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In vitro Antagonistic Activity of Fungal and Bacterialbio Control Agents against Chilli Fruit Rot Incited by *Colletotrichum capsici*

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A B S T R A C T

In this study, antagonistic effect of 6 isolates of *Bacillus subtilis* (Bs-1, Bs-2, Bs-3, Bs-4, Bs-5,Bs-6), *Pseudomonas fluorescens* (Pf-1, Pf-2, Pf-3, Pf-4, Pf-5, Pf-6), *Saccharomyces cerevisiae* (Sc-1, Sc-2, Sc-3, Sc-4, Sc-5, Sc-6) and *Trichoderma* spp. (Ts-1, Ts-2, Ts-3, Ts-4, Ts-5, Ts-6) were evaluated against *Colletotrichum capsici*, the causal agent of chilli fruit rot, as potential biocontrol agents under *in vitro* conditions. Fungal and bacterial biocontrol agents were tested against chilli fruit rot pathogen (*C.capsici*) by dual culture plate assay. Among the biocontrol agents tested *Bacillus subtilis* showed 62.22 % inhibition against the target pathogen followed by *Pseudomonas fluorescens* (58.88 %), *Saccharomyces cerevisiae* (56.66 %) and *Trichoderma* spp (54.44 %) in the dual-culture assay under *in vitro* conditions.

Introduction

Chilli is considered as one of the most important commercial spice crops and as it is so widely used it has been given the name wonder spice. Chilli (*Capsium annuum*) belonging to the family Solanaceae is the most commonly cultivated and an indispensable source of vegetable and spice across the tropical and subtropical regions of the world. India is the second largest producer, consumer and exporter of chilli. Chillies are used for various purposes, both green and ripe chilli fruits contains an alkaloid capsaicin, which are used to impart pungency into the various food preparations, it is used in pharmaceutical industries, cosmetics, preparation of oleoresin and other industrial resources (Bosland and Votava, 2003). Chilli is susceptible to many foliar and soil borne fungal diseases among the biotic stress, fruit rot caused by *Colletotrichum capsici* is one of the most destructive disease which causes heavy yield loss in almost all chilli growing areas. The symptoms of the disease were noticed on leaves, flowers and

fruits. The disease incited as dark spot, water-soaked lesions that rapidly expand. In some cases, the lesions are brown, and then turn black due to the formation of setae and sclerotia (Roberts, 2001). Bio control is an effective alternate that provide disease control, while being relatively harmless to humans, selective in mode of action, difficult for pathogens to develop resistance (Singh *et al.*, 2005).In the present study shows the role of fungal and bacterial biocontrol agents against *Colletotrichum capsici* under *in vitro* which will effective in the field level.

Materials and Methods

Colletotrichum capsici isolate was collected from fruit rot infected chilli plants in Chellampatti village of Madurai, Tamil Nadu by tissue segment method (Rangaswami and Mahadevan, 1999).

The isolate was confirmedby morphological appearance and by ITCC (Accession no: 10025.19). A single pure culture was prepared from the isolate and maintained in PDA slants used for further experiments.

Isolation of bacterial and yeast antagonists from phylloplane region of chilli plants

Healthy and fruit rot infected chilli fruit samples, at ripening stage, were collected from different chilli growing areas of Tamil Nadu. For isolation of antagonist, two grams of each sample were surface sterilized and blot dried.

Cut pieces of samples (5mm) were taken into 250 ml Erlenmeyer flask with 20 ml of sterile distilled water. After 24 hr of shaking with an orbital shaker at 150 rpm at 28±2°C, (10^{-1} to 10^{-8}) serial dilutions of the suspension was made. Small aliquots (50 µl) from dilutes of 10^{-7} and 10^{-8}were poured onto Kings B agar, nutrient agar, and Yeast extract peptone dextrose media in Petri dish for isolation of the antagonists.

The plates were incubated at 28±2°C for 24-48 hr or until colony formation. Selection of single bacterial and fungal colonies was done based on morphological variation, colony characters and confirmed by different biochemical tests and after purification they were preserved in refrigerator.

Isolation of fungal antagonist from rhizosphere region

Healthy chilli plants were pulled out gently with intact roots and the excess soil adhering on roots were removed gently. Ten gram of rhizosphere soil was transferred to 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water. After thorough shaking, the antagonist in the suspension was isolated by using serial dilution plate method (Pramer and Schmidt, 1956). From the final dilutions of 10^{-3}, $l0^{-5}$ and 10^{-6} one ml of each aliquot was pipetted out, poured in sterilized Petri plate containing *Trichoderma* selective medium. *Trichoderma* spp. isolated on TSM, were purified and maintained on PDA medium. The pure cultures were maintained on respective agar slants at4 °C.

Effect of bacterial antagonists on the growth of *Colletotrichum capsici* under *in vitro*

Six isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* and were tested for their antagonistic effect on growth of *Colletotrichum capsici* by dual culture technique (Dennis and Webster, 1971). The bacterial isolates were streaked on one side of the Petri dish (1 cm away from the edge of the plate) on PDA medium and a mycelial disc (9 mm diameter) of nine days old *Colletotrichum capsici* culture was placed on the opposite side of the Petri dish

perpendicular to the bacterial streak. The plates were incubated at room temperature (28±2°C) for 9 days. Three replications were maintained for each isolate. After nine days of incubation, the pathogen growth and inhibition zone were measured.

Effect of fungal antagonists on the growth of *Colletotrichum capsici* under *in vitro*

Six isolates of *Trichoderma* spp. and *Saccharomyces cerevisiae* were screened against *Colletotrichum capsici* by dual culture method. A nine mm disc of *Colletotrichum capsici* and test antagonists *viz.*, *Trichoderma* spp. disc was placed and *Saccharomyces cerevisiae* was streaked opposite to pathogen disc near the periphery of the Petri plate and incubated at room temperature (28±2°C). Three replications were maintained for each isolate. The medium inoculated with the pathogen alone served as control. When the plates attained the full growth, the radial growth of the pathogen was measured in the others treatments. After nine days of incubation, mycelial growth of the pathogen and inhibition zone were measured in treated as well as control plates. Percent inhibition (PI) of mycelial growth was calculated using the formula suggested by(Pandey and Vishwakarma,1998).

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc = Average diameter of fungal growth (cm) in control
Dt = Average diameter of fungal growth (cm) in treatment.

The growth of antagonist over the pathogen was measured nine days after incubation. The growth and zone of inhibition was measured and expressed in cm.

Results and Discussion

Effect of *Bacillus subtilis* on the growth of *Colletotrichum capsici* under *in vitro*

Among the six isolates of *B. subtilis* (Bs-3) isolate was found to record maximum growth reduction of *Colletotrichum capsici* by 62.22 percent over control which was followed by *B.subtilis* (Bs- 4), which recorded the growth reduction of 55.55 per cent. The least mycelial growth reduction was recorded by *B.subtilis* (Bs-5) with 36.67 per cent (Table 1; Plate 1).

Effect of *Pseudomonas fluorescens* on the growth of *Colletotrichum capsici* under *in vitro*

Six *P.fluorescens* isolates were tested against the growth of *Colletotrichum capsici* by dual culture experiment. Among the isolates, *P. fluorescens* (Ps-1) resulted maximum growth reduction of *Colletotrichum capsici* by 58.88 per cent over control which was followed by *P. fluorescens* (Ps-6) which recorded the growth reduction of 55.55 per cent over control. The minimum growth reduction of 40.00 per cent was observed in *P. fluorescens* (Ps-2) (Table 2; Plate 2).

Effect of *Saccharomyces cerevisiae* on the growth of *Colletotrichum capsici* under *in vitro*

The effect of six isolates of *Saccharomyces cerevisiae* was tested against *Colletotrichum capsici* under *in vitro* conditions. *Saccharomyces cerevisiae* (Sc-3) was found to be effective by recording maximum mycelial growth reduction of 56.66 per cent over control which was followed by (Sc-1) which recorded the growth reduction of 54.44 per cent. The least mycelial growth reduction was recorded by (Sc-6) with 33.33 per cent (Table 3; Plate 3).

Effect of *Trichoderma* spp. on the growth of *Colletotrichum capsiciin vitro*

The effect of six isolates of *Trichoderma* spp. were tested against *Colletotrichum capsici* under *in vitro* conditions. Among the antagonists tested, *Trichoderma* spp. (Ts-4) was found to be effective by recording maximum mycelial growth reduction of 56.66 per cent over control which was followed by *Trichoderma* spp. (Ts-2) which recorded the growth reduction of 52.22 per cent. The least mycelial growth reduction was recorded by *Trichoderma* spp. (Ts-3) with 43.33 per cent (Table 4; Plate 4).

Effect of bacterial antagonists on the growth of *Colletotrichum capsici* isolate under *in vitro*

In the present study, *B. subtilis*(Bs-3) isolate was found to record maximum growth reduction of *Colletotrichum capsici* by 62.22 percent over control which was followed by *B.subtilis* (Bs- 4), which recorded the growth reduction of 55.55 percent. Jeyalakshmi *et al.*,(1998) reported that *Bacillus subtilis* was used to control chilli fruit rot and die back.A novel *Bacillus* spp. AB1, with strong antifungal activity, was obtained from coffee phyllosphere of the Nile River in India by (Nair *et al.*, 2002). Thirty *B. subtilis* isolates were evaluated under *in vitro* against *C. capsici.* All the isolates recorded the varied level of inhibition of mycelial growth of *C. capsici*. Among different isolates, BS16 showed maximum inhibition of 63.42 per cent followed by BS 30 (57.40 %) and minimum was 11.98 per cent (Rajkumar *et al.*, 2018).

In the present investigation, *P.fluorescens* isolates were tested against the growth of *Colletotrichum capsici* by dual culture experiment. Among the isolates, *P. fluorescens* (Ps-6) resulted maximum growth reduction of *Colletotrichum capsici* by 58.88 per cent over control which was followed by *P. fluorescens* (Ps-1) which recorded the growth reduction of 55.55 per cent over control.

Similar results were reported by Birari *et al.*, (2018) and showed that *Pseudomonas flourescens* have90% of the radial growth inhibition of the pathogen *Colletotrichum capsici.* Linu *et al.*, (2006) stated that the antagonistic effect of the bacterial isolates was tested against the chilli anthracnose pathogen *Colletotrichum capsici* by the standard dual culture method.

Table.1 Effect of *Bacillus subtilis* against the growth of *Colletotrichum capsici* under *in vitro* conditions

S.No	Isolate code	Radial mycelial growth (cm)*	Per cent inhibition over control
1	Bs - 1	5.00	44.44
2	Bs - 2	4.40	52.22
3	Bs – 3	3.80	62.22
4	Bs - 4	4.00	55.55
5	Bs – 5	5.70	36.67
6	Bs - 6	5.50	38.88
7	Control	9.00	0.00
	CD (P=0.05)	**0.25**	

*Mean of three replications

Table.2 Effect of *Pseudomonas fluorescens* against the growth of *Colletotrichum capsici* under *in vitro* conditions

S.No	Isolate code	Radial mycelial growth(cm)*	Per cent inhibition over control
1	Pf – 1	3.70	58.88
2	Pf – 2	5.60	37.77
3	Pf – 3	5.00	44.44
4	Pf – 4	4.30	52.22
5	Pf – 5	4.50	50.00
6	Pf – 6	4.00	55.55
7	Control	9.00	0.00
	CD (P=0.05)	**0.20**	

*Mean of three replications

Table.3 Effect of *Saccharomyces cerevisiae* against the growth of *Colletotrichum capsici* under in vitro conditions

S.No	Isolate code	Radial mycelial growth (cm)*	Per cent inhibition over control
1	Sc – 1	4.10	54.44
2	Sc – 2	5.00	44.44
3	Sc – 3	3.90	56.66
4	Sc – 4	5.40	40.00
5	Sc – 5	4.30	52.22
6	Sc – 6	6.00	33.33
7	Control	9.00	0.00
	CD (P=0.05)	**0.95**	

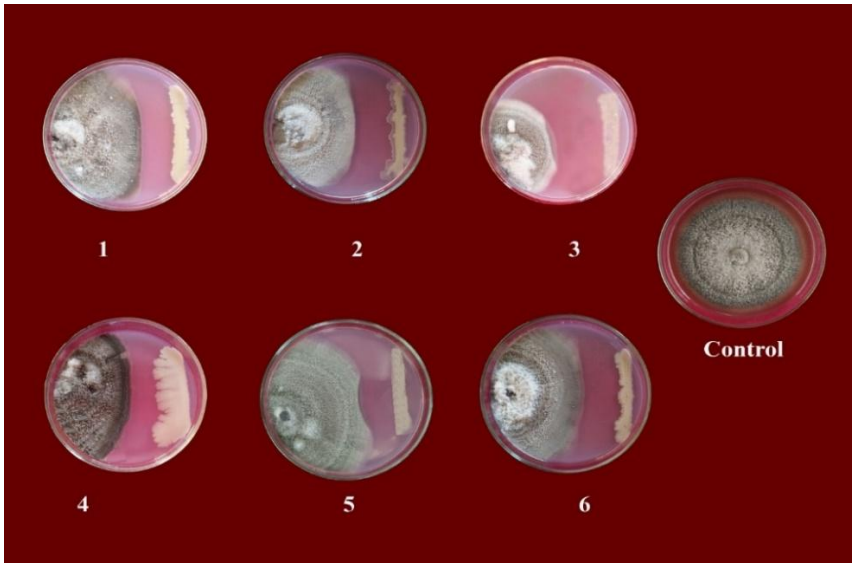
*Mean of three replications

Table.4 Effect of *Trichoderma* spp. against the growth of *Colletotrichum capsici* under *in vitro* conditions

S.No	Isolate code	Radial mycelial growth (cm)*	Per cent inhibition over control
1	Ts – 1	4.90	45.55
2	Ts – 2	4.30	52.22
3	Ts – 3	5.10	43.33
4	Ts – 4	4.10	54.44
5	Ts – 5	4.80	46.66
6	Ts – 6	5.00	44.44
7	Control	9.00	-
	CD (P=0.05)	**0.22**	

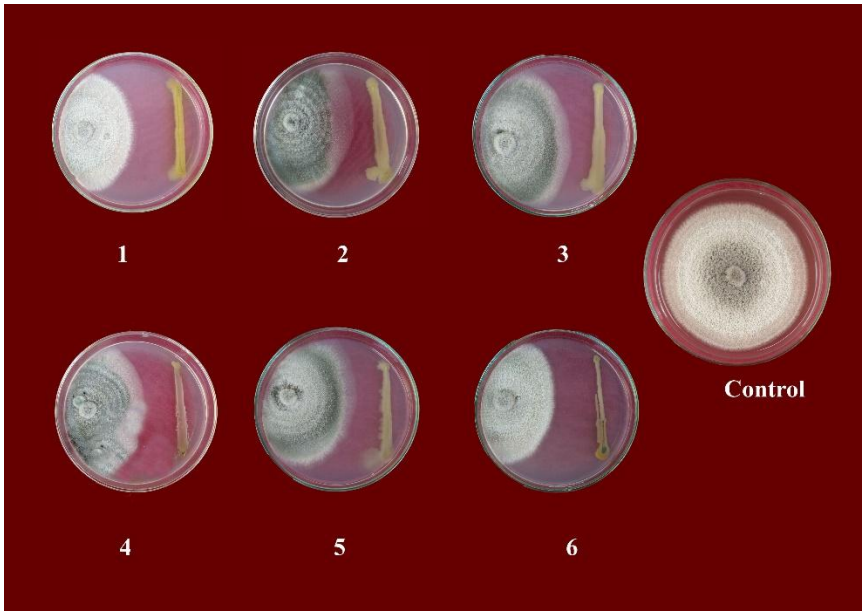
*Mean of three replications

Plate.1 Effect of *Bacillus subtilis* on the growth of *Colletotrichum capsici*



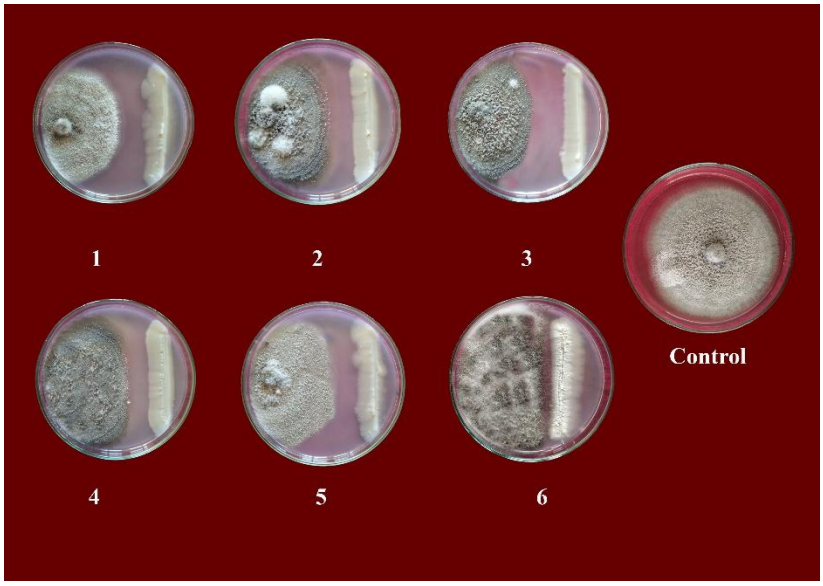
1. Bs - 1 2.Bs - 2 3.Bs - 3
4. Bs - 4 5.Bs - 5 6.Bs - 6
7. Control

Plate.2 Effect of *Pseudomonas fluorescens* on the growth of *Colletotrichum capsici*



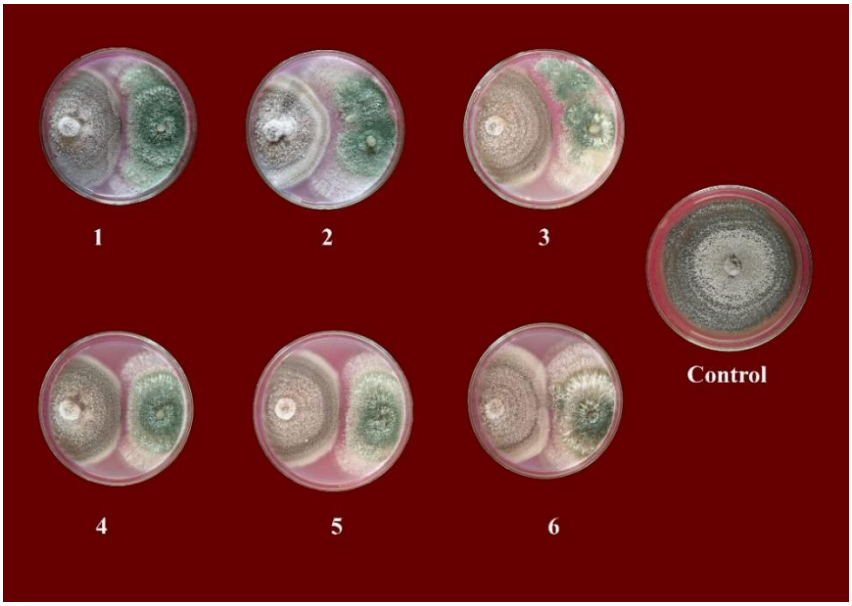
1. Ps - 1 2.Ps - 3 3.Ps - 3
4. Ps - 4 5.Ps - 5 6. Ps - 6
7. Control

Plate.3 Effect of *Saccharomyces cerevisiae* on the growth of *Colletotrichum capsici*



1. Sc - 1 2.Sc - 2 3.Sc– 3
4. Sc - 45.Sc - 5 6. Sc – 6
7.Control

Plate.4 Effect of *Trichoderma* spp. on the growth of *Colletotrichum capsici*



1. Ts - 1 2.Ts - 2 3.Ts - 3
4. Ts - 4 5.Ts - 5 6. Ts - 6
7. Control

The results revealed that among the isolates PS 2 showed maximum inhibition of 93.41% whereas the other isolate PS 3 showed 72.5% of inhibition against *Colletotrichum capsici* after 7 days of incubation.The biological control of three *Colletotrichum*

lindemuthianum races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens WCS365*(Bardas *et al.,* 2009).

The plants sprayed with only *C. capsici* cultures, recorded the highest disease intensity and *P. fluorescens* sprayed plant recorded least infection, this suppression of disease was attributed either to the activity of antifungal compounds produced by the microbe or the hyper parasitism on the pathogen or by ISR in the host plant which combat the pathogen infection. The rapid defense exerted by the treatment at the site of fungal entry delayed the infection process (Ramkumar *et al.,* 2012).

Effect of *Saccharomyces cerevisiae* on the growth of *Colletotrichum capsici* under *in vitro*

Biological control agents such as *Saccharomyces cerevisiae* have been reported as antagonistic to *C. capsici* (Jayelakshmi and Seetharaman, 1998). The results of present experiment revealed that *Saccharomyces cerevisiae* (Sc-3) was found to be effective by recording maximum mycelial growth reduction of 56.66 per cent over control which was followed by (Sc-1) which recorded the growth reduction of 54.44 per cent. Lopes *et al.,* (2015)Co-cultured *C. acutatum* in Petri dishes with each *S. cerevisiae* yeast isolate and observed that all yeast isolates inhibited mycelial growth of *C. acutatum*. The ACB-CAT1 and ACB-CR1 isolates promoted the most inhibitions values correspond to 71% and 67%, respectively. Moreover, the ACB-PE2 and ACB-K1 isolates were the least effective. Three yeast isolates were isolated and showed antagonistic effect of mycelial growth of *C. gloeosporioides* and showed different Inhibition ability relative to the control treatment when cocultured with *C. gloeosporioides*. GA8 isolate exhibited highest inhibition values of 79.29%. Moreover, L24 and LFA802 isolates were less effective (Liu *et al.,* 2018).

Effect of *Trichoderma* spp. on the growth of *Colletotrichum capsici in vitro*

In the present study, the fungal antagonist *Trichoderma* spp. (Ts-4) was found to be the most effective by recording maximum mycelial growth reduction of 56.66 per cent at 9 DAI. Azad *et al.,* (2013) found that *Trichoderma viride* gives 77.60% growth inhibition against *C. gloeosporioides.* The inhibitory effect of volatile and non-volatile compounds released by *T. saturnisporum, T. harzianum, T. viride* and *T. reesei* against *Colletotrichum capsici,* a fungal pathogen responsible for anthracnose disease in bell peppers (Ajith and Lakshmidevi, 2010). Evaluated different *Trichoderma* strains against *Colletotrichum capsici* under laboratory conditions by different techniques and found the *T. harzianum* as potential antagonist for inhibition of the mycelial growth, conidial germination, germ tube elongation and disease severity of *C. capsici* (Rahman *et al.,* 2013).

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