Prospecting efficacy of native bacterial bioagents against blastdisease and their potential for plant growth promotion in finger millet

ABSTRACT

Thirty-seven isolates of *pseudomonas* and *bacillus* were isolated from the phylloplane of healthy finger millet leaves. Two of the bacterial isolates showing maximum inhibition against the blast pathogen under *invitro* conditions were characterized by biochemical test and molecular characterization using 16SrRNA sequencing and also evaluated for hydrolytic enzyme activity under growth promotion activity. The isolates were identified to be *Pseudomonas fluorescens* (P-9) and *Bacillus subtilis*(B-10) showing 100% nucleotide similarity with reported strains. Field evaluation with microbial consortium (P-9 &B-10) as talc formulation resulted insignificant reduction in leaf blast severity (55.15%), neck blast (44.44%) and finger blast (46.02 %) along with a 35.17 % increase in grain yield over control and highest cost benefit ratio of 1: 1.53 over control.

Keywords: Blast disease, Finger millet, Bio control agents, Phylloplane, *Pseudomonas fluorescens*, *Bacillus subtilis*, Management.

INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaetn.) commonly known as Ragi is an important nutritive cereal crop belonging to the family Poaceae. It is the staple food in many African and South Asian countries and is also one of the most important food crops of South India (Dida *et al.* 2008). It is grown widely in different agroecosystems mainly as rainfed and dry land field crop. More than 25 countries, of the world grow finger millet, and is considered as fourth among millets produced worldwide, after sorghum, pearl millet and foxtail millet (Upadhyaya *et al.* 2007).India is the major producer of finger millet in Asia and it is the staple food for millions of people in the states of Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Maharashtra, and Bihar, with annual production of 2.2 million tons over an area of 1.6 million ha (Gupta *et al.*, 2017). Finger millet crop is affected by a number of diseases among which blast disease, brown spot disease, foot rot disease, Cercospora leaf spot, Green ear disease, smut disease and damping of disease (Kumar and kumar, 2009). Blast disease of finger millet is mainly caused by *Pyricularia grisea* (Cooke) Sacc., which was first identified in India and is the most destructive and widely distributed disease in almost all the finger millet growing regions of the world (Mc Rae, 1920). Though, the crop is known to be one of the hardiest crops, it is affected by many diseases, among which, blast caused by *Pyricularia grisea* is the most devastating disease affecting different aerial parts of the plant at all stages of its growth starting from seedling to grain formation and considered as most destructive disease. The average yield loss due to blast disease was recorded to be around 28 per cent (Viswanath *et al.*, 1997), but under favourable conditions it may go up to 80-90 per cent. However, plant disease resistance though remains the most effective strategy of disease management at the end of farmers, identifying of stable resistance to the pathogen remain challenge. In spite of a great deal of research on the pathogen and on the disease, blast still remains a serious constraint to finger millet production in areas with conducive environments where susceptible cultivars are grown. Since finger millet is predominantly grown as rain fed crop by small and marginal farmers, disease management by chemical means is found to be effective. Plant protection measures using chemicals are uneconomical and

oftendisregardedby resource poor farmers. However, reliance on pesticides to manage disease and pest problems has aggravated environment decline and caused serious health effects on agricultural workers and rural communities. Pesticides residues also raise food safety concerns among domestic consumers and pose trade impediments for export crops. Also, their irrelevant and indiscriminative use enhanced resistance in phytopathogens and causing environmental and groundwater pollution. The problems associated with indiscriminate use of chemical pesticides in agriculture have led to increasing interest in the use of native and non-native beneficial microorganisms to improve plant health and to increase crop productivity while ensuring food safety and environmental protection (Hemalatha *et al.*, 2017; Sharma *et al.*, 2017; Schütz *et al*., 2018). The use of bio-inoculants and the exploitation of novel beneficial plant microbes offer promising, sustainable and eco-friendly strategies in conventional and organic agriculture systems worldwide (Negi *et al.*, 2015; Gopalakrishnan *et al.*, 2016; Zhou *et al*., 2016). Among the plant-associated microbes, Pseudomonads are the dominant bacteria known to protect plants against several phytopathogens (Sekhar and Prabavathy, 2014; Wang *et al*., 2015), and to promote plant growth under several abiotic stress conditions The biocontrol ability of pseudomonads is directly correlated with production of various antibiotics such as 2, 4- diacetylphloroglucinol (2, 4- DAPG), Phenazines, Pyrrolnitrin (PRN), Pyoluteorin (PLT), Hydrogen cyanide (HCN), and lytic enzymes (Jegan *et al.*, 2015, Ganga *et al.*, 2016, Sekhar *et al*., 2018). In this context, bio control against plant pathogens in millet crops by native bioagents like, *Pseudomonas fluorescens*, *Bacillus* spp. have recently become the focus of research and resources in many countries (Mageshwaran*et al.*, 2023).Though many biocontrol strains were reported for biological control of blast disease and demonstrate successful performance under labconditions, but exhibit variable performance in greenhouse and field trials. Hence, the present study was undertaken to identify suitable crop and region specific bioagents for blast disease management in finger millet.

MATERIALS AND METHODS

Isolation and maintenance of the pathogen:

Blast pathogen was isolated from the finger millet leaves collected from the field at Agricultural Research Station, Perumallapalle showing typical symptoms of blast disease. The pathogen was isolated and purified by single spore isolation and further maintained on oat meal agar. The pathogen was identified based on its morphological growth pattern, spore shape, conidia and septation by comparing with standard descriptions given by Gashaw *et al*., (2014). Pathogenicity of the fungus was proved by artificial inoculation of conidial suspension (1x 10^5 conidia/ml) on 21 days old finger millet seedlings under greenhouse conditions.

Isolation of bacterial bioagents and testing the antagonistic activity against blast pathogen.

Bacterial strains were isolated from the phylloplane of finger millet plants collected from finger millet growing regions of Chittoor district and the fields of Agricultural Research Station, Perumallapalle using serial dilution method. Serial dilutions were made up to10^{-6} dilution. King's medium B (KMB) and Bacillus Agar (BA) was used for selective isolation of *Pseudomonas* and *Bacillus* isolates. The colonies showing yellow-green fluorescence were

picked and streaked on fresh KMB plates while the colonies with bright cream colour were picked and maintained on bacillus agar. Isolates were maintained at 4 °C for further use, while mother cultures were maintained at −20 °C in half-strength KMB and BA.

The antagonistic activity of the bacterial bio-agents was evaluated against the blast pathogen, *P. grisea* by the dual culture technique on Nutrient Agar medium. Mycelial disc of 6 mm diameter from 10 days old blast culture was streaked at one end of the agar plates and incubated at 28◦C for 48 h. Later, a loopful of an actively growing culture of the bacterial bioagent was inoculated at another end of the agar plate. Plates with only fungal culture served as control. The plates were incubated at 28◦C for 10 days and examined for inhibition of fungal growth by the bacterial bioagents.

The potential bioagents were examined for PGPR activities like HCN production, Siderophore production, protease, phosphatase,cellulase and chitinase activity.

HCN production: For qualitative determination of production of hydrogen cyanide (HCN), Bakker and Schippers (1987) method was followed. The two bacterial isolates were inoculated on nutrient agar medium supplemented with glycine at the rate of 4.4 g/l. A Whitman filter paper no.1 soaked in 2% sodium carbonate and 0.5% picric acid was placed in the lid of each Petri dish. The plates were sealed with parafilm and incubated at 30 °C for 4 days. A change in colour of the filter paper from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++) or strong (+++) reaction, respectively, for the production of HCN.

Chitinase activity: The two potential bacterial strains, *Pseudomonas* isolate-9 and *Bacillus* isolate-10 were tested for the production of chitinase as described by Kole and Altosaar (1985) in Dworkin-Foster (DF) salts minimal medium containing 2.5% (w/v) colloidal chitin.

Cellulase activity: Cellulolytic ability of bacteria was assessed by using Mendels and Reese medium (Mendels and Reese, 1957) containing CMC salt. All the inoculated plates were stained with 1% Congo red solution for 15 min after incubation at 28 °C for 48 h, and distained with 1 M NaCl for 15 min (Teather and Wood 1982). The degradation zones around the bacteria indicated positive for cellulose production

Proteolytic activity was assessed using skimmed milk agar (Hi Media, India) (Wikström, 1983).

Amylase production: Amylase activity of was determined by inoculating in starch agar (Hi Media, India) plates containing starch as the only carbon source. After incubation at 28◦C for 48 h, plates were stained with Gram's iodine solution and the formation of a clear halo zone in the starch agar around the colony indicated amylase production (Cappuccino and Sherman, 1992).

The two strains were assessed for inorganic phosphate solubilization by inoculating the freshly grown culture in the National Botanical Research Institute's phosphate (NBRIP) agar medium containing glucose, 10 g l^{-1}, Ca3(PO4)2, 5 g l^{-1}, MgCl2·6H2O, 5 g l^{-1}, MgSO4·7H2O, 0.25 g l^{-1} ,KCl, 0.2 g l^{-1}, (NH4)2SO4, 0.1 g l^{-1}, and agar 18 g l^{-1}(Nautiyal, 1999). The plates were incubated at 28◦C for 5 days and the diameter of a clear halo zone around the bacterial colony indicating solubilization of mineral phosphate was measured.

Siderophores Production: The two bacterial strains (P-9 and B-10) were inoculated into King's broth and Nutrient agar respectively, incubated for overnight and spotted on Blue Agar Chromeazurol "S" plates and kept at 28◦C. After incubation, production of siderophores was

detected by the appearance of orange-halo zones against a blue background (Louden *et al*., 2011).

Bio-efficacy of bacterial antagonists against blast pathogen (*Pyricularia grisea*)under *in-vitro:*

Mycelial disc of 6 mm diameter from 10 days old blast culture was placed at one end of the agar plates and incubated at 28◦C for 48 h. Later, a loopful of an actively growing culture of the bacterial bioagent was streaked at another end of the agar plate. Plates with only fungal culture served as control. The plates were incubated at 28◦C for 10 days and examined for inhibition of fungal growth by the bacterial bioagents. The per cent growth inhibition was calculated as per formula

$$I (\%) = C\text{-}T/C \times 100$$

Where, I, Per cent mycelial growth inhibition; C, Colony diameter of *Pyricularia grisea* in control; T, Colony diameter of *Pyricularia grisea*in treatment.

Molecular characterization of the potential bacterial bio-agents by 16SrRNA analysis:

DNA was isolated from the two potential bacterial isolates using HiPurA Bacterial Genomic DNA Purification Spin Kit (HIMEDIA.). Bacterial DNA was amplified using 16SrRNA primers ie., 27F GAGTTGATCCTGGCTCA 3' and 1492R: 5' ACGGCTACCTTGACTT 3'. PCR analysis was carried out using PCR cocktail (50 µl) with 50 pM primers, 50 ng genomic DNA, 1X Taq DNA polymerase buffer, 0.2 mM of each dNTP, 1.5 mM Mgcl2. Amplification was performed in Eppendorf Gradient thermal cycler (PCR) machine, programmed with an initial denaturation at 94°C for 30 minutes followed by 30 cycles of denaturation of 10 secs at 94°C, 1 minute at 45°C and 30 secs at 72°C and final elongation was done at 72°C for 5 minutes. A 5 µl aliquot of amplified product was electrophoresed on a 1.2 per cent agrose gel in 1 x TAE at 100 V for one hour. The gel was stained with ethidium bromide and the PCR products were visualized with UV transilluminator. The PCR product was purified and sequenced at M/s Eurofins Ltd, Banglore. The DNA sequences corresponding to 16S rRNA gene, obtained from bacteria were reverse complemented using software Bio edit. Further DNA sequences were aligned using the clustal X software. The aligned DNA sequences were compared using National Center for Biotechnology Information (NCBI) data base for identification of bacteria homology searches with 16S rDNA sequences in GenBank. Phylloplane bacteria were identified based on the closest relative showing 95-99 per cent identity on NCBI.

Field evaluation of potential bacterial bioagents:

Talc formulations of P-9 and B-10 were prepared by procedures followed by Vidhyasekharan*et al.,* 1997. Field studies were conducted to study the efficacy of native strains of *Pseudomonas sp.* (P-9) and *Bacillus sp.* (B-10) @ 1% against the blast disease in finger millet during Kharif and Rabi, 2022-23 at Agricultural Research Station, Perumallapalle. Blast susceptible variety, VR-708 was used in the present study. The crop was sown in 3 x 2.25 m^2 plots following the recommended package of practices. The bio-agents were applied as seed treatment, seedling dip and two foliar sprays at tillering and 50% flowering stages. Chemical treatment with carbendazim as seed treatment @ 2g/kg seed and another treatment with seed treatment followed by foliar spray @1g/lit was also included as it is the recommended practice for blast disease management while spray with water served as check. Observations regarding leaf, neck and finger blast incidence were recorded separately. Leaf blast severity was recorded on 1-9 scale at seedling stage while, neck blast and finger blast incidence were recorded by counting the number of infected panicles and fingers from

total number of plants and fingers (Kumar *et al.*, 2007, Hemalatha *et al.*, 2017). Neck blast and finger blast severity was recorded at physiological maturity stage. The grain yield and fodder yield were recorded after harvesting of crop from individual plots. The influence of PGPR activity of the two bacterial bioagents was evaluated based on plant growth and yield related parameters like Plant height, no. of productive tillers, no.of ears per plant, ear length, days taken for maturity etc.

Statistical Analysis:The mean difference between each treatment was found by using ANOVA and the mean was compared with Duncan's Multiple Range Test by using SPSS 16.0 software

RESULTS

Isolation and Identification of potential biocontrol agents against blast pathogen:

Blast pathogen was isolated from the finger millet leaves and the pathogenicity test was proved under greenhouse conditions.In the study, thirty-seven phylloplane bacterial antagonists (27 *Pseudomonas* isolates and 10 *Bacillus* isolates) were isolated from the healthy leaves of finger millet and were tested for their antagonistic activity against blast pathogen. *M. grisea* by following dual culture assay. Among the twenty-seven fluorescent pseudomonads screened against *P.grisea*, P9 isolate showed maximum mycelial inhibition of 90.44 per cent followed by *Bacillus*sp.isolate, B-10 which showed 85.54 percent inhibition of the blast pathogen. These two isolates were characterized using biochemical and molecular characterization.

Biochemical tests for identification of phylloplane bacterial antagonist:

The two potential bacterial antagonists, P-9 and B-10 were found to be gram negative and gram positive respectively.Both the isolates were able to hydrolyse the starch which in turn produced the clear zone around the bacterial colony and showed the phosphate solubilizing activity in Pikovskaya's agar medium.The isolates were noted for proteolytic activity as they produced clear zone around the bacterial colony on casein agar. And also, they were found to be strong producers of siderophore (iron chelating agent which is orange in colour). The results of the biochemical testsof the two bacterial isolates used in the studyare mentioned in the table 1 and fig.1.

Table 1: Biochemical characterization of promising Bacterial Isolates P-9 and B-10.

S.No	Biochemical test	Bacterial isolates	
		P9	B10
1	Gram staining	-	+
2	Hydrogen sulphide Production	+	-
3	Gelatin liquefaction	+	+
4	Urease	+	-
5	Catalase	+	+
6	Starch hydrolysis	+	+
7	Carbohydrate fermentation	+	+
8	Citrate utilization	+	-

+: Positive -: Negative

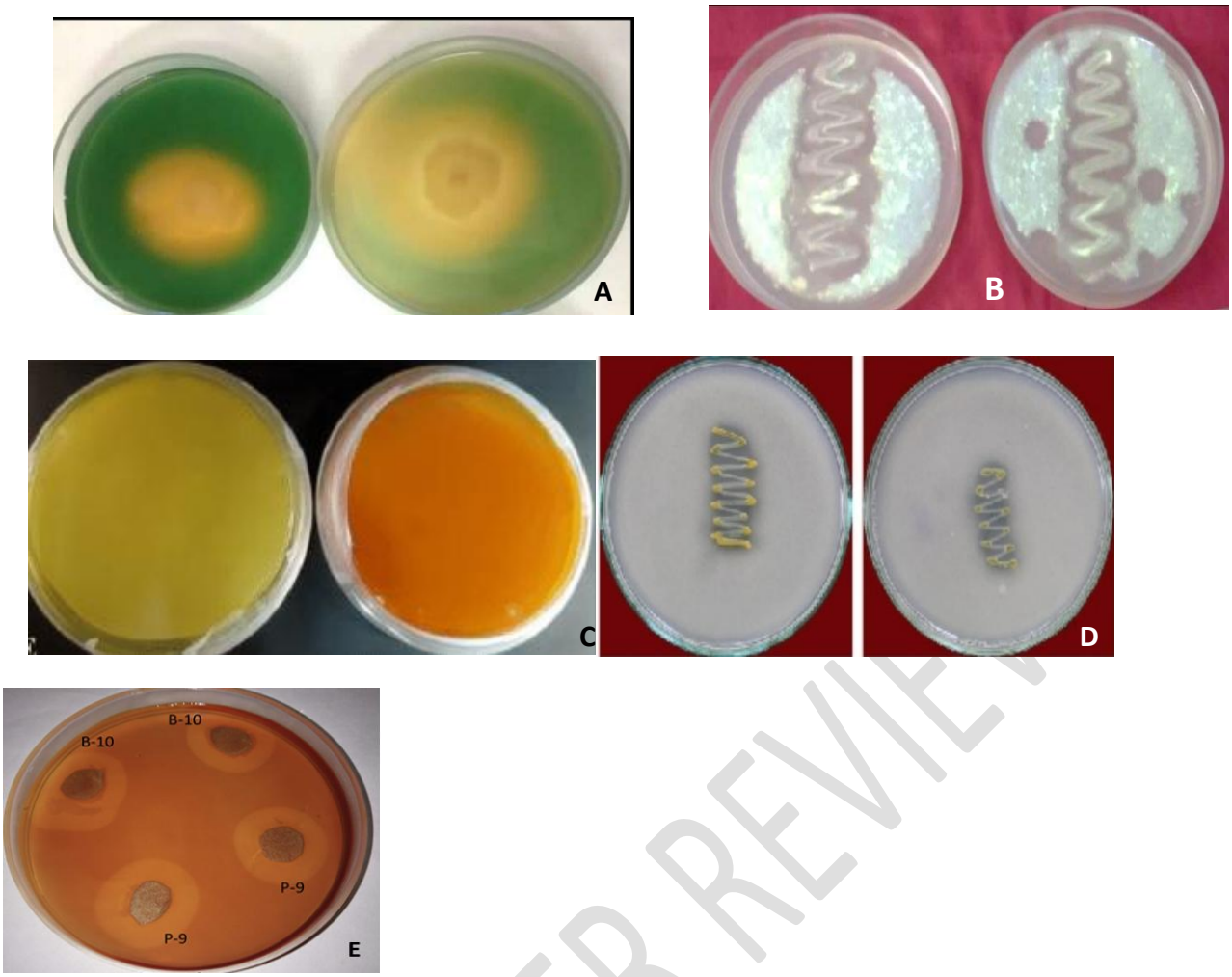
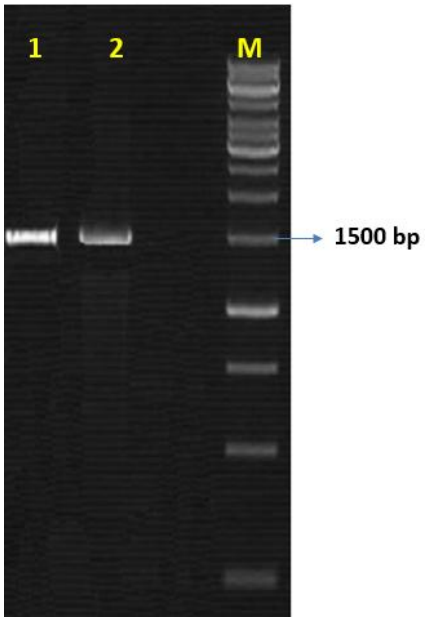


Fig.1.PGPR activity tests and biochemical characterization of the bacterial isolates under the study. A-Siderophore production B-Protease activity, C-HCN Production, D- Phosphatase activity, E-Cellulase activity

Molecular Identification of Bacterial antagonists:

Amplification of the 16S rRNA region from isolates of *Pseudomonas* sp, P-9 and *Bacillus* sp, B-10 with primers 27F and 1492R yielded products of approximately 1500 base pairs. (Fig. 2) and the purified PCR products (50 µl) were sent to Eurofins, Bangalore for sequencing. Sequencing of the PCR product followed by BLAST searches with both the isolates ie., *Pseudomonas*sp(P-9) and *Bacillus*sp (B-10) showed 99 per cent similarity with *Pseudomonas fluorescens* and *Bacillus subtilis* respectively. The nucleotide sequences of both the isolates were deposited in NCBI,with accession no. OR002035 and OQ954773.



Lane M: Marker
Lane 1: *P. fluorescens*
Lane 2: *B.subtilis*

Fig 2: PCR amplification of 1400bp of 16SrDNAregion of the bacterial isolates

Field efficacy of the biocontrol agents in reducing the blast disease:

Kharif, 2022: The results from the field study revealed that among the seven treatments, T4 (Seed treatment followed by seedling dip and foliar spray @0.1%)recorded least leaf blast severity of 34.79% which was significantly superior over the other treatments. The leaf blast severity up to 79.94% was recorded in the control treatment. Least incidence of neck blast (23.76%) and finger blast (25.41%) was recorded in the same treatment ie., T4 while in control neck blast incidence up to 54.51% and finger blast incidence up to53.63 was observed. Maximum grain yield of 28.29 q/ha and fodder yield of 7.89 t/ha was obtained in T4 treatment which was numerically superior over all treatments but statistically on par with T6 treatment with grain yield (26.52Q/ha) and fodder yield (7.78t/ha).Least grain yield of 17.56 q/ha and fodder yield of 6.25 t/ha was recorded in control treatment.

Rabi, 2022: During rabi, 2022, T4 treatment recorded least leaf blast severity (33.27%), neck blast incidence (15.45%) and finger blast incidence (16.24%) compared to control with leaf blast severity of 71.33%, neck blast incidence of 31.02% and finger blast incidence of 37.19%. Highest grain yield of 28.96q/ha was obtained in the same treatment while 19.56q/ha was obtained in control treatment.

From the pooled results, it was evident that in all the treatments there was significant reduction of the leaf blast intensity as compared to the control. Among the seven treatments, T4 treatment showed least incidence of leaf blast severity (34.03%) which was numerically superior over other treatments but was significantly on par with T5 and T6 treatments with chemical treatment ie., seed treatment and foliar spray with carbendazim @ 0.1% (Table.2). Leastneck blast (22.46%) and finger blast incidence (23.17%) were recorded in the T4 treatment which was significantly superior over the control treatment and significantly on par with the chemical treatments ie., T5 and T6 (Fig.3). Highest grain yield (28.63 q/ha) and fodder yield (7.91 t/ha) was obtained in T4 treatment which was significantly superior over the control treatment and was on par with T6 treatment with seed treatment, seedling dip and foliar spray with carbendazim.

The cost benefit economics was calculated by considering the profit increase over control of different treatments. Highest cost benefit ratio was recoded in T4 treatment with 1:1.53 followed by seed treatment with carbendazim @2g/lit+ Foliar application of carbendazim @ 1 g/lit (at tillering and flowering stage with B: C ratio of 1:1.42 The yield and yield related parameters were evaluated at the time of harvest (Table.3) and it was evident from the results that highest plant height (112.50 cm), highest tiller no (2.83), finger length (9.53), root length (18.72 cm) and 1000 seed weight (3.77g) was recorded was recorded in T4 treatment while the control treatment recorded plant height (86.25 cm), tiller no (1.77), No. of finger length (7.75 cm) root length (12.66 cm) and 1000 seed weight (2.44g).

Discussion:

Control of blast disease employing biological agents:

In the present study, two bacterial isolates (P-9 and B-10) were found to be potential against the blast pathogen showing maximum inhibition of 90.4 and 85.54% respectively under *in vitro* conditions.Similarly, Shanmugapackiam*et al* (2021) studied the efficacy of forty-six*Pseudomonas* and *Bacillus* isolates collected from the finger millet fields in inhibitingthe mycelial growth of *M. grisea* under *in vitro*.Among them, nine *Pseudomonas* isolates viz., Pf1, FmP3, FmP4, FmP6, FmP9, FmP17, FmP21, FmP22, FmP23 and four *Bacillus* isolates viz., EPCO5, FmB9, FmB13 and FmB15 recorded more than 50.0 per cent inhibition against *M. grisea* isolates and found that the Pf1 and EPCO5 recorded a significantly higher inhibition of *M. Grisea*. Negi *etal*., (2015) isolated seventy rhizospheric pseudomonads from different annual plants and tested their efficacy against the *M.grisea* causing blast disease in finger millet. Of them, 10 isolates showed good inhibition ranging between 48.65 to 88.43 % of the test pathogen.Getachew *et al*., 2014 and kumar (2011) also demonstrated that *Pseudomonas* spp. and *Bacillus* spp. can inhibit blast pathogen in foxtail millet and finger millet. Similarly, Raja and Mahalakshmi (2014) isolated nine phylloplane microbes from the rice crop andradj tested their antagonistic activity by dual culture assay. The results obtained from the dual culture showed that maximum mycelial growth inhibition was recorded with *Pseudomonas fluorescens* followed by *Bacillus subtilis*. Chakraborty *et al.,* (2021) studied the antagonistic activity of several microbes and reported that *Bacillus cereus* II 14, *B. firmus* E65 and *Pseudomonas aeruginosa* C32b inhibited the mycelial growth of *Magnaporthe grisea* under in vitro conditions.Additionally native strains may posess adaptive characteristics that enhance their effectiveness in specific geographic regions. Furthermore, some biocontrol agents often exhibit multiple beneficial plant growth promoting traits beyond disease control, including plant growth promotion.

Role of antibiotics and secondary metabolites in blast disease control in finger millet:

It is evident from most of the studies that inhibition of growth of the blast pathogen is attributed due to some diffusible substances produced by the pseudomonads sp. which might be the secondary metabolites. A wide range of secondary metabolites such as siderophores, antibiotics, and enzymes are known to be produced by different rhizobacteria, which are inhibitory to different phytopathogens (Negi *et al.*, 2011; Lin *et al*. 2014). Both the biocontrol strains used in the present study were strong producers of siderophores which enable them to defend against the blast pathogen. Production of fungal cell wall degrading enzymes such as proteases, pectinases, and chitinase by fluorescent pseudomonads has been reported by several workers (Gohel *et al*. 2006; Ayyadurai *et al*. 2007). Martins *et al.,* (2020) characterized six bacterial isolates from both phylloplane and rhizosphere regions and subjected to different biochemical tests. From the biochemical screening, they distinguished

the bacterial isolates into gram positive and gram negative, siderophore producers and non-producers and molecularly confirmed to identify the genus and species.

Chitinase assay revealed that the two potential bacterial isolates (P-9 &B-10) produced prominent clearance zone around their colonies on CCA medium. Chitinase enzymes attack on fungal cell wall and cause lysis by degrading chitin and hence, considered as one of the most important mechanisms of biocontrol of pathogens. Fluorescent pseudomonads have also been reported to produce antifungal enzymes which expel them as successful bioagents (Chang *et al.* 2003;macommare*et al.,* 2004; Pankaj *et al.* 2012).Leelasuphakul*et al.,* (2006) characterizedan extracellular enzyme β-1,3-glucanase from *B. subtilis* strain, NSRS 89-24, effective against blast fungus in rice which showed an excellent antagonistic effect including abnormal hyphal swelling, lysis and complete degradation of the hyphal tip as well as interfered with the germination of conidia.

Sekar *et al*. (2018) reported that a reduction in the incidence of finger millet blast disease by fluorescent Pseudomonas isolate MSSRFD41 was mainly due to its plant promoting as well as fungicidal activities which includes fungal cell wall hydrolysing enzymes viz., chitinases, proteases as well as 2,4-DAPG production. Seed priming with the pseudomonas isolate showed induction of disease resistance in finger millet plants against *M. grisea* infection. **The phosphate solubilizing ability** of the two isolates identified under the study **helps inenhancing** the availability of **plant-utilizable forms** of **phosphate, iron, potassium** and **zinc** via solubilisation and mobilization. Treating the seed with these bioinoculants directly **increases nutrient availability** in soil, thus helps in **increasing soil fertility, plant growth, crop productivity** and also **nutrient content of the grain**

Efficacy of the bioagents in reducing the blast disease and enhancement of grain yield:

Several workers reported that the efficacy of the microbial consortium was achieved through establishment of these beneficial microbes in the rhizosphere through seed priming and seedling root dip which provided protection to the finger millet against blast disease in the initial growth stage and foliar spray at later growth stage through induced systematic resistance. This may be due to inherent ability of the bioinoculants indigenous to the host plant and high success rate in the establishment, significant growth promotion, and disease control (Santhanam *et al.,* 2015; Zahid *et al.,* 2015).Similarly, Kumar and Kumar (2011) revealed that seed treatment and two spraysof *P.fluorescencs,* Pf-2 @0.6 per cent recorded lowest neck and finger blast incidence with maximum yield of 2312.34 kg/ha with remarkable percent increase of grain yield by 81.69 over the control. Seed treatment and foliar application of *P. fluorescens* @ 0.6% at 10 days interval recorded least incidence of leaf blast (1%), neck blast (18.3%) and finger blast (27.8%) with higher yield (3288 kg/ha) as compared to other treatments (Patroo*et al.,* 2008).

Waghunde*et al.,*(2013) reported that spraying of *P. aeruginosa*Rambhas Strain at 0.6% (2×109 cfu/ml) for three times at 15 days interval, starting at 21 days of transplanting was found significantly effective in the management of the leaf blast of finger millet by lowering disease intensity and increasing grain yield as well as fodder.Kumar and Kumar (2011) suggested that seed treatment with *Pseudomonas fluorescens,*Pf2 (0.6%) along with two foliar sprays (0.6%) was found significantly most effective in reducing blast disease of finger millet. Sekhar *et al.,*(2018) revealed that inoculation with the native Pseudomonas millet strain MSSRFD41 from finger millet resulted in maximum disease reduction up to 53.07%, which was significantly better than other treatments including a chemical fungicide.

CONCLUSION:

In the present study, we have isolated 37 phylloplane bacteria and identified two potential isolates (*Pseudomonas fluorescens* ARS PPL strain (OR002035)and *Bacillus subtilis* ARS PPL strain (OQ954773)with strong antagonistic activity against blast pathogen and enhanced PGPR activity promoting the plant growth and yield parameters. Hence, the use of these biocontrol agents provides a promising and sustainable alternative method for management of blast disease in finger millet andcan be included in organic farming practices there by making the environment free from hazardous for environment and human health.

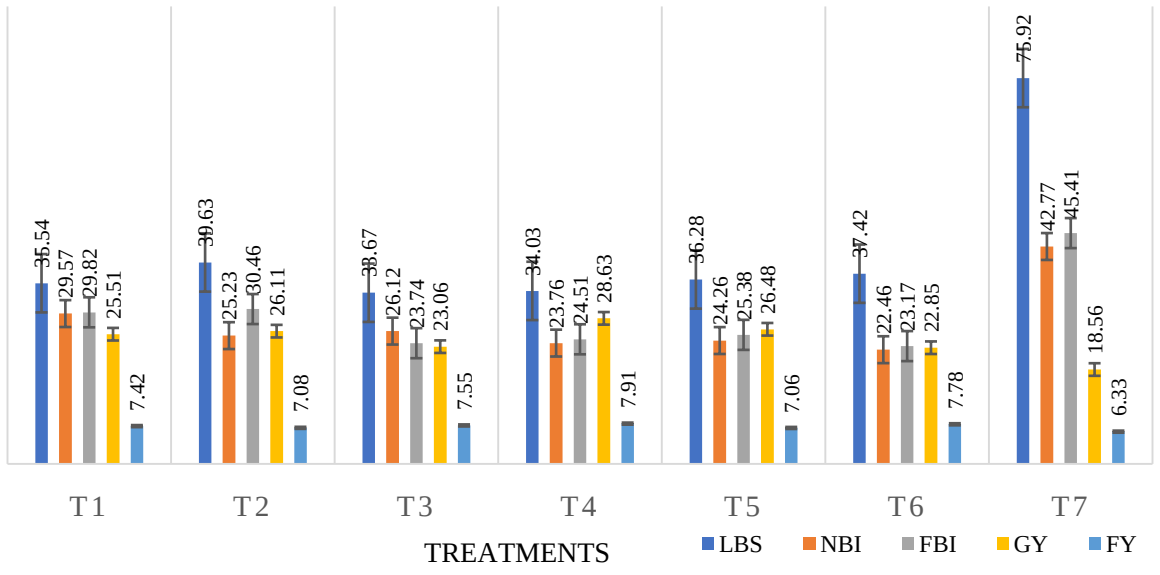
List of Abbreviations: Not applicable in this section.

Table.2: Efficacy of treatments on finger millet blast disease and yield

S.No	Treatments	Leaf blast severity (%)	Neck blast incidence (%)	Finger blast incidence (%)	Grain yield (q/ha)	Fodder yield (t/ha)	B:C cost ratio
1	T1: Seed treatment with microbial consortium @ 10g/kg seed	35.54^{bc}	29.57^{b}	29.82^{b}	25.51^{ab}	7.42^{c}	1.37
2	T2: Seedling dip with microbial consortium @ 10 g/lit water)	39.63^{b}	25.23^{cd}	30.46^{b}	26.11^{ab}	7.08^{d}	1.40
3	T3: Foliar spray with microbial consortium @10g/lit water (at tillering and flowering stage)	33.67^{c}	26.12^{c}	23.74^{c}	23.06^{b}	7.55^{bc}	1.24
4	T4: T1+T2+T3	34.03^{c}	23.76^{cd}	24.51^{c}	28.63^{a}	7.91^{a}	1.53
5	T5: Seed treatment with Carbendazim @ 2g/kg seed.	36.28^{bc}	24.26^{cd}	25.38^{c}	26.48^{a}	7.06^{d}	1.23
6	T6: T5+ Foliar application of carbendazim @ 1 g/lit (at tillering and flowering stage).	37.42^{bc}	22.46^{d}	23.17^{c}	22.85^{b}	7.78^{ab}	1.42
7	T7: Control	75.92^{a}	42.77^{a}	45.41^{a}	18.56^{c}	6.33^{e}	-

Each value represents the mean ± SD (n = 3) and within column different letters were assigned when values were significantly different according to the Duncan's Multiple Range Test ($P < 0.05$)

FIG 3: EFFICACY OF MICROBIAL CONSORTIUM ON GRAIN YIELD, FODDER YIELD AND THE BLAST DISEASE INCIDENCE



LBS: Leaf Blast Severity (%), NBI: Neck Blast Incidence (%), FBI:Finger Blast Incidence (%), Grain Yield (Q/ha); FY: Fodder Yield (t/ha)

Table.3: Efficacy of microbial consortium in improving plant and yield related parameters in finger millet.

S.No	Treatments	Plant height (cm)	No. of tillers	Finger length (cm)	Root length (cm)	1000 Seed weight(g)
1	T1: Seed treatment with microbial consortium @ 10g/kg seed	108.93^{b}	2.35^{b}	8.65^{b}	15.39^{b}	2.69^{bc}
2	T2: Seedling dip with microbial consortium @ 10 g/lit water)	105.83^{c}	2.43^{b}	8.73^{b}	15.65^{b}	2.77^{b}
3	T3: Foliar spray with microbial consortium @10g/lit water (at tillering and flowering stage)	98.42^{d}	2.30^{b}	8.13^{c}	13.97^{d}	2.64^{bc}
4	T4: T1+T2+T3	112.50^{a}	2.83^{a}	9.53^{a}	18.72^{a}	3.77^{a}
5	T5: Seed treatment with Carbendazim @ 2g/kg seed.	93.73^{e}	2.83^{a}	8.10^{c}	14.18^{d}	2.56^{cd}
6	T6: T5+ Foliar application of carbendazim @ 1 g/lit (at tillering and flowering stage).	88.97^{f}	2.35^{b}	8.77^{b}	14.84^{c}	2.74^{bc}
7	T7: Control	86.25^{f}	1.77^{c}	7.75^{d}	12.66^{e}	2.44^{d}

Each value represents the mean ± SD (n = 3) and within column different letters were assigned when values were significantly different according to the Duncan's Multiple Range Test ($P < 0.05$)

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