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Evaluation of the fluorescent *Pseudomonas* isolates for antagonistic potential against *Colletotrichum* spp.

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Abstract
Fluorescent *Pseudomonas* is such an extensively used biocontrol agent against many plant pathogens. Use of bioagents having biocontrol and plant growth promoters' activities has been considered environmentally acceptable alternatives to minimize the use of chemicals. All 30 isolates of fluorescent *Pseudomonas* significantly reduced the fungal mycelia growth of *Colletotrichum truncatum* (C10), *C. capsici* (C5), of *C. coccodes* (C9), *C. cajani* (C1), *C. acutatum* (C6), *C. capsici* (C7), *C. gloesporiodes* (C4), *C. lindemuthianum* (C3), *C. capsici* (C2), *C. destructivum* (C8), fluorescent *Pseudomonas* isolate i.e. P23(10 mm), P3(10 mm), P16(10 mm), P4 (12 mm), P5 (14 mm) was maximum in reduced the fungal mycelial growth respectively. The maximum average % inhibition were recorded with the fluorescent *Pseudomonas* isolate of P5 (73.2%) and minimum average % inhibition were recorded with the fluorescent *Pseudomonas* isolate of P29 (59.6%).

Keywords: Soybean, *Colletotrichum*, biological control, fluorescent *Pseudomonas*

Introduction
The genus *Colletotrichum* includes a number of plant pathogens of major importance, causing diseases of a wide variety of woody and herbaceous plants. It has a primarily tropical and subtropical distribution, although there are some high-profile species affecting temperate crops. Soybean is susceptible to *Colletotrichum truncatum*, at all stages of development particularly from bloom to pod fill. The disease causes considerable damage by reducing plant stand, seed quality, seed germination and yield (Vyas *et al*., 1997) [9]. Raddish brown spot appears on the pods and later they turn black. Fruiting bodies (Acervulli) on infected pods resemble small pin cushions surrounded by minute blackish brown setae. The disease mainly appears on pods, but also on leaves and stem due to early infection. Since, it affects the pods there is damage to seeds and affects the seed yield and germination.

Judicious use of chemicals also offers an alternative for the management of anthracnose. Chemicals are necessary at present, but are not a long term method to crop health. Biological control can be attained either through introduction of biocontrol agents directly or by adopting practices which favors build- up of biocontrol agents under natural conditions (Viswanathan R and Samiyappan R 1999; Vivekananthan *et al.* 2004) [7, 1]. Several fungal and bacterial biocontrol agents have been used for achieving disease control of various plant species. Among bacteria fluorescent *Pseudomonas* is such an extensively used biocontrol agent against many plant pathogens. Use of bioagents having biocontrol and plant growth promoters' activities has been considered environmentally acceptable alternatives to minimize the use of chemicals (Srinivas *et al.* 2006; Shovan *et al.* 2008) [6, 5].

Methods and Materials
Collection of diseased specimens, single spore isolation and maintenance of culture
During the field survey carried out in *Kharif* 2017-18, a large number of pod blight infected soybean samples were collected from different locations of Raipur (C.G.). After isolation of single spore progenies were cultured on potato dextrose agar medium slants and maintained at 26±1 ºC in BOD incubator for further use.

Evaluation of Bio-agents against *Colletotrichum* spp.
Isolation of bacterial antagonist
30 isolate of Fluorescens *Pseudomonas* were isolated from the rhizospheric soil of healthy crop cultivating fields. A sample of 10 g soil was suspended in 100 ml of sterile physiological water and shaken vigorously at 28 °C for 30 min. Serial dilutions were plated on king's B medium, and each dilution was incubated at 30 °C until colonies were observed.

***In vitro* evaluation of antagonistic effect of fluorescent *Pseudomonas* against *Colletotrichum* spp.**
Thirty isolate of fluorescent *Pseudomonas* (P1, P2, P3, P4, P5, P6, P7, P8, P10, P11, P12, P13, P14, P16, P17, P18, P19, P21, P22, P23, P24, P25, P27, P28, P29, P30, P66, P141, P200, P260) were tested against *C. truncatum* on the potato dextrose agar + king's B (50:50) medium using Confrontations assays technique (Kotasthane *et al.*, 2017) [4]. 20 ml melted sterilized potato dextrose agar (PDA) poured in sterilized Petri dishes. A heavy inoculums from an actively growing fluorescent *Pseudomonas* was inoculated at 1 cm away from the edges of the plate and the mycelial disc of the pathogens were placed at the centre of Petri- plates. Control plates were inoculated only with phyto-pathogens but not with fluorescent *Pseudomonas* isolates and mycelial growth (mm) recorded in three, five and sevenday's interval. Bharathi *et al* 2004 [1].

Result and Discussion
***In-vitro* evaluation of the fluorescent *Pseudomonas* isolates for antagonistic potential against *Colletotrichum* spp.**
Thirty isolate of fluorescent *Pseudomonas* (P1, P2, P3, P4, P5, P6, P7, P8, P10, P11, P12, P13, P14, P16, P17, P18, P19, P21, P22, P23, P24, P25, P27, P28, P29, P30, P66, P141, P200, P260) were tested against *C. truncatum* on the potato dextrose agar + king's B (50:50) medium using confrontations assays technique (Kotasthane *et al.*, 2017) [4].
The 30 isolates of fluorescent *Pseudomonas* are assayed under *in vitro* were evaluated for their antifungal activity on the mycelium growth of *Colletotrichum* spp. at 7th day after inoculation (DAI) it is clear from the data, (Table 2) that all isolates of fluorescent *Pseudomonas* significantly reduced the fungal mycelia growth of *Colletotrichum truncatum* (C10)over untreated (control). The fluorescent *Pseudomonas* isolate P23 was recorded with minimum mycelium growth (10 mm) which is statistically at par with the isolates of P5(11mm), P4(22mm), P12(24 mm), P24(26mm), P141 (27mm), P19(28mm), P21(28 mm), P200 (28 mm), P18 (29mm), P8(29 mm), P30(29 mm), P66(30 mm), P16(31 mm), P25(31 mm), P7, P13, P17(32 mm) and followed by the isolates of P2,P3 and P11(33 mm), P6 (34 mm) P10 (35 mm), P1 (36 mm), P28 (36 mm), P29 (39 mm), P14 (39 mm), P27 (40 mm). The maximum mycelia growth was recorded with control treatment P260 (50 mm) and control 66mm.

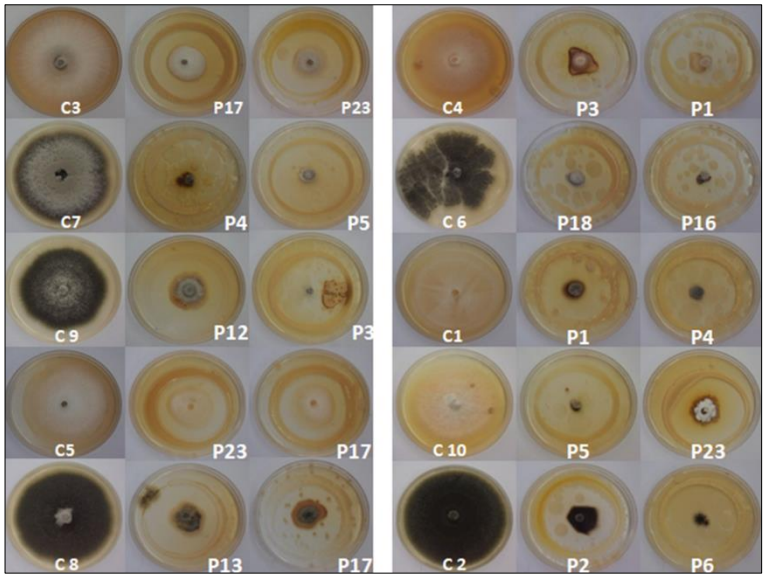

Fig 1: *In vitro* evaluation of the fluorescent *Pseudomonas* isolates for antagonistic potential against *Colletotrichum* spp.

Where - C1- *C. cajani*
C2- *C. capsici*
C3- *C. lindemuthianum*
C4- *C. gloesporiodes*
C5- *C. capsici*
C6- *C. acutatum*
C7- *C. capsici*
C8- *C. destructivum*
C9- *C. coccodes*
C10- *C. truncatum*

At 7th DAI, all the fluorescent *Pseudomonas* isolates significantly reduced the mycelia growth of *C. capsici* (C5) over untreated treatment. Fluorescent *Pseudomonas* isolate i.e. P17 was maximum in reduced the fungal mycelia growth (30 mm), which is statistically at par with the isolates P24 (31 mm). The maximum mycelia growth was recorded P29 (58 mm) and untreated (control) treatment (67 mm). The significantly reduced the mycelia growth of *C. coccodes* (C9) Fluorescent *Pseudomonas* isolate i.e. P3 was maximum in reduced the fungal mycelia growth (10 mm), which is statistically at par with the isolates P2 (12 mm), P12 (20 mm). The maximum mycelia growth was recorded P22 (46 mm) and untreated (control) treatment (66 mm). Fluorescent *Pseudomonas* isolate against *C. cajani* (C1), maximum in reduced the mycelia growth i.e. P4 (12 mm), P1 (15 mm) and minimum in reduced the mycelia growth recorded P200 (46 mm) and untreated (67 mm). Same case in *C. acutatum* (C6), *C. capsici* (C7), *C. gloesporiodes* (C4), *C. lindemuthianum* (C3), *C. capsici* (C2), *C. destructivum* (C8), fluorescent *Pseudomonas* isolate i.e. P16(10 mm), P5 (14 mm), P1 (16 mm), P17 (28 mm), P6 (15 mm), P 17 (25 mm) was maximum in reduced the fungal mycelial growth respectively. The maximum average% inhibition were recorded with the

fluorescent *Pseudomonas* isolate of P5 (73.2%), P1 (71.5%), P2 (71.4%), P4 (71.3%), P3 (69.1%) and P23 (67.5%) and minimum average % inhibition were recorded with the fluorescent *Pseudomonas* isolate of P29 (59.6%).

Table 1: Fluorescent *pseudomonas* expressing specificity to inhibit host specific *Colletotrichum* spp. isolates

Treatment	ISO	Host						
		BR	PP	MB	SB	BP	DF	TU
		% Inhibition						
T01	P1						80	
T02	p2	88						
T03	p3	90	80				84	
T04	p4		88			84		
T05	p5				89	86		
T06	p6							85
T11	p12	80						
T14	p16			90				
T16	p18			88				
T18	p21			80				
T20	p23				90			

Table 2: Average inhibition of *Colletotrichum* spp. isolates

S. No.	Isolates of *Pseudomonas*	Average % inhibition
1	p1	71.5
2	p2	71.4
3	p3	69.1
4	p4	71.3
5	p5	73.2
6	p6	66.7
7	p7	64.3
8	p8	63.9
9	p10	62.8
10	p11	61.9
11	p12	66.7
12	p13	66.6
13	p14	61.2
14	p16	66.7
15	p17	66.5
16	p18	67.4
17	p19	60.7
18	p21	64.2
19	p22	60.7
20	p23	67.5
21	p24	67.4
22	p25	63.1
23	p27	61.5
24	p28	61.1
25	p29	59.6
26	p30	61.5
27	p141	65.1
29	p66	62.5
29	p200	61.4
30	p260	60

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