Journal of Biological Control, 36(4): 234-241, 2022, DOI: 10.18311/jbc/2023/33792

Research Article

Bioprospecting: An innovative technology for the management of coffee collar rot disease

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ABSTRACT: Coffee is an important beverage crop in India. The soil-borne pathogen, *Rhizoctonia solani* Khun is known to cause collar rot of coffee seedlings in the nursery itself and accounts for 10-25% mortality under conducive environmental conditions. This paper presents the efficacy of various microbial antagonists used in the form of biocapsules for management of coffee collar rot. Among the various antagonists used, *Bacillus subtilis* resulted in the maximum reduction of collar rot incidence compared to *Trichoderma harzianum* and *Pseudomonas fluorescens*.

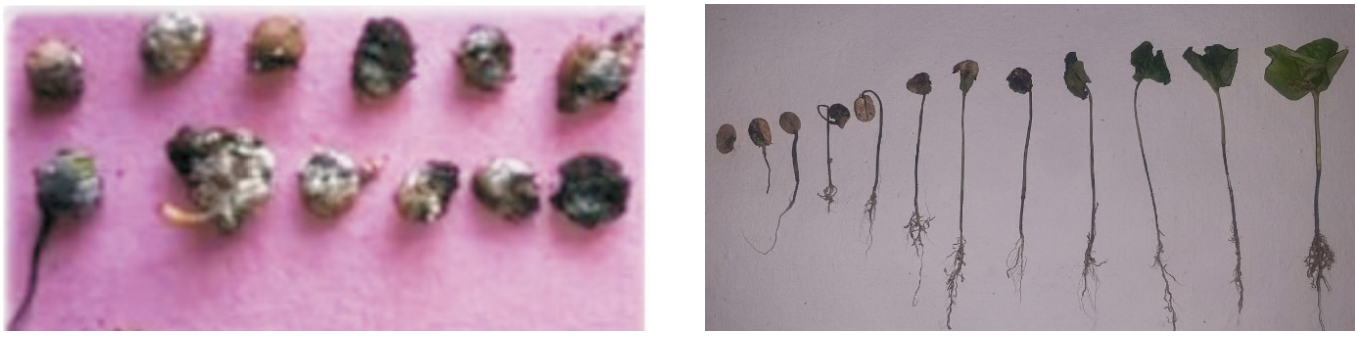
KEYWORDS: *Bacillus subtilis,* biocapsules, *Pseudomonas fluorescens, Rhizoctonia solani*, *Trichoderma harzianum*

(Article chronicle: Received: 21-10-2022; Revised: 28-12-2022; Accepted: 30-12-2022)

INTRODUCTION

The coffee (*Coffea* sp.), belonging to the family Rubaceae, is the most economically important beverage plant worldwide. *Coffea arabica*, *Coffea canephora* and *Coffea liberica* are the important commercialized species belonging to *Coffea.* The *Coffea arabica* accounts for 75-80% of the world's coffee production, and roughly 20% comes from *C. canephora* (Mussatto *et al.,* 2011; Castellanos *et al.,* 2013). Coffee is the most consumed beverage in the world (Masi *et al.,* 2015). It has a global market and regarded as the most traded commodity after oil (Chanakya and De Alwis, 2004). Tamil Nadu has approximately 33,904 acres of land under coffee farming, with an average annual production equal to 18,289 metric tonnes with a productivity of 539 kg per hectare (Annual report, 2023).

Long-term cultivation of coffee results in a buildup of pests and the emergence of diseases. Biotic stress caused by insects and pathogens are the main obstacles that affect production and productivity. *Rhizoctonia solani* Khun causes collar rot in coffee plants and is a common disease in traditional coffee-growing areas. The disease incidence was recorded as high as 25 to 30 percent in the months of April-May (Sudha *et al*., 2006). Weather conditions required for the coffee seedling establishment are positively correlated with collar rot disease development. Valdez and Acedo (1963) showed that the moisture content in the nursery beds directly influences collar rot disease development. Irrigation frequency, viz. twice irrigation per day and single time irrigation in a day, predispose the *R. solani* disease development between 45 - 50 days. Under conducive environmental conditions, seed rotting symptoms are also noticed in the field. However, when the coffee plant nears maturity, the stem region turns into a brown area and becomes harder which leads to failure in the pathogen establishment and its subsequent infection. Since the *R. solani*, is a soil-borne pathogen, it has a wide host range, and the pathogen successfully overwinters in the soil in the form of sclerotia which undergoes the myceliogenous type germination under favorable environmental conditions. Disease development takes place at two stages of the crop growth. The first phase of infection takes place at the seed germination phase, which leads to rotting and failure in seedling establishment. The second phase of the infection takes place at the post-emergence stage where black discoloration at the collar region of the stem leads to the rotting of the tissues. Wilting and the death of the growing tips of the infected seedling were observed in the infected plants (Jayalakshmi *et al.,* 2021).



(a) **(b)**

Figure 1 (a). Pre emergence damping off, **(b).** Post emergence damping off.

Coffee collar rot (*Rhizoctonia solani* Khun) belongs to the Phylum: Basidiomycota, Class: Agaricomycetes, Order: Cantherallales and Family: Ceratobasidiaceae. Collar rot causes both pre-emergence and post-emergence damping off (Figure 1). In pre-emergence, damping off the entire seed will rot and fail to germinate. Embryo and endosperm are invaded by the fungus before germination and the radical during germination resulting in seed rot and disintegration. In case of post-emergence, damping off the collar region near the soil level is infected leading to the rotting of tissues and death of seedlings. Post emergence damping off occur in the field between 45-50 days after sowing. Seedlings show brownish discoloration on the stem near the ground level leading to rotting of the tissue. The principal inoculum for the pathogen comes from the sclerotia that live in the soil. The pathogen spreads as sclerotia, and these sclerotia migrate between host plants via wind, water, or soil movement.

The application of chemical fungicides is not desirable as it leads to environmental pollution and the development of resistant strains and suppress the activity of beneficial microorganisms in the soil (Vinale *et al.,* 2008). However, the use of beneficial biocontrol agents is a highly feasible strategy to overcome disease development and is safe and non-hazardous for human, farm animals and environment. Exploiting antagonistic microorganisms has proved to be successful in controlling various plant diseases in many countries (Janisiewicz *et al.,* 2000).

MATERIALS AND METHODS

Fungal and bacterial biocontrol agents

The fungal biocontrol agent (*Trichoderma harzianum*) and the bacterial biocontrol agents (*Bacillus subtilis* and *Pseuodomonas fluorescens*) were isolated from the rhizosphere soils of the coffee plant by adopting the serial dilutions (10^{-6} for *B. subtilis* and *P. fluorescens* 10^{-4} for *T. harzianum*) and the pour plate technique procedures following the procedures adopted by Society for general microbiology. The nutrient agar, King's B agar, and potato dextrose agar were used for *B. subtilis*, *P. fluorescens* and *T. harzianum,* respectively. The colonies of the biocontrol agent were maintained at 10^7cfu/ml with the help of a Malassez hemocytometer (Rajan, 2019).

Mass multiplication of biocontrol agents

After the culture establishment of the above antagonistic microorganisms, the mass production of these organisms was initiated. To initiate the mass production nutrient broth, King's B broth and potato dextrose broth were prepared in a conical flask and the loopful of *B. subtilis*, *P. fluorescens* and *T. harzianum* were inoculated respectively which were kept at room temperature for 2-5 days for bacterial antagonists and 7 days for fungal antagonists. Once these antagonistic organisms had attained optimal growth in the respective media, they were mixed with talc powder in a ratio of 1:2 and shade dried below 8% moisture content. To increase the adhesive properties of antagonistic microorganisms the sticking agent Carboxy methyl cellulose should be added in the quantity of 10 g per kg of talc powder (Rajan, 2019).

Encapsulation of antagonistic microorganisms in capsules (Biopriming)

The antagonistic microorganisms are bioprimed in the quantity of 8 g/kg of seed (Figure 2). Certain food supplements required for the growth of antagonistic microorganisms, *viz.* carbohydrates, protein and fat were also added in the concentration of 0.01% along with antagonistic microorganisms. In addition to the food supplements, chitin and carboxy methyl cellulose were also added. The above ingredients were encapsulated in the coffee biocapsules which is made up of cellulose and weighed about 0.4 g. The antagonistic microorganisms along with food supplements and coffee seed weighed around 1.0 and 0.5 g, respectively. Thus 1.5 g was the weight of the biocapsule. Similarly, biocapsules were made by filling talc-based formulations

of antagonistic microorganisms along with CMC and chitin (Figure 3). The capsules for soil application were given at the doses of 1 g, 2 g and 3 g capsules of each microorganism with carbendazim 3 g capsule as a treatment check (Table 1).

Biocapsule (BC) applicator

The Biocapsule applicator (Figure 4) was made from PVC pipe mounted with a bottle at the neck region. The PVC pipe is made with 17 holes for the purpose of delivering biocapsules, jeevamrutham, fish amino acid and panchakavya at different altitudes in the rhizosphere region of the coffee plant. BC applicator is also used for sowing coffee biocapsules in the field. The encapsulated material is made up of cellulosic material, which is highly degradable in nature when it is infused with jeevamrutham, fish amino acid and panchakavya during application.

RESULTS AND DISCUSSION

***In vitro* antagonism assay**

A dual culture technique was conducted to identify the efficacy of antagonistic microorganisms against the collar rot pathogen under *in vitro* conditions. The maximum reduction in mycelial growth of *R. solani* was observed with *Bacillus subtilis.* However, the degree of antagonism was highest when *B. subtilis* was isolated from native coffee rhizosphere soil than the distantly extracted isolate (Plate 1). *Trichoderma harzianum* also significantly suppressed the mycelial growth reduction of collar rot-causing pathogen (Plate 2). Almost all the isolates showed statistically on par with carbendazim fungicide.

Bio-priming of coffee seeds with antagonistic microorganisms

A significant reduction in collar rot disease severity was noticed when the coffee seeds were treated with antagonists, *B. subtilis*, *T. harzianum, P. fluorescens*. The disease severity reduction was recorded as 58.65% with *B. subtilis*, 55.10% with *T. harzianum* and 54.52% with *P. fluorescens*. The findings of Rini and Sulochana (2007) also showed that *Trichoderma harzianum* and *T. pseudokoningii* and *P. fluorescens* were more effective against *R. solani.* The results were in line with microscopic photographs which revealed that among three antagonists maximum bacterial accumulation was observed in *B. subtilis*, followed by *P. fluorescens* and *T. harzianum*.

Performance of coffee seedling height, weight, and collar rot incidence

The assessment of efficacy of biocapsules on coffee seedlings was observed at three different crop growing stages viz., coffee seedling stage (Topee), butterfly stage of coffee seedling (BF) and three months old coffee seedling (3M). The maximum seedling height and weight were observed when

(a) **(b)** **(c)**

Figure 2. Biopriming of coffee seeds with **(a).** *B. subtilis***, (b)**. *P. fluorescens* and **(c).** *T. harzianum***.**

Figure 3. Biocapsules and coffee biocapsules along with antagonistic microorganisms **(***Trichoderma harzianum***,** *Bacillus subtilis* and *Pseudomonas fluorescens***).**

Table 1. Treatment details

S. No	Treatment Details
1.	T_1- *Pseudomonas fluorescenc*e as Soil application at 1 g of capsule
2.	T_2- *Pseudomonas fluorescence* as Soil application at 2 g of capsule
3.	T_3- *Pseudomonas fluorescence* as Soil application at 3 g of capsule
4.	T_4- *Trichoderma harizianum* as Soil application at 1 g of capsule
5.	T_5- *Trichoderma harizian*um as Soil application at 2 g of capsule
6.	T_6- *Trichoderma harizianum* as Soil application at 3 g of capsule
7.	T_7- *Bacillius subtilis* as Soil application at 1 g of capsule
8.	T_8- *Bacillius subtilis* as Soil application at 2 g of capsule
9.	T_9- *Bacillius subtilis* as Soil application at 3 g of capsule
10.	T_{10}- Carbendazim 3 g of capsule
11.	T_{11}-Control

Figure 4. Biocapsule applicator delivers coffee Biocapsules (BC) along with jeevamrutham at different rhizosphere regions of the coffee plant.

the *B. subtilis* and *T. harzianum* were used at 3 g/capsule at all three different crop-growing stages (Table 2, Figures 5 and 6). Similarly, the least incidence of collar rot was recorded from the coffee plant treated with the *B. subtilis* filled biocapsules followed by *T. harzianum* filled biocapsules at the rate of 3 g/capsule (Figure 7) at the seedling stage, butterfly cropping stage and the three-month-old coffee seedling stage. The obtained results showed a non-significant relationship with carbendazim-treated coffee seeds which denotes that these antagonists showed equal efficacy with the fungicides at the three-month-old seedling stage.

The mechanism by which *Trichoderma* sp. suppresses plant pathogens through three mechanisms *viz.,* direct competition for space or nutrients (Elad and Baker, 1985), the production of antibiotic biomolecules, whether volatile or non-volatile (Chen *et al.,* 1997) and direct parasitism. Moreover, the genus *Trichoderma* possesses good qualities for controlling soilborne pathogens as well as obligate pathogens especially *Macrophomina phaseolina*, *Phytopthora*, *Rhizoctonia* and *Ustilaginoidea virens* (Sathiyaseelan *et al.,* 2009; Howell, 2003 and Anbazhagan *et al.,* 2022) *Pythium*, *Fusarium* (Sathiyaseelan *et al.,* 2009). In addition to the

Table 2. Performance of coffee seedling height against with coffee bio-capsules

Treatment	Plant Height (cm)			Plant Weight (g)		
	Topee	BF	3M	Topee	BF	3M
T_1- P.f- at 1 g of biocapsule	$6.20^{cde} \pm 0.13$	$7.20^{de} \pm 0.15$	$14^{fg} \pm 0.29$	$1.80^{g} \pm 0.04$	$5.00^{fg} \pm 0.10$	$10.50^{e} \pm 0.22$
T_2- P.f- at 2 g of biocapsule	$6.35^{bcde} \pm 0.13$	$7.50^{cd} \pm 0.16$	$14.3^{efg} \pm 0.30$	$2.10^{ef} \pm 0.04$	$5.20^{ef} \pm 0.11$	$11.50^{d} \pm 0.24$
T_3- P.f- at 3 g of biocapsule	$6.64^{bc} \pm 0.14$	$8.15^{ab} \pm 0.17$	$16^{abc} \pm 0.33$	$2.70^{c} \pm 0.06$	$6.00^{bc} \pm 0.13$	$12.60^{abc} \pm 0.26$
T_4- T.h- at 1 g of biocapsule	$6.00^{e} \pm 0.13$	$7.00^{e} \pm 0.15$	$13.8^{fg} \pm 0.29$	$1.70^{gh} \pm 0.04$	$4.80^{g} \pm 0.10$	$10.20^{e} \pm 0.21$
T_5- T.h- at 2 g of biocapsule	$6.45^{bcde} \pm 0.13$	$8.00^{ab} \pm 0.17$	$15.2^{cde} \pm 0.32$	$2.50^{d} \pm 0.05$	$5.50^{de} \pm 0.11$	$12.20^{cd} \pm 0.26$
T_6- T.h- at 3 g of biocapsule	**$6.79^{ab} \pm 0.14$**	**$8.25^{ab} \pm 0.17$**	**$16.503^{ab} \pm 0.34$**	**$3.00^{b} \pm 0.06$**	**$6.20^{b} \pm 0.13$**	**$13.00^{ab} \pm 0.27$**
T_7- B.s- at 1 g of biocapsule	$6.25^{cde} \pm 0.13$	$7.40^{cde} \pm 0.15$	$14.2^{efg} \pm 0.30$	$2.00^{f} \pm 0.04$	$5.10^{fg} \pm 0.10$	$10.75^{e} \pm 0.22$
T_8- B.s- at 2 g of biocapsule	$6.40^{bcde} \pm 0.13$	$7.80^{bc} \pm 0.16$	$14.8^{def} \pm 0.31$	$2.20^{e} \pm 0.05$	$5.30^{ef} \pm 0.11$	$12.00^{cd} \pm 0.25$
T_9- B.s- at 3 g of biocapsule	**$7.14^{a} \pm 0.14$**	**$8.43^{a} \pm 0.18$**	**$17.01^{a} \pm 0.36$**	**$3.50^{a} \pm 0.07$**	**$7.00^{a} \pm 0.15$**	**$13.20^{a} \pm 0.28$**
T_{10}- Carbendazim 3 g	$6.50^{bcd} \pm 0.13$	$8.10^{ab} \pm 0.17$	$15.503^{bcd} \pm 0.32$	$2.60^{cd} \pm 0.05$	$5.70^{cd} \pm 0.12$	$12.30^{bc} \pm 0.25$
T_{11}- Control	$6.10^{de} \pm 0.13$	$7.00^{e} \pm 0.15$	$13.503^{g} \pm 0.28$	$1.60^{h} \pm 0.03$	$1.60^{h} \pm 0.03$	$4.60^{f} \pm 0.10$
C.D.	0.416	0.423	0.969	0.154	0.344	0.732
SE(d)	0.198	0.201	0.461	0.073	0.164	0.349
C.V.	3.763	3.197	3.769	3.833	3.845	3.823

Topee – Coffee seedling stage, BF: Butter fly stage of coffee seedlings, 3M: 3 month old coffee seedlings

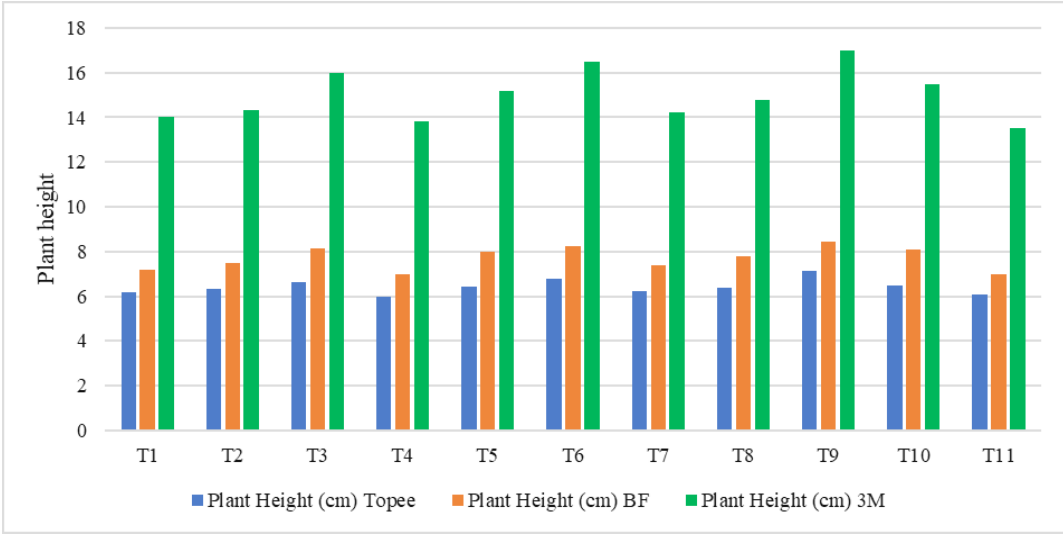


Figure 5. Performance of coffee seedling height against with coffee bio-capsules.

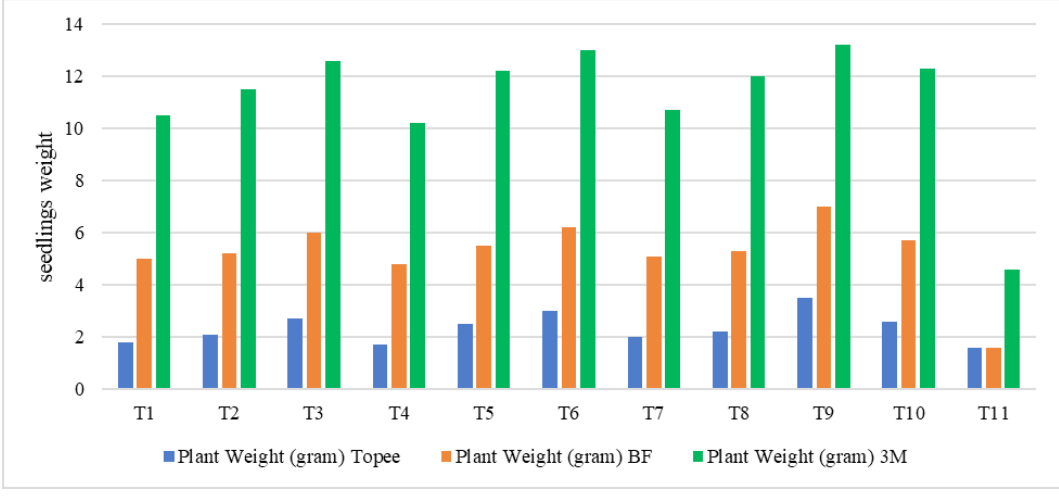


Figure 6. Performance of coffee seedlings weight against coffee bio-capsules.

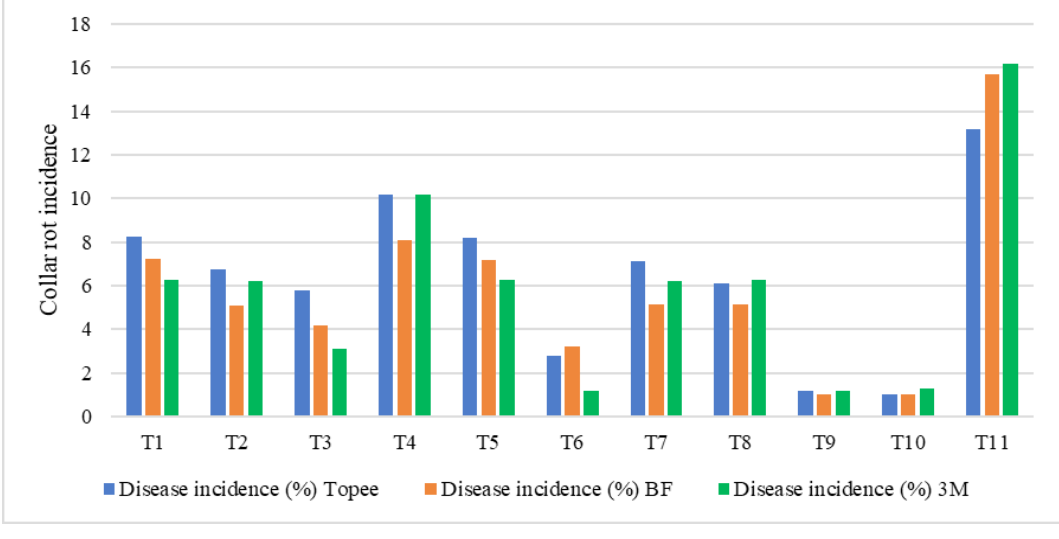


Figure 7. Coffee collar rot incidence against coffee bio-capsules.

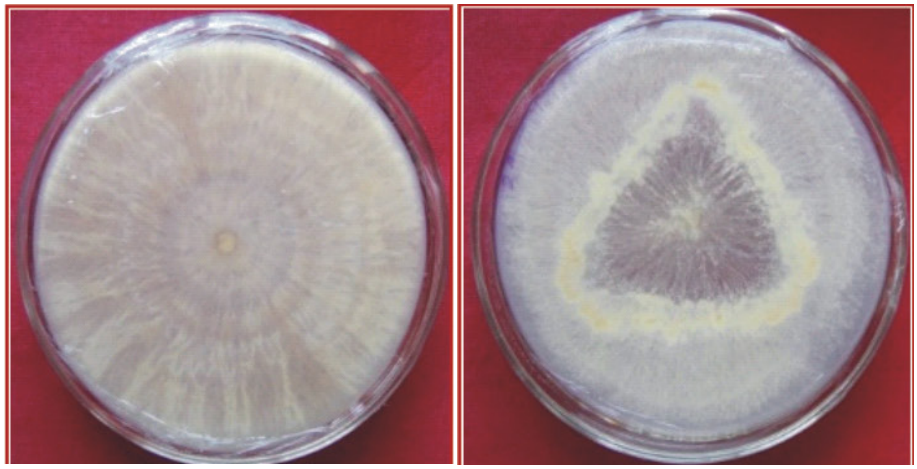


Plate 1. Dual culture technique - *Bacillus subtilis* (Native).

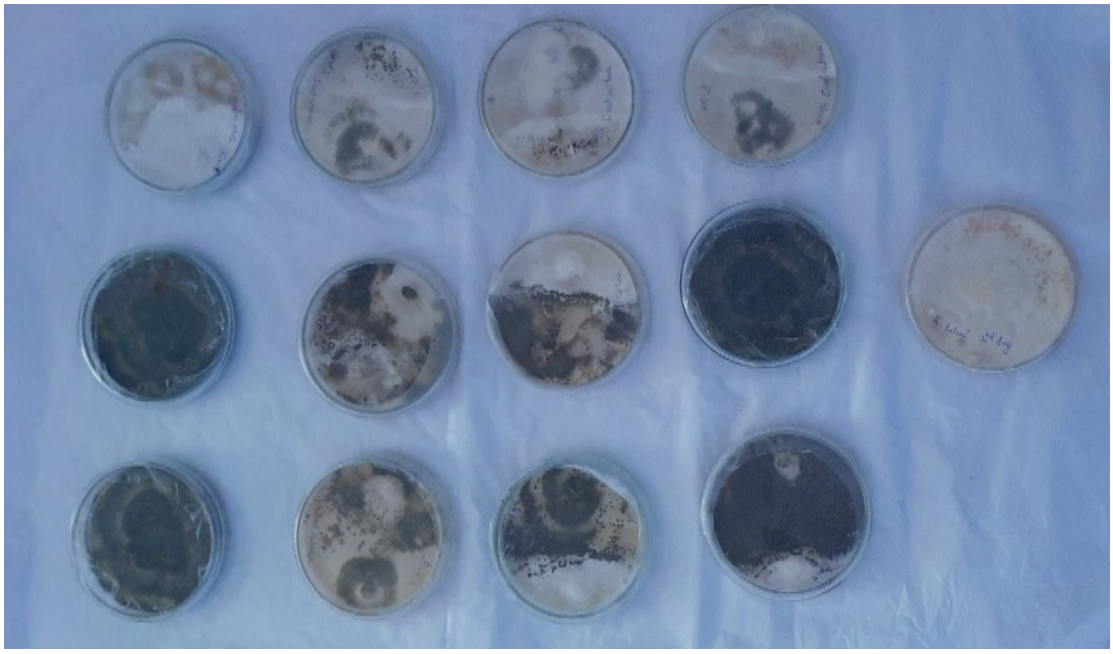


Plate 2. *Trichoderma harzianum* antagonist effect on 7th day.

above the *Trichoderma harzianum* effectively mitigate the drought stress tolerance in tomato (Anbazhagan *et al.,* 2020).

CONCLUSION

Exploiting biocontrol agents is an ideal alternative method in controlling coffee collar rot disease. In an attempt to mitigate the collar rot pathogen biologically, fungal and bacterial biocontrol agents were used *in vitro* and *in vivo* against *Rhizoctonia solani*. Results showed that among three antagonistic microorganisms *Bacillus subtilis* significantly suppressed the mycelial growth than remaining biocontrol agents under *in vitro* conditions. Under field conditions also the antagonistic microorganisms applied at different coffee seedling growth stages were able to suppress the diseases besides improving plant growth. The maximum reduction in collar rot incidence was recorded with carbendazim filled in biocapsules followed by *Bacillus subtilis* at 3 g biocapsule treatment and *Trichoderma harzianum* at 3 g biocapsule. The obtained results amply proved that an alternative way for fungicide application in reducing collar rot incidence can be implemented with the use of coffee biocapsule of *B. subtilis* and *T. harzianum*. This inoculum-efficient culture of *B. subtilis* is a simple low cost and novel technology that contributes to vigorous and healthy coffee seedlings by the coffee capsule method.

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