Halo tolerance of Biocontrol Agents against Root Rot of Mung bean (*Vigna radiata* (L.) Wilczek var. *radiata)* Caused by *Macrophomina phaseolina* (Tassi) Goid in Salt Affected Soils

P.T. Sharavanan [1*], V. K.Satya [2] and M. Rajesh [2]

[1]Centre of Excellence in Millets

TNAU, Thiruvannamalai- 606 603, India

[2] Dept of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute

TNAU, Tiruchirapallai -620 027, India

.

ABSTRACT

Root rot of mung bean [*Vigna radiata* (L.) Wilczek var. *radiata*] is major disease and claims huge yield loss if they occur in the field. The pathogen is basically soil borne and survivability may vary depends on soil condition. The fungicide chemicals are available to manage the disease; however, the biocontrol agents are nowadays available for the disease management and the microbial activity of the biocontrol agents is influenced by existing soil condition including soil pH. Hence, a study was conducted to find out the halo tolerance capacity of the biocontrol agents against root rot disease in salt affected soils under *in vitro, in vivo* and field condition. The root rot pathogen *Macrophomina phaseolina* was isolated from infected root. Efficacy of biocontrol agents against

[1, 2] Assistant Professors (Plant Pathology)

growth of *M. phaseolina* was assessed *in vitro.* The results revealed that TNAU strain of *Bacillus subtilis* reduced the mycelial growth of the *M. phaseolina* significantly when media supplemented with NaCl at 5% (1.4 cm), 7.5% (1.5 cm), 10% (1.6cm) and 12.5% (1.6 cm) and without NaCl (1.2 cm) and similar trend of reduction also expressed by BCA1 strain of *B. subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride* under *in vitro*. The performance of the biocontrol agents against the pathogen is slightly reduced when media supplemented with NaCl. The reduction of mycelia weight of *M.phaeolina* was more in media added with TNAU strain of *B.subtilis* and the performance of TNAU strain of *B.subtilis* on reduction of mycelial weight of *M.phaseolina* is reduced when the broth added with NaCl at 5% (3.15g), 7.5% (3.25g), 10% (3.32g) and 12.5%(3.65g) level and which is followed by *P. fluorescens,* BCA 1 strain of *B. subtilis* and *Trichoderma viride.* Under pot culture conditions, the effect of talc formulated biocontrol agents and challenge inoculation with pathogen was assessed against root rot incidence. It was found that the soil application of TNAU strain of *B.subtilis* performed better in reducing the root rot incidence at pH of 7.0 (2.37%), 7.5 (4.50%), 8.0 (5.53%) and 8.7 (6.57%) and followed by BCA 1 of *B.subtilis* in all pH level. Among the biocontrol agents, TNAU strain of *B.subtilis* applied as seed as well as soil application expressed more population in the rhizosphere in all pH level. The biocontrol agents applied as soil application had more populations of the agents in the soil when compared to seed treatment. The halo tolerance performance of the biocontrol agents was also assessed under field condition in pH of 7.5 and 8.7 during 2019-20 and 2020-21. It was found that the minimum root rot incidence and maximum yield was observed from soil application of TNAU strain of *B subtilis* at 2.5 kg/ha but the effect is on par with soil application of BCA1 strain of *B.subtilis* at 2.5 kg/ha.

Key words: *Vigna radiata, Macrophomina phaseolina, Biocontrol agents, Halo tolerance, Salt affected soils*

1. INTRODUCTION

Mung bean (*Vigna radiate* L) is one of the important pulse crop grown in India and the growth and yield are being affected by various diseases. Among the diseases in Mung bean, dry root rot

caused by *Macrophomina phaseolina* claims yield loss of 10–44% in India [1]. The pathogen is a soil borne and survives in soil for long period and survivability varied due to salinity of soil. Although chemicals are available to manage the disease, the biocontrol agents are nowadays available to contain the pathogenic growth in soil effectively. Beneficial bacteria used as biocontrol agents in disease management can prevent damage caused by plant pathogens by means of antagonism, induction of systemic resistance, competition for nutrients and ecological niches, nutrient mobilisation, phytohormone production and plant growth acceleration [2]. In general, a variety of biotic and abiotic stresses affect crop productivity, including extreme weather factors, the presence of toxic metals and organic contaminants in the environment, saline condition and various plant pathogens [3]. Drought and soil salinity served as limiting factors for crop development and production, especially in arid and semi-arid environments [4], and had a negative impact on soil microbial complexity, variety, composition, and functions [5]. In soil, a range of interactions among physical, chemical, and biological variables play a vital influence in microorganism metabolic activities and are a driving factor in fundamental metabolic cycles where many enzyme activities occur [6]. The microbial activity of biocontrol agents and pathogens is frequently influenced by soil salinity and the soil salt level exceeded, the overall population of bacteria is reduced [7]. The introduced biocontrol agents in soil ecosystem against plant disease should have capacity to withstand even adverse soil condition. Most of the *in vivo* as well as field experiments for the development of biocontrol agents against plant disease were carried out in soil with neutral pH or slightly acidic or basic pH level. The salt stress affects both the metabolic activity of plant cells and increases the vulnerability of the host plant to phytopathogen [8]. So, the efficiency of the agents was influenced by salt condition of soil. *P. fluorescens*, *P. trivialis*, *P. putida*, *P. chlororaphis* and *P. extremorientalis* had an antagonistic effect on *F.solani* in tomato, and the strains produced more indole acetic acid even under saline conditions, stimulating root growth of the crop against saline conditions [9]. Hence, an elaborate study was conducted in order to find out the halo tolerance of the biocontrol agents on root rot incidence of mung bean in salt affected soils.

2. MATERIALS AND METHODS

2.1 Isolation of *M.phaseolina*

Root rot infected mung bean plants were collected from the Farm of Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli, India. The roots showing root rot infection were initially washed with sterile water and roots were cut into small pieces using a sterile blade and the pieces were surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec followed by washing in several changes of sterile distilled water. The surface sterilized pieces were inoculated in the sterile Petri plates added with sterilized potato dextrose agar (PDA) (20% potato extract, 2% dextrose, and 1.5% agar) medium under aseptic condition. The inoculated Petri plates were incubated at room temperature (28 ± 2°C) temperature for five days. After that, the plates observed for the presence of *M .phaseolina.* Then, the fungus was purified by single spore isolation technique by transferring a single spore to PDA medium. The purified culture was used for further studies.

2.2 Preparation of sand maize inoculum of *M.phaseolina*

The pathogen *M.phaseolina* was multiplied in sand maize medium. Well dried maize grain was grinded to powder level. The sand was mixed with maize powder @19:1 ratio and sterilized. The sterilized medium was inoculated with a disc of *M.phaseolina* and incubated for 15 days at room temperature 28±2ºC. Then the well grown medium was used in the study.

2.3 Isolation of *Bacillus* from rhizosphere of mung bean

Rhizosphere soil from mung bean grown in soil with pH of 8.7 in the Farm of Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli was used for isolation of *Bacillus* spp. Initially healthy mung bean was pulled and shaken vigorously. Then 10 g of root segments were excised and taken in a flask containing 100 ml of sterile and shaken for 15 min. The bacteria was isolated by following serial dilution technique. 0.1 ml of solution was taken in a sterilized Petri plate and poured with Nutrient Agar medium. The plates were kept for incubation under room temperature for 36 hours. The growth of the bacteria was observed and colonies of *B. subtilis* was identified.

2.4 Screening of halophytic capacity of biocontrol agents

Screening of salt tolerant capacity of biocontrol agents was carried out under laboratory condition. TNAU strain of *B. subtilis* and *P. fluorescens* was collected from Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli and used in the study. BCA 1 strain of *B.subtilis* was isolated from rhizosphere of mung bean grown in soil with pH of 8.7. Potato dextrose broth mixed with different concentration of NaCl used for this study. The sterilized Potato dextrose broth with various concentration of NaCl added with five ml of 48 hours old liquid culture of *B.subtilis* or *P.fluorescens* or five days old *T.viride* liquid culture. Then 8 mm disc of *M.phaseolina* was added in the broth and incubated under room temperature (28 ± 2 º C) for seven days. After that the mycelial growth of *M.phaseolina* was taken from the broth and air dried under shade condition. Then the weight of the mycelial growth was calculated and expressed as gram of mycelial weight.

2.5 Dual culture plate assay

The well grown pure culture of the root rot pathogen *M. phaseolina* cultivated on Petri dishes using standard PDA medium was used in the study. An 8 mm culture disc of the *M.phaseolina* was inoculated on one side of sterile Petri plate poured with sterilised PDA medium and another side of plate inoculated with either streaked with bacterial biocontrol agents or 8 mm disc of fungal biocontrol agents. Then the plates were kept under room temperature (28 ± 2 º C) for 5-7 days. The growth of *M. phaseolina* was measured after incubation and expressed as cm.

2.6 Collection of soil samples

Soil samples at different pH level collected from farm of Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli using standard procedure. Initially soil from surface layer removed by a spade. A “V’’ shape cut was made to a depth of 15 cm in the sampling area using spade to remove 1 to 2 cm slice of soil. Then the soil was collected and taken in a clean bucket. Likewise, soil samples were collected from four spotted area in a field. Then soil collected at bucket taken on a clean paper of cloth and mixed thoroughly. The soil was spread evenly and divided it into four quarters. Two opposite quarters were discarded and the soil from remaining two sides again mixed thoroughly

likewise till soil sample comes to level of 0.5 kg. The soil samples were packed and taken to laboratory for further studies.

2.7 Preparation of talc formulation of the biocontrol agents

For assessment of efficacy of the biocontrol agents, talc-based formulation of the biocontrol agents was prepared as per method [10]. Initially sterilized Kings' B medium broth (proteose peptone 20 g, K_2HPO_4 1.5 g, Mg $SO_4.7H_2O$ 1.5 g, glycerol 20 ml, water 1000 ml, pH 7.2) inoculated with loopful of the bacterial biocontrol agents and incubated for 48 hours at room temperature (26±2°C) in a rotary shaker at 150 rpm/min. Then the well grown bacterial culture was mixed with talc powder @ 2.5 kg/ litre of culture. In order to keep the pH at 7.0, $CaCO_3$ was added @ 15g /kg of talc powder and talc formulation containing 10^8 cfu /ml. At time of preparation, the population of bacteria in the formulations was 10^8 cfu /g of talc powder and fungal biocontrol agents was 10^6cfu/g of talc powder.

2.8 *In vivo* assessment of efficacy of the biocontrol agents against root rot pathogen in salt affected soils

The soil with different pH level was utilized for pot culture experiments under *in vivo*. The collected soils were sterilized in autoclave at 137.9 kPa for 20 min. The soil was taken in pot @ 12.5 kg/pot and the seeds of mung bean were sown in the pot soil as per the treatment.

2.8.1 Seed treatment of the biocontrol agents

The seeds of mung bean were treated with the biocontrol agents @ 10 g/kg of seed in case of *B.subtilis* and *P.fluorescens* and 4g/kg of seed in case of *T.viride* before sowing. The talc formulation of the biocontrol agents applied @ 5 g/ 12.5 kg of pot soil as soil application of biocontrol agents. Twelve numbers of treated seeds were sown in Pot.

2.8.2 Soil application of the biocontrol agents

The sand maize inoculum of *M.phaeolina* was also inoculated in the pot @ 5 g/12.5 kg of pot soil. The talc formulation of the biocontrol agents applied @ 5 g/ 12.5 kg of pot soil as soil application of biocontrol agents. Twelve numbers of untreated seeds were sown in Pot and they kept in shade net house and regular watering was done. Each replication contain three pots and four replications were maintained. The disease development and growth of the crop were recorded at 10 days interval. The root rot incidence was recorded as per cent disease incidence.

2.8.3 Population of the biocontrol agents

The population of biocontrol agents in the rhizosphere region of the crop was assessed at 15 days intervals upto 45 days after sowing using serial dilution technique. The population of the biocontrol agents was expressed as x 10^6 cfu/g of rhizosphere soil in case of *T.viride* and x 10^8 cfu/g of rhizosphere soil in case of *B.subtilis* and *P. fluorescens*.

2.9 Efficacy of the biocontrol agents against root rot of mung bean in salt affected soil

In order to assess the efficacy of the biocontrol agents, two field trials were conducted in soil with pH 7.5 and 8.7 level at the Farm, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli during 2019-20 and 2020-21. Following treatments were applied in the trial and the mung bean variety VBN 5 used in the study.

Treatment details

T1: Control
T2: Seed treatment with carbendazim at 2g/kg of seed
T3: Soil drench with copper oxy chloride at 0.3% when infection noticed
T4: Seed treatment with BCA1 strain of *B.subtilis* at 10g/kg of seed
T5: Seed treatment with TNAU strain of *B. subtilis* at 10g/kg of seed
T6: Seed treatment with *T.viride* at 10g/kg of seed
T7: Soil application of BCA1 strain of *B.subtilis* at 2.5 kg/ha

T8: Soil application of TNAU strain of *B subtilis* at 2.5 kg/ha
T9: Soil application of *T.viride* at 2.5 kg/ha

The crop management practices for mung bean regularly applied in the trial. The crop was monitored for root rot infection regularly and recorded.

2.10 Statistical analysis

The data collected from the experiments were subjected to statistical analysis. The per cent data are arcsine transformed before statistical analysis. The significance difference between the treatments (P≥ 0.05%) was examined using analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Efficacy of the biocontrol agents on *M.phaseolina* in vitro

An elaborate study was undertaken on the efficiency of biocontrol agents on management of root rot of mung bean under salt affected soils. Initially root rot pathogen *M. phaseolina* was isolated from root infected with pathogens collected from field having pH of 8.7. The biocontrol agent's viz., *T. viride*, The performance of the biocontrol agents were assessed against the growth of *M.phaseolina* under *in vitro* and the results presented in Table.1 and 2. The results revealed that the TNAU strain of *B. subtilis* reduced the mycelial growth of *M.phaseolina* when media supplemented with NaCl at 5% (1.4 cm), 7.5% (1.5 cm), 10% (1.6cm) and 12.5% (1.6 cm) and without concentration of NaCl (1.2cm) which is followed by BCA1 of *B.subtilis*, *P. fluorescens* and *T. viride*. The performance of the biocontrol agents against the pathogen is slightly reduced when media supplemented with NaCl. The growth of the *M. phaseolina* also reduced when the pathogen grown as control in media supplemented with NaCl.

Similarly, reduction of mycelial weight of *M.phaeolina* was more in media added with TNAU strain of *B.subtilis* and the performance of TNAU strain of *B.subtilis* on reduction of mycelial weight of

190 *M.phaseolina* is reduced when the broth added with NaCl at 5% (3.15 g), 7.5% (3.25 g), 10% (3.32 g)
191 and 12.5% (3.65 g) level and which is followed by *P. fluorescens,* BCA 1 of *B. subtilis* and *T.viride.*
192 The salinity and drought tolerance was well observed in *Bacillus* strains compared
193 to *Pseudomonas* due to spore forming capacity of *Bacillus* spp [11]. The endospores produced by
194 *Bacillus* are extremely resistant dormant structures can withstand unfavorable environmental
195 conditions [12]. The spore forming *Bacillus* expressed antagonistic effect on *M.phaseolina* [13]
196 However, the growth promoting effect of *P. fluorescens* and *P. aeruginosa* on tomato observed even
197 at 6% NaCl [14]. *T. virens* and *T. atroviride* created IAA compound when cultivated in medium
198 containing 100 mM NaCl and enhanced the growth of Arabidopsis seedlings [15]. *Trichoderma* spp.
199 enhanced expression of genes linked to salt tolerance, osmoprotection, and ascorbic acid (AA)
200 synthesis when Arabidopsis and cucumber roots were subjected to salt stress and inoculated with
201 *Trichoderma* spp. [16].
202
203 **3.2. Efficacy of the biocontrol agents on root rot incidence in different soil pH under**
204 **pot culture condition**
205
206 Effect of talc formulation of the biocontrol agents on root rot incidence was assessed under
207 pot culture condition using soil having different pH. The biocontrol agents applied as seed treatment
208 @ 4g/kg of seed in case of fungal biocontrol agent and 10g/kg of seed in case of bacterial biocontrol
209 agents and soil application @ 5 g/12.5 kg of pot soil. The results were given in the Table 3 indicated
210 that soil application of TNAU strain of *B.subtilis* performed better in reducing the root rot incidence at
211 pH of 7.0 (2.37%), 7.5 (4.50%), 8.0 (5.53%) and 8.7 (6.57%) which was followed by BCA 1 of
212 *B.subtilis* in all pH except neutral pH. Among the seed treatment of biocontrol agents, *P. fluorescens*
213 had lower incidence of root rot in soil pH 7 (5.08%), 7.5 (5.10%) and TNAU of *B.subtilis* in soil pH 8
214 (6.68%) and 8.7 (8.83%). The efficiency of the biocontrol agents was reduced against root rot of mung
215 bean when soil pH increased. However when the pathogen alone inoculated, the incidence of root rot
216 was increased when pH of pot soil increased. Halo tolerant *B.subtilis* suppressed the root rot as well
217 as wilt incidence in Mung bean [17]. In Mung bean, application of *B. subtilis* showed a marked
218 reduction of disease incidence caused by *M. phaseolina* and the survival rate of healthy plants was
219 also increased to 82.14% [18].

3.3 Population dynamic of the biocontrol agents in the rhizosphere of mung bean

The population of the biocontrol agents was assessed from the experiments. The results given in Table 3. indicated that the populations of the biocontrol agents in rhizosphere of mung bean are varied significantly when applied as seed as well as soil treatment. In case of seed treatment, the population of biocontrol agents was shown as increasing trend over period of time in soil with pH 7.0 and 7.5. Whereas, the population of the agents in the rhizosphere with pH 8.0 and 8.7 was shown as decreasing trend from 15 DAS over period of time. Among the four biocontrol agents, TNAU strain of *B.subtilis* applied as seed as well as soil application expressed more population in the rhizosphere in all pH level. The biocontrol agents applied as soil application had more populations of the agents in the soil when compared to seed treatment. In our experiments, the population of the biocontrol agents is reduced when soil pH was increased. Microbial toxicity is a direct effect of sodium chloride in the soil on the microbial community in the rhizosphere structure [19]. Several workers reported the deleterious effect of salinity on the soil microbial communities and its activities [20,21]. But, the ability of the salt tolerant *Pseudomonas* strain on root colonisation was not affected by higher salinity in soil [22].

3.4. Halo tolerance of biocontrol agents on root rot of mung bean under field condition

Field efficacy of the biocontrol agents was also studied in two field trials during 2019-20 and 2020-21. The root rot incidence as well as yield parameters were recorded from the trials and the results were presented in Table 4. The results revealed that all the treatments were significantly reduced the level of root rot incidence and increased the yield of the crop. Among the treatments, soil application of TNAU strain of *B.subtilis* at 2.5 kg/ha was found to be superior in reducing the root rot incidence in pH of 7.5 (6.63% in 2019-20 and 8.22 % in 2020-21) and 8.7 (8.60% in 2019-20 and 8.02 % in 2020-21) but the effect is on par with soil application of BCA1 strain of *B.subtilis* at 2.5 kg/ha. The efficiency of soil application of biocontrol agents was more than that of seed treatment of the same in the trials. The data also revealed that the maximum yield of crop was obtained from soil

application of TNAU strain of *B. subtilis* at 2.5 kg/ha (928.48 kg/ha and 872.13 in soil pH 7.5 and 8.7 respectively during 2019-20 and 950.88 kg/ha and 910.56 kg/ha in soil pH 7.5 and 8.7 respectively during 2020-21) and which was followed by soil application of BCA1 strain of *B.subtilis* at 2.5 kg/ha. *B. subtilis* enhanced the plant growth and disease resistance under normal and *M.phaseolina* infected conditions [18]. Plant growth promoting rhizobacteria Genera including *Pseudomonas* and *Bacillus* are performed well in improving crop productivity even under saline conditions [23,24,25]. The characteristics of PGPR include the production of phytohormones [26], produce secondary compounds [27], synthesize ACC deaminase [28] and osmolytes [29] and activation of plant's antioxidative enzymes under salt stress [30] are involved in plant disease defense mechanisms. Among this, activity of ACC deaminase enzyme is a common phenomenon especially when plants exposed to high salt stress. Besides, ACC deaminase activity of rhizobacteria, they also increased the survivability survival in saline soils but also increased the productivity of the crop. Under drought and salt stress, PGPR had an effect on the host cell's membrane stability, the creation of biocompatible solutes, and the production of photosynthetic pigments [31]. Plant health was improved by *P. fluorescens* and *P. migulae* strains generating the ACC deaminase enzyme, which impacted the physiological parameters of the plants under salt stress [32]. The PGPR like *Bacillus* and *Pseudomonas* stimulated growth of maize under saline conditions [33]. In our studies, *Trichoderma* also found to be best performing agent against root rot of mung bean in salt affected soils. *Trichoderma* spp. also having traits of salt tolerance and expressed suitable mechanisms in salt stress condition [16].

4. CONCLUSION

The biocontrol agents used in the study viz., BCA 1 strain of *B.subtilis*, TNAU strain of *B.subtilis*, *P. fluorescens, T. viride* possessed the halo tolerance capacity in high soil pH and TNAU strain of *B.subtilis* suppressed the root rot incidence of mung bean even in high soil pH very effectively and soil application of TNAU strain of *B.subtilis* at 2.5 kg/ha was found to be superior in reducing the root rot incidence.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Plant Protection and Department of Agronomy, Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirapallai for providing the Lab and Field facility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kaushik CD, Chand JN. Seedborne nature of *Rhizoctonia bataticola* causing leaf blight of mung bean. *J. Mycol. Plant Pathol.* 1987; 17: 154–157.

2. Sharf W, Javaid A, Shoaib A, Khan IH. Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis.* Egyptian Journal of Biological Pest Control. 2021*;* 31: 16.

3. Ahmad P, Hashem A, Abd Allah EF, Alqarawi AA, John R, Egamberdieva D, Gucel S.Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. Front. Plant Sci. 2015; 6: 868.10.3389/fpls.2015.00868

4. Ahmad P, Hakeem KR, Kumar A, Ashraf M, Akram NA. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). Afr. J. Biotech. 2012; 11: 2694-2703.

5. Zhang Wen Wen, Wang Chong, Xue Rui, Wang Li Jie. Effects of salinity on the soil microbial community and soil fertility. Journal of Integrative Agriculture, 2019; 18 (6): 1360-1368

6. Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellarar G, Renella G. Microbial diversity and soil functions. European Journal of Soil Science. 2003; 54, 655-659

7. Egamberdieva D, Kucharova Z, Davranov K, Berg G, Makarova N, Azarova T. Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils. Biol. Fertil. Soil. 2011; 47: 197-205.

8. Dileo MV, Pye MF, Roubtsova TV, Duniway JM, Macdonald JD, Rizzo DM. Abscisic acid in salt stress predispositions to Phytophthora root and crown rot in tomato and chrysanthemum. Phytopathology. 2010; 100: 871-879

9. Dilfuza Egamberdieva, Gabriele Berg, Vladimir Chebotar, Igor Tikhonovich, Faina Kamilova, Shamil Z Validov, Ben Lugtenberg. Plant Growth Promoting Rhizobacteria for sustainable agriculture, In Proceedings of the 2nd Asian PGPR Conference, August 21-24, 2011, Beijing, P.R. China page 75-79

10. Vidhyasekaran P, Muthamilan M. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Dis. 1995; 79: 782-786

11. Praveen Kumar G, Mir Hassan Ahmed SK, Suseelendra Desai, Leo Daniel, Amalraj E, Abdul Rasul. *In Vitro* Screening for Abiotic Stress Tolerance in Potent Biocontrol and Plant Growth Promoting Strains of *Pseudomonas* and *Bacillus* spp. 2014; Accessed 21 November 2021. Available: https://doi.org/10.1155/2014/195946

12. Piggot PJ, Hilbert DW. Sporulation of *Bacillus subtilis*, Current Opinion in Microbiology, 2004; 7 (6): 579–586.

13. Stefany Castaldi, Claudia Petrillo, Giuliana Donadio, Fabrizio Dal Piaz, Alessio Cimmino, Marco Masi, Antonio Evidente and Rachele Isticato. Plant Growth Promotion Function of *Bacillus* sp. Strains Isolated from Salt-Pan Rhizosphere and Their Biocontrol Potential against

Macrophomina phaseolina. Int. J. Mol.Sci. 2021; Accessed 21 November 2021. Available: https://doi.org/10.3390/ijms22073324

14. Tank N, Saraf M. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. Journal of Plant Interactions, 2010; 5(1): 51–58

15. Hexon Angel Contreras-Cornejo, Lourdes Macías-Rodríguez, Ruth Alfaro-Cuevas and José López-Bucio. *Trichoderma* spp. improve Growth of *Arabidopsis* Seedlings Under Salt Stress Through Enhanced Root Development, Osmolite Production, and Na+ Elimination Through Root Exudates, MPMI. 2014; 27(6): 503–514.

16. Brotman Y, Landau U, Cuadros-Inostroza Á, Tohge T, Fernie AR, Chet I, Viterbo A, Willmitzer L. *Trichoderma*-plant root colonization: Escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. PLoS Pathog. 2013; 9:e1003221. Published online.

17. Patel RR, Disha D, Patel, Parth Thakor, Bhavika Patel, Vasudev R, Thakkar. Alleviation of salt stress in germination of *Vigna radiata* L. by two halotolerant *Bacilli* sp. isolated from saline habitats of Gujarat. Plant Growth Regul. 2014; Accessed 21 November 2021. Available: DOI 10.1007/s10725-014-0008-8

18. Hashem A, Elsayed Fathi Abd_Allah, Abdulaziz A, Alqarawi, Ramalingam Radhakrishnan, Ashwani Kumar. Plant defense approach of *Bacillus subtilis* (BERA 71) against *Macrophomina phaseolina* (Tassi) Goid in mung bean. Journal of Plant Interactions. 2017;12(1):390-401.

19. Nelson DR, Mele PM. Subtle changes in rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. Soil Biol. Biochem. 2007; 39(1):340–351.

20. Rietz DN, Haynes RJ. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol. Biochem. 2003; 35(6):845–854.

21. Sardinha M, Muller T, Schmeisky H, Joergensen RG. Microbial performance in soils along a salinity gradient under acidic conditions. Appl. Soil Ecol. 2003; 23(3):237–244.

22. Paul D, Nair S. Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. J. Basic. Microbiol. 2008; 48(5):378–384.

23. Sharma S, Kulkarni J, Jha B. Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut. Front. Microbiol. 2016; 7:1600.

24. Singh RP, Jha PN. The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). PLoS One. 2016; 11:e0155026. Accessed 22 November 2021. Available: doi: 10.1371/journal.pone.0155026

25. Sarkar A, Ghosh PK, Pramanik K, Mitra S, Soren T, Pandey S. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Microbiological Research. 2018; 169: 20–32.

26. Dodd IC, Zinovkina NY, Safronova VI, Belimov AA. Rhizobacterial mediation of plant hormone status. Annals of Applied Biology. 2010; 157: 361–379.

27. Timmusk S, El-Daim IAA, Copolovici L, Tanilas T, Känaste A, Behers L. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 2014; 9:e96086. Accessed 22 November 2021. Available: doi: 10.1371/journal.pone.0096086

28. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B. Promotion of plant growth by bacterial ACC deaminase. Cri. Rev. Pla. Sci. 2007; 26: 227–242.

29. Bano A, Fatima M. Salt tolerance in *Zea mays* (L). following inoculation with *Rhizobium* and *Pseudomonas*. Biol. Fertil. Soils. 2009; 45: 405–413.

30. Hashem A, Abd_Allah EF, Alqarawi AA, Al-Huqail AA, Wirth S, Egamberdieva D. The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. Front. Microbiol. 2016; 7:1089.

31. Tiwari G, Duraivadivel P, Sharma S, Hariprasad P. 1-Aminocyclopropane-1-carboxylic acid deaminase producing beneficial rhizobacteria ameliorate the biomass characters of *Panicum maximum* Jacq. by mitigating drought and salt stress. Sci. Rep. 2018; 8:17513.

32. Ali S, Charles TC, Glick BR. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol. Biochem. 2014; 80:160–167.

33. Aslam F, Ali B. Halotolerant bacterial diversity associated with *Suaeda fruticosa* (L.) forssk. improved growth of maize under salinity stress. Agronomy. 2018. 8:131-135.

Table 1. Efficacy of the biocontrol agents on the growth of *M.phaseolina in vitro*

Sl.No	Name of the biocontrol agents	*Mycelial growth of *M.phaseolina* (cm) in media with NaCl					*Mycelial weight of *M.phaseolina* (g) in broth with NaCl				
		0 %	5%	7.5%	10%	12.5%	0 %	5%	7.5%	10%	12.5%
1	Control (Only *M.phaseolina*)	7.6	7.2	6.8	6.7	6.2	12.35	11.75	10.23	9.75	9.50
2	BCA1 strain of *B. subtilis*	1.3	1.4	1.6	1.6	1.8	3.12	3.29	3.40	3.55	4.15
3	TNAU strain of *B. subtilis*	1.2	1.4	1.5	1.6	1.6	2.76	3.15	3.25	3.32	3.65
4	*P. fluorescens*	1.7	1.8	2.0	2.2	2.4	2.83	3.21	3.37	3.52	3.74
5	*T.viride*	1.6	1.6	2.3	2.5	2.5	4.32	4.62	4.86	4.91	5.03
	SEd	0.078	0.081	0.076	0.169	0.056	0.090	0.221	0.194	0.095	0.099
	CD (P ≥ 0.01)	0.18**	0.18**	0.17**	0.37**	0.19**	0.20**	0.49**	0.43**	0.21**	0.22**

* Mean of four replications, each replication contain three petri plates

** Mean of four replications, each replication contains three number of 250 ml conical flask containing 200 ml broth medium.

Table 2. Efficacy of the biocontrol agents on root rot of mung bean under *in vivo* condition

Treatment No	Name of the treatments	*Per cent root rot disease incidence			
		At 7.0 pH level	At 7.5 pH level	At 8.0 pH level	At 8.7 pH level
T1	Control	0.00 (0.91)	0.00 (0.91)	0.0 (0.91)	0.00 (0.91)
T2	Sand maize inoculum of *M.phaseolina* @ 5 g/pot	28.67 (32.36)	32.50 (34.74)	34.73 (36.09)	38.89 (38.52)
T3	Seed treatment with BCA1 strain of *B.subtilis* @ 10 g/kg of seed	4.73 (12.51)	5.90 (13.99)	11.77 (20.04)	13.43 (21.47)
T4	Seed treatment with TNAU strain of *B. subtilis* @ 10 g/kg of seed	5.23 (13.22)	5.13 (13.09)	6.63 (14.86)	8.83 (17.26)
T5	Seed treatment with *P. fluorescens* @ 10 g/kg of seed	5.08 (13.02)	5.10 (13.05)	6.71 (14.96)	11.76 (20.03)
T6	Seed treatment with *T. viride* @ 4g/kg of seed	6.73 (14.99)	10.93 (19.28)	13.47 (21.51)	19.00 (21.36)
T7	Soil application of BCA1 strain of *B.subtilis* @ 5 g/ 12.5 kg of pot soil	2.90 (9.78)	4.71 (12.53)	5.93 (14.03)	6.84 (15.10)
T8	Soil application of TNAU strain of *B.subtilis* @ 5 g/ 12.5 kg of pot soil	2.37 (8.84)	4.50 (12.21)	5.53 (13.58)	6.57 (14.79)
T9	Soil application of *P. fluorescens* @ 5 g/ 12.5 kg of pot soil	2.47 (9.03)	5.40 (13.43)	7.03 (15.29)	6.60 (14.85)
T10	Soil application of *T.viride* @ 5 g/ 12.5 kg of pot soil	4.50 (12.21)	5.03 (12.95)	6.77 (15.02)	7.42 (15.81)
	SEd	0.53	0.49	0.48	0.51
	CD (P ≥ 0.05)	0.86**	0.97**	0.81**	0.87**

*Mean of four replications. Values in parenthesis are arcsine transformed values.

Table 3. Population dynamic of the biocontrol agents in rhizosphere of mung bean under *in vivo* condition

Treatment No	Name of the treatments	*Population of biocontrol agents											
		At 7.0 pH level			At 7.5 pH level			At 8.0 pH level			At 8.7 pH level		
		15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
T1	Control (x 10^8 cfu/g of rhizosphere soil)#	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T2	Sand maize inoculum of *M.phaseolina* @ 5 g/pot (x 10^8 cfu/g of rhizosphere soil)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	Seed treatment with BCA1 strain of *B.subtilis* @ 10 g/kg of seed (x 10^8 cfu/g of rhizosphere soil)	6.06	6.51	6.50	5.82	6.08	6.05	4.69	3.92	3.35	4.32	3.88	2.61
T4	Seed treatment with TNAU strain of *B. subtilis* @ 10 g/kg of seed (x 10^8 cfu/g of rhizosphere soil)	7.57	8.35	8.37	6.77	7.40	8.17	6.21	5.91	5.44	5.41	5.28	5.11
T5	Seed treatment with *P. fluorescens* @ 10 g/kg of seed (x 10^8 cfu/g of rhizosphere soil)	7.04	7.73	8.24	6.74	7.59	7.96	5.81	5.46	4.20	5.25	4.87	4.18
T6	Seed treatment with *T. viride* @ 4g/kg of seed (x 10^6 cfu/g of rhizosphere soil)	4.15	4.82	4.32	4.84	4.40	3.97	3.12	3.06	2.93	2.94	2.07	1.99
T7	Soil application of BCA1 strain of *B.subtilis* @ 5 g/ 12.5 kg of pot soil (x 10^8 cfu/g of rhizosphere soil)	8.30	8.77	8.95	7.27	7.89	7.07	7.01	6.75	6.56	6.34	6.11	5.79
T8	Soil application of TNAU strain of *B. subtilis* @ 5 g/ 12.5 kg of pot soil (x 10^8 cfu/g of rhizosphere soil)	9.56	9.75	10.27	9.53	10.09	10.48	9.46	9.75	9.85	8.32	8.63	7.61
T9	Soil application of *P. fluorescens* @ 5 g/ 12.5 kg of pot soil (x 10^8 cfu/g of rhizosphere soil)	8.79	9.25	9.62	9.38	9.88	10.35	9.20	9.37	9.01	8.26	8.30	6.94
T10	Soil application of *T.viride* @ 5 g/ 12.5 kg of pot soil	5.20	5.67	6.22	5.60	6.36	6.91	5.27	4.75	4.67	5.22	4.87	4.18
	SEd	0.014	0.112	0.017	0.134	0.021	0.162	0.024	0.113	0.156	0.029	0.123	0.139
	CD (P ≥ 0.05)	0.21**	0.26**	0.25**	0.31**	0.36**	0.39**	0.42**	0.39**	0.37**	0.52**	0.56**	0.44**

DAS- Days after sowing

*****Mean of four replications.

#Control: there is no application of biocontrol agents and pathogen.

In every pot, sand maize inoculum of pathogen were inoculated in the pot @ 5 g/12.5 kg of pot soil.

Table 4. Halo tolerance effect of biocontrol agents on root rot and yield of mung bean under field condition during 2019-20 and 2020-21

Treatment No	Name of the treatments	Per cent root rot disease incidence				Yield (kg/ha)			
		2019-20		2020-21		2019-20		2020-21	
		At 7.5 pH level	At 8.7 pH level	At 7.5 pH level	At 8.7 pH level	At 7.5 pH level	At 8.7 pH level	At 7.5 pH level	At 8.7 pH level
T1	Control	20.16 (26.68)	23.48 (28.99)	17.93(24.95)	19.98(26.55)	769.25	724.08	794.85	718.11
T2	Seed treatment with carbendazim at 2g/kg of seed	8.11 (16.53)	12.31 (20.53)	11.62(19.23)	13.09 (21.33)	817.76	784.21	838.80	782.32
T3	Soil drench with copper oxy chloride at 0.3% when infection noticed	12.88 (21.03)	13.83 (21.83)	12.85 (21.00)	13.20 (21.30)	800.93	744.11	817.79	756.82
T4	Seed treatment with BCA1 strain of *B.subtilis* at 10g/kg of seed	10.43 (18.81)	12.55 (20.74)	9.70 (18.14)	11.61 (19.92)	828.51	803.17	833.83	803.20
T5	Seed treatment with TNAU strain of *B. subtilis* at 10g/kg of seed	8.70 (17.12)	10.29 (18.73)	8.97 (17.42)	9.91 (18.34)	894.96	826.37	890.40	871.36
T6	Seed treatment with *T.viride* at 10g/kg of seed	12.97 (21.08)	13.75 (21.76)	10.18 (18.60)	12.87 (21.22)	829.20	792.57	835.55	771.12
T7	Soil application of BCA1 strain of *B.subtilis* at 2.5 kg/ha	7.45 (15.67)	8.72 (17.17)	8.52(16.94)	8.61 (17.06)	931.04	845.04	952.43	878.02
T8	Soil application of TNAU strain of *B subtilis* at 2.5 kg/ha	6.61 (14.88)	8.60 (17.04)	8.22(16.58)	8.02 (16.44)	928.48	872.13	950.88	910.56
T9	Soil application of *T.viride* at 2.5 kg/ha	8.24 (16.66)	9.83 (18.26)	9.83(18.27)	10.07 (18.49)	913.39	833.87	911.92	869.49
	SEd	0.75	0.44	0.39	0.89	12.06	11.15	11.69	10.88
	CD (P ≥ 0.05)	1.61**	0.93**	1.16**	1.22**	25.46**	23.64**	24.80**	23.06**