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Research Note

In vitro evaluation of fungal and bacterial antagonists against *Colletotrichum falcatum* Went

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ABSTRACT: Red rot of sugarcane caused by *Colletotrichum falcatum* is a major constraint in sugarcane production causing substantial loss in yield and quality. Use of biocontrol agents is an effective, eco-friendly and non-hazardous possibility for red rot management. In the present investigation potential bio-control agents were screened against *C. falcatum* on sugarcane. Among the 15 species/ isolates of bacterial and fungal antagonistic organisms screened against *C. falcatum* in dual culture, *Pseudomonas fluorescens* isolate-1 and *Trichoderma viride* isolate-3 showed maximum inhibitory effect on *C. falcatum*. These two isolates identified as more potential biocontrol agents in the present investigation can be exploited to control red rot of sugarcane.

KEY WORDS: Antagonist, *Colletotrichum falcatum,* dual culture, red rot, sugarcane.

Sugarcane is one of the major cash crops cultivated in both tropical and subtropical region all over the world. Sugarcane cultivation all over the world is hindered by many problems, of which the most important one is red rot disease, called "Cancer of sugarcane", and caused by *Colletotrichum falcatum* Went. Among the different management practices, planting of resistant variety is the best way to overcome this problem. However, due to the development of new variants of this fungus, the newly released resistant varieties often become susceptible after some years of cultivation and make breeding resistant varieties a routine process. Management of this disease with fungicide though attempted, is not preferred due to bio-safety consideration. So, biological control is a distinct alternate possibility and eco-friendly approach for its management. Therefore, the present investigation was carried out to evaluate various bacterial and fungal antagonists against *Colletotrichum falcatum* causing red rot of sugarcane.

The bacterial and fungal antagonists isolated from the rhizosphere soil of sugarcane (Table 1 and 2) were tested against *C. falcatum* by following dual culture (Dennis and Webster, 1971). Eight mm actively growing culture disc of *C. falcatum* was placed onto sterilized Petri-dish containing previously plated and solidified Czapek's Dox agar medium at 1.5 cm away from the edge of the plate. Eight mm fresh culture disc of fungal antagonistic organism was placed opposite to *C. falcatum* disc. Czapek's Dox agar medium inoculated with pathogen alone served as control. Three replications were maintained for each test. The plates were incubated at room temperature (28 ± 2°C). The radial growth of pathogen in each plate was measured when the control plate showed full growth. The results were expressed as per cent inhibition of the mycelial growth of pathogen over control (Vincent, 1927) as furnished below:

$$I = \frac{100\ (C - T)}{C}$$

Where,

I = Per cent inhibition over control
C = Growth of pathogen in control
T = Growth of pathogen in treatment

An eight mm actively growing mycelial disc of the pathogen was placed on nutrient agar (NA) medium 1.5cm away from the edge of the plate and incubated at room temperature for 48 hours. Then the actively growing 48 hours old cultures of the respective bacterial antagonist were separately streaked onto the medium at the opposite side of pathogen disc. The plates were incubated at room

Table 1. Effect of antagonists on the growth of *C. falcatum* (Dual culture technique)

S. No	Antagonistic organisms	Mycelial growth (cm)	Per cent inhibition over control *
1.	*Chaetomium globosum*		43.18 (41.30)
2.	*Trichoderma virens*	4.9	44.31 (41.73)
3.	*T. harzianum*	4.4	50.00 (45.00)
4.	*T. longibrachiatum*	6.7	23.87 (29.02)
5.	*T. reesi*	5.2	40.90 (39.76)
6.	*T. viride* isolate-1	3.9	55.68 (48.22)
7.	*T. viride* isolate-2	4.4	50.00 (45.00)
8.	*T. viride* isolate-3	3.3	62.50 (52.24)
9.	*T. viride* isolate-4	5.3	39.71 (39.76)
10.	*T. viride* isolate-5	5.8	34.09 (35.67)
11.	*B. subtilis* isolate-1	5.2	40.09 (39.76)
12.	*B. subtilis* isolate-2	5.4	38.63 (38.41)
13.	*P. fluorescens* isolate-1	2.8	68.18 (55.61)
14.	*P. fluorescens* isolate-2	4.3	51.13 (45.25)
15.	*P. fluorescens* isolate-3	4.9	44.31 (41.73)
16.	Control	8.8	00.00 (0.513)
CD (P = 0.05)			2.35

*Mean of three replications; data in parentheses are arc sine transformed values

Table 2. Effect of culture filtrates on mycelial biomass production and spore germination

S. No	Antagonistic organism	Mycelial biomass production		Spore germination	
		Mycelial dry weight (mg)*	Per cent inhibition over control	Spore germination (%) *	Per cent inhibition over control
1.	*Chaetomium globosum*	652	22.47 (29.80)	45.57 (42.27)	50.48 (45.46)
2.	*Trichoderma virens*	602	28.42 (32.20)	41.77 (40.27)	54.61 (47.64)
3.	*T. harzianum*	638	24.14 (29.40)	47.30 (43.45)	48.60 (42.20)
4.	*T. longibrachiatum*	696	17.24 (24.50)	50.37 (45.21)	45.26 (45.25)
5.	*T. reesi*	616	28.75 (32.39)	42.47 (40.66)	53.85 (47.18)
6.	*T. viride* isolate-1	515	38.76 (38.47)	41.60 (40.19)	54.79 (47.70)
7.	*T. viride* isolate-2	525	37.57 (37.76)	40.43 (55.95)	56.06 (48.45)
8.	*T. viride* isolate-3	418	50.30 (45.17)	39.67 (39.06)	56.89 (48.91)
9.	*T. viride* isolate-4	553	34.24 (35.79)	50.16 (45.09)	45.49 (42.36)
10.	*T. viride* isolate-5	639	24.02 (29.33)	48.27 (44.19)	47.54 (43.75)
11.	*B. subtilis* isolate-1	639	24.02 (29.33)	43.50 (41.23)	52.73 (46.55)
12.	*B. subtilis* isolate-2	658	21.76 (27.76)	41.50 (33.24)	54.90 (47.81)
13	*P. fluorescens* isolate-1	403	52.08 (46.15)	30.07 (33.24)	67.32 (55.12)
14.	*P. fluorescens* isolate-2	516	38.64 (38.41)	36.80 (37.34)	60.01 (50.27)
15.	*P. fluorescens* isolate-3	531	36.80 (37.35)	39.10 (38.70)	57.51 (49.31)
16.	Control	841	0.00 (0.513)	92.03 (75.97)	0.00 (0.513)
CD (P=0.05)		2.41			3.46

*Mean of three replications; data in parentheses are arc sine transformed values

temperature (28 ± 2°C). The plates inoculated with the pathogen alone served as control. Three replications were maintained for each antagonist. The radial growth of the pathogen was measured two days after inoculation and the results were expressed as per cent growth inhibition over control.

Fifty ml of Czapek's Dox and nutrient agar broth inoculated with the fungal and bacterial antagonists, respectively, were taken in 250 ml conical flasks. The flasks were then incubated at room temperature (28 ± 2°C) in shake culture for 14 and 21 days, respectively. The cultures were filtered by coarse filtration using Buckner funnel and then through millipore filters. The culture filtrates of antagonistic organisms were tested against the growth of pathogen *C. falcatum* under in vitro condition.

Eight mm fungal pathogen disc was inoculated into 250ml Erlenmeyer flasks containing 40ml of sterilized Czapek's Dox broth and 10ml of culture filtrate of antagonistic organisms. The broth without antagonistic culture filtrate served as control. The inoculated flasks were incubated at room temperature (28 ± 2°C) for 10 days. After the incubation period, the mycelial mats of *C. falcatum* were separated and collected on a previously weighed filter paper. The mycelial mats were dried at 80ºC for 48 hours in a hot air oven and cooled in a desiccator. The weight of mycelial biomass of fungus in each treatment was taken and per cent inhibition of mycelial dry weight over control was calculated.

Spore germination assay was conducted to find out the effect of bacterial and fungal antagonistic culture filtrates on spore germination of red rot pathogen *C. falcatum.* Fifty µl of the antagonistic culture filtrate and a drop of conidial suspension (4 x 10^6 spores ml^{-1}) of *C. falcatum* prepared in sterile distilled water were mixed together in a cavity slide and incubated in a Petri dish glass bridge moist chamber. The spore suspension in sterile distilled water served as control. Three replications were maintained in each treatment. Spore germination was counted after 48 hours after incubation and the results were expressed in percentage.

Among the antagonists, *Pseudomonas fluorescens* isolate–1 and *Trichoderma viride* isolate–3 showed significantly higher mycelial inhibition of *C. falcatum* in dual culture (Table 1). The mycelial growth inhibitions in the above plates were 68.18 and 62.50 per cent respectively, over control. *T. viride* isolate–1 was the next best isolate in inhibiting the growth of *C. falcatum* to the extent of 55.68 per cent. *Trichoderma harzianum* and *P. fluorescens* isolate–2 were the other two isolates which inhibited the pathogen growth to 50.00 and 51.13 per cent, respectively. The mycelial growth inhibition by other isolates was less than 50 per cent. *T. longibrachiatum* was identified as a poor competitor against *C. falcatum* with the growth inhibition of only 23.87 per cent over control. The results presented in Table 2 indicate that the culture filtrates of *P. fluorescens* isolate-1 and *T. viride* isolate-3 inhibited the growth of *C. falcatum*. The mycelial dry weights of *C. falcatum* in the liquid medium incorporated with the culture filtrate of the antagonist were (403 and 418 mg) followed by *T. viride* isolates-1 and *P. fluorescens* isolate–2 with 515 and 516 mg dry weight respectively, while it was 841 mg in control. The growth inhibition of *C. falcatum* ranged from 17.24 per cent in *T. longibrachiatum* and 52.08 per cent in *P. fluorescens* isolate–1.

The culture filtrate of all the antagonists inhibited the *C. falcatum* spore germination (Table 2), ranged from 45.26 to 67.32 per cent over control. Maximum inhibition was observed in culture filtrate of *P. fluorescens* isolate–1 (67.32 per cent) followed by *P. fluorescens* isolate–2 (60.01 per cent). The bacterial antagonist *P. fluorescens* isolate-3 was the next best in inhibiting the spore germination of *C. falcatum* to the extent of 57.51 per cent. The antagonist *viz*., *T. viride* isolate-3, *T. viride* isolate-2, *T. viride* isolate-1 *T. virens, Bacillus subtilis* isolate–2 and *T. reesi* also showed appreciable inhibitory effect on *C. falcatum* spore germination to the extent of 56.89, 56.06, 54.79, 54.61, 54.90 and 53.85 per cent respectively over control. In vitro selection of antagonist against *C. falcatum* was carried out with ten fungal antagonists and five bacterial antagonists. The study indicated that, *P. fluorescens* isolate–1 was the best among the antagonists screened against *C. falcatum* under in vitro. This antagonist exhibited maximum inhibitory action on mycelial growth, mycelial biomass production and spore germination of *C. falcatum* and it was on par with *T. viride* isolate–3. The results were similar to the findings of Revathi *et al*., (1997) and indicated that *T. viride* was effective in inhibiting the growth of *C. falcatum,* whereas its efficacy increased in the field when combined with *T. harzianum*. Anilkumar and Satyavir (1998) found that *T. harzianum, T. viride* and *C. globosum* were strong competitors against *C. falcatum.* The culture filtrates of *T. harzianum* and *C. globosum* were found to inhibit the spore germination of *C. falcatum* to the extent of 55 per cent and 43 per cent, respectively. Senthil *et al*., (2000) stated that *P. fluorescens* was effective in inhibiting the mycelial growth, biomass production and spore germination of *C. falcatum in vitro.* They also reported poor inhibitory action by species of *Trichoderma* other than *T. viride* as observed in the present study. Patel and Anahosur (2001) found that

T. harzianum was effective against *S. rolfsi*, *Fusarium* sp., and *Colletotrichum* sp. *in vitro*. Jayakumar (2004) suggested that biological control is an option for managing this disease and bioagents like *T. harzianum, T. viride, C. globosum, T. virens* and *P. fluorescens* were found to be strong competitors against *C. falcatum* under *in vitro* conditions. Kaur *et al*. (2006) suggested that volatile metabolites produced by *Trichoderma* species suppressed the growth of *C. capsici in vitro*. Vivekananthan *et al*. (2006) suggested the lytic enzyme induced by *P. fluorescens* and other biocontrol organism mediate defense against the *C. gloeosporioides* Present studies showed that *P. fluorescens* isolate 1 and *T. viride* isolate 3 can be exploited to control the red rot of sugarcane, as a non–chemical method. Among the antagonists tested, *Pseudomonas fluorescen*s isolate 1 exhibited maximum growth inhibition of *C. falcatum* in both solid (68.18 per cent) and liquid medium (52.08 per cent) and inhibited the spore germination to the extent of 67.32 per cent.

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