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Evaluation of Antagonistic Potential of *Bacillus subtilis* against Plant Pathogenic Fungi

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A B S T R A C T

The purpose of this research was to evaluate antagonistic activity of *Bacillus subtilis* isolated from Phylloplane and rhizospheric soil of different field crops. Bacteria are able to synthesis a wide range of secondary metabolites with fungicidal capabilities. The antagonistic potential of *Bacillus subtilis* was assessed *in vitro* against some foliar and soil borne plant pathogenic fungi. The result indicated that tested phylloplane *Bacillus subtilis* isolates exhibited antagonistic potential in the range of 76.13-85.59% against foliar pathogenic fungi and also showed that rhizosphere *Bacillus subtilis* recorded between 57.16- 71.89% against soil borne pathogenic fungi. Thus results indicated that tested bacterial species can be used as promising biocontrol agent.

Introduction

Biological control is an environmentally-friendly alternative to chemical pesticides and it is an attractive method protecting the plants from pathogens, because the wide usage of chemicals has a negative impact on the environment and human health. Many biocontrol agents were isolated by screening of the large number of soil or plant-associated microorganisms for antagonism against phytopathogens *in vitro* or in planta (Berg *et al.*, 2001). A number of bacterial species with antagonistic activity were isolated from the rhizosphere of different plants. Among those, bacilli and pseudomonads are the most common isolates. It is known that various species of the Bacillus genus are able to stimulate the plant growth (Cazorla *et al.*, 2007). Bacteria can promote plant growth through a number of mechanisms, such as improvement of plant nutrition; induction of systemic resistance; toxicity to pests and antagonism pathogens. The antagonistic activity of *Bacillus* is associated with the synthesis of various antimicrobial peptides, secreted enzymes, proteins and volatile organic compounds (VOCs). Many *Bacillus* isolates were shown to have antifungal activity against phytopathogenic fungi that make them good biocontrol candidates Cyclic lipopeptide fengycin plays an important role in this process (Mardanova *et al.,* 2017). The strains of the species *B. subtilis* can vary considerably both phenotypically and genetically and that affects their antagonistic potential. Comparative analysis of proteomes of two *B. subtilis* strains with antagonistic potential highlighted the major differences in the composition of intracellular and extracellular proteins (Tan *et al.,* 2013), some of which can be associated with antimicrobial properties. Because of their fast growth, ability to effectively grow in low cost media and to sporulate under undesirable conditions *Bacillus* isolates are the attractive candidates for application as the biocontrol agents. There is a growing demand for such agents since it is expected that global market for biopesticides will significantly expand in the next 3 - 5 years (Wu *et al.,* 2015).

The aim of this study was to directly compare two antagonistic strains of *Bacillus subtilis* isolated from phylloplane and rhizosphere soil of different field crops, in their ability to suppress phytopathogenic fungi through production of cyclic lipopeptides, hydrolytic enzymes and siderophores.

Materials and Methods

Collection and isolation of *B. subtilis* isolates

Thirty isolates of *B. subtilis* were collected from different phylloplane and rhizosphere soil samples of cotton, soybean, pegionpea, paddy, green gram, sorghum, bean and chickpea, crops of Vidarbha region of Maharashtra agro ecosystem by serial dilution technique on nutrient agar medium and designated as (PBs-1 to 15 and RBs-1-15) simultaneously.

Collection of diseased samples for isolation of foliar and soil borne fungal pathogens

Collected diseased crop plant samples from various field crops are as follows.

In vitro screening of *Bacillus subtilis* isolates against plant pathogenic fungi

The isolates of *B. subtilis* were evaluated *in vitro* for their antagonistic properties against foliar and soil borne fungal pathogen of different field crops, by using dual culture technique. The bio-agent and the pathogen were inoculated side by side in a single Petri plate containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen. The plates were incubated for 7 days at 28 ± 1 ^{o}C. The mycelial diameter of pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of test pathogen was calculated using the following equation (Vincent, 1927).

Where;

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

I = Per cent inhibition of mycelium
C = Growth of fungal mycelium in control.
T = Growth of fungal mycelium in treatment.

Results and Discussion

Collection of phylloplane and rhizospheric samples and isolation of *Bacillus subtilis*

Plant and soil samples were collected from phylloplane and rhizosphere of *kharif* and *rabi* crops *viz*. cotton, soybean, pigeonpea, sorghum, green gram, paddy, and chickpea etc. from various locations of Vidarbha region of Maharashtra (Table 1). Collected samples were processed in the laboratory for isolation of *Bacillus subtilis* on nutrient agar medium by serial dilution technique.

Milky white colonies were observed on nutrient agar medium which after three days of incubation picked and streaked on fresh nutrient agar medium for pure culture and used for further study. The data presented in Table 2 and 3, Plate 1 shows the crop wise isolates of *B. subtilis* obtained from phylloplane and rhizospheric samples of different field crops.

The isolates are obtained confirme the existence of *B. subtilis* in the phylloplane of different crops which have been reported earlier by many workers. Patro *et al.,* (2002) recorded three isolates of phylloplane bacteria (Plb) (*Bacillus* spp.) from mungbean leaves. Brian (2004) isolated and reported that *Bacillus subtilis* most abundant bacteria cultured from the phylloplane of soybean. Mohammadipour *et al.,* (2009) studied the characterization of surfactin producing 290 phylloplane isolates of *B. subtilis* which was collected from different ecological zones of Iran. Similarly, Theodulos *et al.,* (2003) reported that *Bacillus subtilis* as a natural inhabitants of the tomato phylloplane. Killani *et al.,* (2011) isolated *Bacillus subtilis* from the soil sample collected from cowpea rhizosphere. Jadhav *et al.,* (2014) studied five isolates of *Bacillus subtilis* from rhizospheric soil of wilt infected pigeonpea plants by serial dilution method.

Amin *et al.,* (2015) collected fifty soil samples from different sites of Bahmanshir riverside in Abadan city, Iran and analyzed for presence of *Bacillus* species. Abdalla *et al.,* (2015), Saha *et al.,* (2015) and Ashwini and Srividya (2016) isolated *Bacillus subtilis* from the rhizosphere of tomato, egg plant and chilli. Mardanova *et al.,* (2017) isolated *Bacillus* strains from the rhizosphere soil of potato roots and Smitha *et al.,* (2017) also isolated *Bacillus* culture CaB5 and *Bacillus subtilis* strain CcB7 from the chickpea and pigeonpea rhizospheric soil by serial dilution plate technique. A total of five *Bacillus* spp. (TB1, TB2, TB3, TB4 and TB5) were isolated from rhizhospheric soil samples of tomato as reported by Karthick *et al.,* (2017) and also thirty isolates of *B. subtilis* collected from different rhizosphere soil samples of chilli, chickpea, cotton, groundnut, onion, marigold, mustard, niger, pegionpea, paddy, sorghum, sunflower and wheat of Bagalkot, Ballari, Raichur and Koppal parts of North Eastern Karnataka by serial dilution and plate count technique on Nutrient agar medium (Rajkumar *et al.,* 2018).

Isolation of fungal pathogens

Isolation of different fungal pathogens from diseased phylloplane and rhizospheric samples was done on PDA by tissue isolation method.

Efficacy of *Bacillus subtilis* isolates against plant pathogenic fungi by dual culture technique

Efficacy of phylloplane *Bacillus subtilis* isolates against foliar fungal pathogens

Data presented in the Table 5 and 6 indicated that all the isolates inhibited the growth of all tested foliar fungal pathogen in the range of 76.13 - 85.59% against PBs13 isolate. The minimum mycelial growth (12.50 mm) with maximum mycelial growth inhibition (81.81%) of *Myrothecium roridum, Colletotrichum dematium, Alternaria alternata* (soybean), *Colletotrichum truncatum, Curvularia lunata, Phoma* *medicaginis* and *Alternaria alternata* (cotton) was recorded in *B. subtilis* isolate PBs13 followed by PBs3 (72.81%), PBs14 (69.45%) and PBs4 (67.34%) respectively. The other isolates of *B. subtilis i.e*. PBs15, PBs10, PBs6 and PBs7 were found least effective against all the fungal pathogens.

The results of present investigations are in agreement with the findings of Amaresan *et al.,* (2012) who reported *B. subtilis* isolates were effective against *Colletotrichum capsici* in chilli**.** However, the suppression of mycelial growth of *Colletotrichum gloeosporiodes* causing anthracnose in *Dendrobium* with crude extract of antifungal compound produced by *B. subtilis* was also reported by Prapagdee *et al.,* (2012). The inhibition of *Colletotrichum lindemuthianum* causing anthracnose of cowpea with different strains of *B. subtilis* (Bs-21, Bs-22 and Bs-23) was recorded by Adebanjo and Bankole (2004). Laha and Venkantaraman (2001), Muralidharan *et al.,* (2004) and Singh and Sinha (2004) reported inhibition of *Curvularia lunata* causing black kernel in rice with *B. subtilis* (97.77%).

Table.1 Particulars of collected diseased samples

Sr.No.	Crops	Diseased plant part used for isolation	Symptoms observed	Fungal pathogens Isolated
1	Cotton	Leaves	Lesion with concentric necrotic rings with sporodochia	*Myrothecium roridum*
2		Leaves	Small,circular to irregular, necrotic, spot	*Alternaria alternata*
3	Pigeon- pea	Root	Purple band extending upward from the base of stem, split root observed	*Fusarium udum*
4	Soybean	Leaf	Small, circular, necrotic, spot	*Alternaria alternata*
5		Leaf	Brown, irregularly shaped spots on stem, pods and petioles	*Colletotrichum truncatum*
6		Root	Leaves and stem straw coloured, root dark, shraded brittle and devoid of lateral roots	*Rhizoctonia bataticola*
7	Sorghum	Seed	Grain black colour	*Curvularia lunata*
8		Seed	Fluffy white or pinkish colouration	*Fusarium moniliformae*
9	Chickpea	Root	Drooping of petioles, rachis and leaflet	*Fusarium oxysporum* f.sp. *ciceri*
10		Root	Lower portion of stem is affected, Decortication, mycelium and mustard like sclerotia on stem	*Sclerotium rolfsii*
11	Green gram	Leaf	Small, enlarged dark brown to blackish spot	*Phoma medicaginis*
12	Paddy	Stem	Oval or elliptical or irregular greenish grey spot	*Rhizoctonia solani*
13	Bean	Leaf	Small watery, dark green to black spots on pods and leaves	*Colletotrichum dematium*

Table.2 Isolates of phylloplane *Bacillus subtilis* obtained from different *kharif* and *rabi* crops

Sr. No.	Crops	Districts	Locations	*B. subtilis* isolates designated as
1	Cotton	Wardha	Hinganghat	PBs1
2		Yavatmal	Murali	PBs2
3	Soybean	Washim	Amkheda	PBs3
4		Washim	Brahamanwada	PBs4
5		Yavatmal	Yavatmal	PBs5
6	Pigeonpea	Buldhana	Ganeshpur	PBs6
7		Washim	Doangaon	PBs7
8	Sorghum	Akola	Sorghum Research Unit, Dr. P.D.K.V., Akola	PBs8
9		Amravati	RRC, Amravati	PBs9
10	Green gram	Akola	Patur	PBs10
11		Amravati	Warud	PBs11
12	Paddy	Bhandara	Sakoli	PBs12
13		Gondia	Amgaon	PBs13
14	Chickpea	Akola	Akola	PBs14
15		Wardha	Dhanora	PBs15

Table.3 Isolates of rhizospheric *Bacillus subtilis* obtained from different *kharif* and *rabi* crops

Sr. No.	Crops	Districts	Locations	*B. subtilis* isolates designated as
1	Cotton	Amaravati	Paratwada	RBs1
2		Buldhana	Bhadgaon	RBs2
3		Nagpur	College of Agriculture,Nagpur	RBs3
4	Soybean	Yavatmal	Gunj, Pusad	RBs4
5		Akola	Pulses Research Unit Dr. P.D.K.V., Akola	RBs5
6	Pigeonpea	Yavatmal	Takli	RBs6
7		Nagpur	Katol	RBs7
8	Sorghum	Amravati	Tivasa	RBs8
9		Nagpur	Umred	RBs9
10	Chickpea	Buldhana	Sangrampur	RBs10
11		Akola	Umra	RBs11
12	Green gram	Amravati	Pipalkhutta	RBs12
13		Buldhana	Jalgaon jamod	RBs13
14	Paddy	Chandrapur	Sindewahi	RBs14
15		Gadchiroli	Allapalli	RBs15

Table.4 Details of diseased sample used for isolation of fungal pathogens

Sr.No.	**Crops**	**Diseased samples used for isolation**	**Fungal pathogens Isolated**
1	Cotton	Leaves	*Myrothecium roridum*
2	Cotton	Leaves	*Alternaria alternate*
3	Pigeonpea	Roots	*Fusarium udum*
4	Soybean	Leaves	*Alternaria alternate*
5	Soybean	Leaves	*Colletotrichum truncatum*
6	Soybean	Roots	*Rhizoctonia bataticola*
7	Sorghum	Seed	*Curvularia lunata*
8	Sorghum	Seed	*Fusarium moniliformae*
9	Chickpea	Roots	*Fusarium oxysporum* f.sp. *ciceri*
10	Chickpea	Roots	*Sclerotium rolfsii*
11	Green gram	Leaves	*Phoma medicaginis*
12	Paddy	Stem	*Rhizoctonia solani*
13	Bean	Leaves	*Colletotrichum dematium*

Table.5 Efficacy of phylloplane *Bacillus subtilis* isolates against foliar fungal pathogens by dual culture technique

***B.subtilis* host and isolates**		**Foliar fungal pathogens and host**													
		***M. roridum* (Cotton)**		***C. dematium* (Bean)**		***A. alternata* (Soybean)**		***C. truncatum* (Soybean)**		***C. lunata* (Sorghum)**		***P. medicaginis* (Green gram)**		***A.alternata* (Cotton)**	
Host	**Isolates**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**
Cotton	PBs1	16.00	80.40	11.83	79.18	30.17	47.68	22.00	61.85	31.50	62.19	28.00	57.36	32.83	62.12
	PBs2	15.00	81.63	10.67	81.22	29.17	49.41	20.83	63.88	28.83	65.40	28.67	56.34	22.67	73.84
Soybean	PBs3	10.37	87.30	10.17	82.10	21.50	62.71	19.17	66.75	32.33	61.20	20.83	68.28	22.33	74.23
	PBs4	15.33	81.22	11.83	79.18	25.50	55.78	21.17	63.29	33.17	60.19	21.17	67.76	27.33	68.46
	PBs5	16.17	80.20	12.33	78.30	24.33	57.81	20.33	64.74	31.00	62.79	26.00	60.40	28.00	67.69
Pigeon-pea	PBs6	15.67	80.81	12.17	78.58	30.50	47.11	22.17	61.55	31.33	62.40	28.50	56.60	23.20	73.23
	PBs7	15.33	81.22	13.50	76.24	32.17	44.21	19.33	66.48	32.67	60.79	21.17	67.76	30.50	64.80
Sorghum	PBs8	15.50	81.02	13.17	76.82	34.17	40.74	21.17	63.29	25.00	69.99	28.17	57.10	29.50	65.96
	PBs9	14.50	82.24	12.33	78.30	32.17	44.21	22.50	60.98	26.67	67.99	29.17	55.58	29.50	65.96
Green gram	PBs10	13.33	83.67	12.50	78.00	32.17	44.21	24.50	57.51	31.33	62.40	27.00	58.88	30.67	64.61
	PBs11	14.00	82.85	12.00	78.88	29.50	48.84	21.00	63.58	33.50	59.79	27.17	58.62	31.50	63.65
Paddy	PBs12	15.00	81.63	11.50	79.76	26.33	54.34	18.33	68.21	33.50	59.79	31.00	52.79	30.33	65.00
	PBs13	12.00	85.30	10.33	81.82	10.33	82.08	12.50	78.32	12.00	85.59	15.67	76.13	18.67	78.45
Chickpea	PBs14	17.00	79.18	11.83	79.18	29.17	49.41	21.50	62.71	26.83	67.80	22.50	65.73	25.33	70.77
	PBs15	17.50	78.57	33.17	41.63	31.83	44.80	20.17	65.02	26.33	68.40	29.33	55.33	30.50	64.80
	Control	81.67	00.00	56.83	00.00	57.67	00.00	57.67	00.00	83.33	00.00	65.67	00.00	86.67	00.00
	F Test	Sig.	-	Sig.	-	Sig.	-	Sig.	-	Sig.	-	Sig.	-	Sig.	-
	SE(m)±	0.58	-	0.59	-	0.89	-	1.00	-	0.73	-	0.48	-	0.52	-
	CD(P=0.01)	2.26	-	2.34	-	3.34	-	3.89	-	2.81	-	1.87	-	1.92	-

Table.6 Efficacy of phylloplane *Bacillus subtilis* isolates against foliar fungal pathogens by dual culture technique (mean of mycelial growth and inhibition)

***B. subtilis* Isolates**	**Locations**	**Host of *B.subtilis* isolates**	**Mean of mycelial growth of fungi tested (mm)**	**Mean of mycelial growth inhibition of fungi tested (%)**
PBs1	Hinganghat	Cotton	24.61	64.39
PBs2	Murali	Cotton	22.26	67.38
PBs3	Amkheda	Soybean	19.52	71.79
PBs4	Brahamanwada	Soybean	22.21	67.98
PBs5	Yavatmal	Soybean	22.59	67.41
PBs6	Ganeshpur	PigeonPea	23.36	65.75
PBs7	Doangaon	PigeonPea	23.52	65.92
PBs8	Akola sorghum	Sorghum	23.81	64.98
PBs9	RRC, Amravati	Sorghum	23.83	65.03
PBs10	Patur	Green gram	24.50	64.18
PBs11	Varud	Green gram	24.09	65.17
PBs12	Sakoli	Paddy	23.71	65.93
PBs13	Amgaon	Paddy	13.07	81.09
PBs14	Akola	Chickpea	22.02	67.82
PBs15	Dhanora	Chickpea	26.97	59.79
Control	-	-	69.93	00.00

Table.7 Efficacy of rhizospheric *Bacillus subtilis* isolates against soil borne fungal pathogens by dual culture technique

***B.subtilis* host and isolates**		**Soil borne fungal pathogens and host**											
		***F. udum* (Pigeonpea)**		***F. moniliformae* (Sorghum)**		***F. oxysporum* f.sp. *ciceri* (Chickpea)**		***S. rolfsii* (Chickpea)**		***R. bataticola* (Soybean)**		***R. solani* (Rice)**	
Host	**Isolates**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**	**Av.radial mycelial growth (mm)**	**Per cent growth inhibition**
Cotton	RBs1	33.50	52.48	31.50	56.55	31.83	56.39	36.33	59.63	40.50	55.00	33.50	62.77
	RBs2	30.17	57.20	21.20	70.76	26.33	63.93	25.33	71.85	33.67	62.56	25.83	71.30
	RBs3	34.63	50.87	28.50	60.68	29.17	60.04	28.00	68.88	41.50	53.88	35.33	60.74
Soybean	RBs4	35.33	49.88	31.50	56.55	30.33	58.45	34.67	61.47	44.67	50.36	40.67	54.81
	RBs5	36.33	48.46	29.83	58.85	32.83	55.02	34.17	62.03	46.50	48.33	40.50	55.00
Pigeon-pea	RBs6	34.83	50.59	30.33	58.16	36.00	50.68	35.17	60.92	42.83	52.41	41.17	54.25
	RBs7	36.00	48.93	30.00	58.62	31.33	57.08	37.00	58.88	43.17	52.03	40.50	55.00
Sorghum	RBs8	36.83	47.75	32.33	55.40	36.83	49.54	38.33	57.41	43.67	51.47	41.50	53.88
	RBs9	37.00	47.51	32.17	55.62	32.83	55.02	37.50	58.33	45.17	49.81	41.17	54.25
Chickpea	RBs10	34.60	50.92	33.00	54.48	36.17	50.45	32.67	63.70	43.00	52.22	42.67	52.58
	RBs11	37.17	47.27	33.83	53.33	32.17	55.93	35.20	60.88	35.50	60.55	42.17	53.14
Green gram	RBs12	31.50	55.31	23.50	67.59	29.33	59.82	26.07	71.03	37.67	58.11	32.67	63.70
	RBs13	32.17	54.36	22.50	68.96	29.50	59.58	26.83	70.18	34.83	61.30	31.50	65.00
Paddy	RBs14	33.00	53.19	23.00	68.27	30.50	58.21	27.83	69.07	34.67	61.47	31.67	64.81
	RBs15	36.17	48.69	32.33	55.40	31.83	56.39	36.50	59.44	42.33	52.96	39.33	56.30
	Control	70.50	00.00	72.50	00.00	73.00	00.00	90.00	00.00	90.00	00.00	90.00	00.00
	F Test	Sig.	-	Sig.	-	Sig.	-	Sig.	-	Sig.	-	Sig.	-
	SE(m)±	0.04	-	0.07	-	0.06	-	0.12	-	0.09	-	0.05	-
	CD(P=0.01)	0.015	-	0.27	-	0.26	-	0.34	-	0.35	-	0.21	-

Table.8 Efficacy of rhizospheric *Bacillus subtilis* isolates against soil borne fungal pathogens by dual culture technique (mean of mycelial growth and inhibition)

***B.subtilis* Isolates**	**Location**	**Host of *B. subtilis* isolates**	**Mean of mycelial growth of fungi tested (mm)**	**Mean of mycelial growth inhibition of fungi tested (%)**
RBs1	Paratwada	Cotton	34.52	57.13
RBs2	Bhadgaon	Cotton	28.13	65.00
RBs3	COA, Nagpur	Cotton	32.85	59.18
RBs4	Gunj, Pusad	Soybean	36.19	55.25
RBs5	Akola	Soybean	36.69	54.61
RBs6	Takli	Pigeonpea	36.72	54.50
RBs7	Katol	Pigeonpea	36.33	55.09
RBs8	Tivasa	Sorghum	38.16	52.57
RBs9	Umred	Sorghum	37.64	53.42
RBs10	Sangrampur	Chickpea	37.01	54.05
RBs11	Akot	Chickpea	36.00	55.18
RBs12	Pipalkhutta	Green gram	29.06	63.87
RBs13	Jalgaon jamod	Green gram	29.55	63.23
RBs14	Sindewahi	Rice	30.11	62.50
RBs15	Allapalli	Rice	36.41	54.86
Control	-	-	81.00	00.00

Similar findings in respect of mycelial growth inhibitions of *Curvularia geniculata* with antifungal compound produced by *B. subtilis* was reported by Dass and Teyegaga (1996). The strong antifungal effect *in vitro* against *Phoma* spp. with *B. subtilis*, isolated from wheat phylloplane and suppression of *Myrothecium* spp. in watermelon with *B. pumilus* were recorded by Perello et al.(2001) and Lokesh *et al.,* (2007) respectively. The findings reported by different workers regarding antifungal activity of *B. subtilis* against tested fungi confirmed the results of present investigations.

Efficacy of rhizospheric *Bacillus subtilis* isolates against soil borne fungal pathogens by dual culture technique

Data presented in the Table 7 and 8 indicated that all the isolates suppress the growth of all tested soil borne fungal pathogens. Mycelial growth inhibition ranges between 57.16 – 71.89% in RBs2 isolates. Minimum mycelial growth (28.13mm) with maximum mycelial growth inhibition (65.00%) of *Fusarium udum, Fusarium moniliformae, Fusarium oxysporum* f.sp. *ciceri, Sclerotium rolfsii, Rhizoctonia bataticola* and *Rhizoctonia solani* was recorded in *B. subtilis* RBs2 followed by RBs12 (63.60%), RBs13 (63.35%) and RBs14 (63.08%) isolates. The other isolates of *B. subtilis* i.e. RBs8, RBs9, RBs10 and RBs6 were found least effective against all the tested soil borne fungal pathogens.

The results of present investigation are corroborates the findings of Wijesundera and Herath (1994) who isolated *Bacillus subtilis* from rice phylloplane, which was inhibitory to *R. solani* causal agent of sheath blight of rice. Karimi *et al.,* (2012) recorded antagonistic effects of six rhizosphere isolates of *Pseudomonas* and *Bacillus* spp. obtained from chickpea against *Fusarium oxysporum* f. sp. *ciceris* as potential biocontrol agents *in vitro*. Jadhav *et al.,* (2014) evaluated five isolates of *Bacillus subtilis* from rhizospheric soil of wilt infected pigeonpea plants, viz. BS1, BS2, BS3, BS4, BS5. Isolate BS5 was found most effective against *Fusarium udum* which recorded 48.12% mycelial growth inhibition in dual culture technique. Abou-Aly HE *et al.,* (2015) revealed that among 116 bacterial isolates, nineteen bacterial isolates showed the highest inhibition against *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*. Isolates B103 and B38 showed considerable inhibition against pathogenic fungi. The most potent isolates for bioagent production were chosen and were identified as *Pseudomonas fluorescens* (B103) and *Bacillus subtilis* (B38).

Rajkumar *et al.,* (2018) screened thirty *B. subtilis* isolates *in vitro* against *S. rolfsii*, isolate BS16 inhibited maximum mycelial growth (64.04%) followed by BS 30 (11.98 %). Shifa *et al.,* (2015) recorded *Bacillus subtilis* strain G-1 was the most effective in inhibiting the mycelial growth of *S. rolfsii*. *In vitro* mycelial suppression of *R. bataticola* to the extent of 52.22% with *B.subtilis* was also reported by Veenashri Jainapur *et al.,* (2019). Thus present studies confirm the efficiency of the isolates under investigations.

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