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EVALUATION OF CULTURE FILTRATE OF BIOAGENTS ON THE GROWTH OF *SAROCLADIUM ORYZAE* CAUSING RICE SHEATH ROT DISEASE

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ABSTRACT

Studies on the effect of culture filtrate of five native *Pseudomonas* and five native *Bacillus* isolates were evaluated to test the antagonistic activity against *Sarocladium oryzae* under *in vitro* conditions. The results of the present study showed that the culture filtrate of *Pseudomonas* isolate- PfS5 was found to be more inhibitory to the growth of *S. oryzae* at 20 per cent concentration and the same isolate recorded the maximum germination per cent (91.76%), shoot length (8.89 cm), root length (14.25 cm) and vigour index (2123.32) compared to control (60.51%, 3.39 cm, 6.89 cm; 622.04). Further, the effect of culture filtrate of *Bacillus* showed that the isolate- BsA1 exhibited maximum inhibition (89.15%) on the mycelial growth of *S. oryzae* at 20 per cent concentration under *in vitro* condition followed by the isolate- BsS5, which recorded 85.91 per cent inhibition of mycelial growth over control at 20 percent concentration and recorded the maximum germination per cent (70.83%), shoot length (8.86 cm), root length (14.35cm) and vigour index (2071.02) of rice seedlings by roll towel method.

Key words : Rice, sheath rot, bacterial antagonist, plant growth promotion

Introduction

Rice (*Oryza sativa* L.) is the staple food of nearly half of the world's population and mainly grown and consumed in Asian counties such as China, India, Indonesia, Japan, Thailand, Pakistan, Bangladesh, North and South Koreas, Myanmar, Philippines, Sri Lanka etc. The productivity of rice is threatened by a number of fungal and bacterial diseases attacking the crop and causing enormous yield loss. Among the various diseases of rice, sheath rot caused by *Sarocladium oryzae* (Sawada) Gams and Hawksworth has gained the status of a major disease of rice and reduce the yield considerably all over the rice growing areas of the world. The disease causes empty grain production (Kulwanth and Mathur, 1992) and glume discolouration (Sachan and Agrawal, 1995). It also causes poor grain filling and reduction in seed germination (Vidhyasekaran *et al.*, 1984). Seeds from infected panicles became discoloured and sterile (Mew and Gonzales, 2002). The stem borer or mite damage to the boot or leaf sheaths increases this verity of this disease.

Chemical pesticides are exclusively used for the management of the disease but not considered as a long-term solution because it may cause health and environment hazards, residue persistence and elimination of natural enemies and development of resistance. Therefore, the need for an alternative means of control of sheath rot disease has become imperative. The use of microorganisms as biological control agents to control plant disease has emerged as powerful alternative method (Kulkarni *et al.*, 2007). The rich diversity, complexity of interactions and numerous metabolic pathways makes amazing resource for biological activity (Emmert and Handelsman, 1999; Alabouvette *et al.*, 2006; Raghukumar, 2008).

Several bacterial species namely *Pseudomonas* and *Bacillus* spp were widely used as bio control agents

against various plant pathogens. *Pseudomonas* and *Bacillus* spp play an important role as biocontrol agent in management of several soil borne pathogens (Sakthivel and Gnanamanickm, 1987). Therefore, the present experiment was undertaken to evaluate the best effective mirobial antagonist against the sheath rot pathogen.

Materials and Methods

Isolation and identification of pathogen

The pathogen was isolated from the diseased rice sheaths showing the typical lesions of sheath rot. The edge of the lesions was cut into small pieces by means of a sterile knife. Then the pieces were surface sterilized in 0.1 percent sodium hypochlorite solution for 30 seconds and washed in three repeated changes of sterile distilled water and then plated into sterile Petri dishes containing PDA medium. The plates were then incubated at room temperature 28±2°C. The tip of the hyphal growth radiating from the infected tissue was transferred into PDA slants (Rangaswami, 1972). The fungus was purified again by single hyphal tip method and maintained on PDA slants for the further studies (Fig. 1). A total of 10 isolates (So1 to So 10) were obtained from infected sheath region of rice plants collected from different districts of Tamil Nadu. Based on the pathogenicity studies the highly virulent isolate of So5 alone was used for further studies.

Fig. 1 : Axenic culture of *Sarocladium oryzae*.

Isolation of bacterial antagonists from rice rhizosphere

Antagonistic bacteria were isolated from the rhizosphere soil collected from five different rice growing tracts of Tamil Nadu. The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently. In this procedure 10g of soil from each soil sample was taken in a conical flask of 90 ml saline. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate method. From the final dilutions of 10^{-6} and 10^{-7} one ml of each aliquot was pipetted out, poured in to sterilized Petri plate containing King's B medium (King *et al*., 1984), nutrient agar medium separately and they were gently rotated clock wise and anti-clock wise for uniform distribution and incubated at room temperature (28±2^{0}C) for 48 hours. Colonies with characteristics of *Bacillus* spp, *Fluorescent pseudomonads* were isolated individually and purified by streak plate method (Rangaswami, 1993) on nutrient agar medium and King's B medium, respectively. *Fluorescent pseudomonads* and *Bacilus* spp. were identified based on biochemical test. A list of bio-agents obtained from rice rhizosphere is given below,

S. no.	Location	Crop	Isolates code
a)	**Bio-control agents isolated from different locations**		
I.	***Fluorescent pseudomonads***		
1.	Ambigapuram (Cuddalore)	Rice	PfA1
2.	Annamalainagar (Cuddalore)	Rice	PfAn2
3.	Kalambur (Tiruvannamalai)	Rice	Pfk3
4.	Krishnapuram (Tiruvannamalai)	Rice	PfKp4
5.	Sivapuri (Cuddalore)	Rice	PfS5
II.	***Bacillus subtilis***		
1.	Ambigapuram (Cuddalore)	Rice	BsA1
2.	Annamalainagar (Cuddalore)	Rice	BsAn2
3.	Kalambur (Tiruvannamalai)	Rice	BsK3
4.	Krishnapuram (Tiruvannamalai)	Rice	BsKp4
5.	Sivapuri (Cuddalore)	Rice	BsS5

Bioassay of culture filtrate of bacterial antagonists (*Fluorescent pseudomonads* and *Bacillus* isolates) on the mycelia growth of *S. oryzae* (So5)

Preparation of the culture filtrate of *Fluorescent pseudomonads* and *Bacillus* isolates

Five bacterial isolates were inoculated in to five different Erlenmeyer flasks containing 100 ml of sterile King's B broth (*Fluorescent pseudomonads*), Nutrient broth (*B. subtilis*) and kept on a rotary shaker at 100 rpm for 48 h. Then the cultures were filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Effect of culture filtrate of bacterial antagonists on the mycelial growth of *S.oryzae* (So5)

The culture filtrates of the bacterial antagonists (*Fluorescent pseudomonads* and *Bacillus* isolates) were separately incorporated into sterilized PDA medium at 5, 10,15and 20 per cent concentration by adding the

calculated quantity of the culture filtrate to the medium by means of a sterile pipette. The amended media were transferred in to sterile Petri plates separately @ 15 ml and allowed to solidify. Each plate was inoculated at the centre with 15 day's old (six mm) culture disc of *S. oryzae* grown on PDA. Three replications were maintained for each treatment. The medium without culture filtrates served as control. The linear growth of pathogen (in mm) was measured when the mycelia growth fully covered the control plates.

Efficacy of bacterial antagonist (*Fluorescent pseudomonads* and *Bacillus* isolates) on plant growth promotion of rice

Seed treatment

Ten ml of each isolate antagonistic suspension was taken in a Petri plate. To this, 100 mg of carboxy methyl cellulose (CMC) was added as an adhesive material. Twenty-five rice seeds (var RNR 15048) were soaked in 10 ml of antagonistic suspension for 2 h. and air dried over night in a sterile Petri plate.

Plant growth promotion (Roll towel method)

Five isolates of each bacterial antagonists (*Fluorescent pseudomonads* and *Bacillus*) were assessed based on the seedling vigour index by the standard Roll towel method (ISTA, 1993). Twenty-five treated seeds were kept over the pre-soaked germination paper. The seeds were held in a position by placing another pre-soaked germination paper strip and gently pressed. The polythene sheet along with seeds were then rolled and incubated in growth chamber for10 days. Three replications were maintained for each treatment. The root length and shoot length of individual rice seedlings were measured and the germination percentages of seeds were also calculated.

The vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973).

Vigour Index = (Mean root length + Mean shoot length) × Germination (%)

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

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Statistical analysis

The data were statistically analyzed using the Wasp version 2.0 developed by the Indian Council of Agricultural Research, Goa (Gomez and Gomez, 1976). Data were subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$ and $P< 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Effect of culture filtrate of *Fluorescent pseudomonads* isolates on the mycelia growth of *S. oryzae* (So5)

Generally, an increase in the conc. of the culture filtrate reduced the mycelia growth of pathogen. Among the isolates tested, the isolate-PfS5 was found to bemore inhibitory to the growth of *S. oryzae* at 20 per cent concentration (Table 1). Also reports are that Chanutsa *et al*. (2014) reported that the culture filtrate of *P. fluorescens* showed 100 per cent inhibition on the growth of *S. rolfsii*. The *in vitro* efficacy of culture filtrate of *P. fluorescens* isolate-I7 completely inhibited the mycelial growth of *S. rolfsii* at 15 percent concentration (Muthukumar *et al*., 2019). The growth inhibition might be due to the reduction of antifungal metabolites or antibiotics by *P. fluorescens*. *P. fluorescens* were known to produce a range of low-molecular weight antimicrobial metabolites some of which were potential antifungal agents (Dowling and Gara, 1994). Nielson and Sorensen (1999) demonstrated that isolates of *P. fluorescens* was antagonistic to *P. ultimum* and *R. solani*, which produced endochitinase and chitobiosidase. Several studies indicated the production of lytic enzymes (Jayapala *et al*., 2019), antibiotics (Fridayati and Syafruddin, 2020), siderophores (Subramanium and Sundaram, 2020) and salicylic acid (Suárez- Estrella *et al*., 2019), which was correlated with

Table 1 : Effect of culture filtrate of *Fluorescent pseudomonads* isolates on the linear growth of *S.oryzae* (So5) by poisoned food technique.

S. no.	Treatments	Linear growth of pathogen (mm)							
		5 %	Per cent inhibition over control	10 %	Per cent inhibition over control	15 %	Per cent inhibition over control	20 %	Per cent inhibition over control
1	PfA1	21.05[b] (27.29)	76.11	18.75[c] (25.64)	79.16	14.75[b] (22.57)	83.61	12.40[c] (20.61)	86.22
2	PfAn2	19.50[b] (26.19)	78.33	16.86[b] (24.23)	81.24	12.00[a] (20.25)	86.65	10.37[b] (18.77)	88.16
3	PfK3	25.57[c] (30.36)	69.91	24.36[d] (29.56)	71.34	23.08[c] (28.70)	72.84	22.42[d] (28.25)	73.62
4	PfKp4	30.42[d] (33.46)	64.21	28.02[de] (31.94)	67.03	26.17[d] (30.75)	69.21	24.61[e] (29.72)	71.04
5	PfS5	17.25[a] (24.53)	80.55	14.81[a] (22.61)	83.54	11.88[a] (20.15)	86.80	7.43[a] (15.81)	91.74
6	Control	85.00[e] (67.22)	0.00	85.00[e] (67.28)	0.00	85.00[e] (67.19)	0.00	85.00[f] (67.21)	0.00
	SE(d)	0.688	-	1.030	-	0.319	-	0.529	-
	CD(0.05)	1.925	-	2.741	-	1.936	-	1.409	-

Values are mean of three replications. Values in the column followed by same letters not differ significantly by DMRT(p=0.05)

antagonistic potential of *P. fluorescens* against various soil-borne plant pathogens. The results of the present investigations were confirmed by the above reports.

Effect of culture filtrate of *Fluorescent pseudomonads* isolates on the growth of rice seedlings (Roll towel method)

The culture filtrates of all the antagonists showed increased the germination of rice seeds and induced the plant growth. Among these, the isolate-PfS5 the maximum germination per cent (91.76%), shoot length (8.89 cm), root length (14.25 cm) and vigour index (2123.32).This was followed by PfAn2, PfA1, PfK3 and Pfkp4 in the decreasing order of merit (Table 2). Also reports that Thakur and Tripathi (2015) reported that seed treatment with *P.fluorescens* significantly improved the germination percentage of 93.1 per cent and seedling vigour of 953.33. Shahzaman *et al*. (2016) reported that among the four bacterial isolates, Pf1 recorded maximum germination percentage, increased shoot length, root length and vigour index of chickpea seedlings. Whereas, Kang *et al*. (2019) reported that lettuce and Chinese cabbage seedlings were inoculated with of *P. koreensis* significantly increased the root length, shoot length, dry biomass and chlorophyll content. *P. fluorescens* might have stimulated the plant growth by improving uptake of minerals into the host plants particularly phosphate (Nakkeeran *et al*., 2005; Goswami *et al*., 2015), siderophore mediated iron uptake (Lemanceau *et al*., 2009; Ashraf *et al*., 2013), association with nitrogen fixation (Bashan and De-Bashan, 2005), production of IAA (Liu *et al*., 2013; Gupta *et al*., 2015), production of cytokinin (Ruzzi and Aroca, 2015), regulating ethylene production in roots (Gupta *et al*., 2015). The above findings agreement with the present investigation.

Effect of culture filtrate of *B.subtilis* isolates on the mycelia growth of *S. oryzae* (So5)

The culture filtrate of all the bacterial isolates inhibited the mycelial growth of *S. oryzae*. Generally, an increase in the conc. of the culture filtrate reduced the mycelia growth of pathogen. Among the isolates tested, culture filtrate of the isolate- BsA1 exhibited maximum inhibition (89.15%) on the mycelial growth of *S. oryzae* at 20 per cent concentration under *in vitro* condition followed by the isolate- BsS5, which recorded 85.91 per cent inhibition of mycelial growth over control at 20 percent conc (Table 3, Fig. 2). Also reports that culture filtrate of *B. subtilis* V26 completely reduce the mycelial growth of *R. solani* or inhibit pathogenic fungus (Chen *et al.*, 2016). Wu *et al*. (2019) reported that the destruction of the mycelia growth of *R. solani* by culture filtrate of *B. subtilis* SL-44 was examined by light microscopy. The results showed high vascularization, protoplasm leakage, cell wall damage and breakage in pathogens when compared to the normal

Table 2 : Efficacy of seed treatment with *Fluorescent pseudomonads* isolates on rice seed germination and seedling vigour by Roll towel method.

Treatment	Seed germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	Per cent increase over control
PfA1	84.23^{bc}(66.64)	11.89^{c}	7.35^{c}	1620.58^{c}	79.56
PfAn2	87.66^{b}(69.41)	13.33^{b}	8.17^{b}	1884.69^{b}	85.97
PfK3	82.50^{cd}(65.27)	10.95^{d}	6.61^{d}	1399.20^{d}	72.97
PfKp4	80.08^{d}(63.48)	8.35^{e}	5.54^{e}	1112.31^{e}	68.79
PfS5	91.76^{a}(73.33)	14.25^{a}	8.89^{a}	2123.32^{a}	94.68
Control	60.51^{e}(51.05)	6.89^{f}	3.39^{f}	622.04^{f}	59.56
SE(d)	1.343	0.228	0.211	31.451	-
CD(0.05)	3.812	0.388	0.334	66.435	-

Values are mean of three replications. Values in the column followed by same letters not differ significantly by DMRT(p=0.05)

Table 3 : Effect of culture filtrate of *B. subtilis* isolates on the linear growth of *S. oryzae* (So5) by poisoned food technique.

S. no.	Treatments	Linear growth of pathogen (mm)							
		5 %	Per cent inhibition over control	10 %	Per cent inhibition over control	15 %	Per cent inhibition over control	20 %	Per cent inhibition over control
1	BsA1	19.87^{a}(26.45)	77.92	16.65^{a}(24.07)	81.50	13.00^{a}(21.12)	85.55	9.76^{a}(18.19)	89.15
2	BsAn2	35.39^{d}(36.48)	58.36	33.23^{d}(35.18)	60.90	32.42^{e}(34.69)	61.85	30.70^{e}(33.63)	63.88
3	BsK3	29.80^{c}(33.07)	66.88	29.05^{c}(32.60)	67.72	27.60^{d}(31.67)	69.33	23.40^{d}(28.91)	74.00
4	BsKp4	24.08^{b}(29.37)	73.24	21.50^{b}(27.61)	76.11	19.50^{c}(26.19)	78.33	17.25^{c}(24.52)	80.83
5	BsS5	19.50^{a}(26.19)	78.33	17.25^{a}(24.53)	80.83	15.25b(22.98)	83.05	12.68^{b}(20.84)	85.91
6	Control	85.00^{e}(67.25)	0.0	85.00^{e}(67.19)	0.00	85.00^{f}(67.19)	0.00	85.00^{f}(67.24)	0.00
	SE(d)	0.906	-	0.300	-	0.244	-	0.833	-
	C.D	2.574	-	0.847	-	0.674	-	2.320	-

Values are mean of three replications. Values in the column followed by same letters not differ significantly by DMRT (p=0.05).

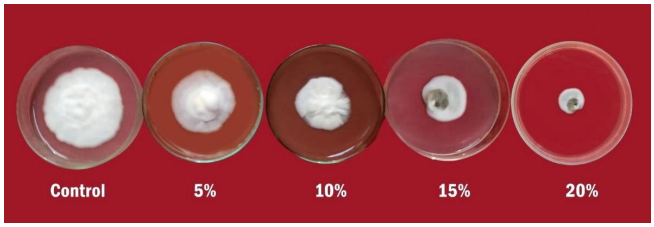

Fig. 2 : Effect of culture filtrates of *Fluorescent pseudomonads* isolate on the growth of *S. oryzae*.

growth of the control. Sanjeev Kumar *et al*. (2020) reported that the culture filtrate of *B. cereus* at 40 percent concentration and above completely, inhibited the mycelia growth of *M. phaseolina*. *B. subtilis* produced several kinds of antimicrobial peptide substances such as subtilin, bacilysin, mycobacillisyn, iturin, oligomycin A, kanosamineandzwittermicin A, which may be responsible for the inhibition of pathogen (Harwood *et al*., 2018;

Fig. 3 : Effect of culture filtrate of *Fluorescent pseudomonads* isolates on the growth of rice seedlings.

Table 4 : Efficacy of seed treatment with *B. subtillis* isolates on rice seed germination and seedling vigour by Roll towel method.

S. no.	Seed germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	Per cent increase over control
BsA1	89.23(70.83)	14.35[a]	8.86[a]	2071.02[a]	88.79
BsAn2	80.92(64.08)	8.39[e]	5.35[e]	1111.84[e]	62.86
BsK3	81.08(64.19)	10.83[d]	6.41[d]	1397.81[d]	75.69
BsKp4	84.32(66.70)	11.79[c]	7.55[c]	1630.74[c]	80.86
BsS5	86.05(68.04)	13.50[b]	8.31[b]	1876.75[b]	82.96
Control	59.86(50.66)	6.59[f]	3.42[f]	599.19[f]	58.26
SE(d)	0.966	0.231	0.192	11.786	-
CD(0.05)	2.127	0.458	0.402	64.320	

Values are mean of three replications. Values in the column followed by same letters not differ significantly by DMRT(p=0.05).

Caulier *et al*., 2019). All these earlier results lend support to the present findings.

Effect of culture filtrate of *B. subtilis* isolates on the growth of rice seedlings (Roll towel method)

The culture filtrates of all the antagonist increased the germination of rice seeds and induced the plant growth (Table 4, Fig. 3). Among these, the isolate BsA1 recorded the maximum germination per cent (70.83%), shoot length (8.86 cm), root length (14.35cm) and vigour index (2071.02). This was followed by Bs S5,BsKp4, BsK3 and BsAn2 in the decreasing order of merit. The untreated control recorded the lowest values in terms of germination per cent (59.86%), shoot length (3.42cm), root length (6.59cm) and vigour index (599.19) Also reports are that, Gowtham *et al*. (2016) reported that two different strains of *B. amyloliquefaciens* significantly reduced the incidence of wilt and also enhanced the vigour of tomato seedlings. Seed treatment with *B. amyloliquefaciens* increased the shoot length, root length and vigour of tomato seedlings (Abo-Elyousr *et al*., 2019). Tomato seeds were treated with cell suspensions of *B. subtilis* OTPB1 showed increased the shoot and root growth, seedling vigour and leaf area of the tomato plant. Higher level of plant growth-promoting hormones (GA_3 and IAA) were observed in treated plants compared with non-treated plants (Chowdappa *et al*., 2013). IAA has an important role in origination and emergence of adventitious roots. It also enhances shoot development by influencing cell expression, division and differentiation (Gardner *et al*., 2009). The GA_3 plays it role in combination with auxin for elongation of plant and leaf bud formation (Srivastava, 2002). Production of cytokinins by PGPR can also lead to enhanced root exudate production by the plant (Ruzzi and Aroca, 2015). These plant growth-promoting hormones enhance the nutrients uptake ability of plants and help the plant to defend against various biotic and abiotic stresses (Ghanashyam and Jain, 2009). All the above reports were in line with the present finding.

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