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Sensitivity of Bio-control agents against *Colletotrichum* spp. isolated from different hosts

Pranjali Sinha, Jahaar Singh, Neelum Chouksey and AK Singh

Abstract
Colletotrichum (teleomorph *Glomerella*) is an important plant microbe that affects agricultural and plantation crops across the world. It adheres to the hemi-biotrophic mode of feeding, in which both biotrophic and necrotrophic stages occur sequentially. Pathogenic variability among *Colletotrichum* species affecting various crops as mango (anthracnose), strawberry (fruit rot), chilli (anthracnose), turmeric (leaf blight), soybean (pod blight), bean (anthracnose) and sugarcane (red rot). These naturally infected seven crops were collected from different growing regions of Chhattisgarh. In the present study, laboratory evaluation of two Bio-control agents (Fungus-*Trichoderma viride* and Bacteria-*Pseudomonas fluorescence*), reponsed differentially to *Colletotrichum* spp. via confrontation assay. Highest mycelial inhibition by *T. viride* was observed in *C. fragariae* (C_7) inhibition of 81.4%, followed by *C. gloeosporioides* (C_6) with 80% and *C. lindemuthianum* (C_4) with 77.4%, respectively however, *P. fluorescencs, C. falcatum*, and *C. fragariae* showed similar inhibition of 51.1%.

Keywords: sensitivity, pathogenic, *Colletotrichum* spp.

Introduction
Genus *Colletotrichum* (teleomorph *Glomerella*) is one of the major plant pathogens of agricultural and plantation crops worldwide. The primary distribution, of this pathogen, lies on the various crops grown in tropical, subtropical and temperate areas (Hyde *et al*, 2009). Various species of this genus as *C. gloeosporiodes* (anthracnose of mango), *C. falcatum* (red rot of sugarcane), *C. curcumae* (leaf spot of turmeric), *C capsici* (anthracnose on chilli) and *C. truncatum* (pod blight of soybean) are more important, among the all-other species, and belong to Kingdom- Fungi, Phylum- Ascomycota, Class– Coelomycetes, Order-Melanconiales, and Family- Melanconiaceae (Hawksworth *et al.*, 1995) [5]. In later stage, after the infection, the hyphae develop and spread in the tissues leading to killing of the host cell. Thus, this genus follows the hemi-biotrophic mode of nutrition where sequential occurrence of the biotrophic and necrotrophic phases take place (Cannon *et al.*,2012) [4]. Fungus *Colletotrichum* produces characteristic acervuli and it causes losses at all stages of growth, as in anthracnose of beans, but few species of C*olletotrichum* may cause tremendous loss by damaging fruits, reducing yields through destruction of blossoms or by affecting leaves and stems. In mango, anthracnose is an important disease that affects plants by killing inflorescence, causing spots on leaves, and dark brown to black decay spots on the fruits at the ripening stage (Agrios, 1969) [1]. Additionally, both scientists and farmers have been concerned about the lack of resistant cultivars of the varied crop, efficient fungicides, and botanicals to manage anthracnose. Finding potent fungicides and plant-based antifungal chemicals is therefore essential to battling this illness (Ranjitha *et al*., 2019) [15]. It has been reported that fungicides and biological agents can be used *in-vitro* to regulate the anthracnose and blight caused by different species of Colletotrichum in a variety of hosts, although no host-pathogen interaction has yet been completely controlled. In the present study, laboratory evaluation of two Bio-control agents (Fungus-*Trichoderma viride* and Bacteria-*Pseudomonas fluroscence*), reponsed differentially to *Colletotrichum* spp. via confrontation assay.

Material and Methods
Fungus *Colletotrichum* spp. studied in this investigation, were isolated from seven different host naturally infected with anthracnose during a survey carried out in kharif (2020 - 2021). Pods of soybean (CoA, Raipur), matured chilli fruit, turmeric leaves, sugarcane stem, mango & strawberry leaves (KVK, Ambikapur) and matured pods of bean (Surajpur) regions of Chhattisgarh.

The individual symptoms, from the plant parts, for isolation, of the fungus was examined directly by placing infected part on the stereoscopic microscope. Only those infected parts were selected showed typical symptom for presence of conidia. The fungus cultures were purified using single spore isolation on 2% agar medium while being maintained on potato dextrose agar (PDA) medium. Single spore isolation was used to subculture, at intervals of 15 days, and samples were stored at a low temperature (4 ℃).

Pathogenicity tests of the fungus was proved (i) on young seedlings grown in between wet blotting papers (ii) on stem cuttings, fruits after making a fissure on the stem or fruits and inoculating with mycelium along with small piece of agar (Wijesekara, 2005) [21] and re-isolation was carried out.

Bioagent *Trichoderma viride* (CFU-2×10^8 CFU/g) and *Psuedomonas fluorescens* were screened, for antagonistic potential against the pathogen following dual culture technique (Morton and Stroube, 1955). Twenty ml of sterilized melted PDA was aseptically poured in a sterilized 90 mm diameter Petri plates and allowed to solidify. Five mm of mycelial disc of seven different species of *Colletotrichum* and test biocontrol agents cut with the help of sterilized cork-borer from the edge of 10 days old culture plates, were placed on solidified PDA in such a manner that they lie just opposite to each other (approximately 6 cm apart from each other). Inoculated Petri plates were incubated at 28 + 1 ℃. The process was replicated three times for seven consecutive days. During final observation the plates were 24, 48, 72, 96, 120, 144 and168 hours old. Periodic observations on the growth of biocontrol agents and the ability of biocontrol agents to colonize the pathogen were recorded.

Valuation of antagonism between *Pseudomonas fluorescens* isolates and *Colletotrichum* spp.

Toothpick were sterilized in autoclave at 121℃ at 15 psi for 20 minutes. Five mm discs were cut from the periphery of actively growing seven days old culture of the test fungus, with the help of sterilized cork-borer. Sterilized toothpick were used to pick *Pseudomonas* culture then spread in such a manner that both pathogen and antagonist lie opposite to each other in Petri plates (9cm diameter) from three sides with PDA amended with King's medium B (in 50:50 ratio, approx. 20 ml/ plate). Three replications were used for each treatment. All the plates were incubated at 28±10 ℃. Petri plates without *Pseudomonas* served as control.

The percent inhibition of radial growth was calculated with following formula:

$$\% \text{ inhibition of radial growth} = \frac{\text{Radial growth in check} - \text{Radial growth in treatment}}{\text{Radial growth in check}} \times 100$$

Result and Discussion

Efficacy of Bioagents

Fungi (*T. viride*) and bacteria (*P. fluorescens*), as bioagents, were evaluated, *in vitro* against seven different *Colletotrichum* spp using confrontation assay on PDA medium. Statistical analysis was carried out in CRD design with seven treatments, one control and three replications to compare the efficacy of bioagent against *Colletotrichum* spp. Both, the test pathogen and bio-agents, were confronted on edges of the plate through dual culture assay. After seven days of incubation at 28 ± 2 °C, observations were recorded by measuring the radial growth of the pathogen and then comparison of the treated plates were done, over control, by calculating the inhibition percent with the use of formula given by Vincent (1947) and data, recorded, are presented in Table 1.

a. Evaluation of the effect of *Trichoderma viride* against *Colletotrichum* spp under *in vitro* condition: In dual culture assay, highest mycelial inhibition was observed in *C. fragariae* (C_7) inhibition of 81.4%, followed by *C. gloeosporioides* (C_6) with 80% and *C. lindemuthianum* (C_4) with 77.4%, respectively, but remained statistically at par to other samples as *C. falcatum* (C_5) with 75.5% and *C. capsici* (C_1) also. On the other hand, lowest mycelial inhibition, by *T. viride*, was recorded in *C. acutatum* (C_2) with inhibition of 67.4% that remained statistically at par to *C. truncatum* (C_3) with the inhibition of 65.9% (Plate 1).

b. Evaluation of the effect of *Pseudomonas fluorescence* against *Colletotrichum* spp under *in vitro* condition: In dual culture assay, highest mycelium inhibition, by *P. fluorescence*, has been achieved in *C. falcatum* (C_5), with inhibition of 51.1%, followed by, but statistically at par to, *C. fragariae* (C_7), with 50.3%. On the other hand, *C. truncatum* (C_3), *C capsici* (C_1) and *C. acutatum* (C_2) recorded 44.4%, 43.7% and 42.2% growth inhibition, respectively, but remained statistically at par to each other, followed by *C. lindemuthianum* (C_4) with 40.7%. Lowest mycelial growth suppression, by *P. fluorescence*, was observed in *C. gloeosporioides* (C_6) with 35.6% inhibition (Plate 2).

Table 1: Impact of *T. viride* and *P. fluorescence* against *Colletotrichum* spp.

SN	Sample	Treatment	Growth inhibition %	
			T. viride	***P. fluorescence***
01	*C. capsici*	C_1	74.8	43.7
02	*C. acutatum*	C_2	67.4	42.2
03	*C. truncatum*	C_3	65.9	44.4
04	*C. indemuthianum*	C_4	77.0	40.7
05	*C. falcatum*	C_5	75.5	51.1
06	*C. gloeosporioides*	C_6	80.0	35.6
07	*C. fragariae*	C_7	81.4	50.3
Control		-	0.00	0.00
CV (%)		-	5.20	8.01
CD (5%)		-	5.90	5.40
SEm		-	1.90	1.80

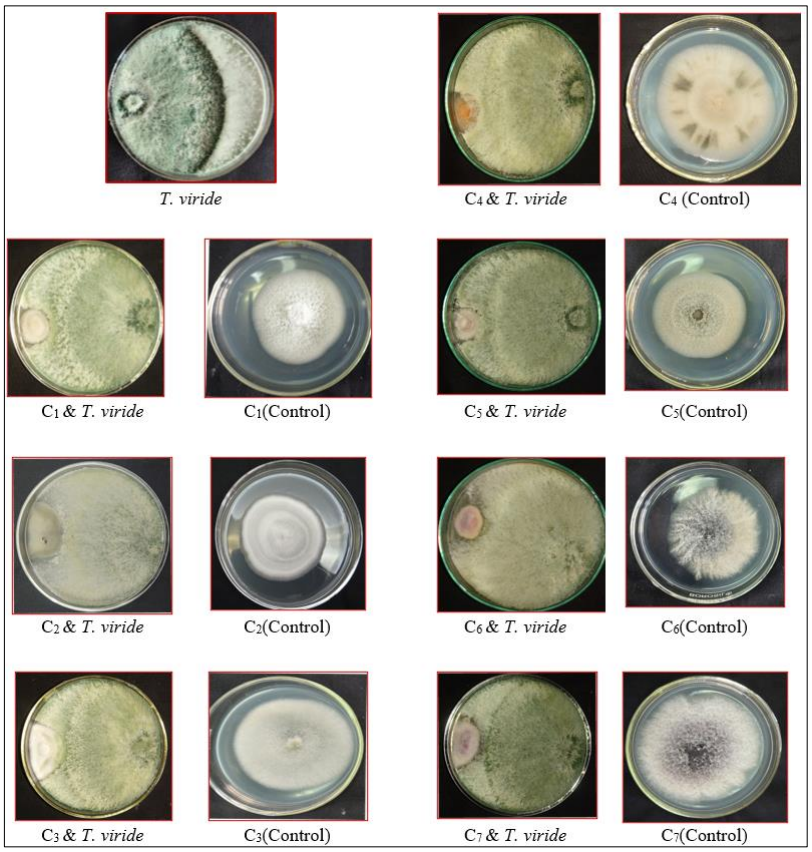

Plate 1: Confrontation assay to evaluate the efficacy of seven *Colletotrichum* spp and *T. viride*

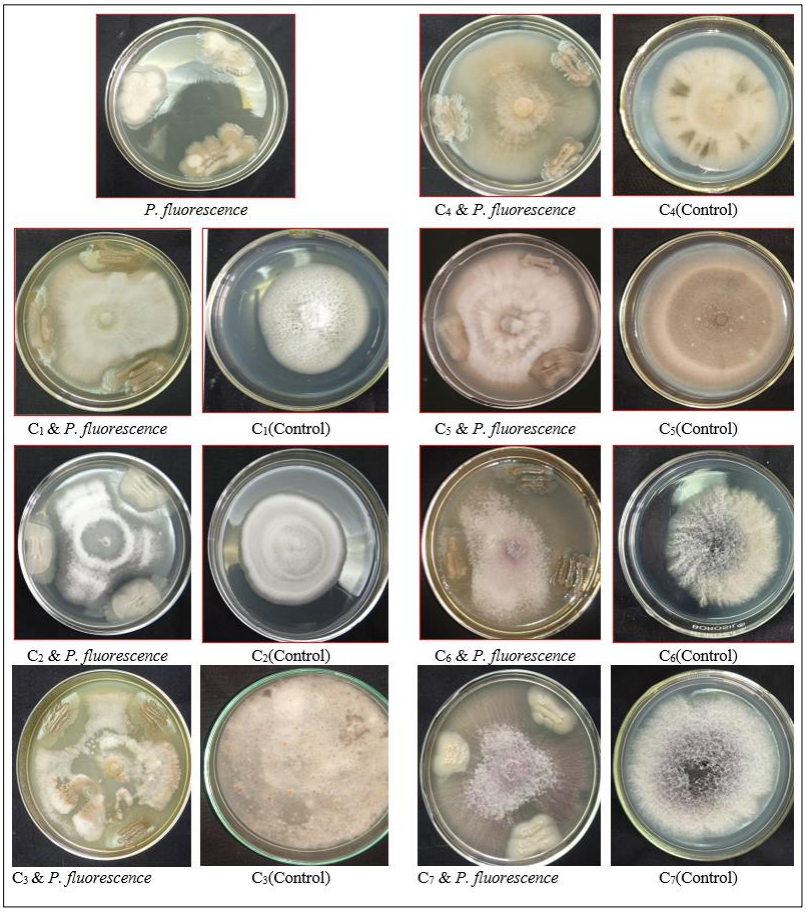

Plate 2: Confrontation assay to evaluate the efficacy of seven *Colletotrichum* spp and *P. fluorescence*

Data, on the present investigation, showed highest mycelial growth suppression by *T. viride* was recorded in *C. gloeosporioides* and *C. fragariae* with 81.0%. Various researchers reported that culture filtrate, prepared from *T. viride*, completely inhibited radial growth at 25 and 50% concentration and also reduced the spore germination of the *C. capsici* (Padder *et al.*, 2010; Kaur *et al.*, 2006; Rahman *et al.* 2012: Ranasinghe *et al.*, 2013) [9, 6, 11, 14], *C. falcatum* (Singh *et al*., 2008) [18], C. *lindemuthianum (*Patil *et al.*, 2009) [10], *C. dematium* (Bhujbal *et al*., 2016; Somwanshi *et al*., 2016; Kothikar *et al.*, 2017) [3, 19, 7]. Thus, present finding is in the affirmation of above researchers with variable degree of reduction. In case of bacteria as bio-agent, highest mycelial suppression, by *P. flouresence*, was recorded in *C. gloeosporioides* and *C. falcatum* (each of them 51.0%) and in *C. capsici* with 43.7%. Significant bio-control, of fungal pathogens, have been recorded by bacterial bioagent that inhibited the mycelial growth of *C. capsici, in vitro*, and also reduced the incidence of fruit rot in chilli by Ramamoorthy and samiyappan 2001 [13] (44.2%), Begum *et al.*, 2008 [2] (48.6%), and Raj and Christopher, 2009 [12] (52.3%) respectively. Thus, present study on biocontrol, through bacterial bioagent, also satisfy the finding of above researcher positively. Fungal and bacterial bioagents, evaluated *in vitro*, were found fungistatic against *A. helianthi* of sunflower (Waghe *et al.*, 2015) [20], and fungal bioagent (70.2%) was found effective than bacterial bioagent (48.6%), for inhibition of test pathogen are in conformity to those reported earlier by several workers (Meena *et al.*, 2004; Singh *et al.*, 2005; Rao, 2006) [8, 17, 16].

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