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RESEARCH ARTICLE

Study the biochemical and molecular basis of ISR and plant growth promotion in maize under pathogenic stress condition of *Rhizoctonia solani*

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ABSTRACT

The fungus Rhizoctonia solani Kühn causes banded leaf and sheath blight, one of the most common maize diseases. The goal of this study was to evaluate bioagents for plant growth promotion in maize under Rhizoctonia solani pathogenic stress. The bioagents utilised were *Bacillus licheniformis B-02, B. pumilus B-07, B. amyloliquefaciens B-16, B. subtilis B-20, and B. amyloliquefaciens* UI. They produced amylase, HCN, IAA, H2O2, Siderophore, ammonia, chitinase, and protease, starch hydrolysis, and phosphate solubilization. After 30 and 45 days of seeding, *Bacillus subtilis B-20 and B. amyloliquefaciens B-16* colonisation on the root surface and rhizosphere increased total chlorophyll, carbohydrate, and protein content in plant leaves. Plants inoculated with B. subtilis B-20 had the highest PAL activity. The epidermis, vascular bundles, and pericycle of B*. amyloliquefaciens* B-16 inoculated maize leaf and sheath showed maximum lignin deposition.

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INTRODUCTION

Plant diseases produce losses that vary depending on the plant type, pathogen, location, environment, control methods, and combinations of these factors (Agrios, 2005). In Uttar Pradesh, the fungus Rhizoctonia solani Kühn causes banded leaf and sheath blight, an economically important disease in maize. Srilanka was the first to report this ailment as 'Sclerotial'. In the following two decades, it became an epidemic. The disease is now a severe issue in India and numerous other nations in Tropical Asia where maize is produced (Sharma et al, 1993).

Plants fight parasitic fungus and other diseases by triggering localised and systemic reactions. Viruses that infect resistant plants cause localised hypersensitivity. Pathogens propagate via complex molecular pathways (Bakker et al., 2007). As a result of this, synthesis of pathogenesis related (PR) proteins are produced, inhibiting invader growth (Shoresh et al. 2010; Xu et al., 2015). Several plant protein families are linked to ROS control, localised responses, ISR and SAR (Singh, 2008).

Concerns about chemical safety, environmental impact, and expense have increased demand for non-chemical plant disease treatment. Bacillus spp. have showed promise in biological control of R. solani. It has been shown that these bio agents can induce ISR and signalling in a host–pathogen interaction in maize-Rhizoctonia Patho-System. As a result, the practical impact of these selected bioagents in ISR and pathogen defence responses has been mostly unknown.

MATERIALS AND METHODS

Twelve bacterial strains showed highest suppression of mycelial growth in dual plate and in vitro assay screening and were chosen for genotyping and species identification. Sequencing and analysis of DNA sequences were used to identify newly isolated antagonist bacterial strains up to species level. The strains were identified through NCBI Nucleotide Sequence Database. Bacterial antagonists were cultivated for 36 hours at 28°C in a BOD incubator shaker (Humans Lab, India). Extraction buffer was added to the collected cells and centrifuged for 5 minutes at 10000 g. (Sigma, model 3K30). Bacterial DNA was isolated using a kit (B.R. Biochem Life Sciences) and following the instructions. The PCR reaction mixture (50 l) contains 10 l Taq buffer. In order to visualise the reaction mixture, it was electrophoresed in 1.2 percent agarose gel (BioRed, India) and stained with ethidium bromide using a gel documentation system (BioRed, India) (Singh et al., 2016). Xelris sequenced the 16s rRNA gene. The NCBI server analysed DNA sequences and identified through sequence analysis. The strains were identified through NCBI Nucleotide Sequence Database.

The most successful and promising bioagents were in vitro evaluated for PGP characteristics. Using Dey et al techniques selected strains were evaluated for IAA, siderophore, ammonia, and phosphate solubilisation (2004). These enzymes were produced using procedures from Bergey's Manual of Systematic Bacteriology with minor modifications.

Spot inoculations of freshly produced bacterial cultures were cultured for 7 days at 28 °C. A halo zone formed surrounding the colony. In the liquid broth containing zinc compounds, the solubilization rate of zinc was evaluated quantitatively (Sharma et al., 2016). g Zn ml-1 liquid broth

The aseptic preparation of bacterial cell suspensions was disclosed by Chithrashree et al (2011). A chosen Bacillus strain was cultured in pseudomonas broth for 72 hours. After 72 hours, the bacterial cells were extracted by centrifuging at 8000g for 5 minutes. The pellets collected in sterile distilled water and spectrophotometrically calibrated to 108cfu ml-1. To create the P. fluorescens powder formulation, Chithrashree et al. (2011) used aseptic procedures

We tested the impact of bioagents and salicylic acid on the buildup of enzymes linked to induced systemic resistance and disease progression. Soaked in fungicide (Tilt 25 EC, 0.01 percent), salicylic acid (100 ppm) and one SA and bioagent treatment. In this example, pre-soaked seed (100 ppm salicylic acid) was treated with 5g kg-1 B. amyloliquefaciens B-16 talc formulation (as mentioned above). The treated maize seed was seeded in pots pre-inoculated with R. solani. After 30 days of seeding, enzyme activity was evaluated using the aforesaid method. The illness progression was measured on a 1-5 scale.

After 3 days after inoculation, the effect of bioagents and SA application on symptom development and infection was evaluated in vitro. Plant leaves (2' x 2') were placed on moist chamber. Each leaf was inoculated with R. solani and incubated for 3 days at 25°C. A water-soaked lesion formed 24 hours after pathogen inoculation. Grass was decolored with glacial acetic acid and ethanol for 3 days, then stained with lactophenol cotton blue. A compound light microscope was used to observe infection structure growth. The disease intensity is rated 45 days after inoculation (Ahuja and Payak, 1983). Disease in four sheaths; lesions numerous and coalescent. Calculating the %Disease Index (PDI), PDI = (Sum sum of individual rating/ No. of leaves or samples analysed x Maximum disease rating) / 100

The variations in total chlorophyll, carbohydrate, and protein content in plant leaves were measured using Sadasivam and Manickam's methodology (1996). These were measured at 30, 45, and 60 days after seeding using Tiwari et al. (2011) procedures. In nethouse tests, TPC, proline, PAL activity, peroxidase, 1,3 gluconase, chitinase, superoxide dismutase, catalase, and ascorbate peroxidase were assessed 30 and 45 days after seeding.

After 45 days, maize plants were uprooted. Transverse sheath pieces were manually cut and counterstained with safranin. Lignin differential staining was performed by fixing tiny slices in ethanol (10-25-50-75-80-95%).

All experiments were tripled. The data were analysed using a completely randomised design (CRD) for the laboratory trials.

RESULT

In vitro plant growth promotion was studied on all five strains. Their starch hydrolysis and phosphate solubilization abilities were also assessed. The PGP test results for B. licheniformis B-02, B. pumilus B-07, B. amyloliquefaciens B-16, and B. subtilis B-20 were all positive. In vitro, B. amyloliquefaciens UI failed to produce HCN, H2O2 or ammonia (Table 1).

On the 30th day after sowing, different bio inoculants were tested in maize. All five strains were tested in a net house (Table 2). Each of the five strains studied (B. licheniformis B-02, B. pumilus B-07, B. amyloliquefaciens UI) significantly increased seed germination. B. amyloliquefaciens B-16 (94.36%) had the best germination followed by B. licheniformis B-02 (91.25%) and B. subtilis B-20 (91.25%). (89.12 percent). However, only 82.50 percent of control and 85.33 percent of B. pumilus B-07 seeds germinated (Table 2). The highest vigour index I (7043.59) was recorded in seeds treated with B. licheniformis B-02, followed by B. amyloliquefaciens B-16 (574.45) and B. amyloliquefaciens UI (5298.69). (2680.43). After 30 DAS, plants treated with B. subtilis B-20 (607.79) had the highest vigour index II, followed by B. amyloliquefaciens UI (492.96) and B. amyloliquefaciens B-20 (434.05). (Table 2).

In nethouse tests, bioagent colonisation on the root surface and rhizosphere increased total chlorophyll, glucose, and protein content in plants 30 and 45 days after sowing compared to pathogen injected and control plants. After 30 days of sowing, B. subtilis B-20 (79.55 mg g-1 fresh wt.) had the highest chlorophyll content followed by B. licheniformis B-02 (78.45 mg g-1 fresh wt.) and B. amyloliquefaciens UI (72.50 mg g-1 fresh wt.). The plant treated with R. solani alone (49.60 mg g-1 fresh wt.) had the lowest chlorophyll concentration (69.67 mg g-1 of fresh wt.). After 45 days after seeding, plants treated with B. amyloliquefaciens UI had the highest chlorophyll content (87.50 mg g-1 fresh wt.) followed by B. subtilis B-20 (82.59 mg g-1 fresh wt.) and B. licheniformis B-02 (81.25 mg g-1 fresh wt) (Table 3).

Table 2. Effects of *Bacillus* spp. on seed germination in maize

Treatments	Plant growth parameters		
	Germination (%)	vigour index I	vigour index II
R. solani + B. lichaniformis B-02	91.25	7043.59	425.22
R. solani + B. pumilus B-07	85.33	4550.65	418.11
R. solani + B. amyloliquefaciens B-16	94.36	5764.45	434.05
R. solani + B. subtilis B-20	89.12	5192.13	607.79
R. solani + B. amyloliquefaciens UI	87.25	5298.69	492.96
Control	82.50	2680.43	222.75
CD at 0.05%	2.66	12.98	8.56

Table 3. Effects of *Bacillus* spp. on total chlorophyll (mg g^{-1} fresh wt.), carbohydrate and protein content (BSA/g) in maize under pathogenic stress after 30 and 45 days of sowing under net house conditions

Treatments	Biochemical parameters					
	Total chlorophyll		Total carbohydrate		Total protein	
	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS
R. solani (alone)	49.60	52.49	10.12	12.47	1.23	1.96
R. solani + B. lichaniformis B-02	78.45	81.25	16.63	18.21	4.96	5.33
R. solani + B. pumilus B-07	71.50	75.96	16.32	15.33	3.66	4.75
R. solani + B. amyloliquefaciens B-16	69.45	72.67	16.96	15.47	5.45	7.72
R. solani + B. subtilis B-20	79.55	82.59	24.03	24.35	4.97	5.67
R. solani + B. amyloliquefaciens UI	72.50	87.50	15.31	18.92	3.49	4.96
Control	69.67	65.93	12.47	14.96	1.74	2.46
CD at 0.05%	1.57	2.96	1.03	1.66	0.55	0.36

After 30 days of sowing, B. subtilis B-20 (24.03 mg glucose/100g fresh wt.) had the highest carbohydrate content followed by B. amyloliquefaciens B-16 (16.96 mg glucose/100g fresh wt.). After 30 and 45 days of seeding, other treatments exhibited comparable trends (Table 3).

After 30 days of sowing, B. amyloliquefaciens B-16 (5.45 mg protein as BSA/g fresh wt.) was followed by B. subtilis B-20 (4.97 mg protein as BSA/g fresh wt) (Table 3). After 30 days of sowing, R. solani alone (1.23 mg protein as BSA/g fresh wt.) and control plants (1.74 mg protein as BSA/g fresh wt.) had the lowest protein content (Table 3). After 45 days of sowing (Table 3).

Among the three cultivars tested, B. licheniformis B-02 had the highest accumulation of total phenolic in leaves (36.4 g of gallic acid equivalent g-1 fresh wt.), followed by B. subtilis B-20 (30.9 g of gallic acid equivalent fresh wt.) and B. pumilus B-07 (Table 4). After 45 days of seeding, plant leaves showed similar tendencies. However, both control and R. solani treated plants had low phenolic content (Table 5).

After 30 days of planting, B. licheniformis B-02 (4.01 moles per g tissue) and B. amyloliquefaciens B-16 (3.75 moles per g tissue) inoculated maize leaves had considerably higher proline content than control. After 45 days after seeding, the tendency continued (Table 4).

Table 4. Effects of *Bacillus* spp. on total phenolic and proline content in maize under pathogenic stress

Treatments	Biochemical parameters	
	Total phenolic Content	*Proline content*

	30 DAS	45 DAS	30 DAS	45 DAS
R. solani (alone)	18.46	20.75	1.49	1.32
R. solani + B. lichaniformis B-02	36.40	29.31	4.01	3.11
R. solani + B. pumilus B-07	29.37	24.33	3.25	2.66
R. solani + B. amyloliquefaciens B-16	26.45	25.96	3.75	3.01
R. solani + B. subtilis B-20	30.96	34.32	2.87	2.14
R. solani + B. amyloliquefaciens UI	25.96	28.79	2.97	2.56
Control	12.36	16.62	0.72	0.55
CD at 0.05%	1.66	2.75	0.37	0.25

Table 5. Effects of *Bacillus* spp. on induction and activation of enzymes in maize under pathogenic stress after 30 and 45 days of sowing under nethouse conditions

Treatments	Biochemical parameters							
	Phenylalanine ammonia lyase (PAL)		*Peroxidase*		*β 1,3-glucanase*		*Chitinase*	
	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS
R. solani (alone)	19.55	16.75	5.96	4.62	12.46	9.47	12.37	9.62
R. solani + B. lichaniformis B-02	24.55	25.31	11.33	9.32	24.33	20.15	20.55	16.56
R. solani + B. pumilus B-07	30.05	29.15	15.32	11.47	21.55	18.95	18.33	13.63
R. solani + B. amyloliquefaciens B-16	28.76	28.35	18.49	14.32	26.33	24.32	21.41	18.39
R. solani + B. subtilis B-20	33.50	32.46	13.96	9.67	22.36	18.66	17.54	14.32
R. solani + B. amyloliquefaciens UI	26.44	24.32	14.37	12.05	14.35	12.96	22.15	17.96
Control	7.54	9.33	3.01	2.51	3.46	4.26	3.45	5.14
CD at 0.05%	2.55	2.12	0.96	0.55	1.45	2.11	1.66	1.89

Most peroxidase activity was found in B. amyloliquefaciens B-16 (18.49 unit mg-1 min-1 fresh wt.) followed by B. pumilus B-07 (15.32 unit mg-1 min-1 fresh wt.) and B. amyloliquefaciens UI (14.37 unit mg-1 min-1 fresh wt.) treated plants (3.01 unit mg-1 min-1 fresh wt.) and R. solani treated (Table 5). After 45 days of sowing, a similar pattern emerged.

1 glucanase activity (26.33 mol Glc mg-1 protein h-1) in B. amyloliquefaciens B-16 infected maize plants. In contrast, following 30 days of seeding R. solani treated plants (12.46 mol Glc mg-1 protein h-1) had the lowest 1,3 glucanase activity (3.46 mol Glc mg-1 protein h-1). After 45 days after seeding, the tendency held. All treatments have similar chitinase activity (Table 5).

Inoculated plants with B. amyloliquefaciens B-16 increased gallic acid levels the most (52.84 g/l), followed by B. subtilis B-20 (51.09 g/l) and B. pumilus (45.98 g/l). After 30 days of seeding, control plants had a gallic acid level of 25.90g g-1 fresh weight (Table 6).

Similar results were found in leaves treated with B. amyloliquefaciens UI (9.33-unit mg-1 min-1) followed by B. licheniformis B-02 (9.15-unit mg-1 min-1) and B. amyloliquefaciens B-16 (7.33-unit mg-1 min-1) 30 days after planting (Table 8). After 45 days of planting, plants treated with B. licheniformis B-02 (7.01 unit mg-1 min-1 fresh wt.) and B. amyloliquefaciens UI (6.33 unit mg-1 min-1 fresh wt.) had considerably more SOD than R. solani and control plants (Table 7). After 30 days of planting in nethouse trials, catalase activity (mole H2O2 decreased mg-1 prot. min-1) changed considerably between plants treated with bioagents and untreated plants (uninoculated). The highest catalase activity was seen in plant leaves treated with B. amyloliquefaciens B-16 (7.96 mole H2O2 reduced mg-1 prot. min-1) followed by B. subtilis B-20 (6.55 mole H2O2 reduced mg-1 prot. min-1) 30 days after sowing. After 45 days of seeding, ascorbate peroxidase activity in plant leaves revealed a similar trend (Table 7).

Table 6. Effects of *Bacillus* spp. on accumulation of phenolic acids in maize leaves inoculated with *R. solani* under glasshouse conditions

Treatments	Phenolic acids (µg/g fresh wt.)			
	30 days		45 days	
	Gallic acid	Ferulic acid	Gallic acid	Ferulic acid
R. solani	31.45	1.01	41.09	1.55
R. solani + B. lichaniformis B-02	42.15	1.45	52.50	2.33
R. solani + B. pumilus B-07	45.98	1.66	56.26	2.40
R. solani + B. amyloliquefaciens B-16	52.84	2.25	61.94	3.13
R. solani + B. subtilis B-20	51.09	2.55	58.79	2.82
R. solani + B. amyloliquefaciens UI	44.25	1.97	52.96	2.05
Control (untreated)	25.90	0.47	31.56	0.97
CD at 0.05%	2.01	0.15	2.50	0.11

Table 7. Effects of *Bacillus* spp. on SOD, catalase and ascorbate peroxidase activity in maize under pathogenic stress after 30 and 45 days of sowing under net house conditions

Treatments	Biochemical parameters					
	Superoxide dismutase (SOD)		Catalase		Ascorbate peroxidise	
	30 DAS	45 DAS	30 DAS	45 DAS	30 DAS	45 DAS
R. solani (alone)	2.45	1.72	2.97	2.23	1.97	1.77
R. solani + B. lichaniformis B-02	9.15	7.01	5.23	3.55	4.33	3.56
R. solani + B. pumilus B-07	5.27	3.94	4.82	3.96	4.96	3.23
R. solani + B. amyloliquefaciens B-16	7.33	5.92	7.96	6.25	6.03	4.96
R. solani + B. subtilis B-20	6.15	4.23	6.55	4.75	5.05	4.32
R. solani + B. amyloliquefaciens UI	9.33	6.33	5.66	4.96	3.07	2.75
Control	0.75	0.96	0.66	0.67	0.63	0.75

CD at 0.05%	0.25	0.22	0.33	0.23	0.45	0.66

In nethouse trials, bioagents reduced disease compared to pathogen treated plants. After 30 and 45 days of sowing, dramatically reduced disease symptom and average lesion length in nethouse trials (Table 8).

Table 8. Effects of *Bacillus* spp. on disease severity in maize challenged with *R. solani* after 30 and 45 days of sowing under net house conditions

Bacillus spp.	Disease scoring, score rate (1-5)	
	30 DAS	45 DAS
R. solani	4	4
B-02	1	2
B-07	1	2
B-16	1	1
B-20	3	3
UI	2	2
Control	-	-
CD at 5%	2.75	3.66

After 30 and 45 days of planting, B. pumilus B-07 inoculated plants had significantly lower disease index (1 and 1) than R. solani inoculated plants (4 and 4). (Table 8).

Plants treated with different Bacillus spp. strains deposited lignin differently. Compared to other treatments, B. amyloliquefaciens B-16 and B. pumilus B-07 inoculated plant leaf and sheath showed the highest lignin deposition.

Plants infected with R. solani and colonised by bioinoculants containing salicylic acid tend to overproduce and accumulate defence related macromolecules and enzymes. After 30 days of planting in the nethouse, plants inoculated with B. amyloliquefaciens B-16 and SA (29.65) had higher PAL than SA alone (24.35) or R. solani (16.42) and fungicide (Tilt 25 EC) treated plants (10.35). (Table 9). After 30 days of seeding, peroxidase, ascorbate peroxidase, and 1,3 glucanase activities were seen in plant leaves.

After 30 days of seeding, plants treated with B. amyloliquefaciens B-16 and SA showed less illness than other treatments (Table 9). Similarly, plants treated with B. amyloliquefaciens B-16 and SA had better lignification than plants treated with SA and R. solani (Figure 2).

The effects of Bacillus spp. therapy on lesion and infection structure development in excised leaf under moist chamber were studied in vitro after 3 days of inoculation. The largest lesion was seen in an R. solani-only leaf. Comparatively, SA and B. amyloliquefaciens B-16 treated leaves had the least lesion (Figure 3). Similarly, leaves treated with R. solani alone had the most infection cushion. However, additional treatments revealed

comparable patterns after 3 days of inoculation in vitro (Figure 4).

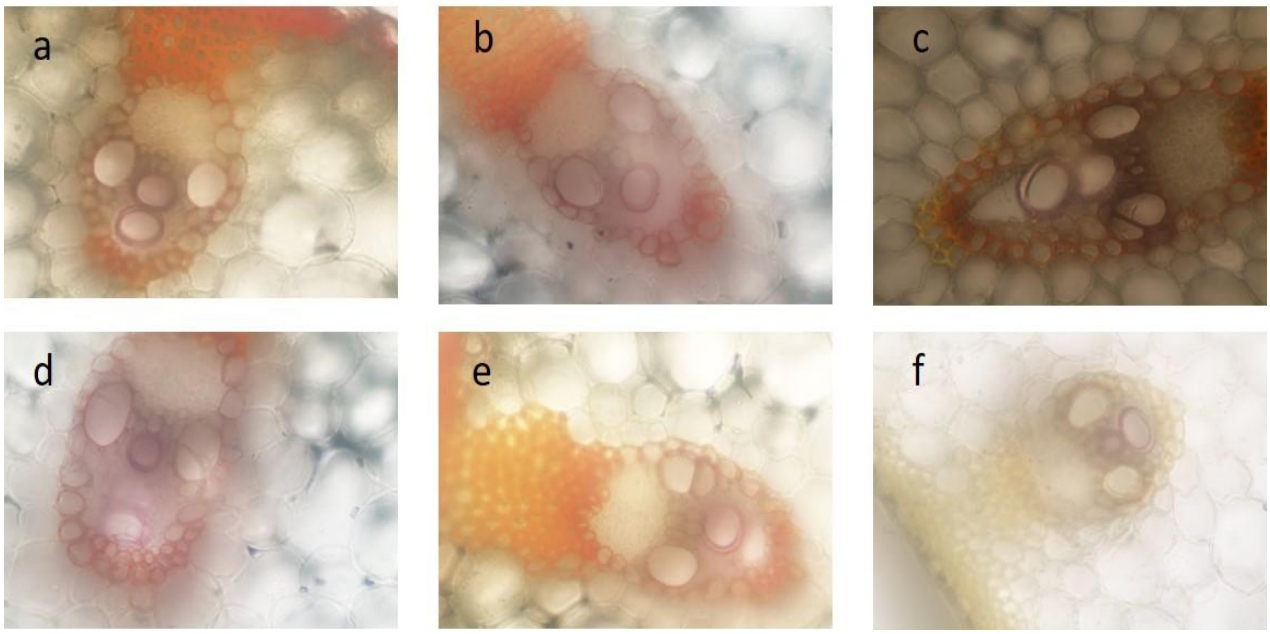

Figure 1. Effects of bioagents treatments on induction of phenylpropanoid pathway and lignification of cells of leaf sheath of maize. Differential staining showed the lignification of xylums, phloems and other cells. Treatments were, (a) *R. solani + B. lichaniformis* B-02, (b) *R. solani + B. pumilus* B-07, (c) *R. solani + B. amyloliquefaciens* B-16, (d) *R. solani + B. subtilis* B-20, (e) *R. solani + B. amyloliquefaciens* UI, and (f) *R. solani* (control)

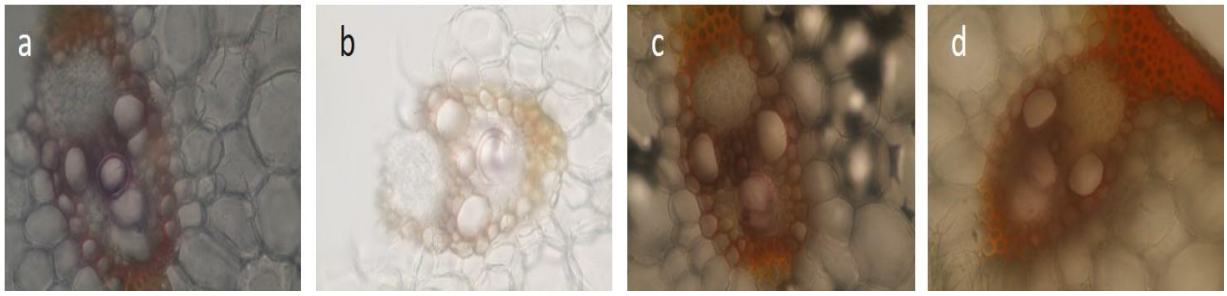

Figure 2. Effects of fungicide (Tilt 25 EC), salicylic acid and *B. amyloliquefaciens* B-16 treatments on induction of phenylpropanoid pathway and lignification of cells of leaf sheath of maize. Differential staining showed the lignification of xylums, phloems and other cells. Treatments were, (a) *R. solani* (alone), (b) *R. solani +* fungicide (Tilt 25 EC), (c) *R. solani +* salicylic acid (SA), and (d) *R. solani +* salicylic acid (SA) + *B. amyloliquefaciens* B-16.

Table 9. Effects of *Bacillus amyloliquefaciens,* fungicide and salicylic acid (SA) application on defence related enzymes and disease scoring in maize under pathogenic stress after 30 days of sowing under net house conditions

Treatments	Biochemical parameters				
	PAL	Peroxidase	Ascorbate peroxidase	B - 1,3 Glucanase	Disease score (scale 1-5)
R. solani (alone)	16.42	5.19	1.55	11.32	4
R. solani + Fungicide	10.35	3.56	0.96	7.22	4
R. solani + SA	24.35	9.67	2.45	16.96	2
R. solani + SA + *B. aamyloliquefaciens* B-16	29.65	11.47	3.79	26.33	1
CD at 0.05%	2.77	0.99	0.25	2.33	-

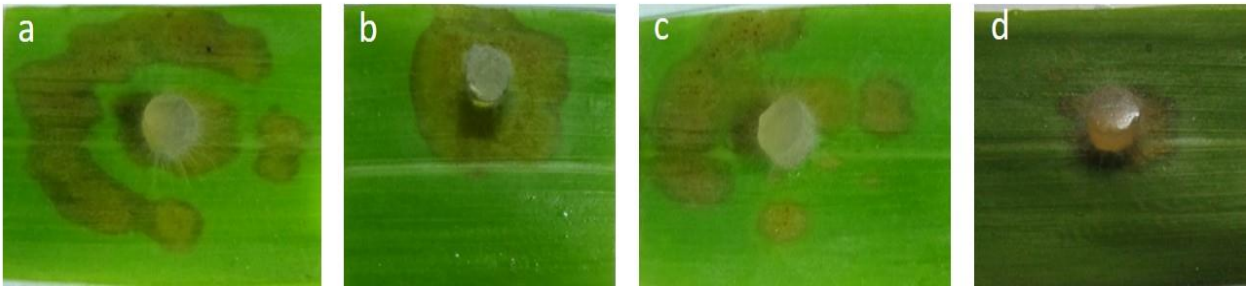

Figure 3. Effects of *B. amyloliquefaciens* B-16 and SA application on symptom development under pathogenic stress after 3 days of inoculation under *in vitro* conditions. Treatments were: (a) *R. solani* (alone), (b) *R. solani* + Fungicide*, (c) R. solani* + SA, and (d) *R. solani* + SA+ *B. amyloliquefaciens* B-16.

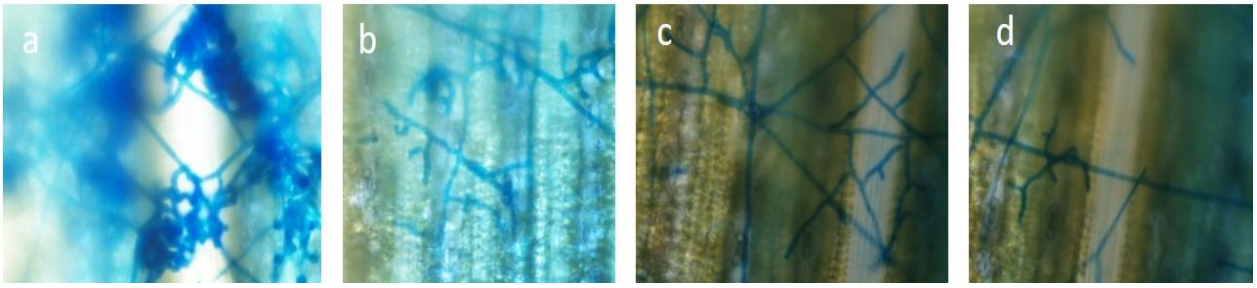

Figure 4. Effects of *B. amyloliquefaciens* B-16 and SA application on infection cushion development under pathogenic stress after 3 days of inoculation under *in vitro* conditions. Treatments were: (a) *R. solani* (alone), (b) *R. solani* + Fungicide*, (c) R. solani* + SA, and (d) *R. solani* + SA+ *B. amyloliquefaciens* B-16.

On the other hand, bioagents enhance the number of nodes, internodes, and leaves per plant between 30 and 45 days after seeding (Table 10 and 11). R. solani infected plants treated with either antagonist had greater health than control plants. After 30 and 45 days of planting, plants inoculated with B.

licheniformis B-02 grew the fastest compared to other bioagents treated plants (Table 10 and 11; Figure 5).

Table 10. Effects of *Bacillus* spp. on plant growth promoting attributes in maize after 30 days of sowing under net house conditions

Treatments (*Bacillus* spp.)	Plant growth promoting attributes								
	Avg. no. of leaf	Avg. no. of nodes	Avg. no. of internodes	Avg. shoot length (cm)	Avg. root length (cm)	Avg. fresh wt. of shoot (gm)	Avg. fresh wt. of root (gm)	Avg. dry wt. of shoot (gm)	Avg. dry wt. of root (gm)
B. lichaniformis B-02	4.06	4.06	3.06	43.03	34.16	11.39	11.00	2.41	2.25
B. pumilus B-07	4.33	4.33	3.33	27.60	25.73	7.06	5.09	2.80	2.10
B. amyloliquefaciens B-16	5.08	5.08	4.08	34.03	27.06	10.60	4.00	2.75	1.85
B. subtilis B-20	4.55	4.55	3.55	31.46	26.80	18.61	8.31	3.87	2.95
B. amyloliquefaciens UI	4.24	4.24	3.24	39.23	21.5	9.16	6.03	3.15	2.50
Control	3.15	3.15	2.15	16.26	16.23	8.6	3.6	1.75	0.95
CD at 0.05%	0.57	0.25	0.33	2.10	1.55	1.01	0.96	0.45	0.33

Table 11. Effects of *Bacillus* spp. on plant growth promoting attributes in maize After 45 days of sowing under net house conditions

Treatments (*Bacillus* spp.)	**Plant growth promoting attributes**								
	Avg. no. of leaf	Avg. no. of nodes	Avg. no. of internodes	Avg. shoot length (cm)	Avg. root length (cm)	Avg. fresh wt. of shoot (gm)	Avg. fresh wt. of root (gm)	Avg. dry wt. of shoot (gm)	Avg. dry wt. of root (gm)
B. lichaniformis B-02	8.96	8.96	7.80	63.23	46.26	21.33	11.00	3.75	3.32
B. pumilus B-07	9.26	9.26	8.26	57.66	45.23	17.66	8.66	3.61	3.13
B. amyloliquefaciens B-16	10.02	10.02	9.02	67.93	47.63	18.66	7.00	3.25	2.58
B. subtilis B-20	10.71	10.71	9.71	62.63	56.83	28.60	14.30	5.41	4.14
B. amyloliquefaciens UI	9.7	9.7	8.7	61.13	43.40	17.66	11.33	4.40	3.28
Control	7.74	7.74	6.74	36.26	25.33	14.10	5.66	2.13	1.25
CD at 0.05%	1.25	0.66	0.54	3.01	2.01	1.21	1.02	0.33	0.25

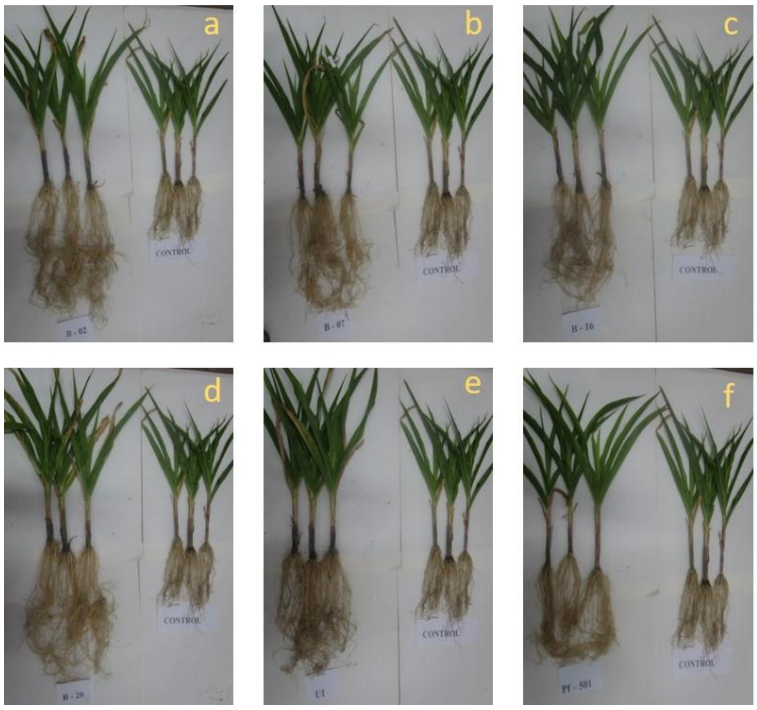

Figure 5. Effects of *Bacillus* spp. on plant growth promotion in maize under net house conditions after 45 days of sowing. A, *B. lichaniformis* B-02; b, *B. pumilus* B-07; c, *B. amyloliquefaciens* B-16; d, *B. subtilis* B-20; e, *B. amyloliquefaciens* UI; f; *Pseudomonas fluorescens* Pf-501 as standard.

DISCUSSION

A virulent R. solani strain infected maize plants was used to assess various defence related macromolecules and enzymes. In the presence of pathogenic pathogens, Bacillus spp. enhanced the expression and accumulation of biomolecules such as phenolics, flavonoids, and antioxidant enzymes (van Wees et al., 1997). A diverse range of insect pests and infections, parasitic plants, parasitic insects, and fungi are effective against induced resistance. It has been reported that systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two different types of induced resistance, evoked by different stressors and crop plants. Plant growth-promoting microbes (PGPM) cause ISR (van Wees et al. 1997). The capacity of PGPM strains to elicit ISR is restricted to some plant species (Durrant and Dong 2004). (Van Wees et al., 1997). SAR is followed by increased endogenous SA levels and the overexpression of many genes, including PR genes for proteins (Ward et al., 1991). Several PR proteins have antifungal action.

Chitinases and -1-3-glucanases are important plant defence enzymes that degrade fungus cell walls (PR2, PR3, PR4, PR8, and PR11). A pathogen has been shown to induce chitinase and -1-3-glucanase in plant leaves (Ying-Zhang et al., 2003; Zhang et al., 2016).

Antioxidant enzymes were only stimulated by pathogenic bioagents in our study. PAL deamines C-phenylalanine to trans-cinnamic acid. That route provides precursors for phenolic and lignin (Singh et al., 2016). Recently, SA therapy that promotes

resistance to FOL (Chandra et al., 2016) has been shown to increase PAL activity. PAL gene expression is increased in pathogen-induced SAR maize plants in this study. PAL, peroxidase, chitinase and other antioxidant enzymes were reported to be pathogen-induced defence responses in maize when salpho-salicylic acid was applied. In this study, inoculation with bioagent strains stimulated expression of the inducible PAL gene, confirming its role in R. solani-induced SAR in maize Many plant species, including wheat (Singh et al., 2016), have exhibited enhanced PAL synthesis in response to root colonisation by bioagents under pathogen infestations (Singh et al., 2016). Pathogens cause accumulation and enhanced activity of the PAL biosynthetic enzyme peroxidase (Singh et al., 2016).

The current study explores the use of native PGPMs to treat this condition (Singh et al., 2016). This method is eco-friendly and maintains soil health. Recent research revealed the existence and interaction of bioagents in several agricultural rhizospheres, with beneficial effects of two bacteria in several crop rhizospheres (Singh et al., 2008). The microorganisms produced IAA, which increased root density by forming adventitious roots and root hairs.

CONCLUSION

In vitro, 25 Bacillus strains were isolated from maize rhizospheres and showed antagonistic potential against Rhizoctonia solani in dual plate assays. The study included five Bacillus strains chosen for their possible antagonistic capabilities against Rhizoctonia solani on dual plate. R. solani mycelial growth was inhibited by B. subtilis B-20 culture filtrateA favourable reaction was observed for amylase, HCN, IAA, H2O2, Siderophore, Ammonia, Chitinase, and Protease synthesis, as well as starch hydrolysis and phosphate solubilization in four strains of B. licheniformis B-02. After 30 and 45 days of seeding, Bacillus subtilis B-20 and B. amyloliquefaciens B-16 colonisation on the root surface and rhizosphere increased total chlorophyll, carbohydrate, and protein content in plant leaves. Plants inoculated with B. subtilis B-20 had the highest PAL activity. After 30 and 45 days of seeding, B. amyloliquefaciens B-16 treated plants had the highest levels of gallic acid and catalase activity in their leaves. The epidermis, vascular bundles, and pericycle of B. amyloliquefaciens B-16 inoculated maize leaf and sheath showed maximum lignin deposition. More nodes, internodes, and leaves per plant were detected in plants inoculated with B. licheniformis B-02 compared to other Bacillus spp. treated plants after 30 and 45 days of sowing.

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