2.1 *In vitro* evaluation of bioagents as seed treatment on dominant seed mycoflora of chilli

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Introduction

Chilli (*Capsicum annuum* L.), belongs to the nightshade family Solanaceae is an important vegetable cum spice crop having diverse commercial and therapeutic value. Seed is the basic and primary material for commercial propagation hence, seed health plays an important role for successful cultivation and higher yield of crop species. Seeds also can be a source of transmission of plant pathogens over long distances (Chamling, 2011).

Sporulating structures emerging out from the dead chilli seedlings serves as the potential source of inoculum for further spread of pathogen in field. Hence, it is of vital importance that seeds must be treated before they are sown in the field. The main aim of the present study is to check the efficacy of seed treatment by bioagents on the seed germination and seedling vigour by controlling the most dominant seed mycoflora (*A. niger*, *Colletotrichum* sp., *Fusarium* sp.) of chilli seeds (var. GVC101, GVC111) by Paper towel method. The main aim of the present study is to check the efficacy of seed treatment by different combination of bioagents on the seed germination and seedling vigour by controlling the most dominant seed mycoflora (*A. niger*, *Colletotrichum* sp., *Fusarium* sp.) of chilli seeds (var. GVC101, GVC111) by Paper towel method.

Materials & Methods

Method used : **Paper towel method**

- After surface sterilization, healthy seeds of GVC-111 and GVC-101 were soaked in the spore suspension of most dominant fungi for 8 hours and spread over the blotter paper for drying.
- Chilli seeds were then mixed in suspension of respective bioagents and again kept for 8 hours and dried.
- Treated seeds were placed equidistantly between two previously wetted germination paper.
- Seed soaked only in spore suspension of corresponding pathogen were kept as control.
- The paper towels were rolled without disturbing the position of the seed and labelled properly. The ends were closed with rubber bands, kept in polythene bag and incubated in upright position. After 7 days of incubation period, they were observed and germination, vigour index were measured and calculated.

The following parameters were observed:

- Germination percentage (%)(Khare and Bhale, 2000)

$$\text{Per cent germination} = \frac{\text{Number of normal seedlings}}{\text{Total planted seeds}} \times 100$$

- Seedling length (cm) (Radical and plumule length)
- Fresh seedling weight (mg)
- Seedling dry weight (mg)
- Vigour index (Abdul-Baki *et al.*, 1973) = Average seedling length on final count (plumule + radical length (cm)) x per cent germination

Treatment No.	Name of bioagents	Dose (g/kg seeds)
T_1	*Trichoderma viride* (Navsari isolate)	5
T_2	*Trichoderma harzianum* (Navsari isolate)	5
T_3	*Pseudomonas fluorescens* (Navsari isolates)	6
T_4	*Bacillus subtilis* (Navsari isolates)	6
T_5	*Trichoderma harzianum*+ *Pseudomonas fluorescens*	5+6
T_6	*Trichoderma viride*+ *Pseudomonas fluorescens*	5+6
T_7	*Pseudomonas fluorescens* + *Bacillus subtilis*	6+6
T_8	Control (treated with respective pathogen only)	-

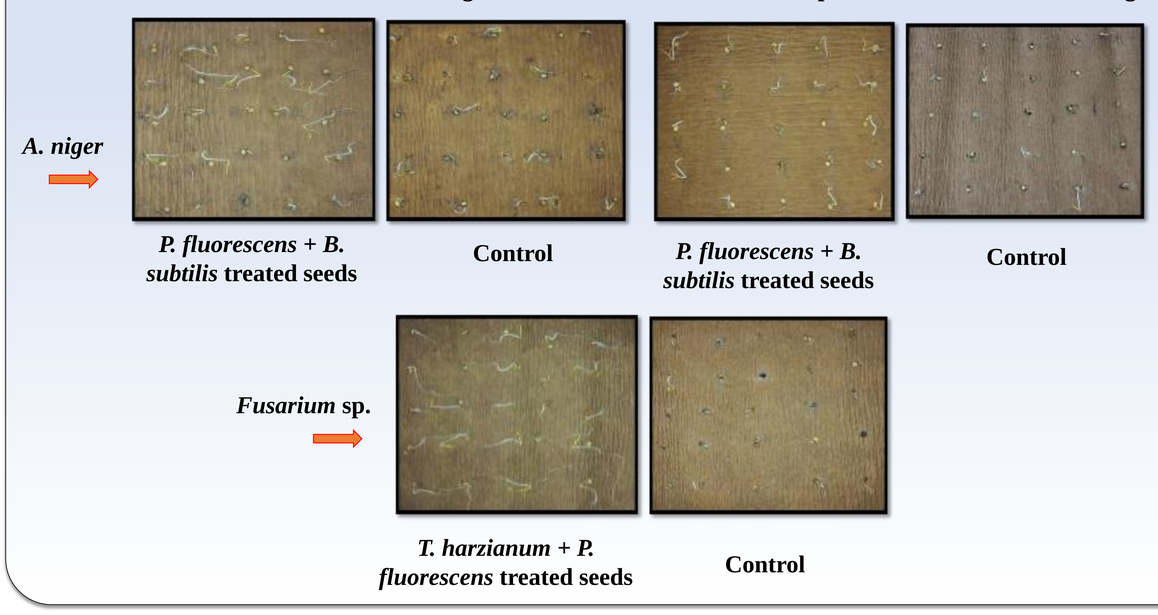
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Results and Discussion

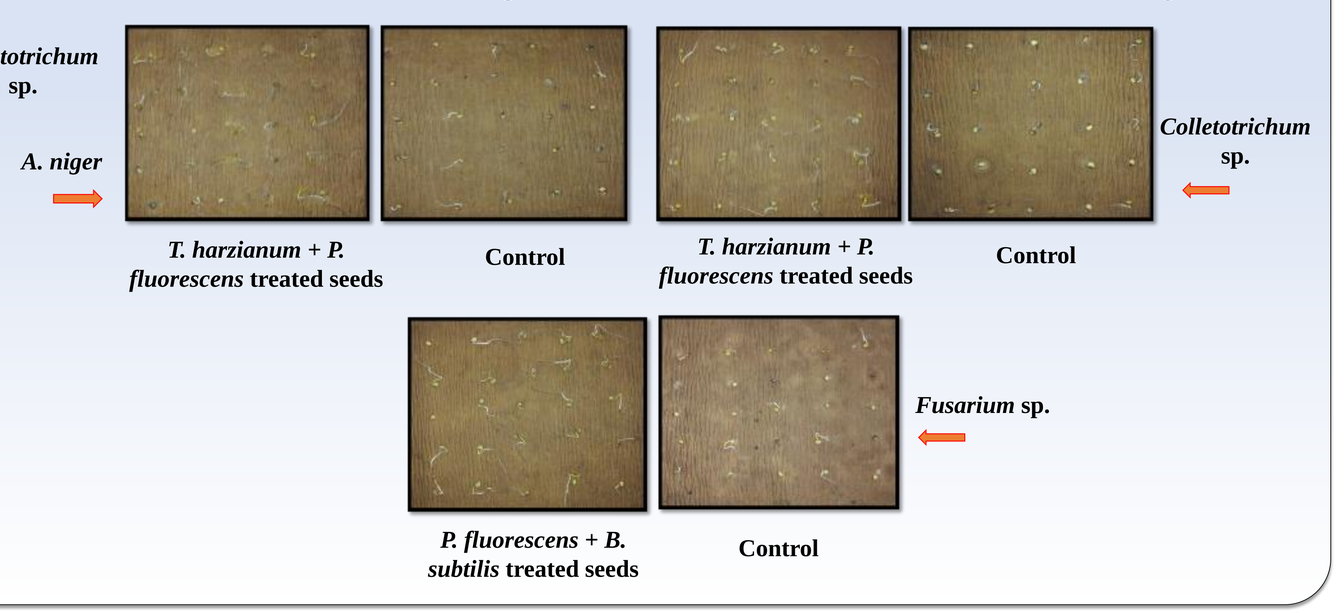
In GVC 101, seed treatment with *P. fluorescens*+*B. subtilis* @ 6+6g/kg seeds and *T. harzianum* + *P. fluorescens* 5+6g/kg seeds found effective in seeds pretreated with *A. niger*, while *P. fluorescens* + *B. subtilis* 6+6g/kg seeds and *T. viride* + *P. fluorescens* 5+6g/kg seeds found effective in seeds pretreated with *Colletotrichum* sp. and *T. harzianum* + *P. fluorescens* 5+6g/kg seeds and *P. fluorescens* 6g/kg seeds found effective in seeds pretreated with *Fusarium* sp.

In GVC 111 seed treatment with *T. harzianum*+*P. fluorescens* 5+6g/kg seeds and *P. fluorescens* 6g/kg seeds found effective in seeds pretreated with *A. niger*, while *T. harzianum* + *P. fluorescens* 5+6g/kg seeds and *P. fluorescens* + *B. subtilis* 6+6g/kg seeds found effective in seeds pretreated with *Colletotrichum* sp. and *P. fluorescens* + *B. subtilis* 6+6g/kg seeds and *T. viride*+*P. fluorescens* 5+6g/kg seeds found effective in seeds pretreated with *Fusarium* sp.

Effect of seed treatment with bioagents on chilli seeds var. GVC 101 pretreated with dominant fungi



Effect of seed treatment with bioagents on chilli seeds var. GVC 111 pretreated with dominant fungi



Effect of seed treatment with bioagents on chilli seeds of var. GVC 101 pretreated with *A. niger*

Tr. No.	Treatment	Dose (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	*T. viride*	5	71.62* (90.00)	8.69 (2.29)	4.25 (0.55)	2.40 (0.18)	205.70
T_2	*T. harzianum*	5	74.53 (92.67)	8.92 (2.41)	5.44 (0.90)	2.51 (0.19)	223.18
T_3	*P. fluorescens*	6	67.55 (85.33)	8.39 (2.13)	3.55 (0.38)	2.14 (0.14)	182.03
T_4	*B. subtilis*	6	63.96 (80.67)	7.63 (1.76)	2.66 (0.22)	1.43 (0.06)	142.19
T_5	*T. harzianum*+*P. fluorescens*	5+6	76.16 (94.00)	9.81 (2.91)	5.49 (0.92)	2.66 (0.22)	273.24
T_6	*T. viride*+*P. fluorescens*	5+6	66.53 (84.00)	7.84 (1.86)	3.47 (0.37)	1.74 (0.09)	155.97
T_7	*P. fluorescens* +*B. subtilis*	6+6	83.44 (98.00)	9.94 (2.98)	5.69 (0.98)	3.17 (0.31)	292.43
T_8	Control (treated with *A. niger* only)	-	52.75 (63.33)	7.33 (1.65)	2.33 (0.17)	1.16 (0.04)	88.72
	S.Em.±		1.98	0.15	0.09	0.10	6.98
	C.D. at 5%		5.95	0.45	0.28	0.29	20.93
	C.V. %		4.94	3.07	3.87	7.67	6.19

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Effect of seed treatment with bioagents on chilli seeds of var. GVC 101 pretreated with *Colletotrichum* sp.

Tr. No.	Treatment	Dose (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	*T. viride*	5	61.15* (76.67)	7.84 (1.86)	3.98 (0.48)	2.19 (0.15)	142.88
T_2	*T. harzianum*	5	58.03 (72.00)	6.92 (1.45)	3.22 (0.32)	1.72 (0.09)	104.61
T_3	*P. fluorescens*	6	57.64 (71.33)	6.76 (1.39)	3.13 (0.30)	1.58 (0.08)	98.99
T_4	*B. subtilis*	6	58.03 (72.67)	7.22 (1.58)	3.47 (0.37)	1.96 (0.12)	115.13
T_5	*T. harzianum*+*P. fluorescens*	5+6	51.93 (62.00)	6.60 (1.32)	3.05 (0.28)	1.32 (0.05)	82.10
T_6	*T. viride*+*P. fluorescens*	5+6	70.41 (88.67)	8.78 (2.33)	4.12 (0.52)	2.52 (0.19)	206.92
T_7	*P. fluorescens* +*B. subtilis*	6+6	71.59 (90.00)	9.29 (2.61)	4.50 (0.62)	2.83 (0.24)	234.63
T_8	Control (treated with *Colletotrichum* sp. only)	-	39.76 (41.00)	6.09 (1.13)	2.49 (0.19)	1.04 (0.03)	46.32
	S.Em.±		1.51	0.09	0.10	0.05	4.44
	C.D. at 5%		4.54	0.26	0.29	0.15	13.31
	C.V. %		4.48	2.04	4.86	4.67	6.01

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Effect of seed treatment with bioagents on chilli seeds of var. GVC 101 pretreated with *Fusarium* sp.

Tr. No.	Treatment	Dose (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	*T. viride*	5	60.22* (75.33)	8.04 (1.96)	3.69 (0.42)	1.75 (0.09)	147.21
T_2	*T. harzianum*	5	59.78 (74.67)	7.47 (1.69)	3.38 (0.35)	1.24 (0.05)	126.29
T_3	*P. fluorescens*	6	73.62 (92.00)	9.57 (2.77)	5.59 (0.95)	2.31 (0.16)	254.62
T_4	*B. subtilis*	6	66.98 (84.67)	8.23 (2.05)	3.99 (0.48)	1.78 (0.10)	173.43
T_5	*T. harzianum*+*P. fluorescens*	5+6	78.68 (96.00)	9.89 (2.95)	6.02 (1.10)	2.52 (0.19)	283.27
T_6	*T. viride*+*P. fluorescens*	5+6	55.55 (68.00)	6.94 (1.46)	3.31 (0.33)	1.19 (0.04)	99.16
T_7	*P. fluorescens* +*B. subtilis*	6+6	70.98 (89.33)	9.33 (2.63)	4.62 (0.65)	1.87 (0.11)	234.99
T_8	Control (treated with *Fusarium* sp.)	-	42.68 (46.00)	6.77 (1.39)	2.86 (0.25)	0.92 (0.03)	63.92
	S.Em.±		1.30	0.14	0.14	0.06	6.04
	C.D. at 5%		3.88	0.42	0.42	0.18	18.12
	C.V. %		3.53	2.95	5.8	5.99	6.06

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Effect of seed treatment with bioagents on chilli seeds of var. GVC 111 pretreated with *A. niger*

Tr. No.	Treatment	Dose (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	*T. viride*	5	53.51* (64.67)	7.30 (1.62)	3.62 (0.40)	1.44 (0.06)	104.53
T_2	*T. harzianum*	5	67.03 (84.67)	8.88 (2.39)	4.74 (0.68)	1.92 (0.11)	201.72
T_3	*P. fluorescens*	6	71.59 (90.00)	9.21 (2.57)	5.39 (0.88)	2.26 (0.16)	231.10
T_4	*B. subtilis*	6	68.03 (86.00)	8.94 (2.42)	5.29 (0.85)	2.21 (0.15)	207.73
T_5	*T. harzianum*+*P. fluorescens*	5+6	75.25 (93.33)	10.01 (3.02)	5.69 (0.98)	2.60 (0.21)	282.29
T_6	*T. viride*+*P. fluorescens*	5+6	64.89 (82.00)	8.75 (2.32)	4.68 (0.67)	1.74 (0.09)	189.87
T_7	*P. fluorescens* +*B. subtilis*	6+6	63.48 (80.00)	8.15 (2.01)	4.19 (0.53)	1.65 (0.08)	161.08
T_8	Control (treated with *A. niger* only)	-	50.36 (59.33)	6.66 (1.35)	3.38 (0.35)	1.23 (0.05)	80.20
	S.Em.±		1.39	0.14	0.10	0.04	6.09
	C.D. at 5%		4.16	0.42	0.29	0.12	18.26
	C.V. %		3.74	2.83	3.64	3.67	5.79

***Figures outside the parentheses indicate arc sine transformation value Figures in parentheses indicate original values**
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Effect of seed treatment with bioagents on chilli seeds of var. GVC 111 pretreated with *Colletotrichum* sp.

Tr. No.	Treatment	Dose (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	*T. viride*	5	43.83* (48.00)	6.18 (1.16)	3.31 (0.33)	1.36 (0.06)	55.63
T_2	*T. harzianum*	5	45.74 (51.33)	7.18 (1.56)	3.49 (0.37)	1.78 (0.10)	80.10
T_3	*P. fluorescens*	6	58.90 (73.33)	7.44 (1.68)	3.55 (0.38)	2.14 (0.14)	122.85
T_4	*B. subtilis*	6	60.22 (75.33)	7.54 (1.72)	3.84 (0.45)	2.24 (0.15)	129.77
T_5	*T. harzianum*+*P. fluorescens*	5+6	69.74 (88.00)	8.65 (2.27)	4.59 (0.64)	3.01 (0.28)	199.37
T_6	*T. viride*+*P. fluorescens*	5+6	63.93 (80.67)	8.08 (1.98)	4.16 (0.53)	2.29 (0.16)	159.73
T_7	*P. fluorescens*+*B. subtilis*	6+6	64.40 (81.33)	8.19 (2.03)	4.40 (0.59)	2.81 (0.24)	165.47
T_8	Control (treated with *Colletotrichum* sp. only)	-	39.97 (41.33)	4.44 (0.60)	2.70 (0.22)	1.19 (0.04)	24.97
	S.Em.±		1.16	0.10	0.08	0.06	3.31
	C.D. at 5%		3.47	0.29	0.25	0.17	9.91
	C.V. %		3.59	2.30	3.77	4.71	4.88

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Effect of seed treatment with bioagents on chilli seeds of var. GVC 111 pretreated with *Fusarium* sp.

Tr. No.	Treatment	Dose (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	*T. viride*	5	63.93* (80.67)	7.83 (1.86)	3.67 (0.41)	1.71 (0.09)	149.62
T_2	*T. harzianum*	5	58.47 (72.67)	6.19 (1.17)	2.33 (0.18)	1.32 (0.05)	84.61
T_3	*P. fluorescens*	6	68.03 (86.00)	8.06 (1.97)	4.05 (0.50)	2.16 (0.14)	169.43
T_4	*B. subtilis*	6	60.27 (75.33)	6.81 (1.41)	3.19 (0.31)	1.48 (0.07)	106.14
T_5	*T. harzianum*+*P. fluorescens*	5+6	71.02 (89.33)	8.79 (2.34)	4.78 (0.70)	2.60 (0.21)	209.16
T_6	*T. viride*+*P. fluorescens*	5+6	75.12 (93.33)	9.56 (2.76)	5.64 (0.97)	2.82 (0.24)	258.05
T_7	*P. fluorescens* +*B. subtilis*	6+6	75.92 (94.00)	9.86 (2.93)	6.20 (1.17)	3.24 (0.32)	275.77
T_8	Control (treated with *Fusarium* sp. only)	-	50.96 (60.33)	6.16 (1.15)	2.45 (0.17)	1.19 (0.04)	69.65
	S.Em.±		1.47	0.12	0.11	0.06	6.05
	C.D. at 5%		4.40	0.36	0.34	0.19	18.13
	C.V. %		3.88	2.64	4.85	5.19	6.33

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Conclusion

From over all study, it can be revealed that *P. fluorescens* + *B. subtilis* @ 6+6g/kg seeds and *T. harzianum* + *P. fluorescens* @ 6+6g/kg seeds were found very effective in controlling or inhibiting the growth of seed infecting fungi of chilli in both variety GVC 101 and GVC 111.Thus, results of seed treatment with bioagents on seed germination and other health parameters of seed were more or less in agreement with earlier works. The difference in results may be due to difference in dose of bioagents and varieties used for investigation.

Discussion

Machenahalli *et al.* (2014) reported that seed treatment with *T. harzianum* 5.0g + *P. fluorescens* 5.0g showed least infection (14.89 %) with highest vigour index (930.74) followed by *P. fluorescens* (10.0g/kg) with 14.94 per cent infection and vigour index of 915.27. Reddy *et al.* (2017) reported that seed dressing with *T. viride* (10g/kg seed) and *T. viride* (5g/kg seed) + *P. fluorescens* (5g/kg seed) decreased the seed mycoflora infection in chilli by 81.8 per cent compared to untreated seed.

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ISOLATION AND SCREENING OF PHYLLOSPHERE BACTERIA AGAINST E. TURCICUM CAUSING LEAF BLIGHT OF MAIZE

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Introduction

Maize (*Zea mays* L.) is one of the important cereal crops of the world and world's third leading cereal crop, after wheat and rice. Among the foliar diseases, turcicum leaf blight incited by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs has become a major threat and results in severe reduction in grain yield to an extent of 28 to 91 per cent. With the development of ecological agriculture, the impact of over use of chemical fungicides on environment and food safety has become a serious concern. Alternate to chemical fungicides phyllosphere microflora can be used as biocontrol agents because of microbial interactions in the phyllosphere suppress and stimulate the colonization and infection of tissues by plant pathogens, increase disease resistance and the productivity of agricultural crops, thus phyllosphere microorganisms can play an important role in growth promotion of plants. Keeping in view the importance of the crop and lack of adequate information on beneficial effects of phyllosphere microflora in the management of turcicum blight of maize, the present study was done.

Materials and methods

Isolation of phyllosphere microflora

Dilution method

Healthy maize plants were collected from different areas of Karimnagar, Mahaboobnagar and Ranga Reddy districts in Telangana region for isolation of phyllosphere microorganisms. From each plant, ten discs of one cm leaf bits were cut with a sterile cork borer. The discs were transferred to sterile distilled water of 100 ml and stirred for one hr. An aliquot of one ml was plated on potato dextrose agar medium and nutrient agar medium.

Leaf imprint method

In order to estimate the bacterial population on adaxial and abaxial leaf surfaces, leaf imprints were made on nutrient agar medium. An intact individual leaf was placed on to nutrient agar plate and was pressed with the smooth end of a sterile glass rod until a clear imprint of the entire leaf was obtained on nutrient agar surface. The plates incubated at 24ºC for 2-5 days until colony formation. Selection of single bacterial colonies was done based on morphological variation.

Isolation of the pathogen

Maize leaves showing typical symptoms were collected from different locations and used for isolation of the pathogen. These leaves were first washed with tap water followed by sterile distilled water. Diseased portions with some healthy portion were cut into small bits of 3-5 mm size, surface sterilize by dipping them in sodium hypochlorite (1%) solution for one minute and then 3-4 bits were transferred aseptically to Petri plates containing potato dextrose agar (PDA) medium and were incubated at 25 ± 1ºC in an incubator (Aneja, 2003). The pathogen was further purified by single spore isolation method as described by Ho and Ko (1997).

Screening of phyllosphere bacteria against *E. turcicum in vitro*

Antagonism test was performed *in vitro* by dual culture method on PDA. One loop of 48 hrs old culture of bacterial isolates were streaked one cm from the outer side of 9 cm PDA plates. Five mm discs of actively growing three-day old fungus was placed at the centre of plates, 2.5 cm apart from the bacteria. Plates inoculated with fungus without bacterial isolates served as control. For each isolate three replicates were maintained. These plates were incubated at 28 ± 2 ºC for 3 days. The growth of turcicum blight pathogen in the presence or absence of any bacterial isolates was measured. Observations regarding the zone of inhibition radius was recorded after 9 day of incubation and calculated as per the formula given below (Vincent, 1947).

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

Where, I = Per cent inhibition over control

C = Radial growth of pathogen in control (mm)

T = Radial growth of pathogen in treatment (mm)

Screening of phyllosphere fungi against *E. turcicum in vitro*

Dual culture technique (Dennis and Webster, 1971) was employed to identify potential fungal antagonists. Seven-day old antagonistic fungal disc of five mm was cut with a sterilized cork borer from the edge and was placed at one end of media on Petri plate. A five mm disc of *E. turcicum* pathogen of five-day old was cut with a sterilized cork borer from the edge and placed at the opposite end. Three replications along with suitable control was maintained by placing only the pathogen on culture medium. The plates were incubated at room temperature (28± 1°C) till mycelial growth in the control plates covered the entire plates. Petri plates were observed daily for recording antagonistic interactions between the pathogen and biocontrol agent. The per cent inhibition of the test pathogen was calculated when growth of the test pathogen was full in control plates by using the formula given below and calculated as per the formula given below (Vincent, 1947).

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

Where, I = Per cent inhibition over control

C = Radial growth of pathogen in control (mm)

T = Radial growth of pathogen in treatment (mm)

Results and Discussion

Isolation of phyllosphere microflora

Twenty-two bacterial cultures and six fungal cultures were isolated from the phyllosphere and designated for bacteria as P1 to P22 and phyllosphere fungi as F1 to F6 respectively.

Screening of phyllosphere bacteria against *E. turcicum*

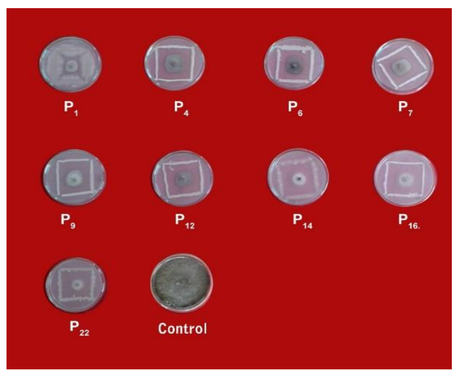
All the twenty-two isolates were used for dual culture test of which nine isolates *i.e*. Isolates P1, P4, P6, P7, P9, P12, P14, P16 and P22 inhibited the radial growth of *E. turcicum*. Among the nine isolates P9 inhibited 24.09 % radial growth of the test fungus *E. turcicum* which was the highest growth inhibition among all the bacterial isolates and it was significantly superior over control (52.33 %). Isolate P16 and isolate P4 which were also effective in inhibiting the radial growth of *E. turcicum* 21.6 per cent and 21.07 per cent radial growth inhibition respectively.

Screening of phyllosphere fungi against *E. turcicum*

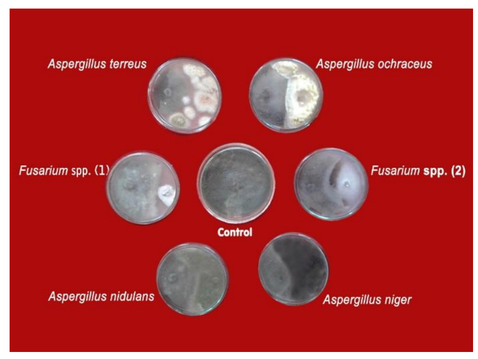
Maximum inhibition of test pathogen was recorded with *Aspergillus niger* (60.6 %) and it was significantly superior compared to other fungal species, followed by *Aspergillus nidulans* (60.0 %). The isolates *Aspergillus terreus* (45.25 %) and *Fusarium* sp. (2) (42.9 %) were on par with each other. Lowest inhibition percentage (20.3 %) was recorded in isolate *Fusarium* sp. (1)

Conclusion

Phyllosphere microorganisms play important role in suppression of the pathogen and induce growth promoting activities in plants. Twenty-two bacterial isolates (P1 to P22) and six fungal (F1 to F6) cultures were isolated from the phyllosphere. Screening of all the twenty two bacterial isolates against *E. turcicum* under *in vitro* conditions by dual culture technique revealed that nine isolates were effective over test pathogen. Among the nine isolates, isolate P9 recorded maximum growth inhibition of 24.09 % among all the bacterial isolates followed by isolate P16 (21.60 %) and isolate P4 (21.07 %). Screening of all the six phyllosphere fungal isolates revealed that highest inhibition of test pathogen was recorded by isolate *Aspergillus niger* (60.6 %) and lowest inhibition percentage was recorded in isolate *Fusarium* sp. (2) (20.3%).



Effect of isolates of phyllosphere bacteria in dual culture technique



Effect of isolates of phyllosphere fungi in dual culture technique

Effect of isolates of phyllosphere bacterial on the mycelial growth of *Exserohilum turcicum* under *in vitro* conditions

Phyllosphere bacterial antagonist isolates	Linear mycelial* growth (mm)	Per cent inhibition of test pathogen over control
P_1	47.36	9.5
P_2	72.1	0.0
P_3	66.4	0.0
P_4	41.30	21.07
P_5	69.7	0.0
P_6	47.40	9.42
P_7	49.60	5.21
P_8	76.1	0.0
P_9	39.70	24.09
P_{10}	65.1	0.0
P_{11}	59.6	0.0
P_{12}	48.96	6.50
P_{13}	55.2	0.0
P_{14}	48.66	7.0
P_{15}	59.2	2.2
P_{16}	41.00	21.6
P_{17}	61.1	0.0
P_{18}	57.2	1.0
P_{19}	55.0	0.0
P_{20}	53.1	0.0
P_{21}	51.1	2.35
P_{22}	44.16	15.6
Control	52.33	
C.D.	2.891	
SE(m) ±	0.973	
C.V.	3.661	

* Average of three replications.

Effect of phyllosphere fungi on the mycelial growth of *Exserohilum turcicum* under *in vitro* condition

Isolates of phyllosphere Fungal antagonists	Linear mycelial* growth (mm)	% inhibition of test pathogen over control
Fusarium sp. (1)	43.76	20.3
Fusarium sp. (2)	30.80	42.9
Aspergillus niger	29.56	60.6
Aspergillus terreus	21.23	45.25
Aspergillus nidulans	21.59	60.0
Aspergillus ochraceus	26.20	51.4
Control	54.0	-
C.D.	3.509	
SE(m) ±	1.146	
C.V.	6.115	

Acknowledgement

I acknowledge Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTSAU for providing necessary facilities to conduct the research work

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2.3 Biological Control of Sheath blight (*Rhizoctonia solani* Kuhn) of Rice by PGPR

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Introduction

Sheath blight caused by Rhizoctonia solani Kuhn is one of the destructive diseases after blast across the rice growing regions of the world. Resent management strategies for sheath blight of rice include application of chemical fungicides like hexaconazole. Concerns on the use of chemical fungicides such as ground water pollution, escalated costs and breakdown of host plant resistance can be overcome by application of plant growth promoting rhizobacteria (PGPR) . Fluorescent pseudomonads representing group of PGPR can promote growth and suppress plant pathogens by multiple mechanisms.

Several strains of *Pseudomonas fluorescens* and *Bacillus* have been successfully used for the biological control of rice sheath blight. In view of the importance of the crop and disease management, the present work was planned with the following objectives.

1. To isolate bacterial microflora from the phylloplane and rhizoplane of rice
2. To screen the bacterial isolates against Rhizoctonia solani under in vitro conditions
3. To screen potential antagonists for their growth promoting activities under laboratory and green house conditions 4. To evaluate potential antagonists against *Rhizoctonia solani* under green house conditions

Material and methods

Morphological characterization : All the isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and Gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewer (1950).

Biochemical characterization: The isolates *in vitro* were characterized biochemically employing IMVIC tests, oxidase test, catalase test, H_2S production, starch hydrolysis, gelatin liquefaction and ammonia production as per the procedure described by Aneja (2001).

Screening of bacterial isolates against Rhizoctonia solani under *in vitro* condition

The antagonistic properties of fluorescent *Pseudomonas* and *Bacillus* spp. were tested against *R. solani* on potato dextrose agar plates by dual culture technique and per cent inhibition was calculated as per the following formula (Dennis and Webster, 1971).

$$I = (C – T) \div C \times 100$$

Where, I = Per cent inhibition over control
C = Radial growth of pathogen in control (mm)
T = Radial growth of pathogen in treatment (mm)

Effect of culture filtrates of antagonistic bacteria on *Rhizoctonia solani*

The antagonistic effect of bacterial filtrates on mycelial growth of R. solani was assessed by agar-well method (Kumar *et al.*, 2012).

Screening of potential antagonists for their growth promoting activities under laboratory and green house conditions

Influence of potential antagonists on seedling vigour index of rice: Rice seeds (cv. Swarna) were surface sterilized with 1% sodium hypochlorite solution for 2 min and used in seed germination assay.

Vigour index = Germination (%) x Seedling length (shoot length + root length)

Evaluation of potential antagonists against Rhizoctonia solani under green house conditions

The efficacy of bacterial antagonists (*Pseudomonas* spp. and *Bacillus* spp.) found effective under in vitro conditions were further evaluated against rice sheath blight under green house conditions.Sheath blight disease severity data was calculated at 20 days after inoculation in terms of relative lesion height (RLH) using the following formula (Sharma *et al.*, 1990)

RLH (%) = (Total lesion height ÷ Total plant height) × 100

Results and Discussion

Twenty-two bacterial cultures and six fungal cultures were isolated from the phyllosphere and designated for bacteria as P1 to P22 and phyllosphere fungi as F1 to F6 respectively.

All the isolates produced ammonia and strong production was seen in the isolates PF2, PF12 and PF14. All the 15 *Bacillus* isolates were screened for plant growth promoting attributes IAA production. All the Bacillus isolates excluding B2, B6 and B9 produced siderophores and strong production was found with the PF2, PF14 and B12 isolates. HCN production was observed in all isolates of *Pseudomonas* and *Bacillus* and strong production was seen in PF2, B7 and B11 isolates.

In dual culture technique, highest per cent inhibition was found with isolate PF2 (54.50%) followed by isolate PF11 (49.54%). The least per cent inhibition was shown by the isolate PF8 (29.5%). Among *Bacillus* isolates, maximum per cent inhibition of 52.20% was shown by isolate B7 followed by isolate B10 (48.50%) and isolate B4 (44.25%). The least per cent inhibition of 30.20% was exhibited by the isolate B9. Few isolates of *P. fluorescens* and some *Bacillus* spp. produced HCN, siderophores which were responsible for their antagonistic activity against R. solani. In agar-well method, PF2 isolate of *Pseudomonas fluorescens* (52.50%) exhibited highest per cent inhibition followed by isolates PF11 (48.60%), PF9 (43.80%), PF1 (37.80%) and least was found with isolate PF8 (29.50%). Among *Bacillus* isolates, per cent inhibition was significantly highest for isolate B7 (50.20%) followed by isolates B10 (48.30%), B4 (42.30%), B6 (39.50%) and least was recorded in isolate B9 (29.60%).

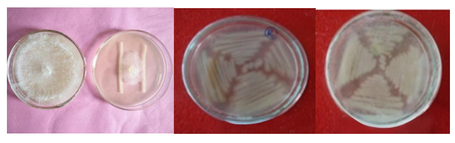
Conclusion

Under pot culture studies, the seedling vigour and germination per cent significantly increased on seed treatment with all the fifteen isolates of *P. fluorescens.* Significantly highest seedling vigour and germination per centage (3200.00 and 96.00% respectively) obtained through seed treatment with PF2 isolate. The seedling vigour and germination per cent significantly increased on seed treatment with all the fifteen isolates of Bacillus spp. Significantly highest seedling vigour and germination per centage (3052.40 and 94.67 respectively) obtained through seed treatment with B7 isolate.

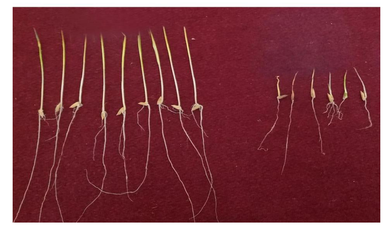
***P. fluorescens* isolate PF2 showed the maximum shoot length (38.20 cm), root weight (1.80 g) and shoot weight (3.70 g). Root length was found non-significant among the different *P. fluorescens* isolates tested. *Bacillus* spp. Isolate B7 shows the maximum shoot length (37.70 cm), root weight (1.70 g) and shoot weight (3.40 g). Root length was found non-significant among the different *Bacillus* isolates tested.**

Under greenhouse conditions, all the nine treatments significantly reduced the sheath blight severity. Significantly highest disease control of 51.28 per cent was obtained with the treatment, T8 (Foliar spray with *Pseudomonas* spp. 24 hours before pathogen inoculation + Foliar spray with Bacillus spp. 10 days after pathogen inoculation).

Isolate no.	Isolate name	Siderophore production	IAA production µg/ml	Ammonia production	HCN production
1	PF1	+	30.60	++	++
2	PF2	+++	50.20	++	++
3	PF3	++	35.60	+	+
4	PF4	++	31.70	++	++
5	PF5	+	30.40	+	+
6	PF6	++	40.20	++	++
7	PF7	+	36.40	+	+
8	PF8	-	38.80	+	+
9	PF9	++	40.00	++	++
10	PF10	++	35.10	+	+
11	PF11	-	33.20	++	+
12	PF12	+++	34.90	+	+
13	PF13	++	39.80	+	+
14	PF14	+++	42.80	++	++
15	PF15	++	25.00	+	+
	S.E(m) ±		0.77		
	CD(P=0.05)		2.20		



Dual culture technique



Effect of seed treatment with *P. fluorescens* (PF2) on growth of rice seedlings under green house conditions

S.No.	Isolate name	Root length (Cm)	Shoot length (Cm)	Root weight (g)	Shoot weight (g)
1	PF1	12.20	35.80	1.50	3.00
2	PF2	14.00	38.20	1.80	3.70
3	PF3	10.70	31.20	1.00	2.60
4	PF4	11.00	32.50	1.30	2.80
5	PF5	10.40	30.20	0.90	2.50
6	PF6	12.80	35.00	1.50	3.20
7	PF7	10.90	32.40	1.20	2.70
8	PF8	10.20	30.50	0.80	2.40
9	PF9	13.00	36.90	1.60	3.20
10	PF10	12.10	34.70	1.40	2.90
11	PF11	13.20	37.60	1.70	3.30
12	PF12	11.20	33.00	1.30	2.90
13	PF13	10.50	31.70	0.90	2.90
14	PF14	11.90	35.40	1.40	3.00
15	PF15	10.60	31.80	1.20	2.70
16	Control	8.80	25.60	0.50	1.90
	S.E(m) ±	0.29	0.35	0.04	0.06
	CD (P=0.05)	N.S	1.02	0.13	0.20

Effect of seed treatment with isolates of *P. fluorescens* on growth parameters of rice seedlings in pot culture studies

Treatments	Relative Lesion Height	Per cent decrease over control
T_1	25.86 (30.40)*	18.08
T_2	30.90 (33.37)	2.20
T_3	22.03 (27.90)	30.21
T_4	20.68 (26.51)	34.52
T_5	23.33 (28.63)	26.10
T_6	16.12 (23.76)	48.93
T_7	20.00 (26.71)	36.64
T_8	15.38 (22.74)	51.28
T_9	31.57 (33.53)	-
S.E(m) ±	1.45	
CD (P=0.05)	4.36	

Effect of foliar spray with bioagents on sheath blight severity under greenhouse conditions

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ANTAGONISTIC PERFORMANCE OF NATIVE STRAINS AND COMMERCIAL FORMULATIONS OF BIO CONTROL AGENTS AGAINST *Rhizoctonia bataticola* (Taub.) Butler, THE CAUSAL AGENT OF DRY ROOT ROT OF GROUNDNUT

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INTRODUCTION AND OBJECTIVE OF THE STUDY:

Dry root rot caused by Rhizoctonia bataticola (Taub.) Butler is responsible for economic losses to an extent of 80% (Rani *et al.*, 2009) in groundnut where dry conditions prevail during the rain fed season. Therefore an attempt was made to identify the best bio control agent for the management of *R. bataticola*, by evaluating native strains of bacterial and fungal bio agents and commercial formulations of *Trichoderma harzianum* and *Pseudomonas flourescens.*

MATERIAL AND METHODS:

Native bacterial (B1 to B9) and fungal (Tr1 to Tr7) bio agents were isolated from the rhizosphere of healthy groundnut plants following serial dilution technique. Native bio agent isolates and commercial formulations of *P. flourescens* (BCF) and *T. harzianum* (TrCF1 and TrCF2) were evaluated *in vitro* for their antagonistic potential against *R. bataticola* by dual culture technique. Potential bio agent was used as seed treatment @ 5g kg^{-1} seed under field conditions.

RESULTS:

Among the bacterial bio agents, isolate BCF (commercial formulation of *P. flourescens*, UAS, Dharwad) showed the highest per cent inhibition (29.00%) followed by B2 (23.33%) and B4 recorded the least (5.77%) while B8 recorded no inhibition of the mycelial growth of *R. bataticola*. Among fungal bio agents,TrCF2 (commercial formulation of *T. harzianum* from IIOR, Hyderabad) recorded the highest inhibition (64.89%) followed by Tr1 (62.22%) while Tr2 (52.78%) showed the least inhibition. The isolate TrCF2 was used a seed treatment under field conditions @5g $^{-1}$ seed.

CONCLUSIONS:

When compared together, the commercial formulations of *P. flourescens* and *T. harzianum* were found to be more effective than the native isolates in inhibiting the pathogen growth which also resulted in significantly higher yield(2031.11 kg ha^{-1}) in field studies when compared to the non treated control (1175.55 $kgha^{-1}$). These results are in agreement with Veenashri *et al.* (2019) who reported that commercial formulation of *T. harzianum* recorded an inhibition of 78.67 per cent against *R. bataticola.* Similarly, Choudhary and Ashraf (2019); Iqbal and Mukhtar (2020) also reported the efficacy of *T. harzianum* in controlling the mycelial growth of *R. bataticola*.

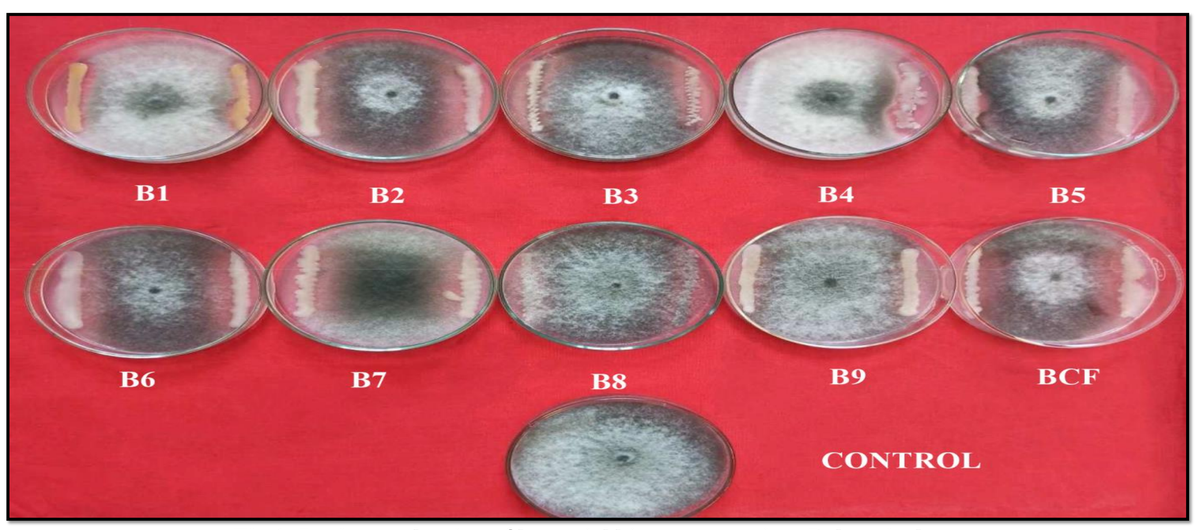

Fig 1. *In vitro* evaluation of bacterial bio agents against *R. bataticola*

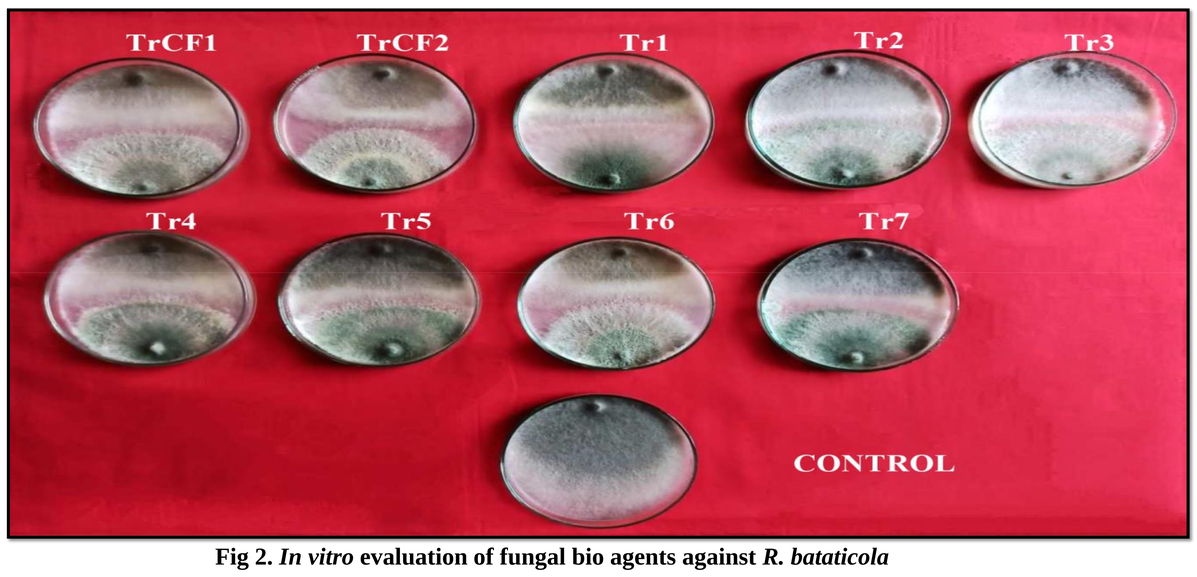

Fig 2. *In vitro* evaluation of fungal bio agents against *R. bataticola*

Table 1. Evaluation of bacterial bio agents against *R. bataticola*

S No.	Bacterial isolate	Mycelial growth (cm)	Per cent inhibition
1	B1	7.33	18.56 (25.50)^{d}
2	B2	6.90	23.33 (28.74)^{b}
3	B3	7.64	15.11 (22.80)^{e}
4	**B4**	**8.48**	**5.77 (13.78)^{g}**
5	B5	7.73	14.11 (22.02)^{e}
6	B6	7.17	20.33 (26.75)^{c}
7	B7	7.36	18.22 (25.20)^{d}
8	**B8**	**9.00**	**0.00 (0.00)^{h}**
9	B9	8.00	11.00 (19.36)^{f}
10	**BCF**	**6.39**	**29.00 (32.00)^{a}**
11	Control	9.00	0.00 (0.00)^{h}
C.D (P=0.05)		0.11	1.14
C.V		0.90	4.83

Table 2. Evaluation of fungal bio agents against *R.bataticola*

S No.	*Trichoderma* isolate	Mycelial growth (cm)	Per cent inhibition
1	TrCF1	3.43	61.90 (51.83)^{bc}
2	**TrCF2**	**3.16**	**64.89 (53.60)^{a}**
3	Tr1	3.40	62.22 (52.05)^{b}
4	**Tr2**	**4.25**	**52.78 (46.55)^{d}**
5	Tr3	4.11	54.33 (47.29)^{d}
6	Tr4	4.16	53.78 (47.10)^{d}
7	Tr5	4.17	53.67 (47.06)^{d}
8	Tr6	4.13	54.11 (47.31)^{d}
9	Tr7	3.56	60.44 (50.98)^{c}
10	Control	9.00	0.00 (0.00)^{e}
C.D(P=0.05)		0.14	1.50
C.V		2.18	1.74

*Figures in parentheses are angular transformed values; values with same alphabet do not differ significantly.

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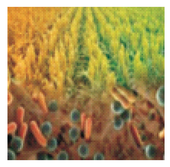


Effect of Pectinase Enzyme on Plant Growth Promotion

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Abstract

Plant growth promoting rhizobacteria (PGPR) are valuable bacteria commercialized as biofertilizer and biopesticides. Any technology that deals with natural environment is always turning to an innovative way and PGPR isolates are categorized as an eco-friendly microbial tool. PGPR inoculants are emerged as a novel technique that reduces the excessive use of chemical fertilizers and pesticide. The industrial requirement for PGPR is progressively increased, as it propounds attractive way to maintain a sustainable agriculture system. The successful commercialization of PGPR strains depend on the association between the scientific organizations and industries. A number of plant growth promoting rhizobacterial isolates has been reported that enhance plant growth either directly or indirectly by inhibiting the growth of plant pathogen. Production of phytohormones (IAA), solubilizing phosphate and nitrogen fixation are some of the mechanism that directly involve in plant growth and promotion. Indirect mechanism for plant growth promotion involves production of antifungal substances, HCN production, production of siderophores and cell wall degrading enzymes.

Introduction

Various types of fungal species have been reported to be employed for production of pectinase. As extracellular pectinase are easier to harvest and thus the scale up is cheaper and simpler. Thus, the waste was used to generate extracellular fungal pectinase from natural sources. Pectinase production by newly isolated fungus strain carried out in submerged fermentation. ***Aspergillus flavus*** produced polygalacturonase (PG) and pectin lyase (Pl) on mixture of **orange peel, banana peel and papaya peel.** These substrate were used as sole source of carbon. Pectinase enzyme which are used in several industries, especially in the clarification process during wine and fruit juice industries. **Pectinase enzyme role in plant Growth promotion or inhibition**. Because pectin is present in middle lamella of plant cell wall. If pectinase producing fungus (***Aspergillus flavus)*** is present in plant root so it is breakdown the middle lamella of plant cell wall and give the chance for PGPR bacteria or fungus. And these PGPR bacteria and fungus are beneficial for the plant growth. If pathogenic fungus or pathogenic bacteria are present in root of the plant, so it is enter to the plant cell wall and inhibit the plant growth.

Mechanism of action

PGPR has a significant impact on plant growth and development in both indirect and direct ways. The direct promotion of plant growth by PGPR generally entails providing the plant with compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment. (Glick, 1995; Glick *et al.*¸1999).

Method and Materials

- An investigation was carried out at art's, science and commerce college Kholwad, Kamrej, Surat; to know Role of pectinase Enzyme in plant growth promotion or inhibition.
- Take a sterile plastic cup.
- Add rhizosphere soil was added in to the cup.
- Than add sterile wheat seeds.
- Fungal culture was added next day.
- The experiments consists of 25 treatment combination of different fungus (pectinase producing fungus+ plant pathogenic fungus P1 and P2).
- Culture was uniformly added at five centimeters depth after putting a thin layer of soil in Inoculum.
- After than 3-5 days measured plant height and note down the result.

Results and Discussion

When we use pectinase enzyme, the growth of plant increase and the observation table is given below.

No	After 3 days	After 4 days (culture inoculation)	After 5- 6 day	After 7-8 day	After 9 - 10 day
1	Sprout	Blank	10 cm	15 cm	22 cm
2	Sprout	Enzyme	13 cm	19 cm	25 cm
3	Sprout	Aspergillus flavus	9 cm	13 cm	18 cm
4	Sprout	Aspergillus niger	8 cm	9 cm	12 cm
5	Sprout	Pathogenic fungus 1	8 cm	12 cm	17 cm
6	Sprout	Pathogenic fungus 2	12 cm	15 cm	22 cm
8	Sprout	Aspergillus flavus + Pathogenic fungus 1	10 cm	13 cm	15 cm
9	Sprout	Aspergillus flavus + Pathogenic fungus 2	7.5 cm	10 cm	18 cm
10	Sprout	Aspergillus niger + Pathogenic fungus 1	5.5 cm	8 cm	10 cm
11	Sprout	Aspergillus niger + Pathogenic fungus 2	6.5 cm	8 cm	9 cm
12	Sprout	Aspergillus flavus + Aspergillus niger + Pathogenic fungus 1 + Pathogenic fungus 2	9 cm	15 cm	16 cm
13	Sprout	Enzyme + Aspergillus flavus	8 cm	14 cm	17 cm
14	Sprout	Enzyme + Aspergillus niger	6 cm	6.5 cm	7 cm
15	Sprout	Enzyme + Pathogenic fungus 1	10 cm	18 cm	22 cm
16	Sprout	Enzyme + Pathogenic fungus 2	8 cm	16 cm	21 cm
17	Sprout	Enzyme + Aspergillus flavus + Aspergillus niger	8 cm	12 cm	15 cm
18	Sprout	Enzyme + Pathogenic fungus 1 + Pathogenic fungus 2	7 cm	10 cm	15 cm
19	Sprout	Enzyme + Aspergillus flavus + Pathogenic fungus 1 + Pathogenic fungus 2	9 cm	14.5 cm	18 cm
20	Sprout	Enzyme + Aspergillus niger + Pathogenic fungus 1 + Pathogenic fungus 2	10 cm	16 cm	20 cm
21	Sprout	Enzyme + Aspergillus flavus + Aspergillus niger + Pathogenic fungus 1 + Pathogenic fungus 2	9 cm	12 cm	20 cm

Conclusion

As our understanding of the complex environment of the rhizosphere, of the mechanisms of action of PGPR The success of these products will depend on our ability to manage rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Rhizosphere management will require consideration of soil and crop cultural practices as well as inoculant formulation and delivery. The use of multi-strain inoculate of PGPR with known functions is of interest as these formulations may increase consistency in the field. They offer the potential to address multiple modes of action, multiple pathogens, and temporal or spatial variability . PGPR offer an environmentally sustainable approach to increase crop production and health. The application of molecular tools is enhancing our ability to understand and manage the rhizosphere and will lead to new products with improved effectiveness.

ACKNOWLEDGMENT

Primarily I would thank God for being able to complete this e-poster with success. Then I would like to thank my microbiology ma'am Dr. Arti Raval, whose valuable guidance has been the once that helped me patch this poster & make it full proof success her suggestion and her instruction has served as the major contributor toward the completion of the poster.

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Screening for Siderophorogenesis of *Trichoderma asperellum* isolates on P-CAS medium and characterization of siderophores

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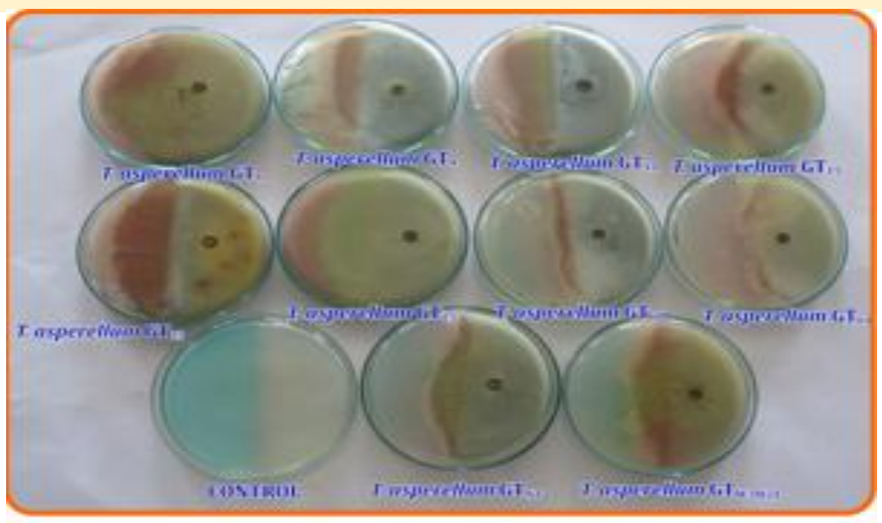
INTRODUCTION: Siderophores are low molecular weight ferric ion specific chelating agents inhibit the growth of phytopathogens by inhibiting the synthesis of nucleic acids and sporulation of the pathogens. In the present investigation on eco-friendly management of stem rot of groundnut pathogen *Sclerotium rolfsii* Sacc ,ten elite *Trichoderma asperellum* isolates isolated from rhizospheric soils of groundnut were screened for siderophorogenesis

METHODS: Ten elite isolates of *T.asperellum*(GT_1,GT_4,GT_{11},GT_{15},GT_{23}.,GT_{25}, GT_{60}, GT_{61},GT_{NT} and $GT_{W38(2)}$ were screened for siderophorogenesis on P-CAS (Potato Dextrose Agar with modified Chrome Azurol S medium)as per Schywn and Neilands(1987).Grimm Allen medium was used for characterization of siderophores biochemically into hydroxamates or catecholates or carboxylataes(Raval and Desai,2015).

RESULTS AND DISCUSSION



Siderophorogeesis y elite *Trichoderma asperellum* isolates of PDA amended modified Chrome /azurol S (Ventral view)

Siderophorogeesis y elite *Trichoderma asperellum* isolates of PDA amended modified Chrome /azurol S (Dorsal view)

Detection of hydroxamate type siderophore

Detection of carboxylate type siderophore

Detection of catecholate type siderophore

1. *T. asperellum* GT_1
2. *T. asperellum* GT_4
3. *T. asperellum* GT_{11}
4. *T. asperellum* GT_{15}
5. *T. asperellum* GT_{23}
6. *T. asperellum* GT_{25}
7. *T. asperellum* GT_{60}
8. *T. asperellum* GT_{61}
9. *T. asperellum* GT_{NT}
10. *T. asperellum* $GT_{W38(2)}$

CONCLUSION:

Changes in the colour from blue to yellow,orange(light or dark),light red indicated siderophorogenesis by *T.asperellum* isolates but no isolate has formed purple colour indicating absence of catecholate.

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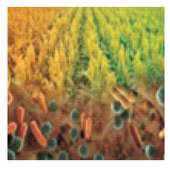


Effect of biocontrol agent(*Pseudomonas fluorescens*) and fungicide on microbial count and enzymatic activities of Rhizospheric soil in soybean cropping system

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Reg.No.– 2.9

Introduction

Fungal infection in soybean crop pose a biotic stress on crop, which disturb the microbial activity that adversely affect the crop yield. To circumvent these biotic stress different type of fungicides are used . Overuse of the chemical fungicide are resulting in the environmental damage, health problems, soil sustainability. Moreover scientist have reported that systemic fungicide carbendazim used in soybean cropping system at its recommended dose(2g/kg of seed) reduce the microbial activity and count. Looking to the negative impact of chemical fungicide the use of biocontrol is becoming a novel alternative. Micro organisms showing efficient bio control and PGPR attributes could be implemented in the agricultural field. This multifarious characteristics will have dual benefit of controlling the biotic stress as well as growth, yield enhancement. This dual approach will aid a value benefit , if the organism is compatible with the applied fungicide. Soil enzymes are the important tools to assess the soil health. Different Microbial enzymes are found in the soil and they release the nutrients in soil and make them available to the plants. The most common type of enzymes present on the soil are represented in fig.o1. In present research, we studied the effect of seed dressing with various combination of *Pseudomonas* and carbendazim on microbial count and enzyme activity in the rhizospheric soil infested with *Rhizoctonia solani*. @ 1 gm/kg of soil

Treatment	Treatment details
T1	Control
T2	*P. fluorescens* @ 10gm/kg of seed
T3	*P. fluorescens* +carbendazim 0.5gm/kg of seed
T4	*P. fluorescens* +carbendazim@1 gm/kg of seed
T5	*P. fluorescens*+ carbendazim@1.5gm/kg of seed
T6	*P. fluorescens*+ carbendazim @ 2gm/kg of seed

Objective :

The present research was accomplished for assessing the soil quality influenced by the ecofriendly integrated disease management approach.

Materials and method

In this experiment we performed the pot analysis with the various treatments (table01) After sowing of the soybean the rhizosphric soil from the different treatments was collected after 60 days .The microbial count and soil enzyme activities was taken as per below given protocol.

Microbial Count

- Prepare serial dilution of soil with normal saline
- Add 0.1 ml of suspension from 10^{-8} dilution and incubate the plate at room temperature.
- After incubation count the number of CFU and calculate microbial count in the soil

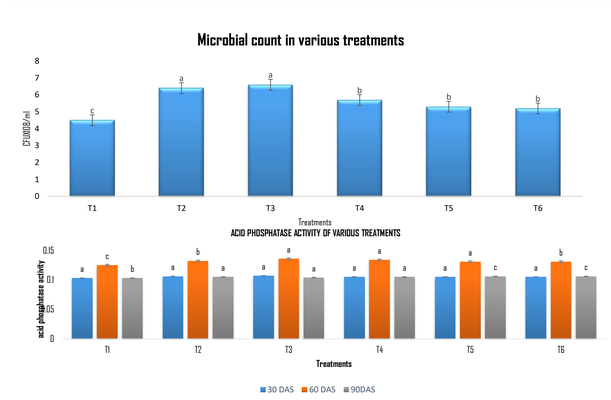
Dehydrogenase activity

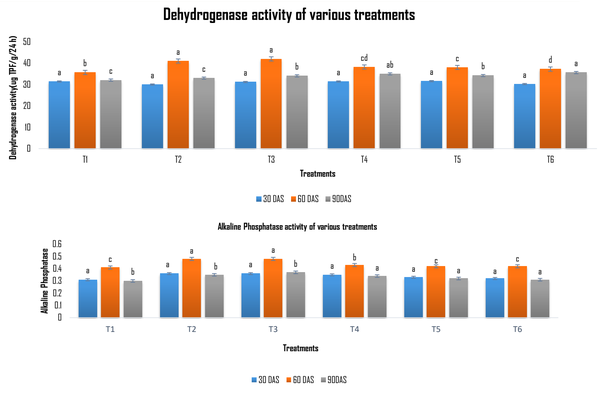
- Add 5 ml of TTC in 5gm of the soil
- Keep the test tube at room temperature for 24 hours
- Take the absorbance at 485 nm and calculate the treatments with the std. TPF graph.

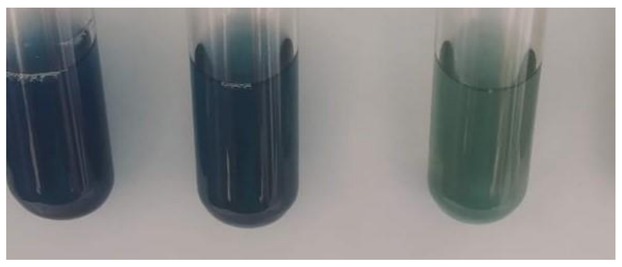
Phosphatase activity

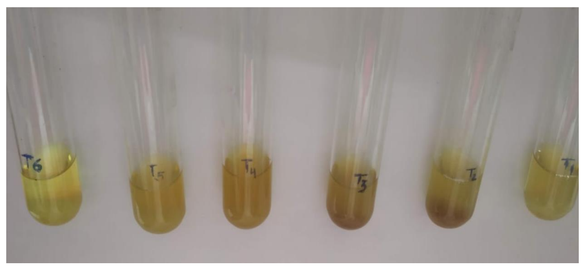
- Take 2 gm of soil sample
- Add 5 ml of 0.5 M CaCl2 ,1 ml of P-nitro phenyl , 1ml of phosphate buffer
- Adjust pH at 3.3 for ACP and 8.5 for ALP
- Take the absorbance at 440 nm and calculate the amount of nitro phenol against the standard graph.

Result and Discussion









Minimum bacterial count(4.5× 10^8CFU gm^{-1})recorded in control. Treatments T2, T3 maximum bacterial population where(6.6× 10^8CFU gm^{-1}) of microorganisms and T5, T6 showed(5.3× 10^8CFU gm^{-1}) bacterial count. (fig2) Similar trend was observed in dehydrogenase and phosphatase enzyme activity. Dehydrogenase and phosphatase activities was observed and color developed (fig6,7) is observed and amount of nitro phenol and triphenyl formazan so formed is observed against the standard graph. The treatment (T5, T6) has shown 6%, 5%, 6.6% increase in Acid Phosphatase, Alkaline Phosphatase, and Dehydrogenase activity respectively as compare to control.(Fig3,4,5) The treatment (T2, T3, and T4) has shown9%, 4.9%, 15.5% increase in Acid, Alkaline Phosphatase and Dehydrogenase activity respectively as compare to control. This enhanced microbial activity in T2, T3 has improved the nutrient supply to the plant, which has improved the growth and yield attributes of cropping soybean.

Conclusion

Integrated disease management is an environmental friendly approach for management of biotic stress. In this experiment we studied that 0.5 gm/kg of seed i.e. 25% of recommended dose (2gm/kg of seed) is best suited treatment tor combating the fungal infection as well as showing significant enhancement in the yield attributes, as compare to control and the treatment T6 containing 2gm/kg of seed

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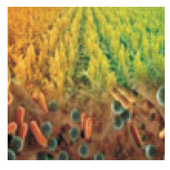


Theme II PGPR and other microbes that confer disease control Registration No 2.10

Antagonistic potential of indigenous rhizobacteria against foliar blight pathogens of wheat *Bipolaris sorokiniana* and *Alternaria triticina*"

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INTRODUCTION

➢The foliar blight is a disease complex includes spot blotch, tan spot and leaf blight caused by *Bipolaris sorokiniana*; *Pyrenophora tritici-repentis* and *Alternaria triticina* respectively.

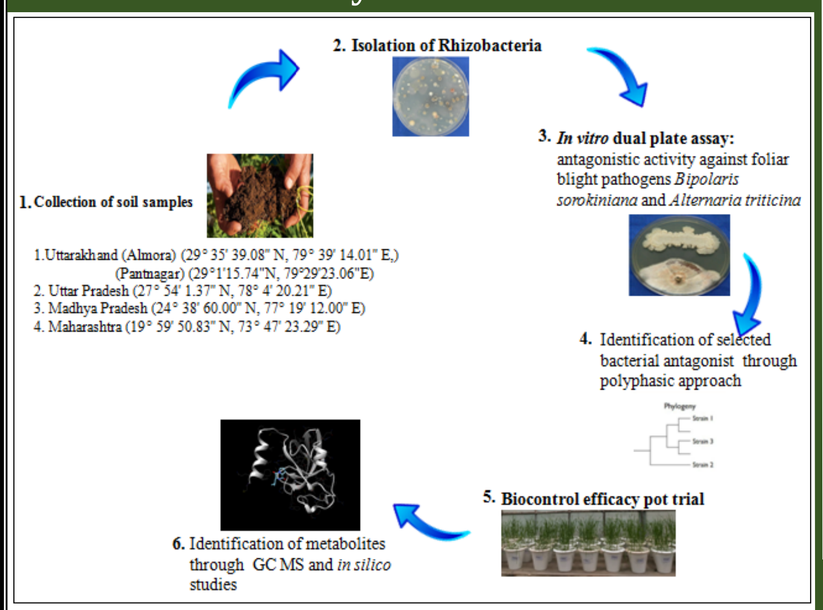
➢In India two diseases spot blotch and leaf blight are prevalent. These two pathogens are reported either individually or in co-occurrence. The disease complex can lead to 15–20% yield loss which at time of heavy infestation increases to 80% **(Kumar *et al.*, 2015)**.

➢ The chemical control is the only effective method to minimize the loss till date. Due to its high cost and detrimental effects on human and soil health researchers are prompted to screen and develop the BCAs for the environmental sustainable disease management **(Sharma *et al.*, 2012).**

Objectives

- Isolation and screening of bacterial antagonists.
- Identification of potential antagonist through polyphasic approach.
- Assessment of biocontrol efficacy through pot trial
- Identification of secondary metabolites produced by bacteria through GCMS and *In silico* studies.

Outline of the study



Methodology

➢Isolation was carried out by the conventional serial dilution method on Nutrient Agar plates. The test fungal pathogens, *B. sorokiniana* (ITCC 4869) and *A. triticina* (ITCC 1186) were used.

➢All rhizobacterial isolates were screened for *in vitro* antagonistic activity by following the method of **(Hazarika *et al.*, 2019)**.

➢Two pot trial was conducted.

Pot trial 1: Effect of Seed bacterization and foliar spray of bacterial strain as booster dose on reduction of foliar blight incidence

Pot trial 2: Effect of foliar spray of bacterial strain after the pathogen infection on reduction of foliar blight incidence

*Both trial were conducted separately for *B. sorokiniana* and *A. triticina.*

➢Leaf samples were drawn at 3rd, 6th and 9th day after infection.

➢They were estimated for following assays

1. Disease incidence percent
2. Protein content
3. Phenol content
4. Antioxidative enzymes: Superoxide dismutase, peroxidase, catalase and phenyl alanine ammonia lyase

➢ Active metabolites were extracted according to the modified method of **Sharma et al., (2016).**

➢GC-MS analysis was carried out at (AIRF), JNU, New Delhi.

Fig 1: Antagonistic activity of bacterial isolates against *B. sorokiniana In vitro*

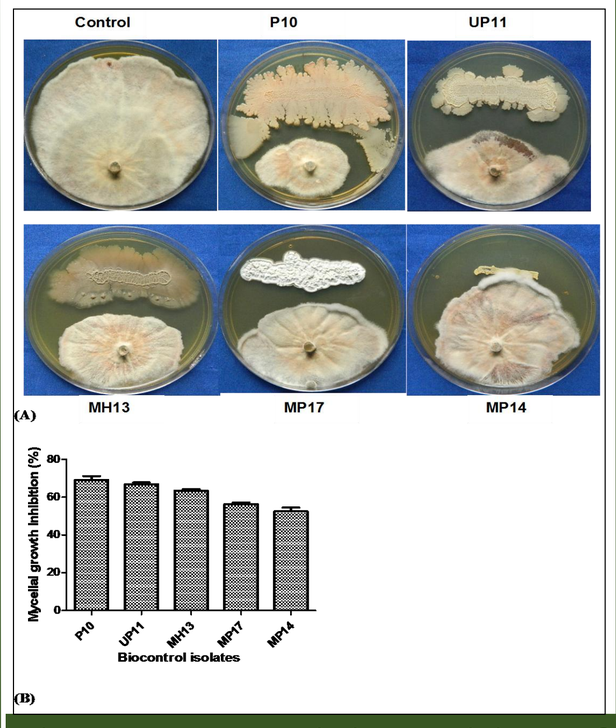


Fig 2: Antagonistic activity of bacterial isolates against *A. triticina In vitro*

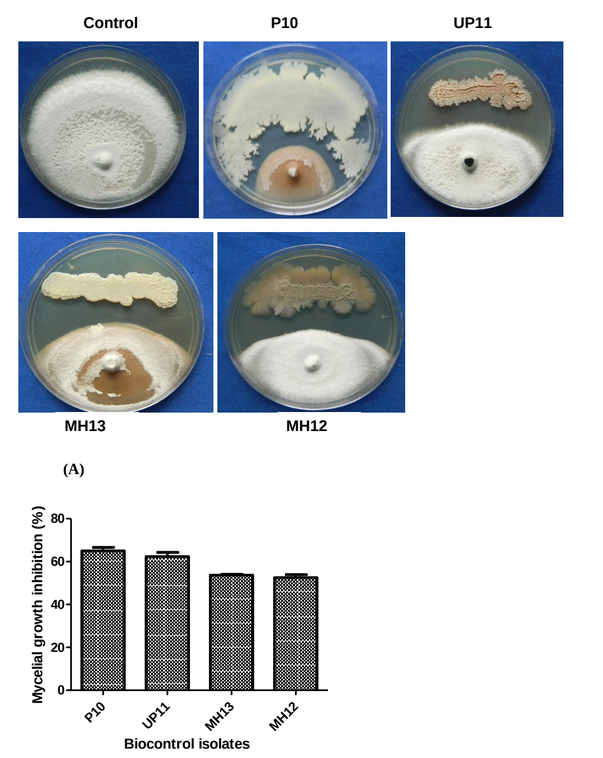


Fig 3: Scanning electron micrograph of *B. sorokiniana* treated with bacterial strain P10, UP11 and MH13

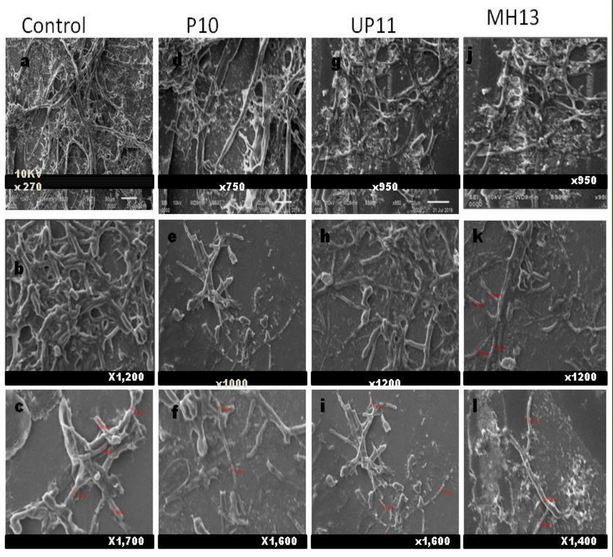
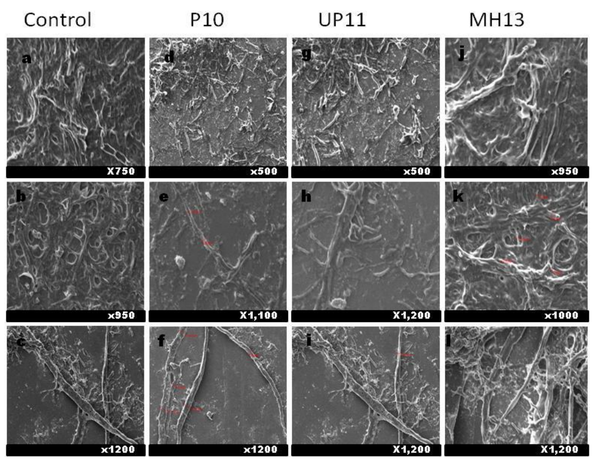


Fig 4: Scanning electron micrograph of *A. triticina* treated with bacterial strain P10, UP11 and MH13



2:Identification of potential bacterial antagonits

Fig. 5: Morphological and cultural characteristics of wheat rhizobacteria

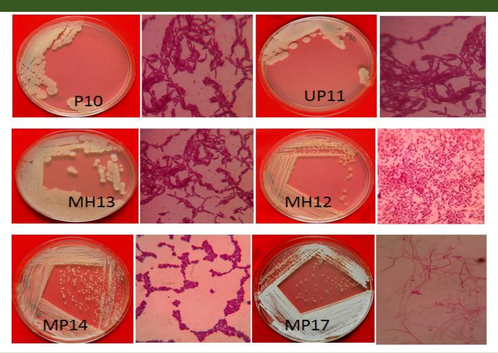
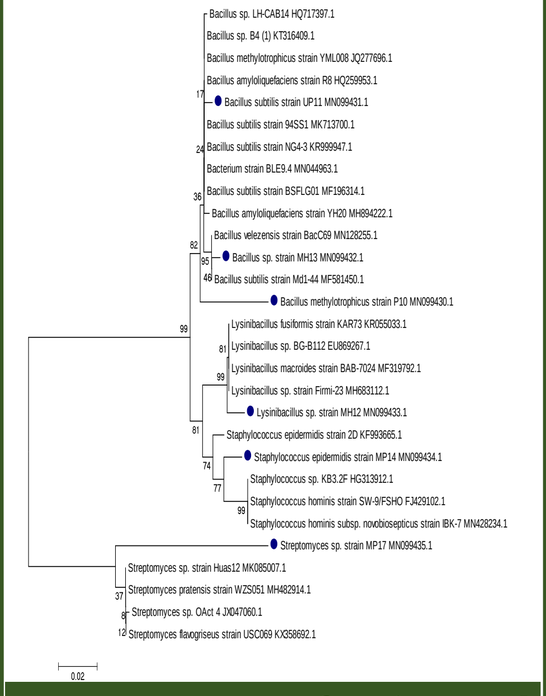


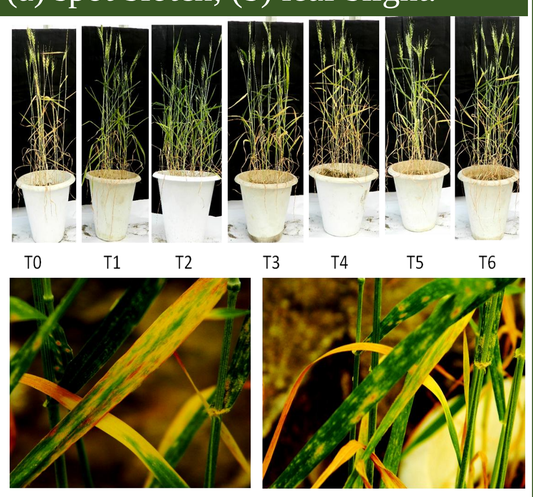
Fig.6 The neighbor joining tree based on 16 S rDNA sequences of bacterial isolates.



3. Assessment of Biocontrol efficacy through pot trial

Fig 7: Pictorial view of pot trial (60 days old wheat plants).

Disease affected wheat leaf sample (a) spot blotch; (b) leaf blight.

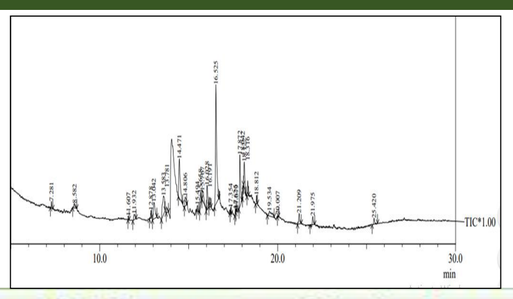


➢Results revealed that trial 1 performed well with respect to trial 2.

➢Treatment with bacterial strains P10 followed by UP11 showed lowest DI% and increased antioxidant enzymes activity.

4. Identification of Secondary metabolites through GC MS and *In silico* studies

Fig 8: *GC-MS* chromatogram of ethyl acetate extract of bacterial strain *Bacillus methylotrophicus P10* and *Bacillus subtilis UP11*



GC MS analysis (Cont..)

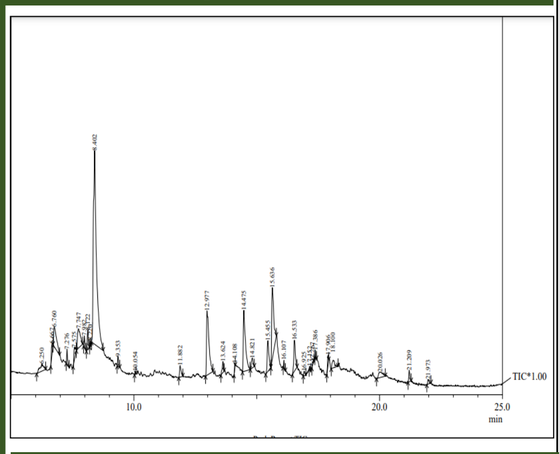
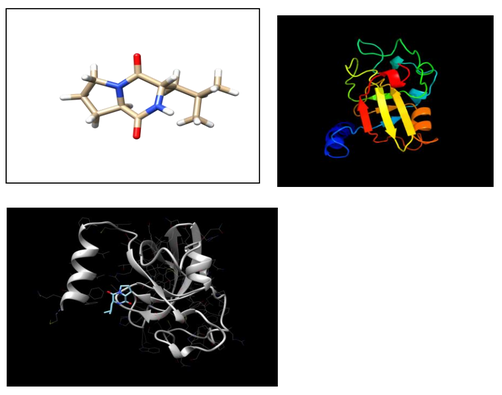


Fig 9: *In silico* study of interaction between isobutylhexahydropyrrolo [1, 2-a] pyrazine-1, 4-dione (ligand) (abundantly produced by both bacterial antagonists) and Ceretoplatinin protein (toxin protein produced by *B. sorokiniana)*



Conclusions

➢ Out of six selected bacterial strains two rhizobacterial strains *Bacillus methylotrophicus* P10 and *B. subtilis* UP11 equally effective to manage the disease against both pathogens. Hence, they have potential to control the foliar blight complex..

➢It was revealed that bacterial antagonist are controlling the disease through Induced systemic resistance.

Future research

Further studies are necessary to test the biocontrol activity under different environmental conditions before these two strains are exploited at commercial scale.

Moreover, research is needed to confirm the ISR mechanism through molecular approach.

References

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Microbial mediated plant protection under stress conditions

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Introduction: Plants are associated with distinct microbial communities, which are key determinant of plant health and productivity and also facilitate plant adaptation under stress conditions. These beneficial microbes elicit physical and chemical changes in plants result in enhanced protection against different biotic and abiotic factors. The use of these microbes in agricultural practices requires precise understanding of plant-microbe interactions and how biotic and abiotic factors influence these interactions. This study is focused on molecular understanding of microbial mediated plant protection under salt stress and pathogen attack.

Aim: Here we aimed to understand how a plant beneficial bacteria (*Sphingobacterium* sp. BHU-AV3) and fungi (*Trichoderma viride* BHU-V2) induce protection and plant growth under salt stress and fungal pathogen (*Sclerotium rolfsii*) attack respectively.

Induced Systemic Tolerance (IST) under Salinity Stress

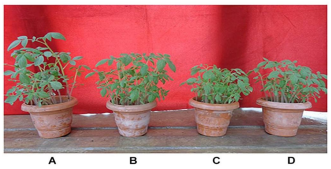
OBJECTIVES

- To evaluate the efficiency of a salt tolerant bacterial strain *Sphingobacterium* sp. BHU-AV3 for growth promotion and salt tolerance in tomato plant.
- To characterize defense related proteins in plants upon bacterial inoculation.
- To investigate histochemical changes in plant tissue under stress condition.

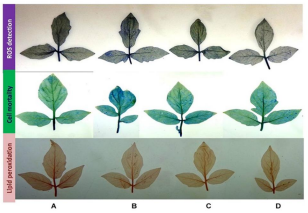
MATERIALS AND METHODS

- Bacterial suspension culture, Pot experiment, 200mM NaCl stress
- Plant growth and measurement
- Protein extraction, isoform expression and MALDI-TOF analysis

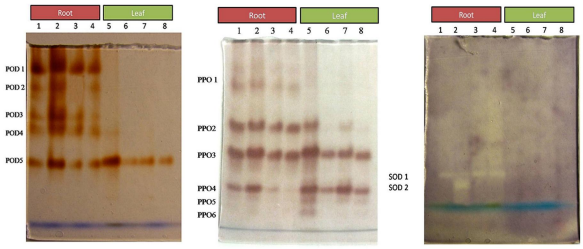
RESULTS



Effect of bacterial (BHU-AV3) inoculation on tomato plant growth under salt stress. **(A)** Control plants; **(B)** bacteria (BHU-AV3)-inoculated plants; **(C)** salt (NaCl) treatment; **(D)** bacterial inoculation + salt.



Histochemical analysis of cell death, ROS production, and lipid peroxidation. ROS detection – blue spots show production of superoxide radicals; cell mortality – light blue spots show cell mortality; lipid peroxidation – red spots show lipid peroxidation.



Zymography of Peroxidase (POD), polyphenol oxidase (PPO) and Superoxide dismutase (SOD) isoforms expressed in root and leaves upon bacterial (BHU-AV3) inoculation under salt stress. Lanes 1 and 5 – salt treatment; Lanes 2 and 6 – bacterial (BHU-AV3) inoculation + salt; Lanes 3 and 7 – bacterial inoculation; Lanes 5 and 8 – Control. POD 1–5 represents number of isoforms expressed.

Differentially expressed proteins in tomato plant roots upon bacterial inoculation (BHU-AV3) under salt stress conditions.

UniProt Accession	Homologous protein	mW (Da)	pI	Molecular function	Biological function
B9TU32	Thiamine biosynthesis protein ThiC variant L1 OS *Solanum lycopersicum* GN thiC PE 3 SV 1	72573	6.0337	ADP-ribose pyrophosphohydrolase activity	Response to vitamin B1
P30264	Catalase isozyme 1 OS *Solanum lycopersicum* GN CAT1 PE 2 SV 1	56470	6.5764	Catalase activity	Hydrogen peroxide catabolic process
Q2MI93	ATP synthase subunit beta chloroplastic OS *Solanum lycopersicum* GN atpB PE 3 SV 1	53433	5.0967	ATP binding	ATP synthesis coupled proton transport
P17786	Elongation factor 1 alpha OS *Solanum lycopersicum* PE 2 SV 1	49256	9.4614	GTPase activity	Translation
P26300	Enolase OS *Solanum lycopersicum* GN PGH1 PE 2 SV 1	47768	5.5746	Phosphopyruvate hydratase activity	Glycolytic process

Induced Systemic Resistance (ISR) under fungal phytopathogen

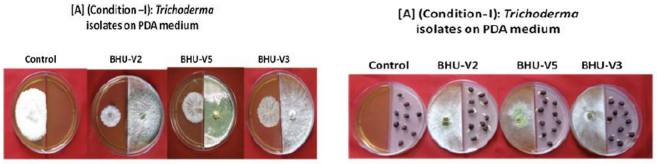
OBJECTIVES

- To evaluate the efficiency of *Trichoderma viride* BHU-V2 producing VOCs for growth inhibition of *Sclerotium rolfsii,* a causative agent of Collor rot.
- To investigate defense mechanism against phytopathogens in Okra by inoculation of BHU-V2.

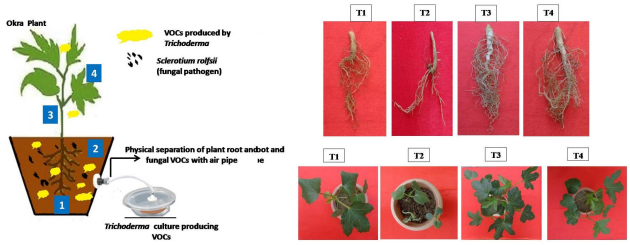
MATERIALS AND METHODS

- *Trichoderma* culture, Fungal pathogen culture, Partite Petri plates
- Belowground experimental setup.
- Analysis of Induced systemic resistance mechanism in plant

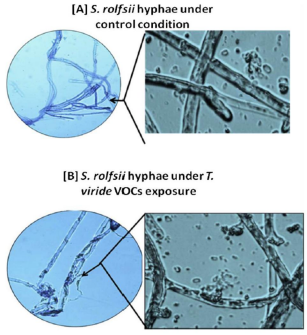
RESULTS



Antifungal and seed germination activity of BHU-V2 in partite petri plate assay.



Effect of VOCs exposure on (A) root morphology and (B) leaf number and area under different treatments. 1) Trichoderma producing VOCs suppress growth of *S. rolfsii* in soil. 2) Okra plant's root perceives VOCs and proliferate more (latera; roots) under stress condition. 3) VOCs induce defense mechanism in okra plant against *S. rolfsii.* 4) VOCs promote expansion of leaf area.



Microscopic analysis of *S. rolfsii* hyphae after exposed under *T. viride* BHU-V2 producing VOCs. (A) Control condition: no exposure of VOCs, shows intact hyphae of *S. rolfsii*; and (B) under VOCs exposure, shows degradation and thinning of *S. rolfsii* hyphae. Photographs were taken on 10µM bar scale.

Conclusion: The IST study results suggested that salt-tolerant PGPRs can boost the potential to decrease the use of agrochemicals on cultivated land and perhaps enhance crop productivity on saline soils around the world. Our ISR study revealed that *T. viride* BHU-V2 can be used as a potent biocontrol agent for soil-inhabiting pathogens and plant growth promoter for sustainable crop production and fungal VOCs could prove as a better alternative to chemical pesticides and fertilizers due to their ecofriendly nature.

Paenibacillus lentimorbus demonstrates biocontrol agents *Sclerotium rolfsii* through autophagy induction

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**Corresponding author:* puneetnbri@gmail.com

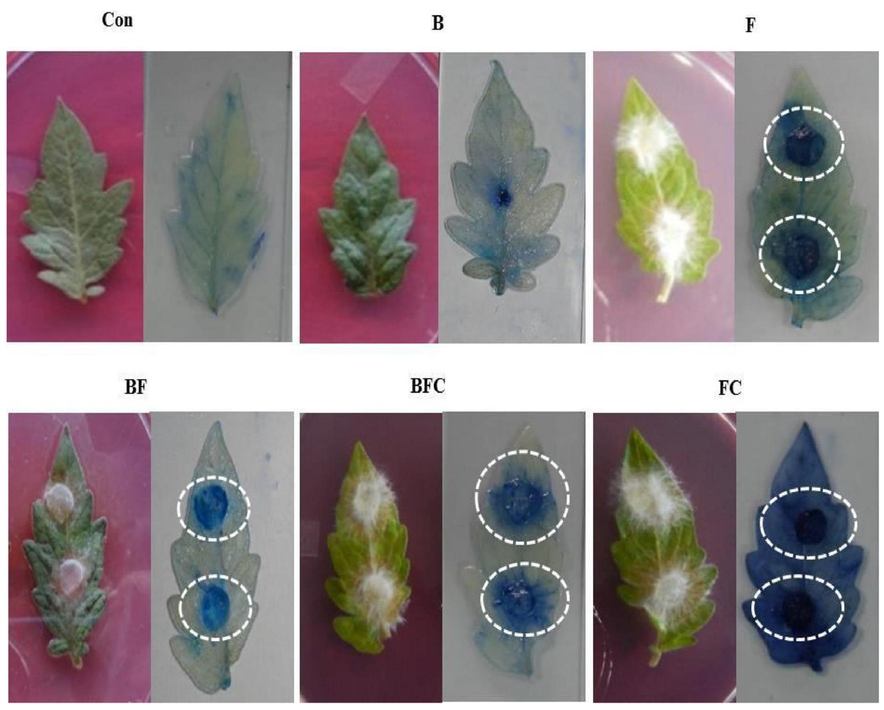
Introduction

❖Plant growth promoting rhizobacteria (PGPR) are known to act as bio-control agents that protect crops against pathogens.
❖PGPR *Paenibacillus lentimorbus* (B-30488) against Sclerotium rolfsii showed previously where several defense-related genes were upregulated with ROS induction in plant.
❖Autophagy is an essential phenomenon for plant disease control and induction of immunity as the infection causes.

Material and Method

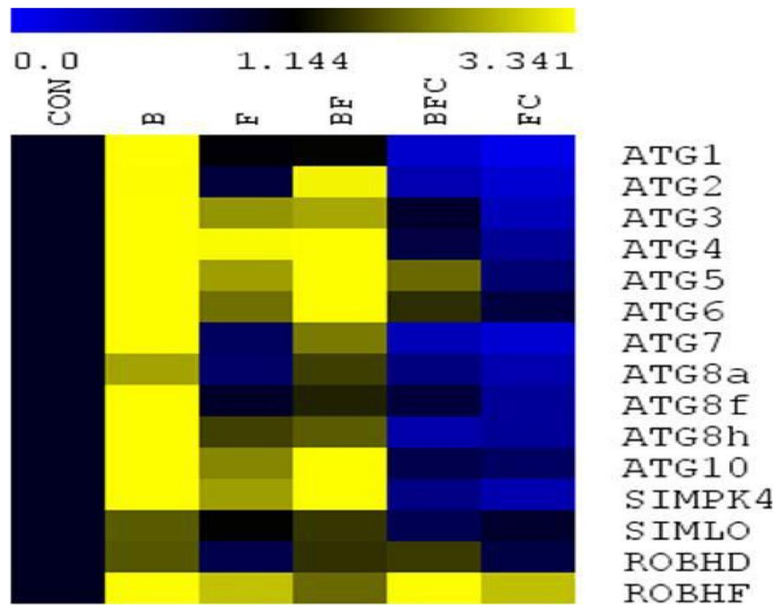
❖Effect of B-30488 on autophagy induction by trypan blue staining are used to measured the growth of *S. rolfsii*.
❖To evaluate the determination of H2O2 accumulation by DAB staining at 72 h post infection in control and treated Leaves.
❖To evaluate the determination of accumulation of autophagosomes using MDC.

Results

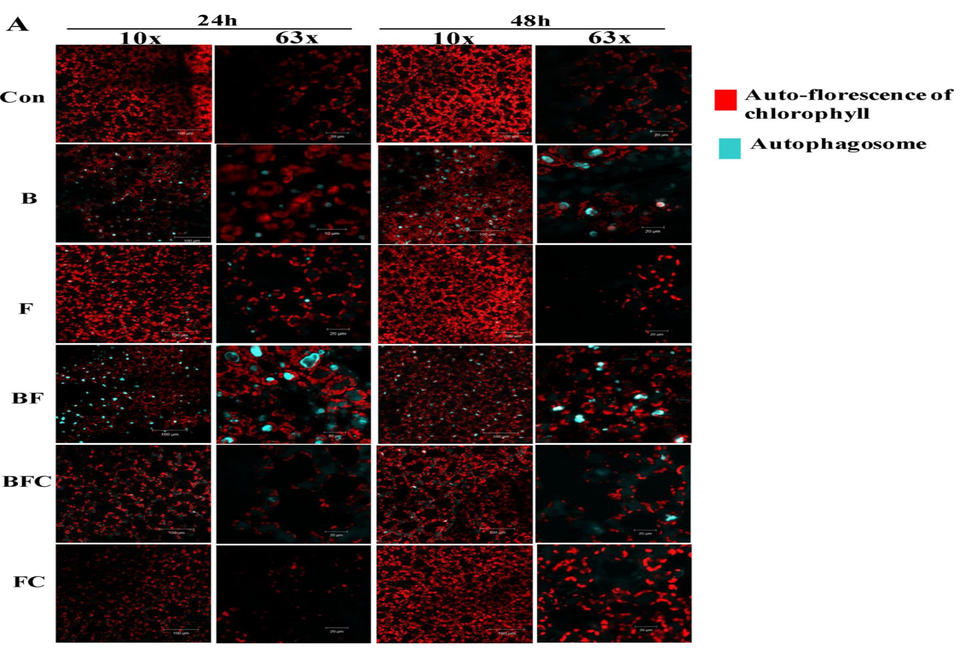


Biocontrol activity of B-30488 against *S. rolfsii*

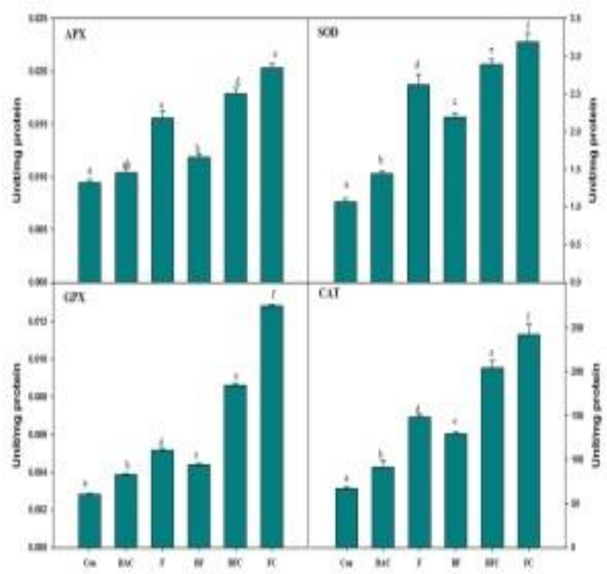
DAB staining. Determination of H2O2 accumulation



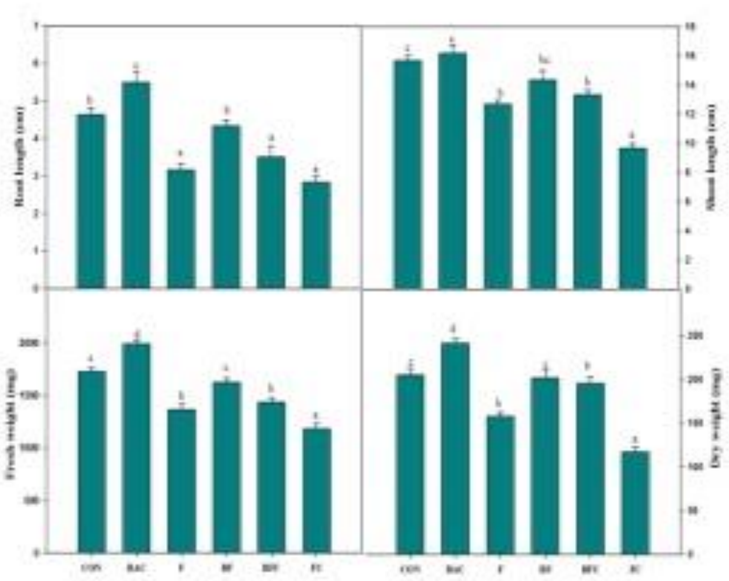
Expression pattern of Autophagy related (ATG) and defense related genes.



Determination of accumulation of autophagosomes using MDC.



APX, GPX, CAT and SOD content was measured in leaves after 72 hours old tomato



Effect of B-30488 inoculations in plant growth under biotic stress conditions

Conclusions

❖MDC staining proved that the strain B-30488 may induce the production of autophagosomes in response to *S. rolfsii*, which play a key role in disease resistance.
❖DAB staining and CAT analysis revealed that B-30488 reduces peroxide buildup in tissue during *S. rolfsii* infection.
❖Due to the biocontrol activity of strain B-30488, there was very minimal localisation of fungal mycelia in plants treated with B-30488 *+ S. rolfsii* revealed by lactophenol cotton blue staining.

6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
(3-4, September 2021)





Autophagy induced by *Paenibacillus lentimorbus* prevents tomato from *Sclerotium rolfsii* infection

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Background

- ❖ **Sclerotium rolfsii is a devastating pathogen of tomato leading to great economic losses in variety of other economically important crops.**
- ❖ **Plants use several defense mechanisms such as production of reactive oxygen species, induction of pathogenesis related proteins and cell death.**
- ❖ **We have previously shown biocontrol activity of PGPR *Paenibacillus lentimorbus* B-30488 against *Sclerotium rolfsii* infection.**

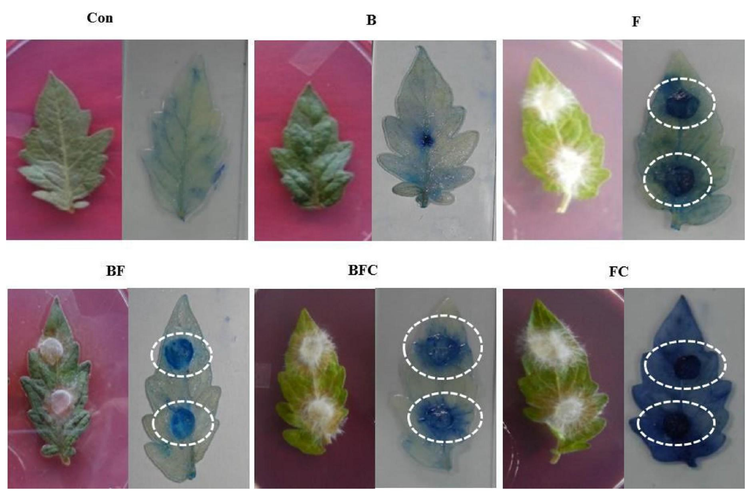
Objectives

❖**We intended to evaluate the role of autophagy in response to biocontrol activity of PGPR *Paenibacillus lentimorbus* B-30488 against *Sclerotium rolfsii* infection in tomato plants.**

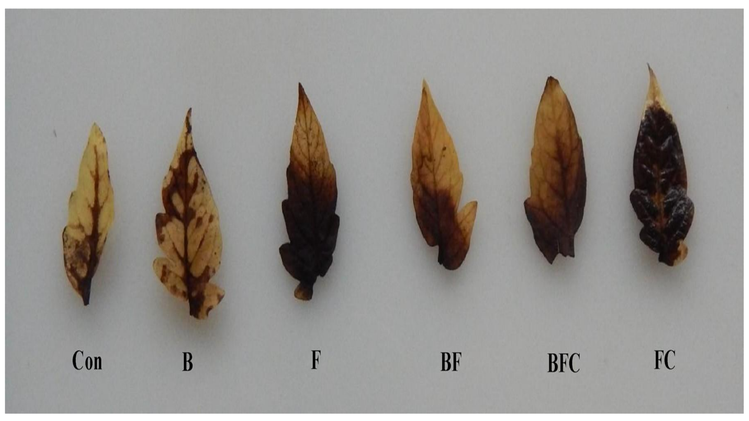
Materials and Methods

- ❖ **Detached leaf assay and Trypan blue staining for assessment of cell death in *S.rolfsii* infected plants.**
- ❖ **Analysis of autophagy by fluorescence microscopy.**
- ❖ **Study of MonoDansyl Cadaverine stained autophagosomal structures through Confocal microscopy.**
- ❖ **Gene expression analysis by Real -Time PCR**

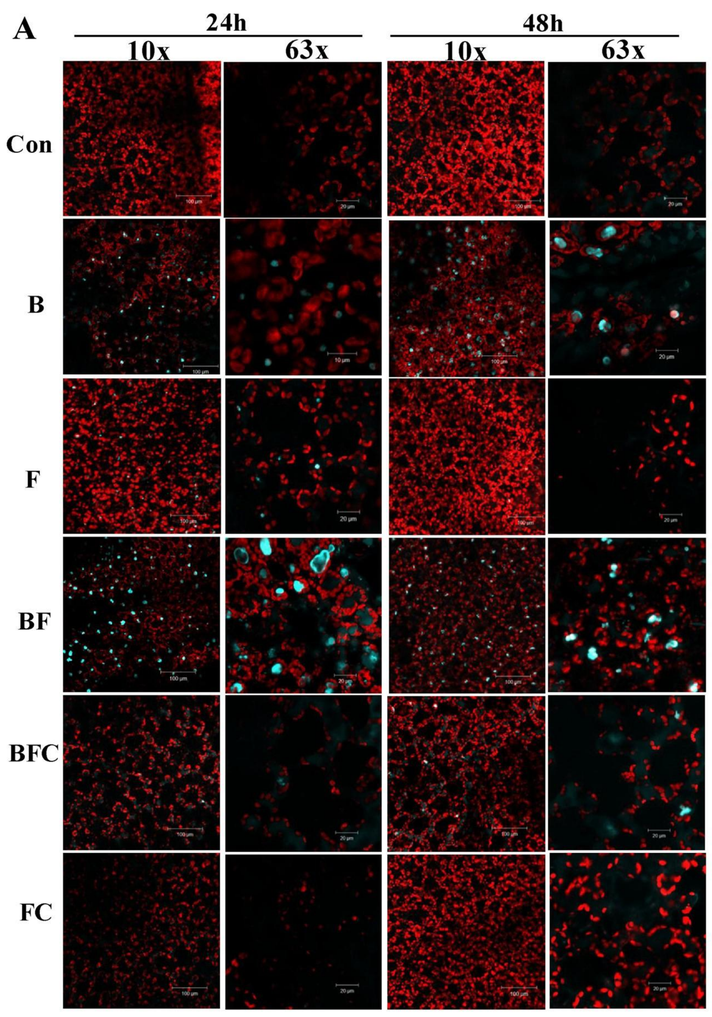
Results

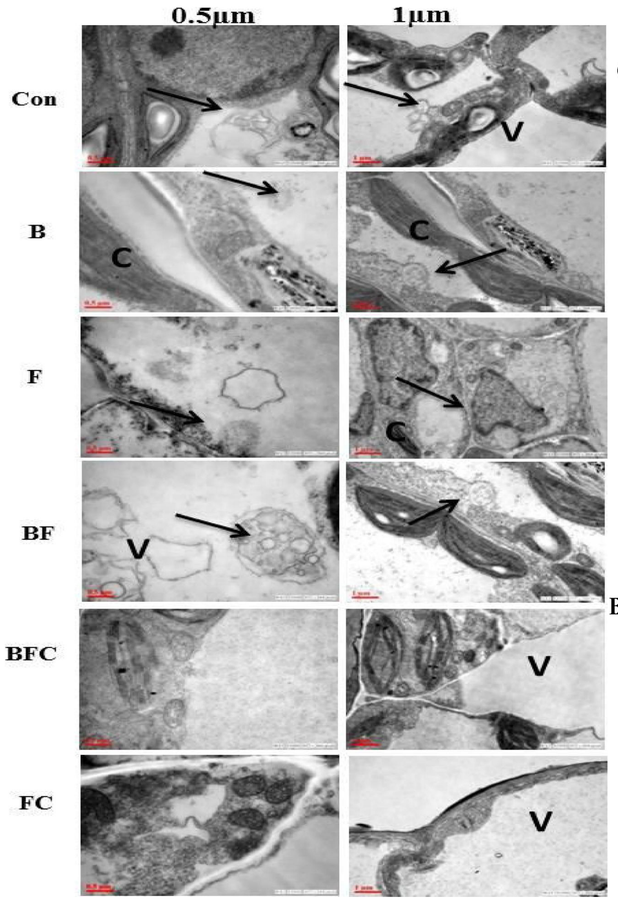


Biocontrol activity of B-30488,study through Detached leaf assay and Trypan Blue staining.

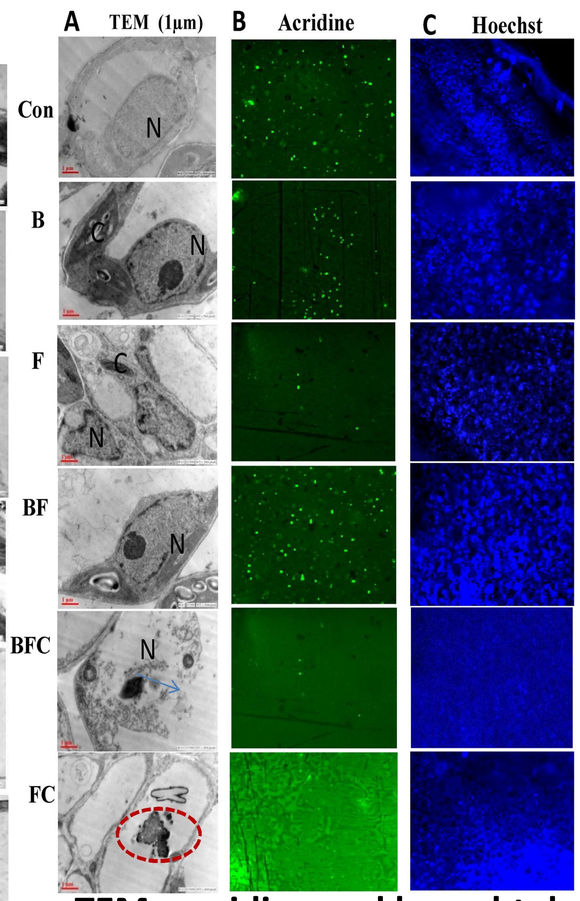


3,3’ diaminobenzidine staining for determination of H202 in treated leaves .

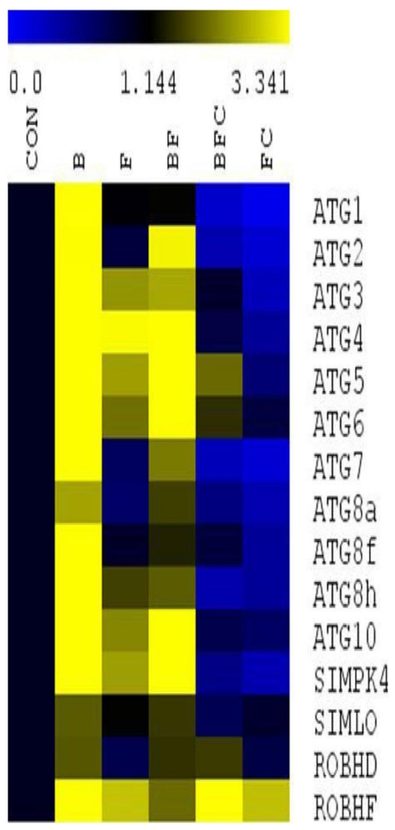




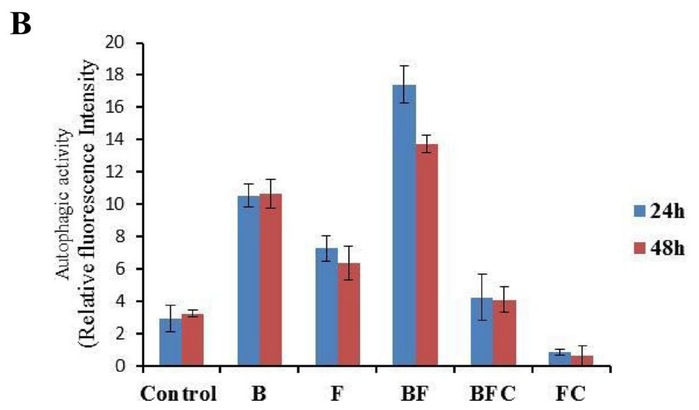
TEM results to detect the presence of autophagosomes



TEM , acridine and hoescht dye used to show chromatin condensation and nuclear degradation



Gene expression analysis of autophagy related genes and defense related genes



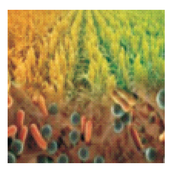
Study of accumulated autophagosomes using MDC and Quantification of MDC-stained autophagosomes

Conclusions

- ❖ **Induction and presence of autophagic structures and autophagic responses on B-30488 treatment during host-plant interaction with *S.roflsii* have been shown.**
- ❖ **B-30488 has biocontrol activity to stimulate resistance by inducing autophagy and enhanced expression of autophagy related genes agaimts necrotrophic fungi *S.rolfsii*.**

6th National Asian PGPR Conference on Advances in PGPR Technology for Betterment of Agriculture and Environment
(3-4, September 2021)




PGPR: A PROMISING APPROACH TOWARDS THE FUNGAL INFECTION CONTROL OF CHILLI PLANT

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Introduction

❖Capsicum (*Capsicum annum*) is among the world's most popular vegetable, grown for its fruits, and are used for its pungency.

❖Chillies are low in sodium and cholesterol free, rich in vitamins A and C, and are a good source of potassium, folic acid and vitamin E.

❖Chilli crop suffers from a large number of viral, fungal, bacterial, nematode diseases. Among them one of the fungal disease anthracnose is very prominently found in chilli. *Colletotrichum sp* is the causal organism of anthracnose and has been found to be the sole reason for the loss of 50%-60% of the total yield worldwide. The plant has also been found to be infected with various other fungal phytopathogens like *Fusarium oxysporum*, *Rhizoctonia solani*.

❖Increasing demand for crop production along with the reduction of chemical fertilizers and pesticides used in the agricultural land is a big challenge for researchers and farmers. In this context, we could say that use of plant growth promoting bacteria (PGPB) as a biofertilizer and biocontrol is a safe and sustainable method for agriculture.

Objectives

❖Collection, isolation and screening of plant growth promoting rhizobacteria of rhizospheric samples from chilli plant of Nimar & Hoshangabad regions.

❖Qualitative screening of bacterial strains for Plant Growth Promoting (PGP) attributes.

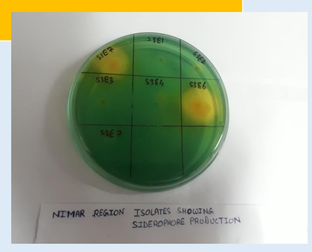
❖Antagonistic activity of potential isolates against Fungal phytopathogens.

Methodology

Collection, isolation and screening of isolates from Nimar and Hoshangabad Regions

Screening of isolates on the basis of PGP attributes

IAA, NBRIP, HCN, Siderophore, Zinc, Ammonia, Potassium Solubilization etc.



Antagonistic activity of isolates against fungal phytopathogens and their percentage inhibition calculation


Nimar isolates showing antagonism against *Colletotrichum gloeosporioides*

•Fig.1- Phosphate solubilization in NBRIP broth

•Fig.2- Indole Acetic Acid Production

•Fig.3-Zinc Solubilization

•Fig.4- HCN Production

•Fig.5-Siderophore Production

•Fig.6- Ammonia Production

•Fig.7-Potassium Solubilization

•Fig.8- Antagonism against *Colletotrichum gloeosporioides*

•Fig.9- Antagonism against *Colletotrichum capsici*

•Fig.10- Antagonism against *Fusarium oxysporum*

•Fig.11- Antagonism against *Rhizoctonia solani*

Results

❖60 Rhizospheric isolates from Nimar region and 56 from Hoshangabad region were isolated from the collected sample of chilli plant.

❖Among them various isolates from both regions had shown promising results for the PGP attributes and *in-vitro* antagonistic activity against four different fungal phytopathogens (*Colletotrichum gloeosporioides, Colletotrichum capsici, Fusarium oxysporum and Rhizoctonia solani)* of Chilli plant.

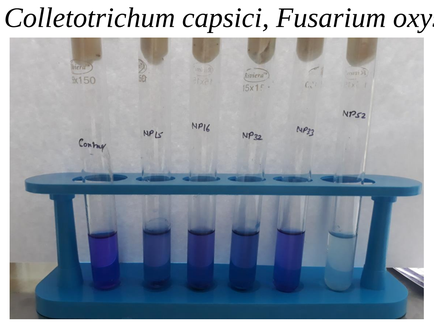


Fig.-1

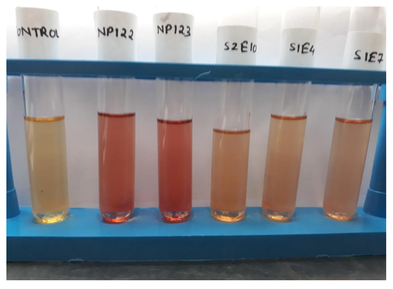


Fig.-2

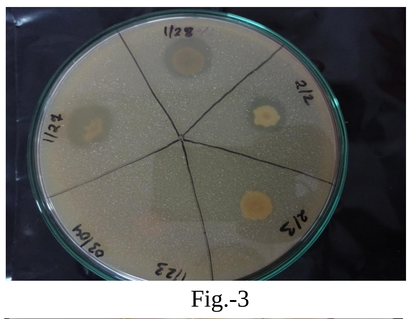


Fig.-3

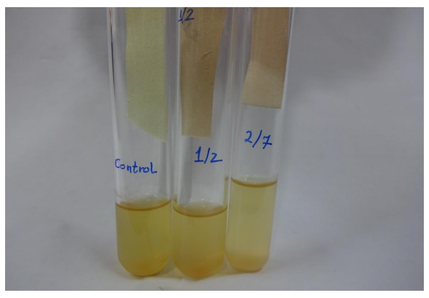


Fig.-4

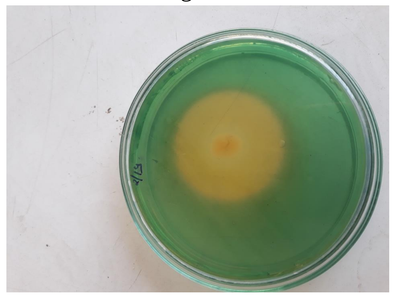


Fig.-5

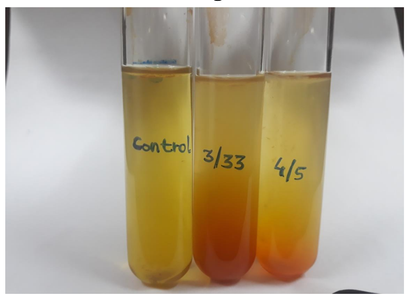


Fig.-6

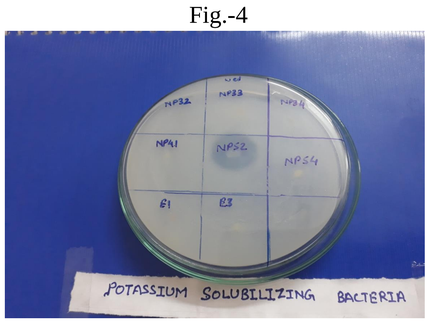


Fig.-7

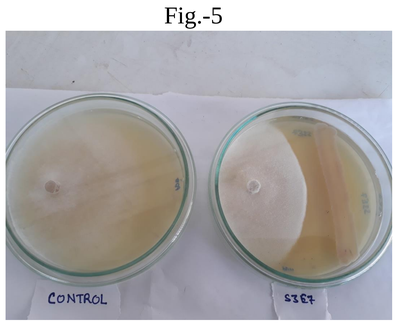


Fig.-8

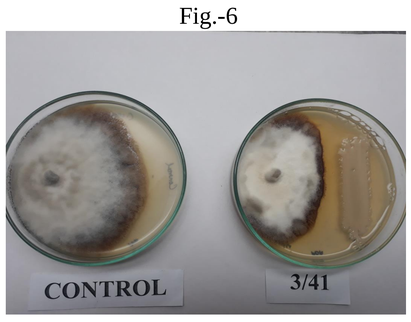


Fig.-9


Fig.-10

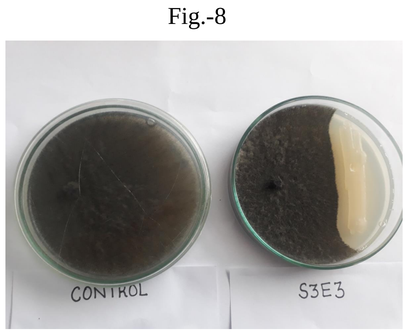


Fig.-11

Conclusion

Study had shown that out of all isolates among Nimar and Hoshangabad Regions, few have shown promising antagonistic activity against fungal phytopathogens. The analyses of growth inhibiting property of the isolates were suggestive of their use as effective microbial biocontrol agents against fungal phytopathogens of Chilli plant.

References

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❖Habazar, T., Yanti, Y., Reflinaldon, Yaherwandi, Nasution, C.R., Felia, S., (2018). *In vitro* characterization of selected indigenous Rhizobacterial strains as biocontrol agent of bacterial wilt disease on chili. *J. Biopest.*, **11**(1):14-24.

❖ Mamta Joshi , Rashmi Shrivastava, Anil Kumar Sharma and Anil Prakash**,** Screening of resistant verities and Antagonistic *Fusarium oxysporum* for Biocontrol of Fusarium Wilt of Chilli (2012), Plant Pathology and Microbiology, **Vol 3**, Issue 5.

Acknowledgement

Author is thankful to MPCOST (Madhya Pradesh Council of Science & Technology), Bhopal for providing the financial assistance and Department of Microbiology, Barkatullah University, Bhopal for providing the facilities to carry out the study.

Registration No.- 2.16





Role of bacterial endophytes in disease suppression and plant growth promotion of *Musa acuminata*

Registration no. – 2.18

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Introduction

Endophytic Bacteria

Endophytic bacteria are plant beneficial bacteria that residing within the plant tissue for whole or part of their life cycle without causing any harmful effect to their host plant (Fakhra Liaqat and Rengin Eltem, 2016).

- ❖ They can benefit host plants

Directly by-

- Improving plant nutrient uptake
- Modulating stress related Phytohormones.

Indirectly by-

- Improve plant health by targeting pests pathogens with antibiotics.
- Secretion of lytic enzymes.
- Induction of plant resistance

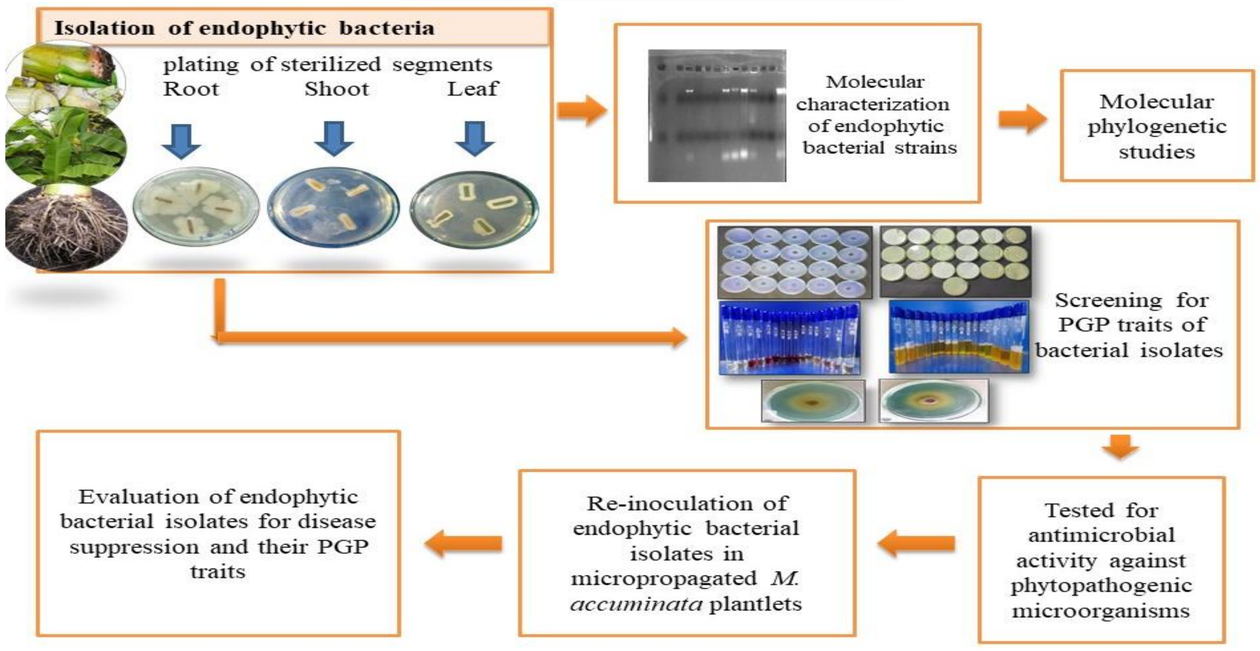
(Pious Thomas and Thyvalappil A. Soly, 2009).

- ❖ Banana grand Nain is one of the most well known variety of Cavendish group. It belongs to AAA genotype which is a triploid variant of the species *M. accuminata* its characteristics medium height and large fruit yield make it deal for commercial cultivation. it is one the most popular fruit and major crop of India and plays a important role in agricultural economy (A. Remakanthan *et al*., 2013).

Objective

Isolation and characterization of entophytic bacteria associated with *Musa accuminata* plant and evaluation of re-inoculation of isolated bacterial endophytes in micro propagated plant growing under Invitro conditions to assess their ability in plant growth promotion and disease suppression.

Methodology



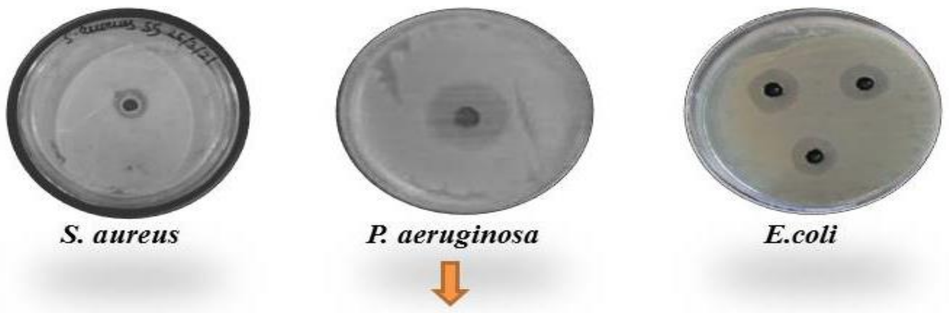
Results

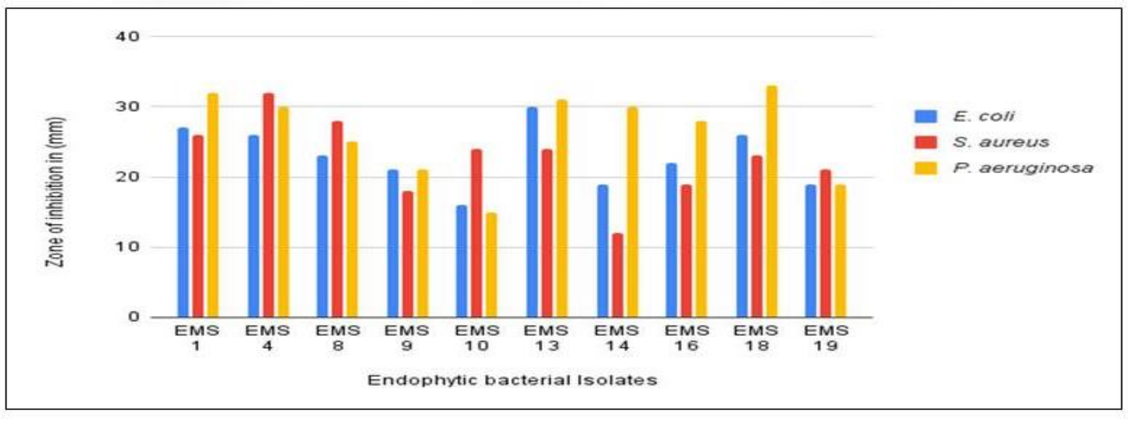
On the basis of plant growth promoting traits, the best PGP properties producing isolates were selected for plant growth promotion and disease suppression of *Musa accuminata* under *in vitro* conditions

S.no.	Isolates	Ammonia test	HCN production	Phosphate solubilization	Siderophore production	IAA
1	EMS 1	+++	++	38 mm	33 mm	0.270 ±.01
2	EMS 2	++	-	24 mm	20 mm	0.114 ±.1
3	EMS 3	-	-	19 mm	21 mm	0.112±.03
4	EMS 4	+++	++	33 mm	31 mm	0.238±.1
5	EMS 5	-	-	20mm	16 mm	0.079 ±.02
6	EMS 6	++	++	23 mm	21mm	0.132±.01
7	EMS 7	-	-	16 mm	18 mm	0.093±.01
8	EMS 8	+	+	19 mm	20 mm	0152±.02
9	EMS 9	+	-	25 mm	22 mm	0.094 ±.01
10	EMS 10	++	-	22 mm	16 mm	0.118±.03
11	EMS 11	+	-	26 mm	20 mm	0.024±.02
12	EMS 12	+	-	19 mm	13 mm	0.123±.01
13	EMS 13	+++	++	20 mm	17 mm	0.063±.02
14	EMS 14	++	-	28 mm	23 mm	0.133±.02
15	EMS 15	++	-	18 mm	16 mm	0.067±.05
16	EMS 16	++	++	31 mm	28 mm	0.166 ±.01
17	EMS 17	-	-	18 mm	21 mm	0.099±.01
18	EMS 18	++	++	30 mm	38mm	0.181±.1
19	EMS 19	+	-	22 mm	19 mm	0.135±.1

Above results shown as Weak (+), Moderate (++), Strong (+++) and Negative (-)

Determination of Antibacterial Activity against pathogenic bacteria

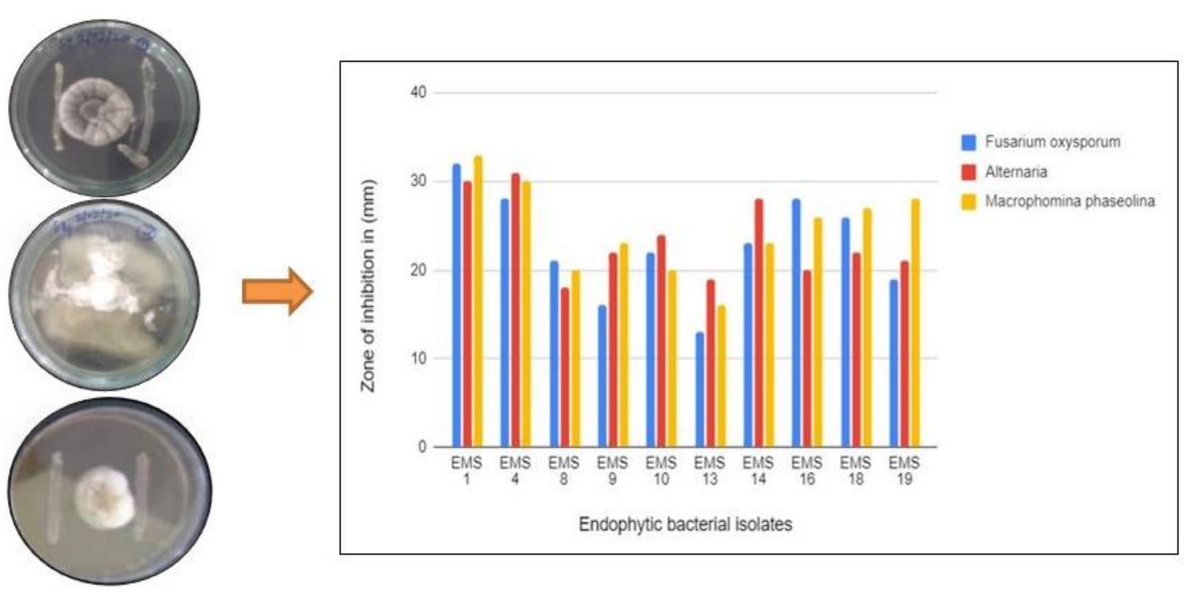




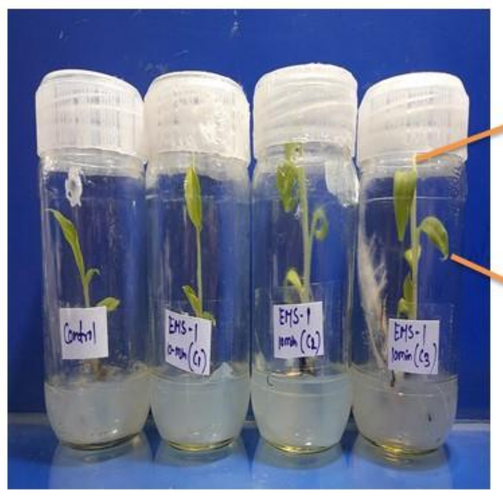
Antibacterial activity of isolates culture supernatant by using well diffusion method against pathogenic bacterial cultures

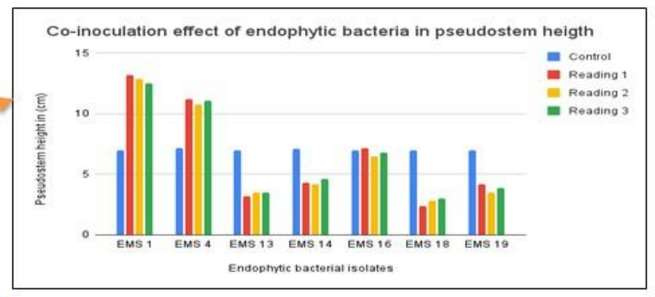
Antagonistic activity of isolates against phytopathogenic fungi

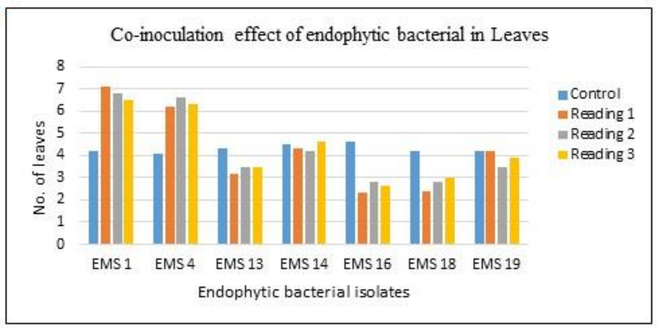
Antagonistic activity of endophytic bacterial isolates were tested for their antifungal activity against isolated fungal cultures from *in vitro* growing contaminated plantlets. Overnight grown bacterial culture was streaked on the two ends of petri dish on PDA (potato dextrose agar) medium and spore of fungal species were spot inoculated at the center of the plates and incubated at 28 ± 2°C for 6-8 days (A. Souza *et al*., 2014).



Evaluation of plant growth promoting potential of bacterial endophytic inoculants induced in micropropagated *Musa accuminata* plantlets







Conclusion

This investigation provides an insight about plant growth promoting properties of endophytic bacteria as potential bioinoculants to control the disease and support the plant growth promotion *under in vitro* conditions. we identified that, **EMS 1 and 4** showing effective results on the basis of selective and evaluated parameters. These bacterial endophytes are helpful in *Musa accuminata* plant growth promotion and disease suppression.

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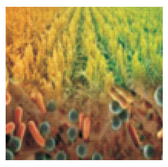


Biocontrol potentials of PGPM of arid zone in managing plant pathogens

2.19

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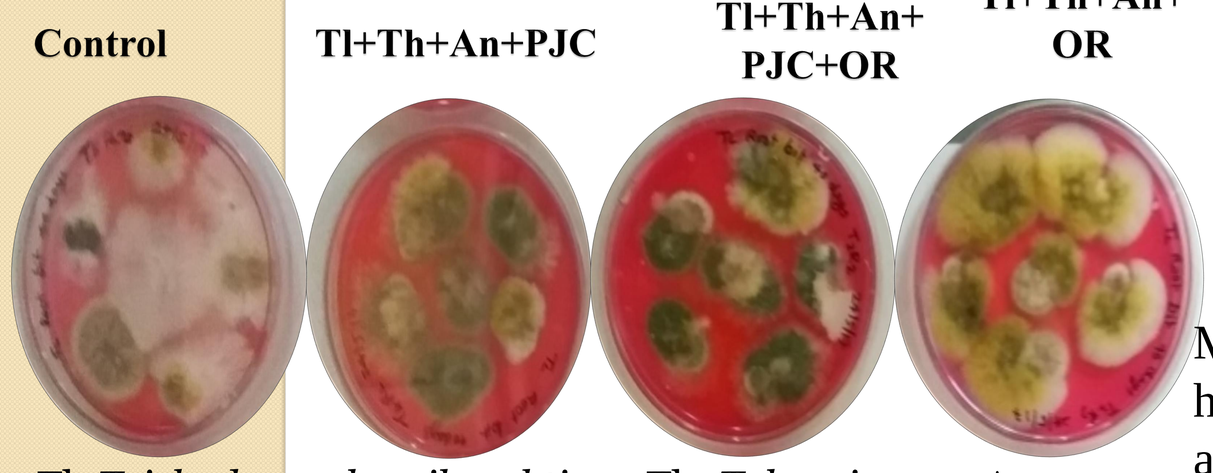
Introduction

Arid soils are inhibited by a large variety of flora and fauna, some of which are beneficial to human beings, while the rest are enemies. Of the soil inhibiting microorganisms that are pathogenic to plants, include bacteria, actinomycetes, nematodes, fungi and some other microbes. Microbes naturally present in soil and root rhizosphere are not always harmful by causing diseases to plants but sometimes serve as biological control agents of various significantly important plant pathogens. The mechanisms through which these bio agents operates is either by direct action against the pathogen i.e. antagonism which includes parasitism, antibiosis, competition and/or indirectly by reducing host susceptibility and includes exudation, altered rhizosphere, induced resistance, hipovirulence, PGPR, etc.

Methods

Soil samples collected from different parts of the arid region led to isolation of native bio-control agents Dual culture test was performed for testing their antagonistic activity. Information was generated for their field efficacy on most commonly grown crops, trees and their effect on resident microflora in order to ascertain whether any bio-agent has adverse effect on native organisms. Cowpea roots (2 cm) were colonized by *Ganoderma* in PDB, Ground-up residues (3%), BCAs and colonized bits were separately mixed in soil maintained at 70% MHC. Colonized bits were retrieved at 20,40 and 60 days interval to assess reduction/viability of *Ganoderma* on PDA.



Tl: *Trichoderma longibrachtium*, Th: *T. harzianum*, An: *Aspergillus nidulans*, PJC: *Prosopis juliflora* compost, OR: Onion residue

Concurrent heat and water stress

Vulnerable growth stage
Susceptible host

Availability of inoculum

Remedial measures

In absence of resistant varieties, development of ecofriendly and cost effective management strategy for important soil-borne plant pathogens of arid legumes, oil seeds and cumin is the only option

Objective

Explore biocontrol potentials of microorganism in order to develop a cost effective and practical management strategy in augmenting disease control

Results

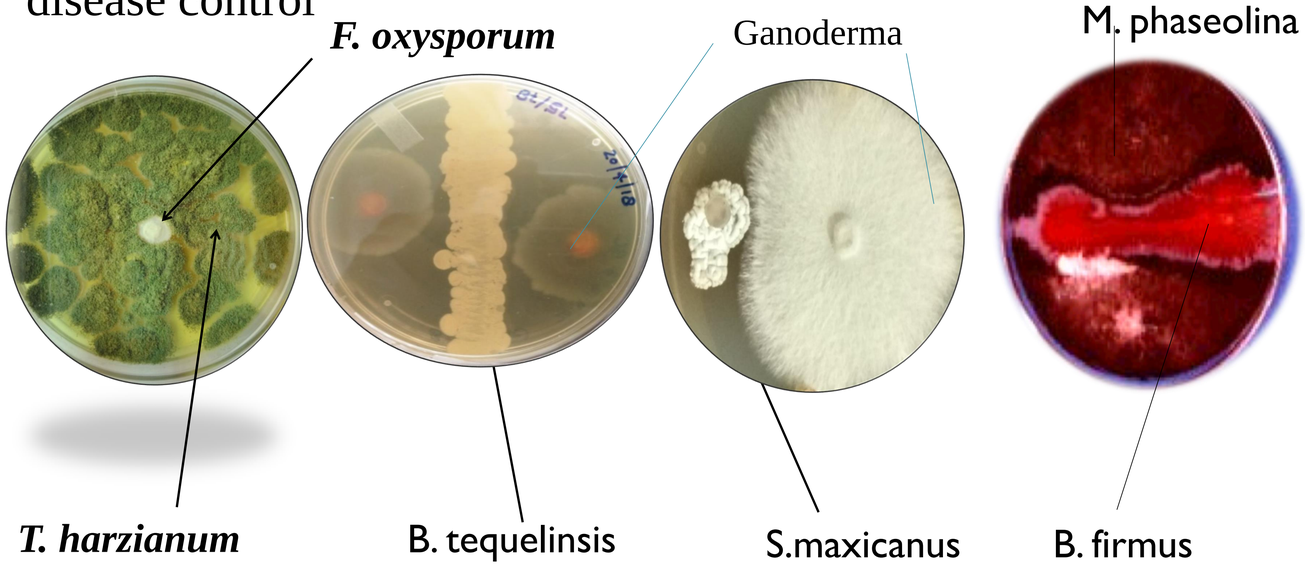


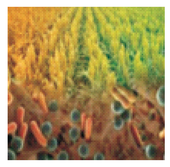
Table: Effect of *BCAs* seed treatment on cowpea roots

Seed treatment	Nodulation	Root coloization 2019	2020	Healthy	Diseased
B.firmus	6.6	6.7	9.5	13.3	5.3
T.harzianum	6.0	9.0	14.7	8.0	6.3
T. harzianum + *B. firmus*	7.3	16.7	39.5	12.6	3.0
Control	2.5	4.0	7.5	2.3	0.0

Conclusion

Many species of *Bacillus* & *Trichoderma* isolated from hot arid region having biocontrol potential against plant pathogenic fungi. These antagonistic strains will be potentially very useful, ecofriendly, yield effective in field studies for management of soil borne plant pathogens




Management of rice blast by using Plant Growth Promoting Rhizobacteria and chemical fungicides

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Objective

- To screen the efficacy of chemical fungicides in combination with bio-pesticides for the management of rice blast
- Assessment of yield
- Testing the presence of blast inoculums in the seed after harvest

Methods

A field trial was laid out in a randomized block design with ASD 16 variety during Rabi season to manage blast of rice using chemical fungicides and biopesticides. Seven treatments were used. All the treatments were applied immediately after the appearance of disease symptom and subsequently two sprays were given at 15 days interval. In all the treatments Percent Disease Index and disease reduction over control were recorded.

Presence of blast inoculums in the harvested seeds of different treatments were tested through agar plate method. For these 25 seed from each treatment was taken and inoculated in the agar plate and incubated under room temperature. Number of blast infected seeds was observed.

Results

A field trial was laid out in a randomized block design with ASD 16 variety during Pishanam season to manage blast of rice using chemical fungicides and biopesticides. All the treatments were applied immediately after the appearance of disease symptom and subsequently two sprays were given at 15 days interval. The disease incidence was found to be reduced substantially in the seeds treated with Pseudomonas fluorescens (liquid formulation) 10 ml/ kg of seed + Azoxystrobin (0.1%) spray (16.32%) whereas the control plot recorded 42.78% incidence. Increased yield was noticed in the Pseudomonas fluorescens (liquid formulation) 10 ml/ kg of seed + Azoxystrobin (0.1%) spray treatment (4980 kg/ha) whereas the control plot recorded comparatively less yield (3700 kg/ha) [Table 1].

Table 1. Management of rice blast using chemical fungicides and bio – pesticides

Treatment Number	Treatments	PDI**	Percent Disease reduction over control	Yield (Kg/ha)
T1	Seed treatment with *Pseudomonas fluorescens* (talc formulation) 10 g/ kg of seed + Azoxystrobin (0.1%) spray *	18.38^{b} (25.38)	57.04	4800 ^{b}
T2	Seed treatment with *Pseudomonas fluorescens* (talc formulation) 10 g/ kg of seed + Metaminastrobin (0.1%) spray*	30.10^{e} (33.27)	29.64	4410 ^{e}
T3	Seed treatment with *Pseudomonas fluorescens* (liquid formulation) 10 ml/ kg of seed + Azoxystrobin (0.1%) spray *	16.32 ^{a} (23.82)	61.85	4980 ^{a}
T4	Seed treatment with *Pseudomonas fluorescens* (liquid formulation) 10 ml/ kg of seed+ Metaminastrobin (0.1%) spray	24.54 ^{d} (29.69)	42.64	4520 ^{d}
T5	Azoxystrobin (0.1%) spray*	20.93 ^{c} (27.22)	51.08	4700 ^{c}
T6	Metaminastrobin (0.1%) spray*	31.69 ^{f} (34.26)	25.92	4356 ^{e}
T7	Seed Treatment with *Pseudomonas fluorescens* (Liquid formulation) + Foliar spray with *Pseudomonas fluorescens* 0.2%	35.43 ^{g} (36.53)	17.18	4100 ^{f}
T8	Control	42.78 ^{h} (40.84)	-	3700 ^{g}
	SEd	0.27	-	26.66
	CD (0.05)	0.57	-	57.18

*Mean of Three replication

Testing the presence of blast inoculums in the seed after harvest

Presence of blast inoculums in the harvested seeds of different treatments were tested through agar plate method. For this, 25 seeds from each treatment was taken and inoculated in the agar plate and incubated under room temperature. Number of blast infected seeds was observed. Percentage of blast infection was found to be reduced substantially in the seeds harvested from the plots treated with Pseudomonas fluorescens (liquid formulation) 10 ml/ kg of seed + Azoxystrobin (0.1%) spray (8 %) whereas the control plot recorded 96 % infection (Table.2).

Table 2. Testing the presence of blast inoculums in the seed after harvest through Agar plate method

Treatment Number	Treatments	Number of seeds taken*	Number of blast infected seed*	Percentage of infection
T1	Seed treatment with *Pseudomonas fluorescens* (talc formulation) 10 g/ kg of seed + Azoxystrobin (0.1%) spray *	25	3	12
T2	Seed treatment with *Pseudomonas fluorescens* (talc formulation) 10 g/ kg of seed + Metaminastrobin (0.1%) spray*	25	11	44
T3	Seed treatment with *Pseudomonas fluorescens* (liquid formulation) 10 ml/ kg of seed + Azoxystrobin (0.1%) spray *	25	2	8
T4	Seed treatment with *Pseudomonas fluorescens* (liquid formulation) 10 ml/ kg of seed+ Metaminastrobin (0.1%) spray	25	8	32
T5	Azoxystrobin (0.1%) spray*	25	5	20
T6	**Metaminastrobin (0.1%) spray***	25	13	52
T7	Seed Treatment with *Pseudomonas fluorescens* (Liquid formulation) + Foliar spray with *Pseudomonas fluorescens* 0.2%	25	15	60
T8	Control	25	24	96

*Mean of Three replication

Conclusion

Among the all the treatments tested, increased yield and reduced blast infection in the harvested seed was recorded in T3 [Pseudomonas fluorescens (liquid formulation) 10 ml/ kg of seed + Azoxystrobin (0.1%)].

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Acknowledgement

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Potential of endophytic bacteria *Pseudomonas florescens* and *Bacillus velezensis* in protecting tomato plants against Fusarium wilt

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Introduction

Fusarium oxysporum ranks among the 10 most devastating fungal plant pathogens worldwide (1). Fo produces micro- and macroconidia and chlamydospores that can remain viable in infected soils for decades, thereby frustrating crop rotation schemes. It is known to affect many crop plants including tomato. Tomato (*Lycopersicon esculentum* L.) is a member of family Solanaceae, cultivated worldwide, ranked first among the processing crops and second as a vegetable crop. India is second largest producer of tomato after China. The yield of tomatoes needs to be increased to meet the growing demands without increasing the amount of chemicals in the soil. It is extremely difficult to control soil-borne fungi via conventional strategies such as the use of synthetic fungicides, etc. Since their spores are able to survive for many years in the soil, biological control strategies for this pathogen should, therefore, be carefully selected and handled in an eco-friendly way instead of using chemical fungicides. The application of microorganisms as biocontrol agents is important, since they may increase beneficial microbial activity which extends for a long period of time.

A wide diversity of plants is associated with endophytic bacteria that colonize host tissues internally without causing damage or eliciting disease symptoms. some bacterial endophytes act as plant growth-promoting bacteria (PGPB), which stimulate plant growth by several mechanisms (2). PGPB can also stimulate plant growth by preventing the deleterious effects of phytopathogenic microorganisms, acting as biological control agents, which can exert their activity by direct antagonistic effects on pathogenic organisms or indirectly, by eliciting plant defence responses (3).

In this study, Bacterial endophytes were isolated from tomato plants and two selected isolates *Pseudomonas florescens* and *Bacillus velezensis were* were screened for their antagonistic potential against *Fusarium oxysporum* in-*vivo*.

Methodology

Isolation of bacterial endophytes from tomato plants (root, stem and leaf)

→ Screening for antagonistic potential against *Fusarium oxysporum* invitro

→ In-vivo analysis of control of Fusarium wilt in tomato plants using selected isolates.

Treatment Plan for pot experiment

Treatment 1	Plant inoculated with pathogen (*Fusarium oxysporum*)
Treatment 2	Plant uninoculated (Healthy Control)
Treatment 3	Plant treated with B1 (*Pseudomonas florescens*) and pathogen
Treatment 4	Plant treated with B2 (*Bacillus velezensis*) and pathogen
Treatment 5	Plant treated with consortia (B1+B2) and pathogen

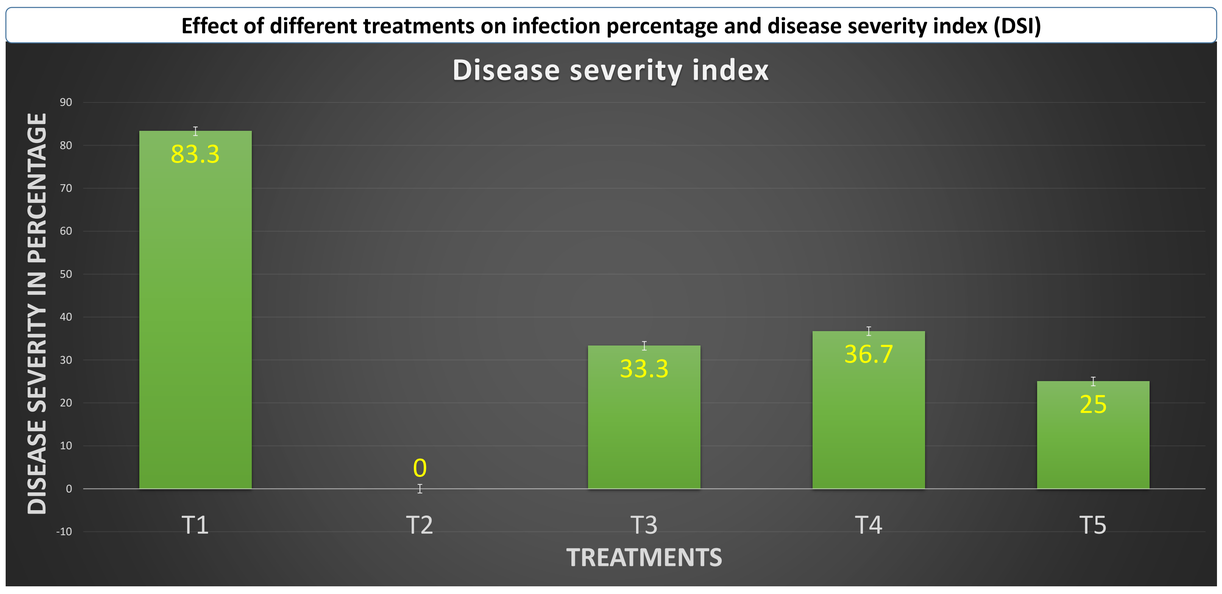
Results

	Control	*Bacillus velezensis*	*Pseudomonas florescens*	Consortia
Germination %age	43.3 ± 1.67^{c}	63.3 ± 1.67^{b}	71.7 ± 3.33^{b}	81.7 ± 1.67^{a}
Seedling length	1.63 ± 0.12^{c}	3.53 ± 0.12^{b}	3.83 ± 0.176^{ab}	4.5 ± 0.208^{a}
Vigour Index	70.5 ± 3.97^{d}	230 ± 11.5^{c}	294 ± 18.9^{b}	374 ± 10.1^{a}

Effect of isolates on germination, seedling length and vigour index

	T1	T2	T3	T4	T5
Infection %age	100 ± 0^{a}	0 ± 0^{d}	73.3 ± 6.67^{b}	66.7 ± 6.67^{b}	33.3 ± 6.67^{c}
DSI	83.3 ± 1.67^{a}	0 ± 0^{c}	33.3 ± 1.67^{b}	36.7 ± 1.67^{b}	25 ± 5^{b}

Effect of different treatments on infection percentage and disease severity index (DSI)



	T1	T2	T3	T4	T5
Root length	6.97 ± 0.24^{d}	18.3 ± 0.0882^{a}	16.4 ± 0.145^{b}	14.5 ± 0.0882^{c}	18.1 ± 0.115^{a}
Shoot length	15.1 ± 0.186^{d}	41.4 ± 0.176^{a}	36.3 ± 0.145^{c}	38.6 ± 0.153^{b}	39.7 ± 0.5^{b}
Fresh weight	2.1 ± 0.115^{d}	9.43 ± 0.133^{b}	7.37 ± 0.0333^{c}	7.43 ± 0.0667^{c}	11.9 ± 0.115^{a}
Dry Weight	0.213 ± 0.00882^{d}	1.8 ± 0.0577^{b}	0.717 ± 0.012^{c}	0.71 ± 0.0115^{c}	2.2 ± 0.0577^{a}

Effect of different treatments on plants after 45 days of treatment

- ✓ Isolates *Pseudomonas florescens* and *Bacillus velezensis* showed maximum inhibition percentage i.e., 67.2% and 69.1% respectively and were chosen for further studies.
- ✓ They showed an enhanced seed germination percentage and vigour index when compared to the uninoculated control after 7 days of incubation.
- ✓ It was observed during the study that treatment T5 significantly reduced the infection percentage by 67% when compared to control followed by treatments T3 and T4 which reduced by 26.7% and 33.3% respectively. Although, there was no significant difference observed in the reduction in disease severity between T3, T4 and T5, but, there is almost three fold reduction in disease severity when compared to the plant inly inoculated with the pathogen (T1).
- ✓ The growth parameters were observed for the plants after 45 days of pathogen inoculation. Maximum root length and shoot length i.e., 18.1±0.11 cm and 39.7±0.5 cm was observed in T5 which is almost equal to that observed in uninoculated or healthy plant (T2). However, a significant increase in fresh weight and dry weight when compared to healthy plant (T2) was clearly observed in plants under treatment T5.

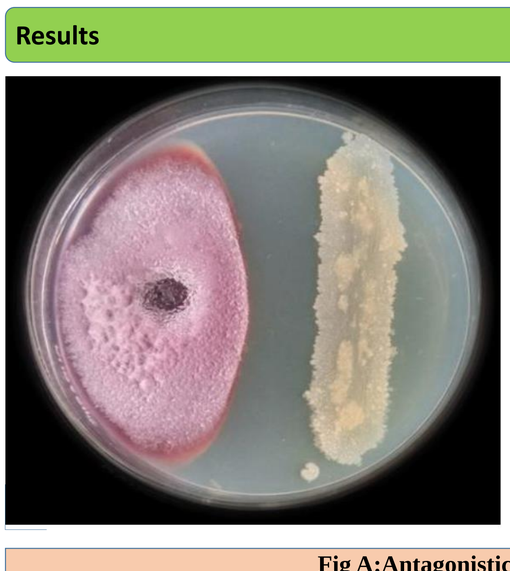


Fig A:Antagonistic activity against *Fusarium oxysporum* in-vitro
Fig B: Plant growth as observed on day 45 under different treatments

Conclusion

The present work was an effort to analyze the antagonistic potential of bacterial endophytes in inhibiting *Fusarium oxysporum* thereby protecting the plant from the deadly Fusarium wilt.The selected isolates *Pseudomonas florescens* and *Bacillus velezensis* have shown promising results for treating fusarium wilt of tomato. They can be used as alternatives to reduce the utilization of chemical fungicides which are harmful for the environment and are also responsible for deteriorating the quality of soil.

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Acknowledgement

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In vitro Estimation of Phytochemicals & flavonoids & study antimicrobial & Antioxidant activity of Flavonoids (*Citrus sinensis*)

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INTRODUCTION

Synthetic drugs have a small number of substantial negative effects, but plant-based drugs have fewer. Orange (*C. sinensis*) peel flavonoids have antibacterial, antioxidant, anticancer, anti-inflammatory, and antiviral effects. However, there is a scarcity of information in the literature about orange peels flavonoids(1,2).

Keyword: Citrus, Flavonoids, Antimicrobial, Antioxidant, MIC

OBJECTIVE

To determine -

- ➢Phytochemical estimation
- ➢Flavonoids separation
- ➢Antimicrobial activity
- ➢Antioxidant activity

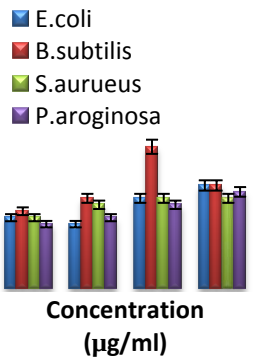
METHOD (3)

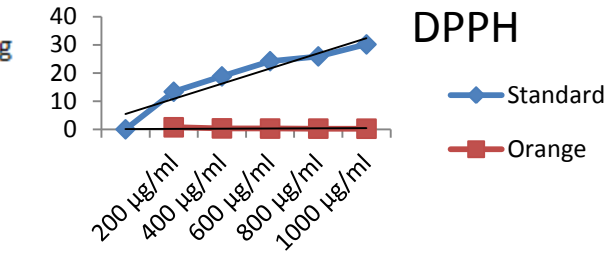
- ✓ Crude extraction
- ✓ Phytochemical estimation
- ✓ Flavonoid separation & Confirmation
- ✓Antimicrobial Activity
- ✓Antioxidant Activity (DPPH)
- ✓Nitric oxide scavenging (NO')

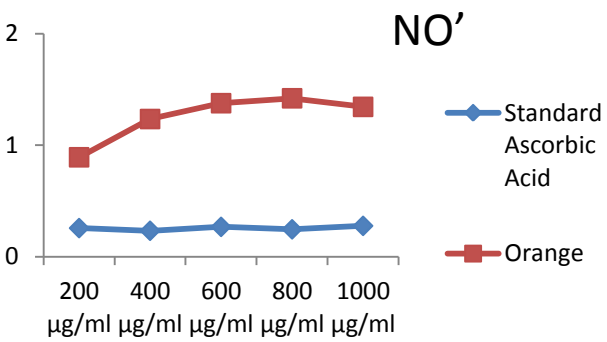
RESULT

In a hydro-methanolic extract of orange peel, phytochemicals were isolated chemically. Column chromatography was being used to segregate the flavonoids, which would then be identified using chemical analysis, TLC, and HPLC. Flavonoids have been found to suppress bacterial growth and to have antioxidant and nitric oxide scavenging properties.

Plant	Plant's part	Extract	TPC	TFC
Orange	peels	26.5gm	32.43µg	62.59µg
Organism	E. coli	B. subtilis	S. aureus	P. aeruginosa
MIC	19.40µg	20.48µg	12.45µg	21.44µg
Antioxidant	**Nitric oxide**			
IC_{50}=121.4µg	IC_{50}=0.88µg			







CONCLUSION

In the current study, *Citrus* peel flavonoids were found to have antibacterial, antioxidant, and nitric oxide scavenging action in methanolic extracts.

This study suggests that *C. sinensis* flavonoids could be exploited as an antibacterial and antioxidant source.

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