seedlings

Diseased collar rot infected samples were collected from nursery at Central Coffee Research Institute, Balehonnuru, Chikkamagaluru District, Karnataka. The infected stem tissue from collar region of seedling was cut into small bits and surface sterilized with one per cent sodium hypochlorite solution for two to three minutes and repeatedly washed in sterilized distilled water. The sterilised infected bits were transferred on to Petri dishes (1-2 bits per Petri dish) containing PDA medium with the help of a sterile forceps under aseptic condition and incubated at 25± 1°C for seven days.

Evaluation of bioagents against R. solani

Based on the morphological characters, growth and development eight out of fifteen *Trichoderma* isolates and ten bacterial isolates were screened to identify the efficient type by adopting dual culture technique (Skidmore and Dickinson, 1976) against collar rot pathogen *R. solani*. About 15 ml PDA medium was poured into Petri plates allowed to solidify. *Trichoderma* culture disc of 5 mm were cut using sterilized cork borer from the periphery of 5 days old actively growing cultures and placed at end of the on PDA plates and same size of the test pathogen was placed at the opposite end of the Petri plate and incubated for 7 days at 25 ±1°C. Five Petri dishes were maintained for each treatment including control devoid of biocontrol agent.

Cultures of fluorescent pseudomonads from single colony selected and streaked on three sides forming a triangle in the Petri dishes containing PDA and at middle the culture disc (5 mm) of the test pathogen was placed. Five replications along with control were maintained. The plates were incubated in an inverted position at room temperature (25 ± 2°C) till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured, and the percentage inhibition was calculated by adopting formula given by Vincent (1927). I = (C-T/C) x 100; where, I = Per cent inhibition of mycelial growth; C = Radial growth of pathogen in control plates (mm); and T = Radial growth of pathogen in dual culture (mm).

RESULTS AND DISCUSSION

Sixty coffee rhizosphere soil samples were collected from different coffee growing regions of Chikkamagaluru district, Karnataka. From these soil samples fifteen *Trichoderma* isolates were isolated on *Trichoderma* selective medium and ten fluorescent pseudomonad isolates were isolated on King's B medium. Based on the morphological characters, growth and development, out of 15 isolates of *Trichoderma* purified only eight isolates and ten isolates of pseudomonads were selected for screening against *R. solani*. All the selected isolates indicated biocontrol ability with different degrees of suppression (Fig. 1). Results showed

Table 1. Antagonistic effect of native *Trichoderma* isolates against *R. solani*

Trichoderma isolates	Mycelia inhibition of *R. solani* (%)
CB-Tr.1	50.74 (45.44)*
CB-Tr.2	37.04 (37.49)
CB-Tr.3	57.78(49.51)
CB-Tr.4	56.67 (48.85)
CB-Tr.5	52.22 (46.29)
CB-Tr.6	52.22 (46.29)
CB-Tr.7	48.15(43.96)
CB-Tr.8	53.33(46.93)
S. Em. ±	0.79
CD @ 1%	3.25

* Figures in parenthesis are Arc sine values

Table 2. Antagonistic effect of native Fluorescent Pseudomonads isolates against *R. solani*

Fluorescent Pseudomonads isolates	Mycelia Inhibition of *R. solani* (%)
CB-FP.1	0.00 (0.00) *
CB-FP.2	13.88 (21.88)
CB-FP.3	0.00 (0.00)
CB-FP.4	0.00 (0.00)
CB-FP.5	10.18 (18.61)
CB-FP.6	0.00 (0.00)
CB-FP.7	13.71 (21.74)
CB-FP.9	0.00 (0.00)
CB-FP.9	0.00 (0.00)
CB-FP.10	65.18 (53.86)
S. Em. ±	0.81
CD @ 1%	0.94

* Figures in parenthesis are Arc sine values

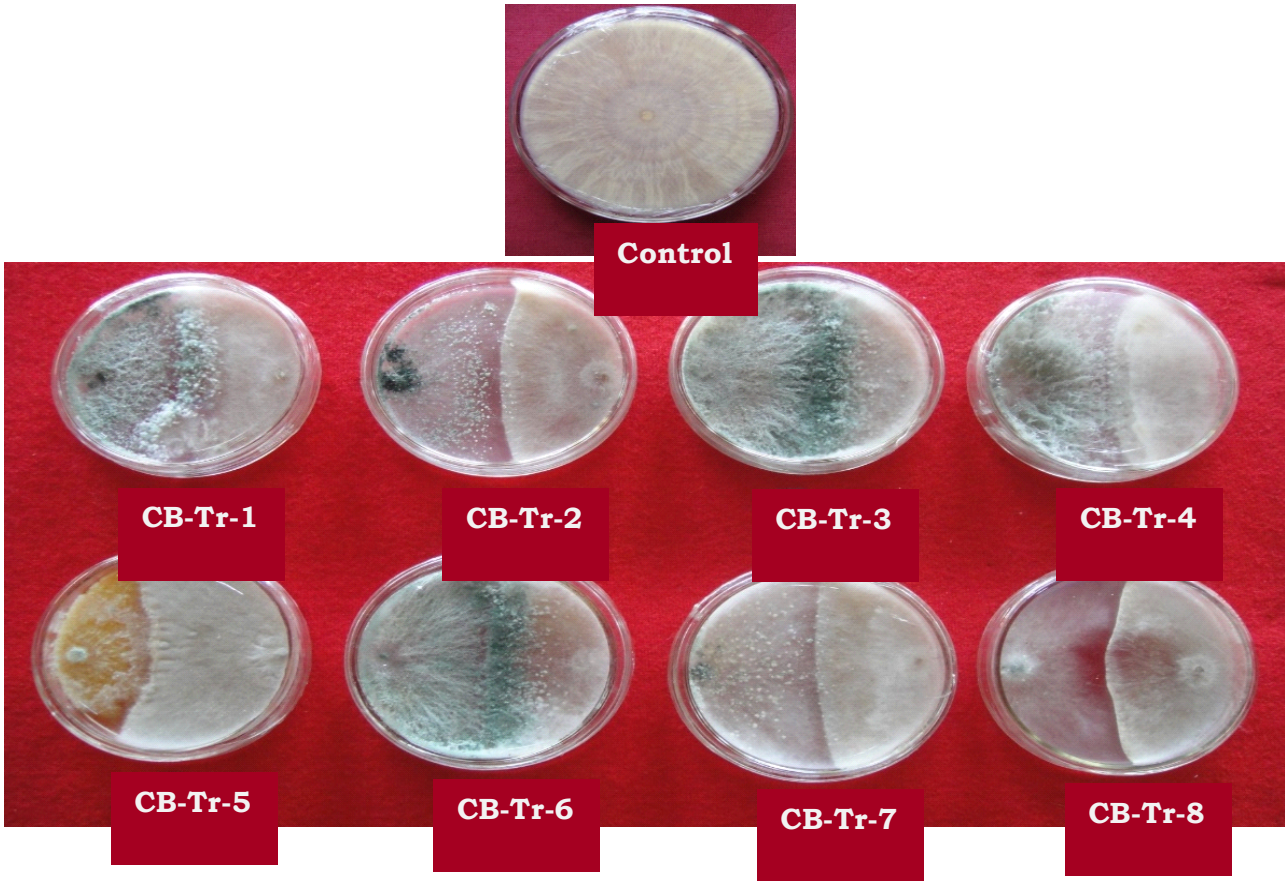

Fig.1 : Antagnostic effect of native *Trichoderma harzianum* isolates on mycelia inhibition of *Rhizoctonia solani*

that all the eight isolates of *Trichoderma* could suppress the mycelia growth of *R. solani* to an extent of 37.04 to 57.78%. Among the different isolates CB-Tr.3 showed maximum mean per cent inhibition (57.78%) of mycelia growth followed by CB-Tr.4 (56.67%) (Table 1 Fig.1). These isolates proved to be the most potent antagonising bio-control agents against *R. solani*. However, after four days of incubation there was clear cut suppression of *R. solani* was observed with respect to the formation of sclerotia at the antagonised region wherein the hyphae of CB-Tr.3 completely over grew on the pathogen. Apart from these fungal isolates, ten different fluorescent

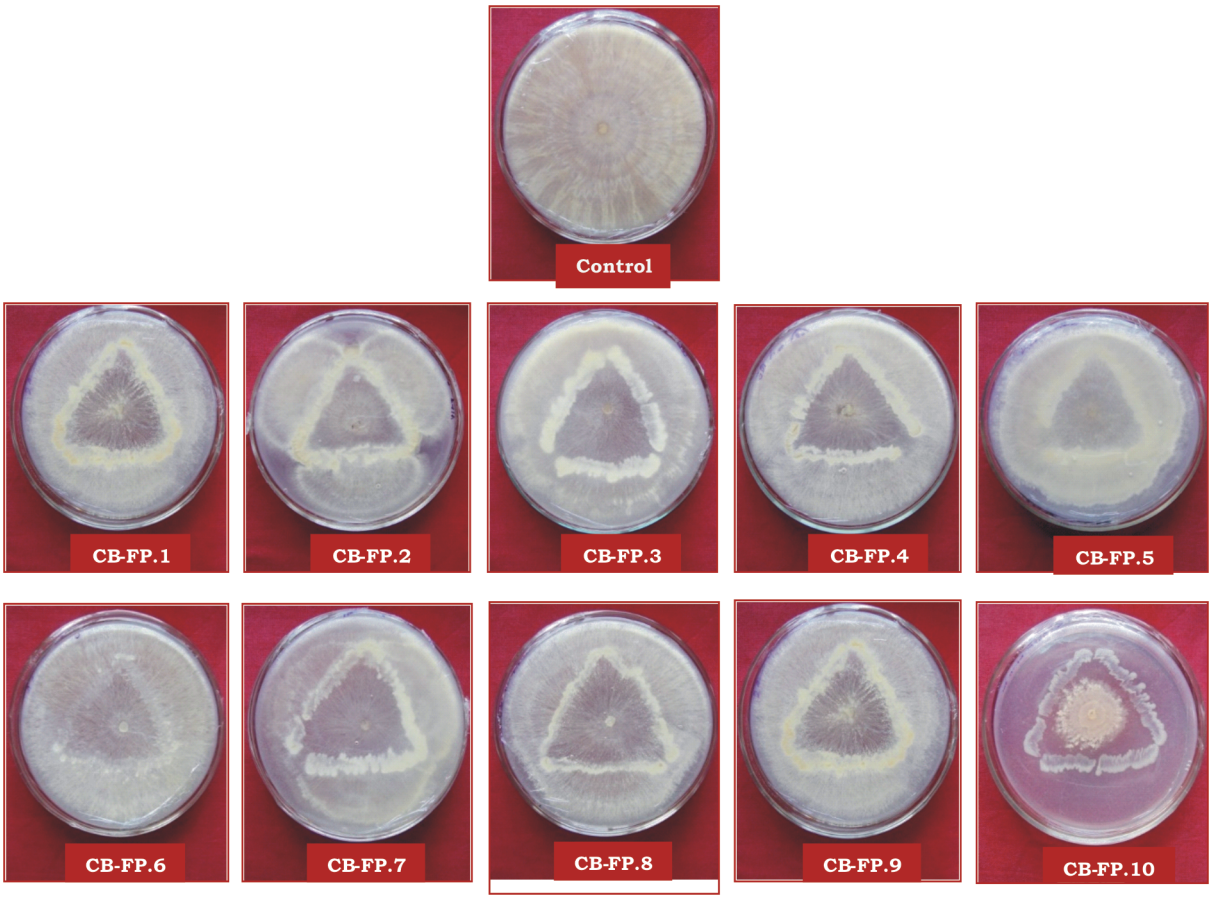

Fig.2. Antagonistic effect of native Fluorescent Pseudomonad isolates on mycelia inhibition of *Rhizoctonia solani*

pseudomonad isolates were also evaluated against *R. solani.* Among them, CB-FP.10 isolate showed maximum inhibition (65.18 %) of mycelial growth of *R. solani* (Table 2, Fig.2).

The mechanisms involved in the control of pathogens by *Trichoderma* spp. are due to antibiosis, lysis, competition for space and nutrients, secretion of chitinolytic enzymes and mycoparasitism. Biological control mechanisms are likely to be specific for antagonists and plant pathogens. Several mechanisms could operate independently or synergistically in any microbial interaction. *Trichoderma* and fluorescent pseudomonads are efficient bioagents, which are commercially produced and applied to suppress the development of several soil borne pathogenic fungi. *Trichoderma* spp. is found to be capable of lysing mycelia of soil-borne fungi like *Sclerotium* and *Rhizoctonia*. Since, *R. solani* causes collar rot/ damping-off in coffee seedlings, efficient antagonistic *Trichoderma* isolate can be used for the effective management of the disease. It has been reported that *T. harzianum* can be used as bioagent to manage the collar rot disease in coffee seedlings. Similarly, studies conducted by others also revealed that fluorescent pseudomonads have better antagonistic effect against R*. solani*.

Trichoderma harzianum can be mass multiplied by using coffee cherry husk a by-product of coffee, which was found to be a good carrier medium, economical, easily available, reliable and can maintain longer shelf life of the fungus up to six months (Sudha *et al.,* 2019). The efficient isolate of *Trichoderma* (CB-Tr.3) was used as coffee seed dresser and also incorporated to the seed beds to assess the per cent germination and growth vigour. Seed germination of 90.52% and high vigour index (1438.06) was observed in CB-Tr.3 treated seeds compared to untreated control (82.13% seed germination & 1178.34 vigour index).

The present study showed that native isolates of *Trichoderma* (CB-Tr.3) and fluorescent pseudomonad (CB-FP.10) can be efficiently used as bio-control agents for the ecofriendly and hazardous chemical free management of coffee collar rot disease in the nurseries.

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