Efficacy of biocontrol agents on *Myrothecium roridum,* the stem necrosis and leaf spot pathogen of coffee seedlings

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ABSTRACT

Myrothecium roridum, pathogen of coffee seedlings causing stem necrosis and leaf spot particularly during the continuous rains results in seedling loss up to 33%. The present study was taken up to know the efficacy of the native bioagents isolated from coffee rhizosphere soil for the control of *M. roridum* under *in vitro* conditions. Efficacy of two *Trichoderma* and six bacteria was tested in the laboratory conditions. The results of the experiment indicated that both the *Trichoderma* isolates: *Trichoderma harzianum* (CRF-1) and *Trichoderma viride* (CRF-2) effectively inhibited the mycelial growth of the pathogen, *M. roridum* up to 100% and 85% respectively whereas among the six bacterial isolates evaluated, two bacteria *viz*., *Bacillus subtilis* (CRB-2) and *Pseudomonas fluorescens* (CRB-5) were more effective in inhibiting the mycelial growth of the pathogen *M. roridum* up to 55.50% and 50.60% respectively. The effective bioagents identified from this study could be utilized for *in vivo* studies to assess their efficacy and to device better control measures for stem necrosis and leaf spot disease of coffee seedlings.

Keywords: Bioagents, Coffee, *Myrothecium*, *Bacillus, Pseudomonas, Trichoderma*

MS History: 02.02.2019 (Received)- 19.05.2019 (Revised)- 21.05.2019 (Accepted).

Citation: Ranjini, A. P. and Raja Naika. 2019. Efficacy of biocontrol agents on *Myrothecium roridum,* the stem necrosis and leaf spot pathogen of coffee seedlings. *Journal of Biopesticides,* **12**(1): 109-113.

INTRODUCTION

Bio Control Agents (BCAs) and biopesticides are slowly replacing the chemical pesticides. BCAs offer the advantages of higher selectivity and lower or no toxicity in comparison to conventional chemical pesticides. Use of fungicides for the control of diseases is costly and also results in environment pollution and health hazards to living beings and adversely affects the beneficial micro flora existing in the soil (Dluzniewska, 2003). Bioagents from rhizosphere of the native plants are effective in controlling the soil borne pathogens. Coffee plants grow within a defined area between the tropics of cancer and Capricorn termed as bean belt or coffee belt. It is a flowering plant which is being cultivated for its grains/seeds. Commercial farming for coffee beans is an excellent business and one can obtain desired profits from ideal crop practices. The coffee tree produces red cherries wherein the seeds of the red cherries are processed to produce the refreshing drink (Anon., 2014). Many coffee products including beverages are made from these coffee beans.

Like many other crops, coffee is also prone to various diseases. In India, the cultivated varieties, arabica and robusta coffee are affected by fungal diseases. The major diseases observed in the field include leaf rust, black rot, anthracnose, root diseases, berry blotch in field conditions. In the nursery, collar rot caused by the fungus *Rhizoctonia solani* Kuhn. and brown eye spot by *Cercospora coffeicola* Berk. & Cke. were considered to be the major diseases affecting coffee seedlings in India till the year 2005. In the recent years, occurrence of stem necrosis and leaf spot on coffee seedlings caused by the soil borne fungus *Myrothecium roridum* Tode ex Fries. was reported to be a major problem in coffee nurseries throughout Karnataka State, causing 5 to 33% mortality of seedlings (Daivasikamani *et al*., 2016). Though, the occurrence of *M. roridum* on coffee seedlings was reported in

India earlier by Nagraj and George (1958), it never caused any economic damage and was considered as minor disease of coffee.

In India, propagation of coffee is mainly through seeds. The success of new planting in perennial crops like coffee depends primarily on planting of vigorous, pest and disease-free seedlings in the field. Therefore, utmost attention is required to raise desirable planting material in the nursery. Stem necrosis and leaf spot of coffee seedlings is caused by the fungus *Myrothecium roridum.* This disease causes severe damage on coffee seedlings especially during the continuous monsoon period from June to September. The pathogen infects both stem and leaves of coffee seedlings. The infected seedling shows constriction of stem that occurs at any place above the soil showing cushion shaped black fruiting bodies of the pathogen. Affected seedlings gradually start wilting and die. The infected leaves initially show water-soaked circular necrotic spots, later spreads gradually to more areas and changes to brown colour with concentric rings. Black fruiting bodies are noticed on the lower surface of the affected leaf all along the concentric rings of the spot (Ranjini and Rajanaika, 2018). The present study was undertaken to find out the effectiveness of different bioagents isolated from coffee rhizosphere against *M. roridum* causing stem necrosis and leaf spot of coffee seedlings to device suitable cost effective and ecofriendly control measures against the disease.

MATERIALS AND METHODS

Isolation of the pathogen

The infected coffee seedlings exhibiting the symptoms of stem necrosis and leaf spot were collected from the nursery of Central Coffee Research Institute, Chikkamagaluru, Karnataka and were examined under stereomicroscope. The pathogen *M. roridum* was isolated by tissue isolation technique. Diseased tissues were washed thoroughly and surface sterilized with 1% sodium hypochlorite solution and then washed with sterilized water 2-3 times. The diseased tissues were placed aseptically on to 90 mm Petri plates containing 20 ml of solidified Potato Dextrose Agar medium (PDA). These inoculated Petri plates were incubated at 25° C for 2-3 days. The isolated fungus was purified for further studies (Jhonston and Booth, 1983; Aneja, 2012).

Isolation of biocontrol agents

The coffee rhizosphere soil samples were collected from the farm of Central Coffee Research Institute (CCRI), Chikkamagaluru, Karnataka. Soil samples were mixed thoroughly and 1g of soil was transferred to test tube containing 9 ml of sterile distilled water. The stock solution was serially diluted up to 10^{-9}. The dilutions of 10^{-4} and 10^{-5} were used for the isolation of fungi and dilutions 10^{-5} and 10^{-6} were used for the isolation of bacteria (Aneja, 2012). Specific culture media were used for the isolation of bioagents *viz.*, Trichoderma Specific Medium (TSM) for the isolation of *Trichoderma*, Kings 'B' medium (KB) for isolation of *Pseudomonas* and Nutrient agar (NA) for isolation of *Bacillus* and *Enterobacter*. Isolated fungi and bacteria were purified. The isolates of bacteria were identified by comparing morphology, staining, cultural and biochemical characteristics on the guidelines of Bergey's Manual of Systematic Bacteriology (Brenner *et al*., 2004) and fungal isolates were identified by morphology, staining, cultural characteristics on the guidelines of Barnett and Barry (1998). Two *Trichoderma* species and six bacterial isolates were used for the studies.

In vitro screening

Basal media for fungi (Potato Dextrose Agar) and bacteria (Nutrient Agar) was prepared. Twenty ml molten medium was poured in each sterile Petri plate. Antagonism of bacteria against *M. roridum* was tested by dual culture method (Aneja, 2012). Five mm mycelial disc of actively growing cultures of *M. roridum* was placed on the centre of the Petri plate and bacterial antagonists was inoculated in triangular position at three cm apart from center of Petri plate. Each treatment was replicated thrice. The inoculated Petri plates were incubated at 28 ±1°C for six days after which observations were recorded.

For fungal antagonist, 5 mm disc of four days old cultures of *Trichoderma* sp. and the test pathogen *M. roridum* was separately cut with the help of a sharp sterilized cork borer from the edge of 4 days old culture and placed in straight line at a distance of 5 mm from edge on PDA medium. Three replicates were maintained for each treatment. The Petri plates were incubated at 28 ±1°C for six days. After incubation, area covered by antagonist and test fungus was recorded. The diameter of mycelial growth was measured on 7 and 14 days after incubation. Index of antagonism was determined and per cent inhibition was calculated and the data were analyzed statistically as suggested by Panse and Sukhatme (1985) for CRD experiment.

RESULTS

Efficacy of different biocontrol agents isolated from coffee rhizosphere and their growth inhibition ability on *M. roridum* are presented (Table 1).

Table 1. Efficacy of biocontrol agents on *Myrothecium roridum*

Biocontrol agents	Growth inhibition of *M. roridum* (%)
Ba. brevis (CRB-1)	2.30*
Ba. subtilis (CRB-2)	55.20
En. intermedius (CRB-3)	8.00
Ba. pumulis (CRB-4)	16.10
Ps. fluorescens (CRB-5)	50.60
Ba. lactolactis (CRB-6)	0.00
Tr. harzianum (CRF-1)	100.00
Tr. viride (CRF-2)	85.90
S.E.	3.01
CD (P=0.01)	12.44

CRB - Coffee rhizosphere bacteria; CRF - Coffee rhizosphere fungi.

From the results, it is clear that there was significant reduction in mycelia growth after confrontation of tested pathogen with four antagonists out of eight tested. The different antagonists exhibited reduction of mycelial growth of *M. roridum* (2.30-100%). The fungal isolates CRF-1 and CRF-2 are the most effective bioagents with pathogen inhibition rate up to 100% and 85.90 % respectively and could show the hyper-parasitism and inhibit the pathogen development. Among bacterial isolates, CRB-2 and CRB-5 could inhibit the growth of the pathogen up to 55.20% and 50.60% respectively. It is also observed that the pathogen did not grow further 30 days after inoculation and mycelia growth was restricted.

DISCUSSION

Myrothecium roridum is a serious pathogen of coffee seedlings and also coffee plants. Very few information is available on the biocontrol of the pathogen. In the present study, evaluation of coffee rhizosphere biocontrol agents revealed the potential of their inhibitory effect on the mycelial growth of the pathogen *M. roridum*. Gunasekaran *et al.* (2003) while working on coconut leaf rot fungi found that *Pseudomonas fluorescens could* inhibit the mycelial growth of all the leaf rot fungi but the leaf rot pathogen *Thielaviopsis paradoxa* was completely inhibited by the bacterial bioagent. Among the biocontrol agents used for the study both the *Trichoderma sp*. was effective in inhibition of the mycelial growth. *Trichoderma* is widely known to be an effective biocontrol agent in controlling many of the soil borne pathogens (Harman *et al.*, 2004).

Murthy *et al.* (2004) reported that the disease caused by *M. roridum* in teak saplings could be effectively controlled by bioagents like *Trichoderma harzianum* and *Pseudomonas fluorescens* that gave the lowest mean percentage of disease incidence. The studies of Sarmah *et al.* (2005) revealed that the fungal bioagents like *Trichoderma harzianum* and *Trichoderma viride* proved their efficacy in controlling diseases like charcoal stump rot, brown root rot and stem diseases of tea. They also reported that the bacterial bio agent *Bacillus subtilis* was efficient in controlling blister bight of tea up to 50% and black rot up to 70%. *Trichoderma* sp. isolate was found most effective species to inhibit the mycelial growth of the fungus *Sclerotium rolfsii* which is a soil borne fungus. The studies of Wells *et al.* (1972); Upadhyay and Mukhopadhay (1986), Patel and Anahosur (2001) also confirmed the potential of *Trichoderma* in controlling the soil borne diseases. Szentes *et al*. (2013) reported that *Psuedomonas fluorescens* BE8 showed 55% control against

Fusarium oxysporum f. sp. *cucumerinum* in an *in vivo* experiment. Durga Prashad *et al.* (2014) found that among eleven isolates of bioagents the fungal isolate *T. koningi* was most effective in inhibiting the growth of pink canker up to 82.60 % and bacterial isolates like *Pseudomonas* sp. provided maximum inhibition zone up to 15.70 mm and an isolate of *Bacillus subtilis* resulted in maximum inhibition zone of 12.30 mm. Najwa *et al.* (2016) studies revealed that the bioagents *T. viride* and *T. harzianum* showed 100% inhibition against citrus pathogens causing gummosis. Shrijana *et al.* (2017) reported that there was 100% inhibition of radial growth of *Sclerotium rolfsii* by three isolates of *Trichoderma*, whereas in case of *Rhizoctonia solani* and *Fusarium solani* the inhibition percentage was found to be 62% and 68% respectively. Neeraja *et al.* (2018) while working on basal stem rot of coconut caused by *Ganoderma* spp. *viz., G. lucidum*, *G. applanatum G. boninense* found that soil application of talc based formulation of 125g of *Trichoderma reesei* + 125 g of *Pseudomonas fluorescens* + 5 kg of Neem cake/palm/year was found effective in reducing the disease index from 28.44 to 4.23 within a period of three years and also found the increasing trend of the nut yield under field conditions.

The present investigation revealed the ability of biocontrol agent's inhibitory effect on the soil borne pathogen *M. roridum* and proved that the identified biocontrol agents could inhibit the growth of the pathogen and hence be useful in the management of stem necrosis and leaf spot disease of coffee seedlings. Biological control seems to the best alternative to disease suppression. Biocontrol agents bring the disease suppression with no environmental hazards. Research has proved that the bio agents trigger the growth of plants. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favours the activity and survival of microbe it contains. Moreover the novel concept of bio control needs a space outside the laboratory to see its fruits in present production systems.

ACKNOWLEDGEMENT

The author's are grateful to the Director of Research, Central Coffee Research Institute (CCRI) for providing facilities to carry out the research work and also to all the Scientists of Plant Pathology Division, CCRI for their co-operation during the study.

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