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Bacillus amyloliquefaciens (RB19): A potential PGPR in managing sugarcane red rot disease

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Abstract
Sugarcane is an important cash crop of India grown in an area of 3.93 million hectares with a production of 167 million tonnes. Most of the popular cultivars of sugarcane were withdrawn from cultivation due to their susceptibility to red rot disease incited by *Colletotrichum falcatum* Went. In Andhra Pradesh, yield losses to a tune of 50% are reported on susceptible cultivars. In the present study an attempt was made to reduce the losses due to red rot disease using PGPR. Different isolates of rhizoplane bacteria were collected from various locations in Visakhapatnam district of Andhra Pradesh, India and cultured by standard protocols. These isolates were tested for their growth promoting traits and antagonistic activity against *C. falcatum* under *in vitro* and *in vivo* conditions. Among the rhizoplane bacteria tested, rhizobacteria 7 and 19 were found promising under *in vitro* conditions and were selected for sett treatment of single noded setts and evaluated for sett germination and seedling growth. Sett treatment with rhizobacteria resulted in increased sett germination and seedling vigour when compared to control. Further, these isolates were tested for red rot management under field conditions by inoculating red rot susceptible genotype, Co 997 pre-treated with PGPR, by plug and cotton swab methods. All the canes in various treatments inoculated with *C. falcatum* pathotype Cf 05 by plug method produced highly susceptible reaction. However, differential reaction was observed in cotton swab method of inoculation. Disease severity was found to be less in treatments where the single noded setts and seedlings were treated with Rhizobacteria 19. The prominent isolate, rhizobacteria 19, was characterized as *Bacillus amyloliquefaciens* using 16S rRNA sequence based homology. Overall, our results suggested the scope and potentiality of PGPR isolated from sugarcane rhizoplane in suppressing the field infection of red rot besides promoting seedling vigour.

Keywords: Biological control, *Colletotrichum*, PGPR, *Bacillus amyloliquefaciens*, red rot

1. Introduction
Sugarcane is an important cash crop grown in tropical and sub-tropical regions of the world. India produces 16 per cent of world sugar production and stands next to Brazil (GOI, 2015) [11]. In Andhra Pradesh, sugarcane is grown in 1.3 lakh hectares with a production of 79.5 lakh tones. However, the sugarcane productivity (71 tonnes/ha) is very low compared to states like Tamil Nadu, West Bengal, Kerala and Karnataka (GOI, 2017) [12]. Productivity of Sugarcane in Andhra Pradesh is majorly hampered by several biotic stresses of which red rot caused by the fungus, *Colletotrichum falcatum* Went, is a serious threat to sugarcane production under irrigated conditions. The pathogen infects mature stalks of sugarcane; leaf midribs and cause rot of planting material which results in substantial losses in crop yield and sugar quality (Rao, 2004) [28]. This disease is responsible for phasing out of several ruling cultivars previously.
Various approaches like cultural methods, use of chemicals, biocontrol agents and thermotherapy were in vogue for the management of sugarcane red rot. Some bioagents like *Trichoderma harzianum*, *T. viride* and *Pseudomonas* species were found promising in the management of red rot of sugarcane by reducing soil borne inoculum of *C. falcatum* (Singh *et al*., 2008; Hassan *et al*., 2011) [32, 16]. Red rot of sugarcane is primarily a sett borne disease that is disseminated through infected setts and irrigation water. The use of seedlings raised in portrays through single node setts is gaining popularity among the farmers and the seed rate used per acre is reduced from 4 tonnes to 1 ton enabling sett treatment with fungicides and bioagents. The objective of the investigation is to identify a potential Plant Growth-Promoting Rhizobacterial (PGPR) strain that promotes seedling growth besides managing red rot disease effectively.

2. Materials and Methods
2.1 Isolation of bacteria from sugarcane rhizoplane
Rhizoplane soil was collected from the sugarcane clones, CoA13326, CoA 06321, CoA 92081,

CoA13324, Co 6907, CoV 92102 and CoA 13322, during winter 2015 and was used for bacterial isolation by serial dilution method on nutrient agar medium (HiMedia). The colonies with different morphology were selected, purified by repeated streaking and maintained on Luria Bertani Agar medium for further studies.

2.2 Identification of elite PGPR

The identity of the elite strain was established through homology analysis of 16 S rRNA gene sequence. The genomic DNA was isolated according to Sambrook and Russell (2001) [29]. The 16 S rRNA gene of elite strain was amplified in a PCR using universal primers, FGPS6-63-GGAGAGTTAGATCTTGGCTCAG and FGPL 132-38-CCCGGTTTCCCCATTCGG (Normand *et al*., 1992) [25]. The thermocyclic conditions included initial denaturation at 95 ^{0}C for 3 min followed by 35 amplification cycles of 95^{0}C for 1 min, 55 ^{0}C for 1 min, 72 ^{0}C for 2 min followed by final extension at 72 ^{0}C for 3 min. The PCR amplified products were reolved in 1% agarose gel in 1X Tris-acetate EDTA, run for 90 min at 100 V, and the amplified products were excised and outsourced (Bioserve Biotechnologies (India) Pvt. Ltd., Hyderabad) for partial sequencing. Similarity of 16 S ribosomal RNA gene sequence was aligned using BLAST Programme of GenBank database (NCBI).

2.3 Antagonistic activity of rhizobacterial isolates against *Colletotrichum falcatum*

The antagonism of bacterial isolates against *C. falcatum* was tested on PDA plates by using dual culture technique. For testing the antagonistic potential of rhizobacterial isolates, an agar block (5 mm diameter) of 5-day-old culture of test pathogen was placed in the centre of Petri dishes (90 mm diameter) containing PDA. A loopful of 24-h-old culture of bacterial isolates was streaked on either side of *C. falcatum* disc at a distance of 2 cm apart. The fungal pathogen culture inoculated centrally on PDA plates, but un-inoculated by bacterial isolates served as control. The inoculated plates were incubated at 25±1 ^{0}C for 5 days and per cent inhibition was calculated as per the following formula given by Dennis and Webster (1971) [8]. Each treatment was replicated thrice.

2.4 Qualitative tests for PGP (plant growth promoting) activity

2.4.1 Protease activity

The protease activity of bacterial isolates was tested on skim milk agar plates by inoculating bacterial isolates followed by incubation at 28 + 1 ^{0}C for 72 hours. Clear zone production around the point of inoculation was correlated for proteolysis.

2.4.2 Phosphate solubilisation

All the bacterial isolates were inoculated on to Pikovaskayas agar plates followed by incubation at 28 $\pm$1 ^{0}C for 3 days to assess the phosphate solubilization ability (Pikovaskaya, 1948) [26]. Appearance of clearing zone around the inoculated bacterial colonies was treated as positive for P solubilization.

2.4.3 Growth on N-free medium

The bacterial isolates were inoculated on to nitrogen free medium (Burk's medium) comprising, 10g dextrose, 0.41g KH_2PO_4, 0.52g K_2HPO_4, 0.05g Na_2SO_4, 0.2g $Cacl_2$, 0.1g $MgSO_4$, 0.005 g $FeSO_4$. $7H_2O$, 0.0025g $Na_2MoO_4.2H_2O$ and 20g agar, pH 7.0 (Wilson and Knight, 1952), to determine the nitrogen fixing ability of the isolates.

2.4.4 Cellulase activity

The cellulase activity of bacterial isolates was tested by inoculating on to carboxymethyl cellulose agar plates followed by incubation at 37^{0}C for 48 hours. The formation of clear zones around the bacterial colonies after application of Gram's iodine solution (Khatiwada *et al*., 2016) [20] was considered as cellulose utilization ability of the isolates.

2.4.5 HCN production

Bakker and Schippers (1987) [4] method was followed to assess HCN production by the bacterial isolates. The bacterial cultures in log phase were streaked on to Luria-Bertani agar plates supplemented with 4.4 g glycine L^{-1}. A filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 was simultaneously placed in the upper lids of each Petri plate along with uninoculated control. The plates were wrapped with parafilm and incubated at 28 $\pm$ 1^{0}C for 4 days and observed for colour change from yellow to brown for putative HCN production.

2.4.6 IAA production

The method of Shrivastava and Kumar (2011) [31] was used for qualitative estimation of IAA production by bacterial isolates. Luria Bertani Agar plate containing 100µg/ml of tryptophan was prepared and poured in Petri plates. Cavities of 5 mm diameter and 0.2 cm depth were made in solidified agar plate using sterile cork borer. Each cavity is filled with 50µl of overnight grown culture and incubated at 30^{0}C for 24 hours. The cavities are filled with two drops of Salkowski reagent after overnight growth. The development of pink colour after addition of Salkowski reagent was considered positive for IAA production.

2.5 Evaluation of elite PGPR isolates for their growth promoting activity on sugarcane seedlings

Single node setts of sugarcane variety, Co 997 were soaked in a solution (10^9cfu/ml) of potent bacterial isolates, with PGP activity and antagonistic potential, for one hour and planted in portrays containing a mixture of cocopit and vermicompost (1:1). The setts soaked in water for one hour served as control. Fifty single noded setts were sown in portray for each treatment. Germination percentage was recorded 40 days after sowing and the seedling vigor index (SVI) was calculated as per the formula, SVI = Germination percentage X Seedling length (Abdul-Baki and Anderson, 1973) [1].

2.6 Evaluation of elite PGPR isolates for red rot disease suppression under field conditions

Single node setts of a red rot susceptible variety, Co 997, was treated with elite PGPR strains (two isolates with better PGP activity and antagonistic potential) for 1 hour and were sown in portrays. At 30 days after sowing (DAS), the seedlings were drenched with a suspension of PGPR strain (10^9cfu/ml), 24 hours prior to transplanting in some of the treatments as indicated below. PGPR was used at a concentration of 10^9cfu/ml and carbendazim at 1g/L of water.

T1: Sett treatment with PGPR1 (Rhizobacteria 19)
T2: Sett treatment with PGPR2 (Rhizobacteria 7)
T3: Sett treatment with PGPR1 + Drenching of seedling tray (DST) with PGPR1 at 30DAS
T4: Sett treatment with PGPR2 + DST with PGPR2 at 30DAS
T5: Sett treatment with PGPR1 + DST with carbendazim at 30DAS
T6: Sett treatment with PGPR2 + DST with carbendazim at 30DAS

T7: Sett treatment with carbendazim + DST with carbendazim at 30DAS
T8: Sett treatment with carbendazim
T9: Control

Thirty days after sowing (DAS), twenty five seedlings in each treatment were transplanted under field conditions and were inoculated with *C. falcatum* pathotype (Cf 05) spore suspension by plug and cotton swab methods at seven months age during 2017-18 crop season in Regional Agricultural Research Station, Anakapalli. Observations on disease severity were recorded at 60 days after inoculation in respect of nature of tops, lesion width, nature of white spots and nodal transgression using 0-9 scale and presented as average disease score (Srinivasan and Bhat, 1961) [33].

3. Results and Discussion

3.1 Isolation of rhizoplane bacteria

The bacteria isolated from the rhizoplane of sugarcane clones, CoA13326, Co A 06321, Co A 92081, CoA13324, Co 6907, CoV92102 and CoA13322, were designated as RB1 to RB 26 (Table 1). Most of the bacterial isolates were found to be Gram positive, rod shaped with opaque to white and irregular edged colonies on LB agar. Few isolates were Gram negative and rod shaped with polar flagella.

3.2 Antagonistic activity of rhizobacteria against *C. falcatum*

The rhizobacterial isolates were evaluated for their antagonistic activity against *C. falcatum* by dual culture method. All the isolates tested (Table 2) were found inhibitory to the mycelial growth of *C. falcatum* under *in vitro* conditions. Highest inhibition (80.56%) of the test pathogen was recorded with rhizobacteria 19 isolated from the rhizoplane of sugarcane clone, CoA 92081. This was followed by rhizobacteria 7 and 10 isolated from CoV 92102 and CoA 06321 with an inhibition of 70.20 and 66.91 per cent, respectively. Least inhibition of mycelial growth of *C. falcatum* was recorded with the rhizobacteria 25 from the rhizosphere of CoA 92081. *Pseudomonas putida* strain NH-50, isolated from sugarcane rhizosphere, was reported as a potent bacterial strain in reducing the red rot severity under field conditions. Production of extracellular metabolites and antibiotics were attributed to the red rot disease concealing ability of the isolate (Hassan *et al*., 2011) [16]. Further, induced systemic resistance (ISH), production of cell wall degrading enzymes, HCN production, aggressive root colonization were also reported as mechanisms of biological control (Ahmad and Kibret, 2014; Haas and Keel, 2003) [3, 15].

3.3 Screening for growth promoting traits

Biochemical characterization of bacterial isolates for plant growth promoting (PGP) traits disclose that most of the isolates could produce protease except RB2, RB3, RB4, RB5, RB 11, RB12, RB13, RB14, RB 15, RB 16, RB 18, RB 22 and RB 24. Only few isolates viz., RB 1, RB4, RB 21 and RB 25 could produce a clear zone around the colonies on Pikovaskayas agar revealing their ability to solubilize phosphate. Differential utilization of phosphate by free living rhizospheric bacterial genera like *Bacillus, Azotobacter, Pseudomonas* and *Mesorhizobium* species was reported by Ahmad and his coworkers (2006) [2]. These phosphate solubilizing bacteria convert the fixed P in the soil and make it available for uptake by the plants, thus promoting their growth.

Only few bacterial isolates (30%) tested could produce cellulase on CMC medium after 48 hours of incubation at 37^0C. The isolates, RB1, RB7 and RB19 could produce maximum level of cellulase compared to other isolates tested. Cellulase production allows the bacterial isolates to hydrolyze cellulose into glucose, thus enabling them to decompose plant biomass that helps in addition of organic matter to the soil. Several fungi, bacteria and actinomycetes are reported as active decomposers of plant biomass rich in cellulose and hemicelluloses (Shrestha *et al*., 2012; Dhapate *et al*., 2018; Puentes-Tellez and Salles, 2019) [30, 9, 27].

Majority of the isolates tested have produced hydrogen cyanide as evidenced by the change in color of the filter paper from yellow to brown (Table 3). Production of HCN by rhizobacteria was postulated as one of the factors for biocontrol of plant diseases. Karmel Reetha *et al*. (2014) [19] compared the HCN production of several strains of *Bacillus subtilis* and *Pseudomonas fluorescens* and their efficacy in repressing the mycelial growth of *Macrophomina phaseolina*. Cyanide secreting strain of *Pseudomonas fluorescens* was reported to suppress tobacco black root rot disease (Stutz *et al*., 1986) [34].

IAA production was observed in the isolates, RB2, RB3, RB7, RB9, RB10 RB11, RB15, RB17, RB 18, RB 19, RB 23, RB24 and RB 25 as indicated by the formation of pink zone around the cavities inoculated with bacterial suspension in the presence of tryptophan. Few isolates *viz*., RB1, RB2, RB3, RB4, RB7, RB9, RB10, RB15, RB16, RB17, RB18 and RB 19 were able to grow on N-free medium and were found to be diazotrophs. However, nitrogen fixation of the isolates has to be quantified by acetylene reduction assay. Nitrogen fixing ability of rhizobacteria associated with important desert plants was studied by Gothwal *et al*. (2008) [13] using nitrogen free malate media containing bromothymol blue and acetylene reduction assay, respectively. Lifishitz and his co-workers (1987) [22] reported enhanced production of IAA by inoculation with rhizobacteria which has promoted root proliferation and thereby increased nutrient uptake by the plant.

3.4 Effect of sett bacterization on seedling vigour index

Soaking the single node setts in bacterial suspension of elite PGPR isolates, rhizobacteria 7 and rhizobacteria 19, for one hour prior to planting in portrays enhanced sett germination and seedling vigour (Table 4) compared to control. Seedling vigour index was higher when the setts were treated with carbendazim and rhizobacterial isolates (7 and 19) when compared to control. The seedling growth increase caused by sett bacterization with rhizobacteria (Isolates 7 and 19) qualifies them as PGPR. The promotion of growth on seed bacterization was attributed to the production of phytohormones like gibberellins, auxins and cytokinins by several authors (Kapoor *et al*., 2016; Bharucha *et al*., 2013 and Grobkinsky *et al*., 2016) [18, 6, 14]. Several physiological processes of the plant like cell division, tissue differentiation and cellular responses to light and gravity are known to be governed by the phytohormones elaborated by the microorganisms that inhabit the rhizosphere of plants (Lambrecht *et al*., 2000) [21]. Production of siderophores that chelate ferric ion and transport into a microbial cell, antibiotics that suppress deleterious microorganisms, ability to solubilize various minerals and induction of resistance against plant pathogens have been reported as beneficial effects of PGPR on plants (Glick, 1995) [10].

3.5 Identification of elite strain

The promising strain (rhizobacteria 19) with growth promoting ability and antagonistic activity against *C. falcatum* was characterized up to species level by amplification of 16S rDNA genes using universal primers, FGPS6-63 and FGPL 132-38 (Normand *et al*., 1992) [25]. Comparison of 16S rDNA amplified genes to sequences of Genbank has shown identity to *B. amyloliquefaciens* (KY354250).

3.6. Evaluation of elite PGPR isolates for red rot disease suppression under field conditions

The setts and seedlings treated with PGPR and carbendazim were transplanted under field conditions and were inoculated with *C. falcatum* under field conditions by plug and cotton swab methods of inoculation. The results pertaining to red rot severity were presented in Table 5. The data revealed that all the canes in various treatments inoculated with *C. falcatum* by plug method produced highly susceptible reaction. In plug method of inoculation, the pathogen is forcibly introduced into the cane by disrupting the physical barrier naturally offered by the rind and other nodal tissues thus enabling the pathogen to penetrate quickly and establish in the cane (Chona, 1954) [7]. This might be the reason for the low efficacy of the bioagents tested in reducing the red rot severity under plug method of inoculation. In addition to this, the application of bioagents as a sett treatment at the time of sowing in portrays followed by drenching with bioagents 30 days after sowing may not be sufficient in maintaining the PGPR population in the plant rhizosphere. Hence, augmentation with PGPR at monthly intervals under field conditions may sustain the PGPR population and may reduce the red rot severity even in plug method of inoculation which needs further experimentation under greenhouse and field conditions.

Differential disease severity was observed in cotton swab method of inoculation, a simulation of natural disease condition, where cotton swab laden with *C. falcatum* spores was wrapped around the young internode. Reduced disease severity was observed in treatments, 1 and 3, where the single noded setts and seedlings were treated with PGPR1 (RB 19) which clearly indicates that prior treatment with potent PGPR isolates can reduce red rot severity under natural disease pressure. Similarly, management of red rot disease through biological control have been addressed by several workers in which *Trichoderma harzianum* strain T 37 and *Pseudomonas* spp. (Malathi and Viswanathan, 2013; Malathi *et al*., 2008; Singh *et al*., 2008; Viswanathan and Samiyappan, 1999) [23, 24, 32] were found promising in red rot disease management.

Table 1: Details of the isolates collected from rhizoplane of sugarcane genotypes

S. No.	Name of the isolate	Place of collection	Sugarcane rhizoplane from which bacteria were isolated
1	RB 1	RARS, Anakapalli	CoA 13322
2	RB 2	RARS, Anakapalli	CoV 92102
3	RB 3	RARS, Anakapalli	CoA 06321
4	RB 4	RARS, Anakapalli	Co 6907
5	RB 5	RARS, Anakapalli	CoA 92081
6	RB 6	RARS, Anakapalli	CoA13324
7	RB 7	RARS, Anakapalli	CoV 92102
8	RB 8	RARS, Anakapalli	CoA 13324
9	RB 9	RARS, Anakapalli	CoV 92102
10	RB 10	RARS, Anakapalli	CoA 06321
11	RB 11	RARS, Anakapalli	Co 6907
12	RB 12	RARS, Anakapalli	CoA13326
13	RB 13	RARS, Anakapalli	CoA13322
14	RB 14	RARS, Anakapalli	Co 6907
15	RB 15	RARS, Anakapalli	CoV 92102
16	RB 16	RARS, Anakapalli	CoA 13324
17	RB 17	RARS, Anakapalli	85A261
18	RB 18	RARS, Anakapalli	CoA13324
19	RB 19	Dibbapalem	CoA 92081
20	RB 20	Dibbapalem	CoA 92081
21	RB 21	Dibbapalem	CoA 92081
22	RB 22	Dibbapalem	CoA 92081
23	RB 23	Dibbapalem	CoA 92081
24	RB 24	Payakaraopet	CoA 92081
25	RB 25	Payakaraopet	CoA 92081
26	RB 26	Anakapalle	CoA 92081

Table 2: *In vitro* antagonism of sugarcane rhizobacteria on *Colletotrichum falcatum*

S. No.	PGPR isolate	Per cent inhibition of mycelial growth
1	RB 1	34.98 (36.23) *
2	RB 2	15.48 (23.12)
3	RB 3	52.12 (46.20)
4	RB 4	4.23 (11.86)
5	RB 5	12.66 (20.78)
6	RB 6	46.00 (42.68)
7	RB 7	70.20 (56.91)
8	RB 8	39.90 (39.16)
9	RB 9	3.77 (11.14)
10	RB 10	66.91 (54.87)

11	RB 11	61.73 (51.77)
12	RB 12	40.59 (39.56)
13	RB 13	57.28 (49.17)
14	RB 14	60.31 (50.94)
15	RB 15	39.20 (38.74)
16	RB 16	39.20 (38.75)
17	RB 17	63.37 (52.74)
18	RB 18	15.48 (23.12)
19	RB 19	80.56 (63.83)
20	RB 20	14.14 (22.08)
21	RB 21	14.14 (21.98)
22	RB 22	13.89 (21.86)
23	RB 23	11.61 (19.91)
24	RB 24	7.83 (16.24)
25	RB 25	1.77 (7.37)
26	RB 26	13.87 (21.86)
	CD (P=0.05)	2.535
	S.Em (±)	0.847
	CV (%)	6.13

*Figures in the parenthesis are angular transformed values

Table 3: Plant growth promoting traits of rhizobacteria isolated from sugarcane

S. No	Isolate	Protease	PSB	Growth on N-free medium	Cellulase	HCN production	IAA production
1	RB 1	++	+	+	+++	+	-
2	RB 2	-	-	+	-	+	+
3	RB 3	-	-	+	-	+	+
4	RB 4	-	+	+	-	+	-
5	RB 5	-	-	-	-	-	-
6	RB 6	+	-	-	-	-	-
7	RB 7	+	-	+	+++	+	+
8	RB 8	+	-	-	-	-	-
9	RB 9	+	-	+	-	+	+
10	RB 10	++	-	+	++	+	+
11	RB 11	-	-	-	-	-	+
12	RB 12	-	-	-	+	-	-
13	RB 13	-	-	-	++	+	-
14	RB 14	-	-	-	-	-	-
15	RB 15	-	-	+	-	+	+
16	RB 16	-	-	+	++	+	-
17	RB 17	+	-	+	-	+	+
18	RB 18	-	-	+	-	+	+
19	RB 19	+	-	+	+++	+	+
20	RB 20	+	-	-	-	+	-
21	RB 21	+	+	-	-	+	-
22	RB 22	-	-	-	-	+	-
23	RB 23	+	-	-	++	+	+
24	RB 24	-	-	-	-	+	+
25	RB 25	+	+	-	-	+	+
26	RB 26	+	-	-	-	+	-

+ (Positive reaction), - (Negative reaction)
In case of protease, phosphate solubilising and cellulose activities the size of the halo has been denoted as +, ++, and +++ based on the halo diameter (+, diameter of the halo upto 30 mm; ++, diameter of the halo from 31-50 mm; +++, diameter of the halo above 50 mm).

Table 4: Effect of rhizobacteria on sugarcane sett germination and seedling growth

Treatment	Particulars	Sett germination %			Seedling vigour index		
		2017-18	2018-19	Average	2017-18	2018-19	Average
T1	Sett treatment with PGPR1	60	65	62.50	17880	16900	17390
T2	Sett treatment with PGPR2	70	60	65.00	18270	15600	16935
T3	Sett treatment with PGPR1+ DST with PGPR1 at 30 DAS	70	70	72.50	20440	18200	19320
T4	Sett treatment with PGPR2+ DST with PGPR2 at 30 DAS	60	65	62.50	16560	17225	16893
T5	Sett treatment with PGPR1+ DST with carbendazim at 30 DAS	55	70	60.00	14430	18900	16665
T6	Sett treatment with PGPR2+ DST with carbendazim at 30 DAS	60	55	57.50	16440	14850	15645
T7	Sett treatment with carbendazim + DST with carbendazim at 30DAS	80	80	77.50	21040	21600	21320
T8	Sett treatment with carbendazim	80	75	80.00	22000	21000	21500

Table 5: Effect of PGPR on red rot severity in sugarcane cultivar, Co 997

Treatment	Particulars	Red rot severity					
		Plug method			Cotton swab method		
		2017-18	2018-19	Average disease score	2017-18	2018-19	Average disease score
T1	Sett treatment with PGPR1	9.0	9.0	9.0	3.3	3.8	3.55
T2	Sett treatment with PGPR2	9.0	9.0	9.0	6.7	6.6	6.65
T3	Sett treatment with PGPR1+ DST with PGPR1 at 30 DAS	8.7	8.7	8.7	3.0	3.8	3.4
T4	Sett treatment with PGPR2+ DST with PGPR2 at 30 DAS	9.0	9.0	9.0	6.3	6.6	6.45
T5	Sett treatment with PGPR1+ DST with carbendazim at 30 DAS	9.0	8.7	8.85	3.7	4.7	4.2
T6	Sett treatment with PGPR2+ DST with carbendazim at 30 DAS	9.0	8.7	8.85	6.3	5.9	6.1
T7	Sett treatment with carbendazim + DST with carbendazim at 30DAS	8.7	9.0	8.85	7.0	7.6	7.3
T8	Sett treatment with carbendazim	9.0	9.0	9.0	6.7	7.5	7.1
T9	Control	9.0	9.0	9.0	7.3	7.7	7.5

4. Conclusion

In the present study, *Bacillus amyloliquefaciens* (RB 19) isolated from the rhizoplane of sugarcane clone, Co A 92081 was found to have a high antagonistic effect against *C. falcatum* under *in vitro* and *in vivo* conditions. Besides disease suppressing ability, this isolate (RB 19) was found superior to all other isolates in promoting plant growth, which is evident by its ability to produce IAA, HCN and protease and to solubilize phosphate and cellulose. Overall, our results suggested the scope and potential of utilizing *B. amyloliquefaciens* in reducing the red rot severity under natural disease conditions besides plant growth promotion.

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