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Biocontrol Activity and PGPR Ability of Different Isolates of *Pseudomonas* and *Bacillus* on Tomato

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ABSTRACT

The present study was to determine the efficacy of bacterial biocontrol agents against Alternaria solani. Among 20-each isolates of Pseudomonas, Bacillus spp. tested for their biocontrol ability against A. solani, Pseudomonas (P28) showed maximum inhibition (74.19%) of the pathogen and Bacillus (B299) showed maximum inhibition (61.94%) of the pathogen. Same set of Pseudomonas and Bacillus isolates were evaluated for their plant growth promoting ability on tomato. There was 100 percent germination in all the treatments of Pseudomonas and Bacillus showing that all the isolates did not possess any deleterious effect towards seed germination. Among Pseudomonas treatments, isolate P43 recorded highest seedling vigour index (1502) followed by isolate P17 (1493). Among Bacillus treatments, isolate B100 recorded highest seedling vigour index (1636) followed by isolate B26 (1586), the lowest being by isolate B27 (996), which was less than control.

Key words: *Biocontrol, PGPR, tomato, Pseudomonas and Bacillus.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) belongs to the family solanaceae and is one of the most remunerable and widely grown vegetable in the world.Tomato (*Lycopersicon esculentum*.L) is one of the most important economic vegetable crops cultivated in India in an area of 7.60 Mha with a production of 18.38Mt and productivity of 24.2 Mt/ha[3]. Tomato crop is usually susceptible to many diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors. Among the fungal diseases, early blight also known as target spot disease caused by *Alternaria solani* (Ellis and Martin) is one of the world's most catastrophic diseases incurring losses both at pre- and post-harvest stages causing 35 to 78 per cent reduction in fruit yield in the tropical and subtropical regions[4]. Plant growth promoting rhizobacteria (PGPR) as originally defined as root-colonizing bacteria (rhizobacteria) are potential plant growth promoters or even used as biological control agents. PGPRs are known to possess biocontrol ability against a wide range of phytopathogens like fungi, bacteria, viruses and nematodes etc.

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Biocontrol agents including, *Bacillus subtilis*, and *Pseudomonas fluorescens* have already been commercially exploited for management of important pathogens like *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum* and other soil borne plant pathogens.

MATERIAL AND METHODS

In vitro efficacy of different isolates of *Pseudomonas* and *Bacillus* against *Alternaria solani*

Efficacy of bacterial biocontrol agents was tested against the test pathogen by dual culture assay on petridishes containing malt extract dextrose agar using bangle method described by Yogesh *et al*[9].

All the test bacterial cultures were grown on TS broth for 24 h and used as inoculum. A bangle of 7 cm diameter was dipped in the test inoculum for 2 min, placed on the solidified medium in a petriplate to inoculate the plate with test biocontrol agent and then the bangle was removed. A five mm diameter disc was cut from the periphery of actively growing colony of seven-day old culture of *A. solani* and kept in the middle of the circle of the plate inoculated with biocontrol agent. Control plates had only pathogen. Petriplates were sealed with parafilm and incubated at 28±2°C in a BOD incubator. Three replications were maintained for each treatment. The radial growth of the pathogen was measured regularly in all the treatments. The results were expressed as percent inhibition of the mycelial growth of the pathogen over control and calculated using following formula as given by Vincent[8].

I %= C - T / C ×100

where,

I = Inhibition of pathogen growth,

C= Pathogen growth in control,

T= Pathogen growth in treatment.

Plant growth promoting (PGP) ability of bio-inoculants on tomato

Tomato seeds were surface sterilized with 1% sodium hypochlorite solution for 2 min and used for further studies. Plant growth promoting (PGP) ability of bio-inoculants (*Pseudomonas, Bacillus* spp.) on tomato plants was evaluated following the method described by Zhender *et al* [10] and is described briefly here. The seeds were pre-coated with microbial suspension of 1x 10^8cfu/ml for bacteria and sown in seedling trays. PGP traits were recorded for treated and control plants 14DAS.Three replications were maintained for each treatment. Seeds soaked in sterile distilled water served as control. Percent seed germination, root and shoot lengths of seedlings were measured after 14DAS and the seedling vigour index was calculated as follows

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

RESULTS AND DISCUSSION

Evaluation of bacterial biocontrol agents

Antagonistic activity of 20 *Pseudomonas* and 20 *Bacillus* strains was tested against *A. solani*. Among *Pseudomonas* strains, P28 exhibited maximum inhibition (74.19%) followed by P35 (69.13%). As seen from the Table 4.1and Fig 4.1, the isolates varied in their antagonistic ability against *A. solani*. While, P28 and P35 showed >65% inhibition, isolates P4, P5, P6, P18 and P19 showed inhibition in the range of 30-50%. P17, P20, P21, P22, P39, P42 and P49 showed least degree of antagonism which was <10%.

Among *Bacillus* strains, B299 exhibited maximum inhibition (61.94%) followed by B33 (58.53%).As seen from the Table 4.1and Fig 4.1, the isolates varied in their antagonistic ability against *A. solani*. While, B299 and B33 showed >55% inhibition, isolates B101, B15, B9, and B5 showed inhibition in >50%. B1, B6, B8 and B27 showed inhibition in the range of 40-50%.B103 and B159 showed least degree of antagonism which was <10%.

The results are in agreement with the findings of Casida and Lukezie[2], who reported that *Pseudomonas* strain 679-2 was able to reduce the severity of the leaf spot disease caused by *A. solani*. Babu *et al*.[1] reported that all the six *P. fluorescens* strains used, significantly inhibited the growth of *A. solani* compared to control and Liu-chienhui *et al*.[6]

reported that *B. subtilis* var. *Globigiii* CBS10 was antagonistic to *A. solani*. The result was also similar to work of Leifort *et al.*[5], who reported high level of bacterial antagonism by fluorescent *Pseudomonas* and *Bacillus* spp. against *A. brassicola.* Ozylmaz and Bnlioglu[7] reported variability among isolates of *Pseudomonas* in management of *Phytophthora* blight of pepper.

Table.1 Evaluation of isolates of *Pseudomonas* spp. and *Bacillus* spp. against *A. solani* on tomato *in vitro*

Treatments		**Per cent inhibition**	
***Pseudomonas* spp**	***Bacillus* spp**		
P4	B1	34.90	46.58
P5	B2	35.91	18.08
P6	B5	43.84	51.19
P17	B6	6.23	45.39
P18	B8	34.23	48.29
P19	B9	30.86	50.51
P20	B15	3.37	51.70
P21	B21	2.86	48.29
P22	B26	7.92	41.46
P28	B27	74.19	40.95
P29	B29	16.86	50
P30	B33	24.78	58.53
P33	B34	17.36	17.57
P35	B98	69.13	25.59
P39	B100	7.25	15.35
P42	B101	3.37	53.41
P43	B103	11.29	2.73
P49	B159	1.18	7.33
P59	B162	61.88	43.17
P64	B299	21.92	61.94
SE (m)		0.17	0.08
SE (d) ±		0.24	0.11
CD		0.49	0.24
CV (%)		0.31	0.18

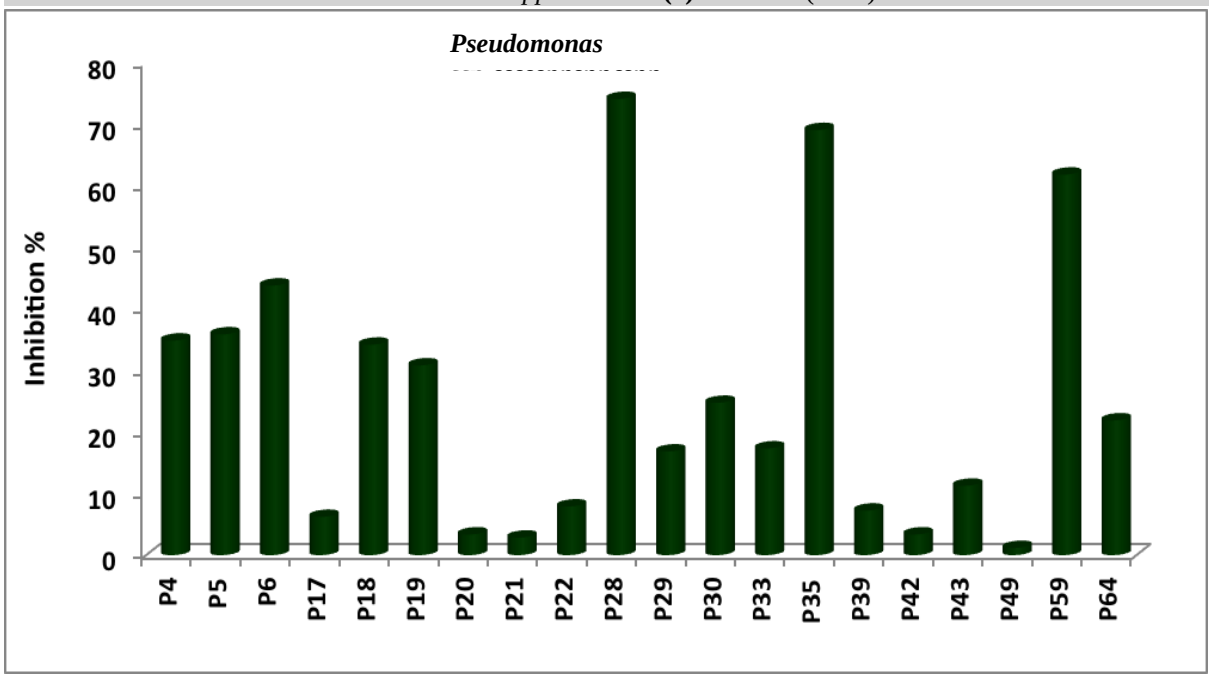


Fig. 1: Evaluation of isolates of *Pseudomonas* spp. against *A. solani* on tomato *in vitro*

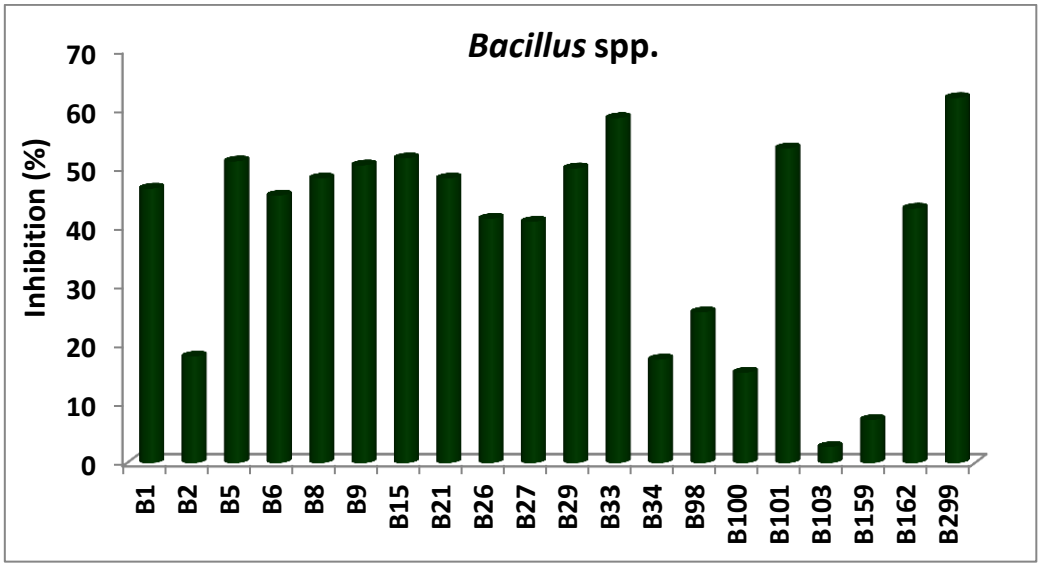
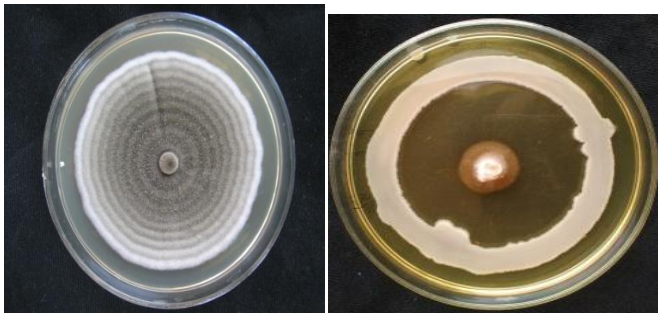
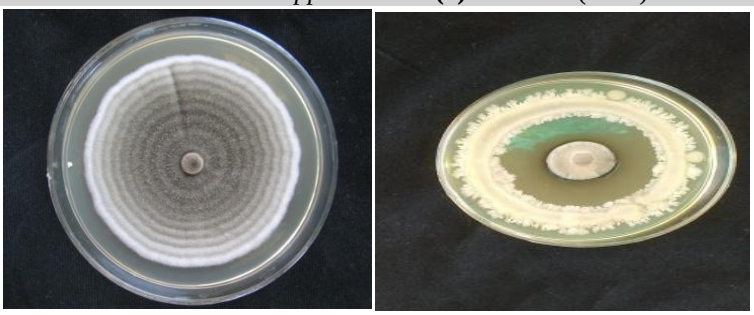


Fig. 2: Evaluation of isolates of *Bacillus* spp. against *A. solani* on tomato *in vitro*



Control **P28**

Plate 1 Effect of *Pseudomonas* (P28) on the radial growth of *Alternaria solani*



Control **B299**

Plate 2 Effect of *Bacillus* (B299) on the radial growth of *Alternaria solani*

Plant growth promoting ability of biocontrol agents

Twenty each of *Pseudomonas* and *Bacillus* isolates were evaluated for their plant growth promoting ability on tomato. The data on germination, seedling vigour, growth parameters like root length, shoot length, fresh biomass and dry biomass were recorded.

There was 100 percent germination in all the treatments of *Pseudomonas* and *Bacillus* showing that all the isolates did not possess any deleterious properties towards seed germination.

Among *Pseudomonas* treatments, the highest root length of 7.86cm was recorded in P43 treatment followed by P59 (7.6cm), which were on a par. As seen from the Table 2 and the isolates varied for their ability to promote root length. Across the isolates, the root length promotion was observed in the range of 3.83 to 7.86 cm, highest being by the isolate P43. The lowest root length was recorded in the P19 treated plants. All other treatments were statistically at par and superior over P5 and P19 isolates. In plants treated with P43, P6, P17 and P59, the root length was >7.0 cm whereas in P18, P20, P28, P33 and P35 treated plants, it was in range of 5.0- 6.5 cm. interestingly, most of the treatments were statistically on a par with control while P5 and P19 were below par than control.

Isolates differed significantly for their ability to promote shoot length. Isolate P20 showed highest shoot length of 8.43cm, which was significantly superior over all other treatments including control unlike in the case of root length where the treatment was on a par with control. The next best treatments were isolatesP33, P35, P5, P6, P17, P19, P22, P30, P33, P35, P39, and P43 where shoot length was recorded in the range of 7.13 to 7.83 cm and all were on a par with P20.While,isolates P4, P5, P9 and P33 showed in the range of >7.5cm. P6, P18, P28, P29 and P42 showed in the range of 5.0- 6.5cm.

Isolate P43 recorded highest seedling vigour index (1502) followed by isolate P17 (1493).The lowest being 932 in case of isolate P21 which was less than control. Isolates P17, P33, P6 and P43 showed seedling vigour index in the range of 1400-1502 while isolatesP21, P19,P5, P29 and P42 showed <1200 seedling vigour index.

Isolate P43 exhibited highest fresh biomass of 0.283g which was significantly superior over all other treatments including control except isolateP17 treatment (0.280g). While isolates P39,P35,P33,P4, P5 and P19 showed shoot fresh biomass in the range of 0.200-0.250g; isolates P21, P28 and isolate P29 showed least fresh biomass, which was < 0.150g. Interestingly, isolates P6, P19, P21, P22, P28, P29, P42, P49 and P64 could not promote shoot biomass more than even control, which again emphasizes that there exists variability among isolates for their plant growth promoting traits.

Isolate P59 exhibited maximum dry biomass of 0.035g followed by isolate P39 (0.032g), isolate P4 (0.031 g), isolate P17 (0.031 g), isolate P43 (0.031 g) and isolate P20 (0.029 g) which were statistically at par. Least dry biomass was recorded in isolates P6, P18, P21 and P28 which was < 0.020g. In case of dry biomass also 14 out of 20 isolates P5, P6, P18, P19, P21, P22, P28, P29, P30, P33, P35, P42, P49 and P64 tested failed to promote dry biomass more than even control.

Among *Bacillus* treatments, the highest root length of 8.43cm was recorded in isolate B98 treatment followed by isolateB100 (8.4cm), which were on a par. As seen from the Table 3 and the isolates varied for their ability to promote root length. Across the isolates, the root length promotion was observed in the range of 3.1 to 8.43cm, highest being by the isolate B98. The lowest root length was recorded in the B29 treated plants. All other treatments were statistically at par and superior over isolate B29 andB5 isolate. In plants treated with isolatesB6, B21, B26 and B98, the root length was > 6.5 cm whereas isolatesB1, B9, B33, B159 and B299 treated plants, it was in range of 4.5-5.5cm. Interestingly, most of the treatments were statistically on par with control while isolatesB29 andB5 were below par than control.

Isolates differed significantly for their ability to promote shoot length. Isolate B1 showed highest shoot length of 8.46cm, which was significantly superior over all other treatments including control unlike in the case of root length where the treatment was on a par with control. The next best treatments were isolatesB1, B2, B5, B6, B8 and B100 where shoot length was recorded in the range of 7.0-8.3cm and all were on a par with B1. While, B27, B33, B101 and B162 showed in the range of 5.0-6.5cm.

Isolate B100 recorded highest seedling vigour index (1636) followed by isolate B26 (1586). The lowest being 996 in case of isolate B27 which was less than control. IsolatesB6, B15, B21 and B98 showed seedling vigour index in the range of (1400-1550) while isolatesB5, B29, B299 and B101 showed <1150 seedling vigour index.

Isolate B100 exhibited highest fresh biomass (0.348g) which was significantly superior over all other treatments including control except isolate B6 treatment (0.314g). While,isolatesB1, B5, B8,B15, B21, B26 and B103 showed shoot fresh biomass in the range of (0.250g-0.300g; isolates B9, B27 and P29, B159 showed least fresh biomass which was < 0.200g. Interestingly, isolates B9, B29, B159 and B101 isolates could not promote shoot biomass more than even control, which again emphasizes that there exists variability among the isolates for their plant promoting traits.

Isolate B2 exhibited maximum dry biomass (0.051g) followed by isolateB26 (0.039g). While isolatesB1, B2, B6 and B21, B98 which were statistically at par Least dry biomass recorded in isolatesB5, B9, B27 and B34 which was < 0.025g.In case of dry biomass also 10 out of 20 isolates B5, B9, B27, B29, B33, B34,B159, B299, B101 and B162) tested failed to promote dry biomass more than even control. Similar observations were made by Agarwal *et al.* who studied the plant growth promoting ability of biocontrol agents. The results showed an increase in shoot and root length as well as enhanced vigour index of tomato seedling as compared to control.

Table 2 Plant growth promoting ability of different isolates of *Pseudomonas* on tomato

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling vigour index	Fresh biomass (g)	Dry biomass (g)
Control	100	5.06	6.73	1179	0.197	0.028
P4	100	5.1	7.63	1273	0.223	0.031
P5	100	3.9	7.5	1140	0.211	0.026
P6	100	7.56	6.8	1436	0.175	0.018
P17	100	7.53	7.4	1493	0.28	0.031
P18	100	5.5	6.66	1216	0.175	0.018
P19	100	3.83	7.6	1143	0.202	0.023
P20	100	5.4	8.43	1383	0.234	0.029
P21	100	3.76	5.56	932	0.117	0.017
P22	100	5.73	7.56	1329	0.189	0.025
P28	100	5.4	6.6	1200	0.13	0.013
P29	100	5.1	6.7	1180	0.151	0.012

P30	100	5.8	7.5	1330	0.206	0.021
P33	100	6.33	7.83	1416	0.209	0.025
P35	100	6.23	7.66	1389	0.235	0.027
P39	100	6.83	7.13	1396	0.244	0.032
P42	100	5.63	6.36	1199	0.161	0.024
P43	100	7.86	7.16	1502	0.283	0.031
P49	100	6.4	6.23	1263	0.16	0.028
P59	100	7.6	6.9	1450	0.25	0.035
P64	100	5.66	6.83	1249	0.189	0.029
SE(m)		1.02	0.51		0.005	0.002
SE(d) ±		1.45	0.72		0.008	0.003
CD		2.92	1.45		0.016	0.006
CV (%)		1.46	0.59		0.707	2.232

Table 3 Plant growth promoting ability of different isolates of *Bacillus* on tomato

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling vigour index	Fresh Biomass (g)	Dry Biomass (g)
Control	100	5.06	6.73	1179	0.197	0.028
B1	100	4.56	8.46	1302	0.26	0.032
B2	100	5.83	7.43	1326	0.306	0.051
B5	100	4.26	7.16	1142	0.256	0.025
B6	100	6.93	7.76	1469	0.314	0.036
B8	100	6.13	7.76	1389	0.247	0.028
B9	100	5.23	6.36	1159	0.184	0.024
B15	100	7	7.23	1423	0.241	0.027
B21	100	6.9	7.3	1420	0.229	0.036
B26	100	8.1	7.76	1586	0.285	0.039
B27	100	4.4	5.56	996	0.117	0.023
B29	100	3.1	7.03	1013	0.136	0.017
B33	100	5.1	6.43	1153	0.208	0.021
B34	100	4.86	6.93	1179	0.202	0.022
B98	100	8.43	7.23	1566	0.219	0.037
B100	100	8.4	7.96	1636	0.348	0.038
B103	100	6	7.13	1313	0.279	0.03
B159	100	5.33	7.2	1253	0.184	0.02
B299	100	5.36	6	1136	0.172	0.026
B101	100	4.81	5.4	1021	0.128	0.027
B162	100	4.83	5.9	1073	0.229	0.027
SE(m)		0.90	0.65		0.003	0.001
SE(d) ±		1.27	0.93		0.005	0.002
CD		2.56	1.88		0.010	0.005
CV (%)		1.30	0.78		0.407	1.536

Plate 3 Plant growth promoting ability of different isolates of *Pseudomonas* on tomato

Plate 4 Plant growth promoting ability of different isolates of *Bacillus* on tomato

CONCLUSION

Early blight of tomato is an economically important disease in Telangana state caused by *A. solani* (Ellis and Martin) Jones and Grout. Under *in vitro* conditions, the disease was well controlled by biocontrol agents. Isolates of *Pseudomonas* and *Bacillus* with a potential to be deployed as biocontrol agents have been identified. As a spin-off, these isolates could also promote growth of tomato plants by enhancing seedling vigour index and thereby invigorate growth.

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