In-Vitro Assessment of Native Plant Growth Promoting Rhizobacterial Isolates Against Diverse Fungal Phytopathogens

Abstract

Although many Plant growth promoting rhizobacteria (PGPR) are used commercially, there is a need to explore more biocontrol agents to combat various pathogens and sustain the productivity of crops. PGPRs inhabit the rhizosphere region of plant and are effective in managing various pathogens. In this study, twenty-six PGPR isolates were screened *in-vitro* against various fungal phytopathogens in the Plant Bacteriological Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia, West Bengal during 2021-22. All the thirteen native *Bacillus* isolates, showed antagonistic activity against *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Pestalotiopsis* sp., *Rhizoctonia solani* and *Sclerotium rolfsii*. Among the thirteen fluorescent *pseudomonads*, all showed antagonistic activity against *A. alternata*, *C. gloeosporioides* and *Pestalotiopsis* sp., eight, CK2LPP, CK2LP8, CK2LP12, GP2, GP8, G11SP37, K11SP4 and S21SP14, against *R. solani,* two, GP2 and GP8, against *S. rolfsii*. BRB 42, BRB 56, PR 18, GP2 and GP8 had the highest antagonistic activity against the fungal pathogens under *in-vitro* condition based on average mycelium inhibition per cent. BRB 56, SM 9 and GP8, showed the maximum inhibition zone against all the phytopathogens.

Keywords: *Bacillus*, fungal pathogens, *In-vitro*, plant growth-promoting rhizobacteria (PGPR), *Pseudomonas*

1. INTRODUCTION

Agriculture is essential for the food security of humans and animals that live on the planet. There is a need to expand the productivity of crops to meet the food demands of expanding populations. The productivity and yield of crop as well as the food quality are severely influenced by various kinds of biotic and abiotic stress [1]. PGPRs are the important biocontrol agents [2,3] and effective in reducing both abiotic and biotic stresses [4,5]. These bacteria have the capability to suppress phytopathogens around plant roots. They competitively colonize the roots of plant and can enhance plant growth [6]. Plant growth promoting rhizobacteria (PGPRs), *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Pantoea*, *Bacillus*, *Serratia* and *Rhizobium,* have shown an ability to improve plant growth [7,8]. Among these, species of *Bacillus* and *Pseudomonas* are predominant because of their distinctive plant growth promoting characteristics [9]. Biofertilization, phytostimulation and biological control are diverse traits of heterogeneous PGPR [10] and can be exploited to develop formulations for management of several phytopathogens, enhancement of yield and food production by using fewer resources and less reliance on the chemical fertilizers and pesticides [11,12]. Because of the broad-host range of pest and pathogens, changing climates, high prices of agrochemicals and ecological crises, devising multi-purpose bio-formulations will be a more practical strategy for integrated pest and nutrient management. Several PGPRs are found to be efficient and used widely against various bacterial and fungal pathogens. Some native rhizobacterial isolates were not evaluated against various regularly occurring pathogens particularly in this agroclimatic region. Despite the significant potential of PGPRs,

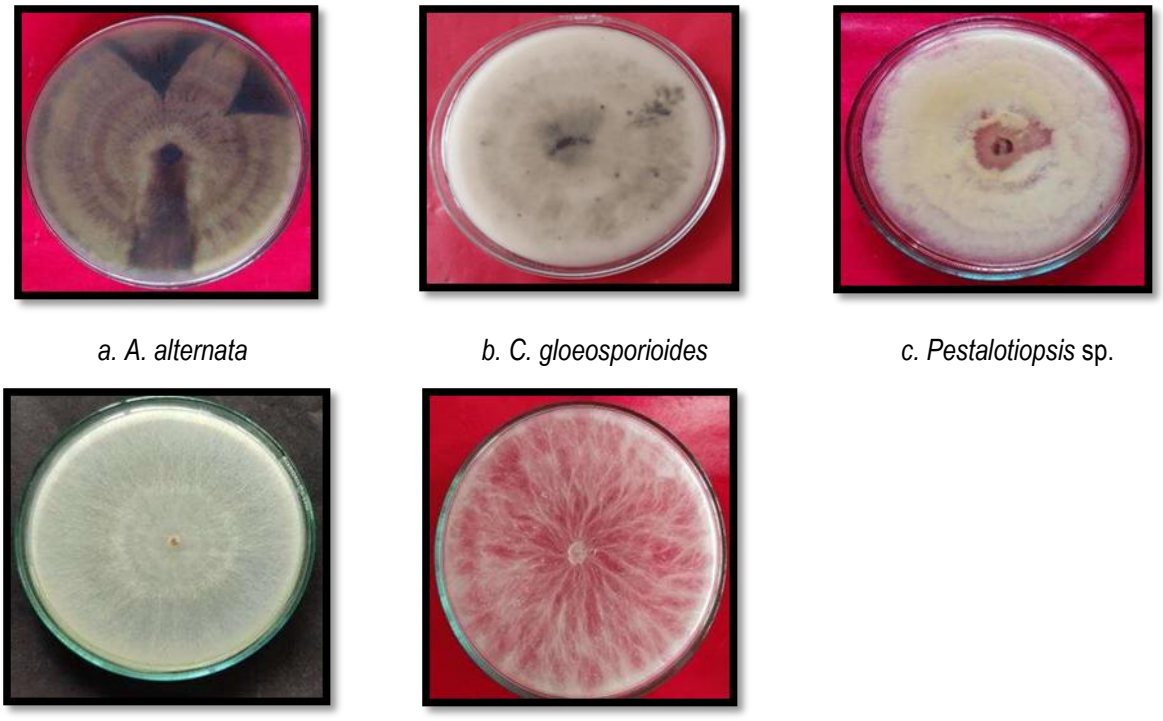
their efficacy against various phytopathogens in specific agroclimatic regions remains largely unexplored. Therefore, this research aims to evaluate the *in-vitro* efficacy of native plant growth-promoting rhizobacterial isolates against different fungal phytopathogens. By understanding the performance of these native isolates in countering prevalent pathogens, we can pave the way for developing region-specific, multi-purpose bio-formulations that integrate pest and nutrient management strategies effectively.

2. MATERIALS AND METHODS

The investigation was carried out *in-vitro* condition in Plant Bacteriological Laboratory, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal during 2021-22. The preparation of media, sterilization, isolation and maintenance of microbial cultures, etc., were done following the method developed by Nene and Thapliyal [13], Dhingra and Sinclair [14] and Aneja [15] with slight modification.

2.1 Collection of fungal phytopathogens

Five fungal pathogens *viz*., *A. alternata*, *C. gloeosporioides*, *Pestalotiopsis* sp., *R. solani* and *S. rolfsii* were collected from the same laboratory and maintained as pure culture at 4°C in the Potato Dextrose Agar (PDA) media plates for further use (Fig. 1).



a. A. alternata *b. C. gloeosporioides* *c. Pestalotiopsis* sp.

d. R. solani *e. S. rolfsii*

Fig. 1. Pure culture of fungal phytopathogens

2.2 Collection of native plant growth promoting rhizobacteria

Thirteen isolates of fluorescent *pseudomonads* and thirteen isolates of *Bacillus* sp. were obtained from the Plant Bacteriological Laboratory, Department of Plant Pathology, BCKV and these strains were maintained by frequent sub-culturing after 30 days interval and stored at 4°C in the test tube slants of NA media. The table labelled as Table 1 presents the identified species of these isolates.

Table 1. List of native rhizobacterial isolates

Native *Bacillus* isolates	Name of Rhizobacteria	Native Fluorescent *Pseudomonads*	Name of Rhizobacteria
BRB 88	*Bacillus subtilis* subsp. *subtilis*	BCLP4	*Pseudomonas fluorescens*
BRB 89	*Bacillus pumilus*	CK2LPP	-----
BRB 35	*Bacillus altitudinis*	CK2LP8	-----
BRB 42	*Bacillus rugosus*	CK2LP12	-----
BRB 52	*Bacillus pumilus*	GP2	*Pseudomonas aeruginosa*
BRB 56	*Bacillus amyloliquefaciens*	GP8	*Pseudomonas aeruginosa*
BRB 74	*Bacillus subtilis*	G11SP37	*Pseudomonas geniculata*
PR 16	*Bacillus australimaris*	G15SP38	*Pseudomonas putida*
PR 18	-----	K11SP4	*Pseudomonas baetica*
PR 19	*Bacillus subtilis* subsp. *subtilis*	K22SP8	*Pseudomonas fluorescens*
PR 20	*Bacillus subtilis*	SS2PP	-----
SM 9	-----	SS2LP	-----
SM 14	-----	S21SP14	*Pseudomonas putida*

The *in-vitro* antagonistic activity of the rhizobacterial isolates against five fugal pathogens *viz*. *A. alternata*, *C. gloeosporioides*, *Pestalotiopsis* sp., *R. solani*, and *S. rolfsii* was conducted through dual culture method.

2.3 *In-vitro* antagonism of the native rhizobacteria against fungal pathogens

Isolated rhizobacteria were tested for their *in-vitro* anti-fungal bio-control potentiality by following the standard protocol of dual culture assay proposed by Shivakumar *et al.* [16]. Fungal phytopathogens *viz*. *A. alternata*, *C. gloeosporioides*, *Pestalotiopsis* sp., *R. solani*, and *S. rolfsii,* were used in the evaluation of rhizobacterial antagonistic activity through dual culture method. The rhizobacterial isolates were streaked by a thin line along both the opposite end of the plates containing sterile PDA media and a 5mm disc of freshly cultured pathogen was placed exactly in the center of the plates. Three replications of each isolate including a control *i.e.*, without inoculation of the antagonist were maintained at 27±1^{0}C for 168 hrs (*A. alternata*), 144 hrs (*C. gloeosporioides* and *Pestalotiopsis* sp.) and 96 hrs (*R. solani* and *S. rolfsii*). The mycelial inhibition percentage was calculated by the formula given by Vincent [17].

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

Where, I= Percentage mycelial inhibition, C= Mycelial growth of the pathogen in control, T= Mycelial growth of

the pathogen in treatments.

2.4 Examination of dual culture assay under Scanning electron microscope (SEM)

To observe morphological changes in the hyphae of fungus at the inhibition zone (in direct contact with the metabolite of the rhizobacteria) in dual culture plates, 0.5 cm pieces of agar medium containing mycelium were taken from the periphery of fungal- antagonistic rhizobacterial interaction zone. The samples for the electron microscopy were prepared following the method as described by Goldstein *et al.* [18]. Also, from the control Fig. (without rhizobacteria), mycelium was taken from the periphery of the plate. The scanning electron microscopy of the prepared samples were done in the New Science Complex, Siksha Bhavan, Visva Bharati University using a LEO 1450 VP scanning electron microscope (ZEISS, Ramsey, New Jersey, USA) and photographed.

The collected data underwent analysis using the standard method of analysis of variance suitable for Completely Randomized Design. At the Department of Agricultural Statistics and Computer Science, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia, West Bengal, the corresponding standard errors (S.Em. ±) were calculated for each case, and the critical difference (C.D.) at the five and one per cent probability levels were determined.

3. Results and discussion

The antagonistic potentiality of twenty-six native rhizobacterial isolates, thirteen native *Bacillus* and thirteen fluorescent *pseudomonads*, were screened against five different fungal phytopathogens *viz*., *A. alternata* (Fig. 2), *C. gloeosporioides* (Fig. 3), *Pestalotiopsis* sp. (Fig. 4), *R. solani* (Fig. 5) and *S. rolfsii* (Fig. 6) by the dual culture plate assay. There was variation in the antifungal activity among the rhizobacterial isolates. Among the native *Bacillus* isolates, BRB 88 exhibited the maximum mycelial inhibition against *A. alternata* (67.41% inhibition) followed by PR 20 (64.44% inhibition) and SM 9 (62.22% inhibition). Among fluorescent *pseudomonads* isolates, GP2 (67.41% inhibition) exhibited the maximum mycelial inhibition followed by GP8 (61.48% inhibition) and CK2LP8 (60% inhibition). The clear zone of inhibition produced in the *in-vitro* experiment was an indicative of antibiosis by native rhizobacterial isolates against the fungal pathogen. The highest zone of inhibition between the pathogen and the rhizobacteria was produced by the *Bacillus* isolate, BRB 88 (16 mm) followed by PR 20 (13.33mm) and by fluorescent *pseudomonads*, GP2 (14.67 mm) followed by GP8 (14.33 mm) (Table 2).

When the native rhizobacterial isolates were evaluated against *C. gloeosporioides*, native *Bacillus* isolate, BRB 88 exhibited the maximum mycelial inhibition (59.26% inhibition) followed by BRB 56 and SM 9 (54.82% inhibition) and PR 18 (54.07% inhibition). Fluorescent *pseudomonads* isolate, GP2 (67.41% inhibition) exhibited the maximum mycelial inhibition followed by GP8 (51.11% inhibition) and K11SP4 (40% inhibition). The highest zone of inhibition between the pathogen and the rhizobacteria was produced by the *Bacillus* isolate, BRB 88 (13.33 mm) followed by BRB 56 (12.67mm) and by fluorescent *pseudomonads*, GP2 (12.67 mm) followed by GP8 (10.33 mm) (Table 3).

Among the native *Bacillus* isolates, BRB 56 exhibited the maximum mycelial inhibition against *Pestalotiopsis* sp. (76.30% inhibition) followed by BRB 88 and PR 18 (71.85% inhibition) and SM 9 (70.37%

inhibition). Among fluorescent *pseudomonads* isolates, CK2LP12 (77.04% inhibition) exhibited the maximum mycelial inhibition followed by SS2LP (75.93% inhibition) and G15SP38 (74.07% inhibition). The highest zone of inhibition between the pathogen and the rhizobacteria was produced by the *Bacillus* isolate, BRB 56 (24.33 mm) followed by BRB 88 (24 mm) and fluorescent *pseudomonads*, CK2LP12 (22.67 mm) followed by SS2LP (19.67 mm) (Table 4).

When the native rhizobacterial isolates were evaluated against *R. solani*, native *Bacillus* isolate, BRB 56 exhibited the maximum mycelial inhibition (59.26% inhibition) followed by PR 18 (48.15% inhibition) and BRB 42 (45.93% inhibition). Fluorescent *pseudomonads* isolate, GP2 (45.93% inhibition) exhibited the maximum mycelial inhibition followed by GP8 (44.44% inhibition). The highest zone of inhibition between the pathogen and the rhizobacteria was produced by the *Bacillus* isolate, BRB 56 (8.67 mm) followed by PR 18 (8.33 mm) and fluorescent *pseudomonads*, GP2 (6.33 mm) followed by GP8 (5.67 mm) (Table 5).

Among the native *Bacillus* isolates, PR 18 exhibited the maximum mycelial inhibition against *S. rolfsii* (66.67% inhibition) followed by BRB 42 (58.42% inhibition) and BRB 52 (57.78% inhibition). Among fluorescent *pseudomonads* isolates, only GP2 (42.22% inhibition) and GP8 (37.04% inhibition) exhibited the mycelial inhibition. The highest zone of inhibition between the pathogen and the rhizobacteria was produced by the *Bacillus* isolate, PR 18 (14.67 mm) followed by BRB 42 (13.67 mm) and fluorescent *pseudomonads*, GP2 (9.67 mm) followed by GP8 (7.67 mm) (Table 6).

The native rhizobacterial isolates were classified on the basis of their average mycelium inhibition percentage against five phytopathogens through hierarchical cluster analysis using average linkage between the groups. The cluster analysis distributed the native *Bacillus* isolates into five clusters (Fig. 7A) and native fluorescent *pseudomonads* isolates into five clusters (Fig. 7B). BRB 42, BRB56 and PR 18, were grouped in one cluster showing the highest mycelial inhibition (Table 7). GP2 and GP8, were grouped in one cluster showing the highest mycelial inhibition (Table 8). Furthermore, the native rhizobacterial isolates were classified based on their average inhibition zone against fungal phytopathogens through hierarchical cluster analysis using average linkage between the groups. The cluster analysis distributed the native *Bacillus* isolates into seven clusters (Fig. 8A). BRB 56 and SM 9 with average inhibition of 14.19 and 13.33 mm, were grouped in one cluster showing the maximum inhibition zone (Table 9). The cluster analysis distributed the native fluorescent *pseudomonads* isolates into four clusters (Fig. 8B). GP8 with average inhibition of 14.19 mm was a single isolate showing the maximum inhibition zone (Table 10).

Variation in the antifungal activity of native *Bacillus* sp. and fluorescent *pseudomonads* isolates was also observed by other workers which support the present findings. The rhizobacteria, *Pseudomonas* and *Bacillus* species could act against phytopathogens in the vicinity of plant root [19]. The members of the genera, *Pseudomonas* and *Bacillus,* have good potential to be used as biocontrol agents due to their various genetic and phenotypic characteristics [20]. *Enterobacter cloacae* subsp. *Cloacae,* ENHKU01, was also reported to possess antagonistic activity against *Colletotrichum capsici*, *Sclerotinia sclerotiorum*, *Alternaria* sp., *Didymella bryoniae* and *Fusarium oxysporum* under *in-vitro* condition by producing the chitinase enzyme, siderophore aerobactin, and enterobactin [21]. Rakh *et al.* [22] reported that *Pseudomonas* cf. *monteilii* 9 had shown strong

antagonistic activity against *S. rolfsii* and produced diffusible antibiotic, volatile metabolites, hydrogen cyanide and siderophore which affect its growth *in-vitro.* Rhizobacteria employed either direct or indirect disease control mechanisms which include plant growth promotion, production of hydrolytic enzymes, siderophore, hydrogen cyanide (HCN) and competition with disease-causing microbes for niches and nutrients [23]. The rhizobacteria developed the induction of systematic resistance in the plants [24].

Table 2. Mycelial inhibition potentialities of different native rhizobacterial isolates against *A. alternata*

Native *Bacillus* Isolates	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %	Native Fluorescent *Pseudomonads*	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %
BRB 88	14.67	16.00	67.41 (55.22) a	BCLP4	19.00	9.67	57.78 (49.48) bcd
BRB 89	21.33	8.33	52.59 (46.49) def	CK2LPP	18.67	10.67	58.52 (49.91) bcd
BRB 35	22.67	7.33	49.63 (44.79) f	CK2LP8	18.00	12.67	60.00 (50.77) bc
BRB 42	18.33	10.67	59.26 (50.35) bc	CK2LP12	25.00	6.33	44.44 (41.81) d
BRB 52	21.33	8.33	52.59 (46.49) def	GP2	14.67	14.67	67.41 (55.20) a
BRB 56	21.67	8.00	51.85 (46.06) def	GP8	17.33	14.33	61.48 (51.64) b
BRB 74	19.00	10.33	57.78 (49.48) cd	G11SP37	24.00	8.00	46.67 (43.08) d
PR 16	22.33	7.67	50.37 (45.21) ef	G15SP38	24.00	6.67	46.67 (43.08) d
PR 18	19.33	9.33	57.04 (49.05) cd	K11SP4	20.67	8.33	54.07 (47.34) d
PR 19	19.00	9.67	57.78 (49.48) cd	K22SP8	19.33	9.33	57.04 (49.05) bcd
PR 20	16.00	13.33	64.44 (53.42) ab	SS2PP	18.33	12.33	59.26 (50.34) bcd
SM 9	17.00	12.33	62.22 (52.09) abc	SS2LP	18.33	10.67	59.26 (50.34) bcd
SM 14	19.67	8.67	56.30 (48.62) cde	S21SP14	20.00	9.00	55.56 (48.20) cd
Control	45	0.00(0.00)	0.00(0.00)	Control	45	0.00(0.00)	0.00(0.00)
SE(m)±	--	0.986374	1.149278194	SE(m)±		0.459158	0.91325491
CD at 5% level	--	2.879023	3.354505536	CD at 5% level		1.340188	2.665602346
CD at 1% level	--	3.901574	4.545935167	CD at 1% level		1.816186	3.612352198

Values are the mean of three replications and the values in the bracket are angular transformed values

Table 3. Mycelial inhibition potentialities of different native rhizobacterial isolates against *C. gloeosporioides*

Native *Bacillus* Isolates	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %	Native Fluorescent *Pseudomonads*	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %
BRB 88	18.33	13.33	59.26 (50.34) a	BCLP4	34.33	1.67	23.70 (29.13) bcde
BRB 89	30.67	0.67	31.85 (34.36) def	CK2LPP	31.67	4.00	29.63 (32.98) f
BRB 35	30.33	1.33	32.59 (34.76) f	CK2LP8	32.33	3.33	28.15 (32.04) f
BRB 42	23.33	10.33	48.15 (43.93) bc	CK2LP12	29.33	4.67	34.82 (36.16) bcde
BRB 52	27.33	5.67	39.26 (38.79) def	GP2	14.67	12.67	67.41 (55.19) a
BRB 56	20.33	12.67	54.82 (47.76) def	GP8	22.00	10.33	51.11 (45.64) bcd
BRB 74	23.67	9.67	47.41 (43.49) cd	G11SP37	28.67	6.33	36.30 (37.04) cde
PR 16	25.33	7.67	43.70 (41.38) ef	G15SP38	28.00	6.67	37.78 (37.92) b
PR 18	20.67	10.67	54.07 (47.34) cd	K11SP4	27.00	8.67	40.00 (39.22) de
PR 19	25.67	7.33	42.96 (40.95) cd	K22SP8	33.00	2.33	26.67 (31.08) f
PR 20	24.00	8.67	46.67 (43.08) ab	SS2PP	32.33	2.67	28.15 (32.03) f
SM 9	20.33	11.00	54.82 (47.77) abc	SS2LP	27.67	8.33	38.52 (38.36) bc
SM 14	29.67	5.00	34.07 (35.68) cde	S21SP14	27.67	7.33	38.52 (38.36) de
Control	45	0.00(0.00)	0.00(0.00)	Control	45	0.00(0.00)	0.00(0.00)
SE(m)±	--	0.63099	1.223456503	SE(m)±		0.918316	0.648910932
CD at 5% level	--	1.841729	3.571016689	CD at 5% level		2.680376	1.894036906
CD at 1% level	--	2.495861	4.839345225	CD at 1% level		3.632372	2.566747583

Values are the mean of three replications and the values in the bracket are angular transformed values

Table 4. Mycelial inhibition potentialities of different native rhizobacterial isolates against *Pestalotiopsis* sp.

Native *Bacillus* Isolates	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %	Native Fluorescent *Pseudomonads*	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %
BRB 88	12.67	24.00	71.85 (57.96) ab	BCLP4	23.83	10.33	47.04 (43.30) g
BRB 89	15.33	12.33	65.93 (54.29) c	CK2LPP	25.83	7.33	42.59 (40.73) h
BRB 35	20.00	10.33	55.56 (48.19) de	CK2LP8	24.17	9.33	46.30 (42.88) g
BRB 42	14.67	15.00	67.41 (55.19) bc	CK2LP12	10.33	22.67	77.04 (61.37) a
BRB 52	17.67	11.67	60.74 (51.22) d	GP2	14.33	14.00	68.15 (55.64) d
BRB 56	10.67	24.33	76.30 (60.90) a	GP8	16.17	12.00	64.07 (53.18) e
BRB 74	17.67	11.33	60.74 (51.22) d	G11SP37	11.83	18.00	73.70 (59.15) c
PR 16	19.33	10.67	57.04 (49.05) de	G15SP38	11.67	18.33	74.07 (59.39) bc
PR 18	12.67	22.67	71.85 (57.96) ab	K11SP4	14.17	16.67	68.52 (55.87) d
PR 19	20.33	8.00	54.81 (47.77) e	K22SP8	24.17	8.33	46.30 (42.88) g
PR 20	14.00	15.33	68.89 (56.13) bc	SS2PP	21.17	10.67	52.96 (46.70) f
SM 9	13.33	20.67	70.37 (57.02) bc	SS2LP	10.83	19.67	75.93 (60.62) ab
SM 14	13.67	19.33	69.63 (56.60) bc	S21SP14	14.33	15.67	68.15 (55.64) d
Control	45	0.00(0.00)	0.00(0.00)	Control	45	0.00(0.00)	0.00(0.00)
SE(m)±	--	1.397749514	0.995827855	SE(m)±		1.218331024	0.434890201
CD at 5% level	--	4.079741968	2.906615707	CD at 5% level		3.556056476	1.269354621
CD at 1% level	--	5.528755963	3.938966986	CD at 1% level		4.819071549	1.720195048

Values are the mean of three replications and the values in the bracket are angular transformed values

Table 5. Mycelial inhibition potentialities of different native rhizobacterial isolates against *R. solani*

Native *Bacillus* Isolates	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %	Native Fluorescent *Pseudomonads*	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %
BRB 88	24.67	7.00	45.19 (42.23) bcd	BCLP4	45.00	0.00	0.00 (0.00) bc
BRB 89	27.33	4.67	39.26 (38.80) e	CK2LPP	32.33	3.00	28.15 (32.04) ab
BRB 35	27.33	3.33	39.26 (38.80) e	CK2LP8	31.33	4.33	30.37 (33.44) ab
BRB 42	24.33	7.67	45.93 (42.66) bc	CK2LP12	30.67	4.67	31.85 (34.36) a
BRB 52	26.00	5.67	42.22 (40.52) cde	GP2	24.33	6.33	45.93 (42.66) a
BRB 56	18.33	8.67	59.26 (50.35) a	GP8	25.00	5.33	44.44 (41.80) a
BRB 74	26.67	5.33	40.74 (39.66) de	G11SP37	31.00	4.67	31.11 (33.90) abc
PR 16	26.67	4.67	40.74 (39.66) de	G15SP38	45.00	0.00	0.00 (0.00) bc
PR 18	23.33	8.33	48.15 (43.94) b	K11SP4	32.00	3.33	28.89 (32.51) abc
PR 19	28.00	3.00	37.78 (37.93) e	K22SP8	45.00	0.00	0.00 (0.00) d
PR 20	25.00	6.33	44.44 (41.81) bcd	SS2PP	45.00	0.00	0.00 (0.00) d
SM 9	26.00	5.67	42.22 (40.52) cde	SS2LP	45.00	0.00	0.00 (0.00) bc
SM 14	24.67	6.67	45.19 (42.23) bcd	S21SP14	30.00	5.00	33.33 (35.26) a
Control	45	0.00(0.00)	0.00(0.00)	Control	45	0.00(0.00)	0.00(0.00)
SE(m)±	--	0.604471	0.783853473	SE(m)±		0.32686	0.431289523
CD at 5% level	--	1.764325	2.287906295	CD at 5% level		0.954037	1.258844986
CD at 1% level	--	2.390965	3.100508726	CD at 1% level		1.292886	1.705952675
Values are the mean of three replications and the values in the bracket are angular transformed values							

Table 6. Mycelial inhibition potentialities of different native rhizobacterial isolates against *S. rolfsii*

Native *Bacillus* Isolates	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %	Native Fluorescent *Pseudomonads*	Mycelial growth (mm)	Inhibition zone (mm)	Inhibition %
BRB 88	24.67	10.00	45.19 (42.24) d	BCLP4	45.00	0.00	0.00 (0.00) c
BRB 89	24.33	7.33	45.93 (42.66) d	CK2LPP	45.00	0.00	0.00 (0.00) c
BRB 35	27.00	7.00	40.00 (39.23) f	CK2LP8	45.00	0.00	0.00 (0.00) c
BRB 42	18.67	13.67	58.52 (49.91) b	CK2LP12	45.00	0.00	0.00 (0.00) c
BRB 52	19.00	13.33	57.78 (49.48) b	GP2	28.00	9.67	42.22 (40.52) a
BRB 56	22.00	10.67	51.11 (45.64) c	GP8	26.33	7.67	37.04 (37.49) b
BRB 74	27.00	4.67	40.00 (39.23) f	G11SP37	45.00	0.00	0.00 (0.00) c
PR 16	26.67	7.33	40.74 (39.66) ef	G15SP38	45.00	0.00	0.00 (0.00) c
PR 18	15.00	14.67	66.67 (54.74) a	K11SP4	45.00	0.00	0.00 (0.00) c
PR 19	32.33	2.33	28.15 (32.03) g	K22SP8	45.00	0.00	0.00 (0.00) c
PR 20	24.67	9.67	45.19 (42.24) d	SS2PP	45.00	0.00	0.00 (0.00) c
SM 9	25.00	8.67	44.44 (41.81) de	SS2LP	45.00	0.00	0.00 (0.00) c
SM 14	25.33	8.33	43.70 (41.38) def	S21SP14	45.00	0.00	0.00 (0.00) c
Control	45	0.00(0.00)	0.00(0.00)	Control	45	0.00(0.00)	0.00(0.00)
SE(m)±	--	0.590765	0.718133	SE(m)±		0.353049	0.247357
CD at 5% level	--	1.72432	2.096083	CD at 5% level		1.030478	0.721983
CD at 1% level	--	2.336752	2.840555	CD at 1% level		1.396476	0.978411

Values are the mean of three replications and the values in the bracket are angular transformed values

Table 7. Effect of different native *Bacillus* sp. on average % mycelial inhibition of phytopathogens

Bacillus **Isolates**	**Average % mycelial inhibition**					
	A. alternata	***C. gloeosporioides***	***Pestalotiopsis*** **sp.**	***R. solani***	***S. rolfsii***	**Average**
BRB 88	67.407	59.259	71.852	45.185	45.185	57.778
BRB 89	52.593	31.852	65.926	39.259	45.926	47.111
BRB 35	49.630	32.593	55.556	39.259	40.000	43.407
BRB 42	59.259	48.148	67.407	45.926	58.519	55.852
BRB 52	52.593	39.259	60.741	42.222	57.778	50.519
BRB 56	51.852	54.815	76.296	59.259	51.111	58.667
BRB 74	57.778	47.408	60.741	40.741	40.000	49.333
PR 16	50.370	43.704	57.037	40.741	40.741	46.519
PR 18	57.037	54.074	71.852	48.148	66.667	59.556
PR 19	57.778	42.963	54.815	37.778	28.148	44.296
PR 20	64.444	46.667	68.889	44.444	45.185	53.926
SM 9	62.222	54.815	70.370	42.222	44.444	54.815
SM 14	56.296	34.074	69.630	45.185	43.704	49.778

Table 8. Effect of different native fluorescent *pseudomonads* on average % mycelial inhibition of phytopathogens

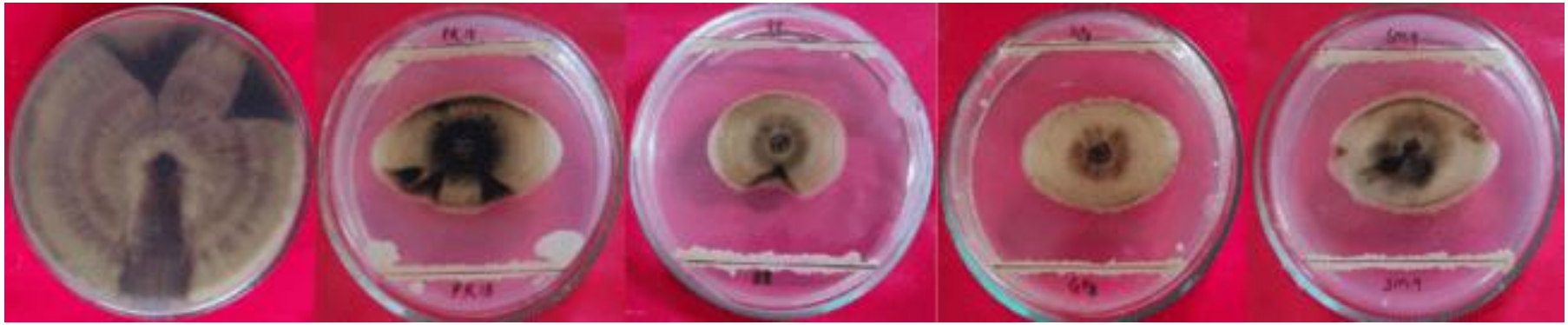
Fluorescent *Pseudomonads* Isolates	Average % mycelial inhibition					
	A. alternata	***C. gloeosporioides***	***Pestalotiopsis* sp.**	***R. solani***	***S. rolfsii***	**Average**
BCLP4	57.778	23.703	47.037	0.000	0.000	25.704
CK2LPP	58.519	29.630	42.593	28.148	0.000	31.778
CK2LP8	60.000	28.148	46.296	30.370	0.000	32.963
CK2LP12	44.444	34.815	77.037	31.852	0.000	37.630
GP2	67.407	67.408	68.148	45.926	42.222	58.222
GP8	61.481	51.111	64.074	44.444	37.037	51.630
G11SP37	46.667	36.297	73.704	31.111	0.000	37.556
G15SP38	46.667	37.778	74.074	0.000	0.000	31.704
K11SP4	54.074	40.000	68.519	28.889	0.000	38.296
K22SP8	57.037	26.667	46.296	0.000	0.000	26.000
SS2PP	59.259	28.148	52.963	0.000	0.000	28.074
SS2LP	59.259	38.519	75.926	0.000	0.000	34.741
S21SP14	55.556	38.519	68.148	33.333	0.000	39.111

Table 9. Effect of different native *Bacillus* sp. on average inhibition zone against fungal pathogens

Bacillus Isolates	Average inhibition zone (mm)					
	A. alternata	*C. gloeosporioides*	*Pestalotiopsis* sp.	*R. solani*	*S. rolfsii*	Average
BRB 88	16.000	13.333	19.333	5.667	9.667	12.800
BRB 89	8.000	1.333	24.333	6.667	7.333	9.533
BRB 35	8.000	0.667	10.000	4.667	4.667	5.600
BRB 42	10.667	5.667	24.000	3.333	10.000	10.733
BRB 52	10.667	5.000	12.333	5.333	13.667	9.400
BRB 56	10.667	12.667	20.667	8.667	10.333	12.600
BRB 74	8.000	7.667	12.000	4.333	2.333	6.866
PR 16	9.000	8.667	12.000	5.333	8.667	8.733
PR 18	13.333	10.333	15.333	5.333	14.667	11.799
PR 19	6.333	5.000	15.000	5.333	2.333	6.799
PR 20	12.333	5.000	11.667	4.667	7.333	8.200
SM 9	10.333	11.000	20.667	7.667	7.000	11.333
SM 14	10.333	1.000	12.333	6.333	8.333	7.666

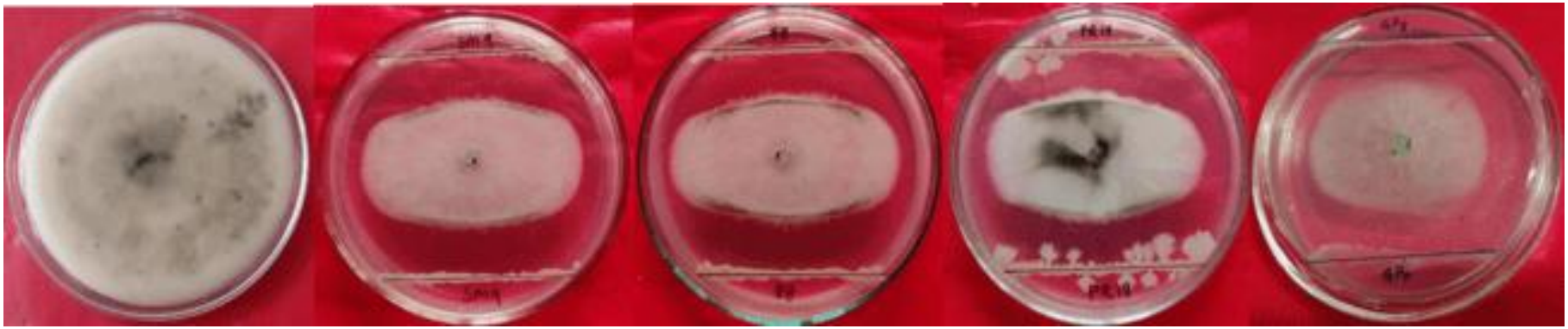
Table 10. Effect of different native fluorescent *Pseudomonads* on average inhibition zone against fungal pathogens

Fluorescent *Pseudomonads* Isolates	Average inhibition zone (mm)					
	A. alternata	*C. gloeosporioides*	*Pestalotiopsis* sp.	*R. solani*	*S. rolfsii*	Average
BCLP4	14.333	4.667	9.333	0.000	0.000	5.666
CK2LPP	14.333	2.667	7.333	3.333	0.000	5.533
CK2LP8	12.333	2.667	10.667	3.333	0.000	5.800
CK2LP12	8.000	4.667	18.000	0.000	0.000	6.133
GP2	12.333	12.667	14.000	5.333	7.667	10.400
GP8	14.667	6.333	22.667	6.333	7.667	11.533
G11SP37	6.667	4.000	18.000	0.000	0.000	5.733
G15SP38	4.333	7.333	16.667	0.000	0.000	5.666
K11SP4	9.333	3.333	18.333	0.000	0.000	6.199
K22SP8	14.667	2.333	8.333	0.000	0.000	5.066
SS2PP	9.000	2.667	12.000	0.000	0.000	4.733
SS2LP	8.333	6.667	15.667	0.000	0.000	6.133
S21SP14	6.667	3.333	18.333	0.000	0.000	5.666



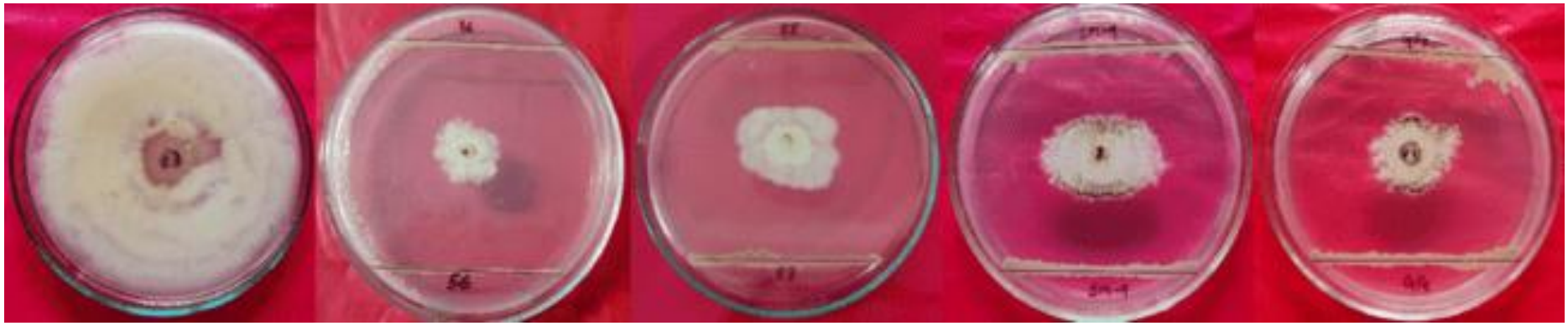
CONTROL PR 18 BRB 88 GP8 SM 9

Fig. 2. Mycelial inhibition of *A. alternata* by native rhizobacteria



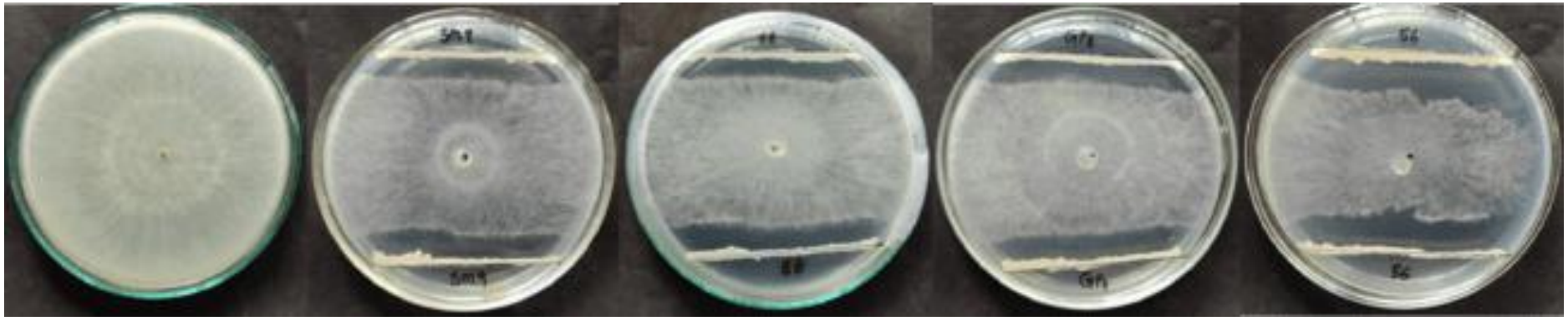
CONTROL SM 9 BRB 88 PR 18 GP 8

Fig. 3. Mycelial inhibition of *C. gloeosporioides* by native rhizobacteria



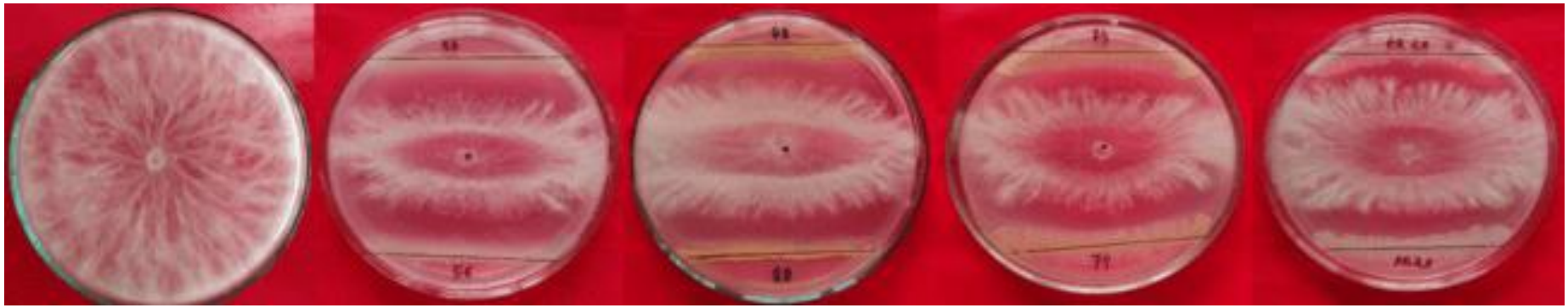
CONTROL BRB 56 BRB 88 SM 9 GP 8

Fig. 4. Mycelial inhibition of *Pestalotiopsis* sp. by native rhizobacteria

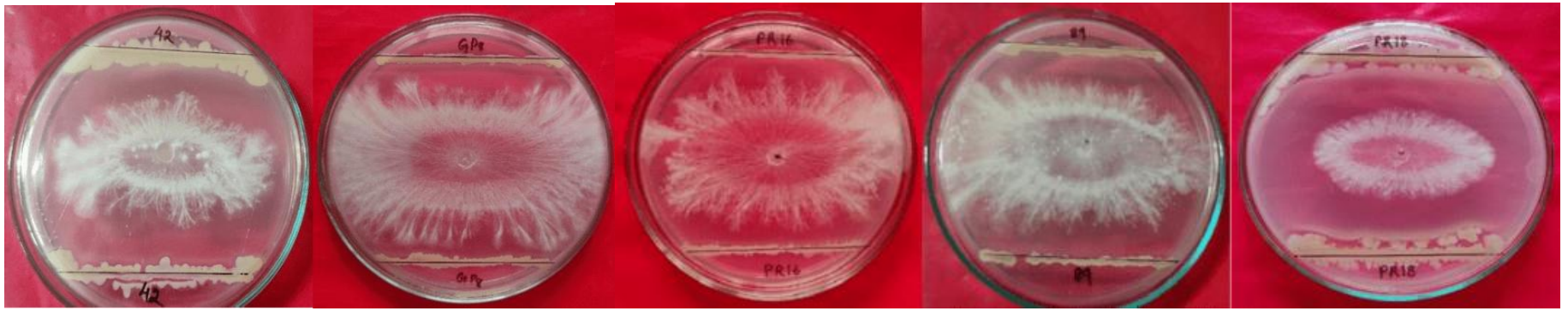


CONTROL SM 9 BRB 88 GP 8 BRB 56

Fig. 5. Mycelial inhibition of *R. solani* by native rhizobacteria



CONTROL BRB56 BRB 88 BRB74 PR20



BRB42 GP8 PR16 BRB89 PR18

Fig. 6. Mycelial inhibition of *S. rolfsii* by native rhizobacteria

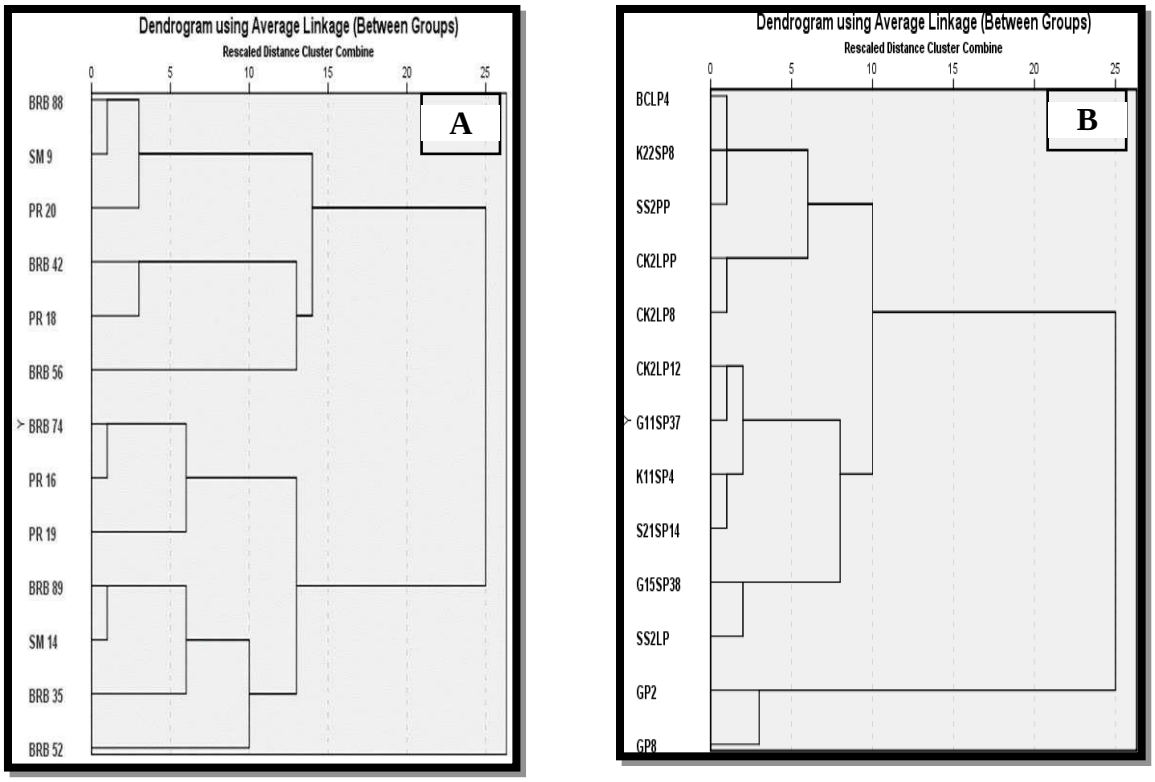


Fig. 7. Dendrogram of effect of (A) Native *Bacillus* sp. and (B) Native fluorescent *pseudomonads* on average % mycelial inhibition of fungal pathogens

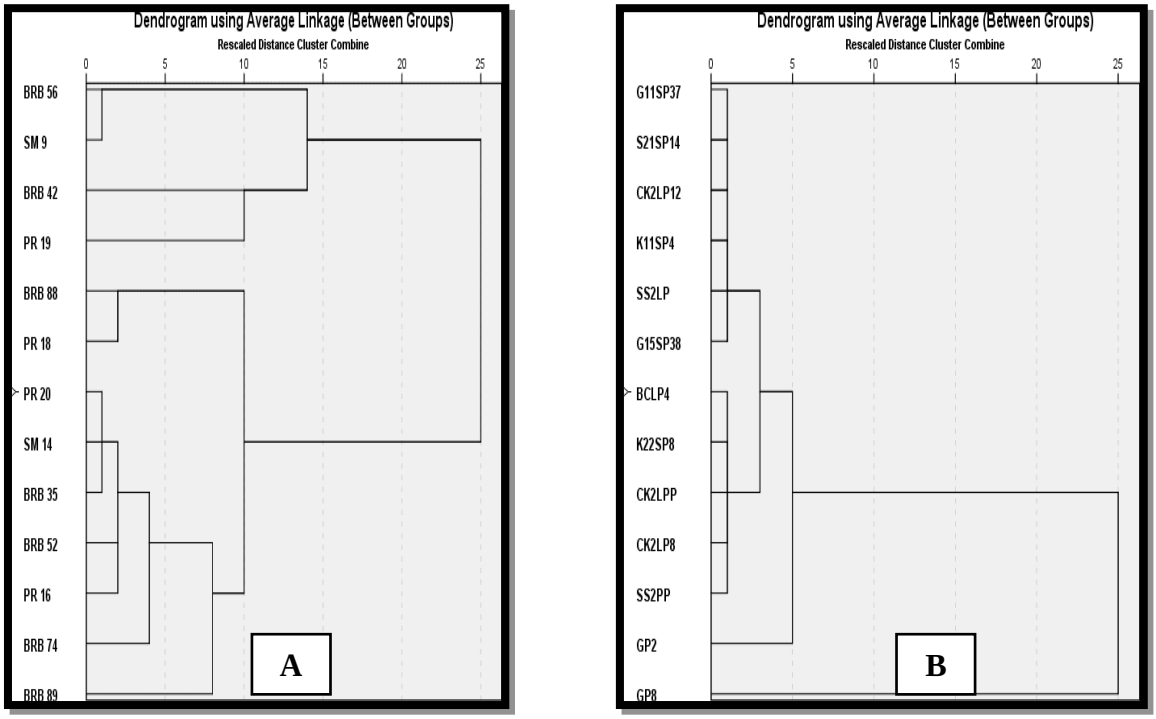


Fig. 8. Dendrogram of effect of (A) Native *Bacillus* sp. and (B) Native fluorescent *pseudomonads* on average inhibition zone against phytopathogens

Scanning electron microscopy (SEM) of dual culture assay

Based on dual culture assay, the native *Bacillus* isolate, PR 18, suppressed the mycelial growth with 66.67% inhibition. A fungal mycelium agar plug was obtained from the edge of *S. rolfsii* colony in the control and within the inhibition zone was examined using scanning electron microscopy (SEM). Those showed that in control, there is typical "net" structure, tubular with smooth surface of hyphae but there were structural changes in the fungal mycelium when antagonistic PR 18 was present. It revealed that the mycelium sample taken from the dual culture assay Fig. (in the presence of PR 18), was degraded (collapsed and deflated) and possibly ruptured (Fig. 9). Similar morphological alterations of fungal mycelia are influenced by metabolites and degrading enzymes and these have been reported in some fungal pathogens. The morphological abnormalities of the mycelia of *Aspergillus* were observed to include deformed and swollen mycelia, when treated with *Pseudomonas* and *Bacillus* bacteria [25].

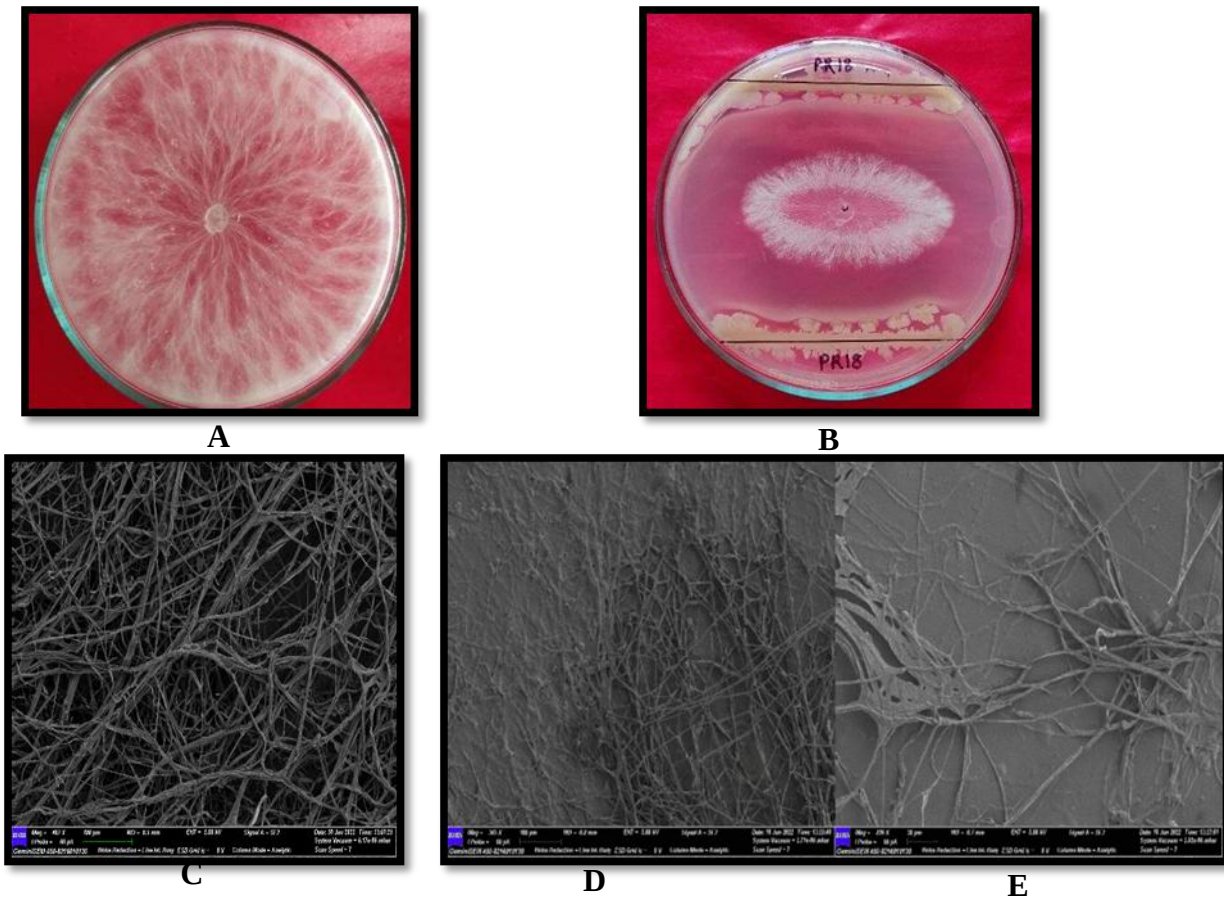


Fig. 9. Antifungal activity tested using dual culture assay (top) and scanning electron microscopy (SEM) micrographs of *S. rolfsii* hyphae (bottom): (A) control fungus; (B) inhibitory effect of isolate PR 18; (C) SEM image of control fungus hyphae; (D, E) SEM image of fungus in the presence of isolate PR 18.

4. CONCLUSION

Native *Bacillus* isolate, BRB 88 and fluorescent *pseudomonads* isolate, GP2, exhibited the maximum mycelial inhibition against *A. alternata.* BRB 88 and GP2, exhibited the maximum mycelial inhibition against *C.*

gloeosporioides. BRB 56 and CK2LP12, exhibited the maximum mycelial inhibition against *Pestalotiopsis* species. BRB 56 and GP2, exhibited the maximum mycelial inhibition against *R. solani*. PR 18 and GP2 exhibited the maximum mycelial inhibition against *S. rolfsii*. BRB 42, BRB 56, PR 18, GP2 and GP8, showed highly antagonist activity against all the phytopathogens.

Author contributions SP conducting the research. MKJ writing, reviewing and editing the manuscript

Conflict of interest No competing interests exist

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