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Interaction effect of *Bacillus* spp. and *Glomus intraradices* on growth parameters of Ragi

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Abstract
Soil microorganisms play a very important role in improving soil fertility and enhancing the plant growth. *Bacillus* and AM Fungi are the important key component of the soil microbial population. So, the aim of the present study was to investigate the effect of single and dual inoculation of *Bacillus* spp with *Glomus intraradices* along with 75% RDF on growth and yield parameters of ragi. The treatment with both strains of *Bacillus* and *G. intraradices* showed the highest plant growth parameters like plant hight, number of tillers, Chlorphyll content, Root length and also the treatment with *Glomus intraradices* showed the highest percent root colonization and spore count at 75 days after transplanting of 90.23% and 178.53% respectively. This is because of the availability of the nutrients uptake, protection of plants against pathogens and mitigation of abiotic stresses like drought, salinity *etc*. through dual inoculation compared to single inoculation of either *Bacillus* spp or *Glomus intraradices* alone.

Keywords: *Bacillus*, *Glomus intraradices*, interaction, root colonization

Introduction
Ragi, also called finger millet (*Eleusine coracana* L. Gaertn), is a major small millet crop grown in India that has the highest productivity among millets. It is a staple crop that is grown in much of India and eastern and central Africa. In India, it is grown in Tamil Nadu, Andhra Pradesh, Maharashtra, Gujarat, Orissa, Jharkhand, and Karnataka. Each year, 29 million hectares of land are planted to millets, with 3.5 million of those acres going solely to small millets.

Over two thirds of the small millets produced (2.8 million tonnes) and 50% of the area are accounted for by finger mill*et al*.one. Finger millet is unique in that it can grow on marginal lands with poor soil fertility and is remarkably adaptable to a variety of agroclimatic conditions. It is also easy to cultivate and resistant to major pests and diseases, drought, and weed growth. Finger millet is now a necessary crop in dry farming systems because of these characteristics. Traditional farmers know finger millet as a crop that requires little fertilizer, but under these conditions, it yields very little. Tropical soils that are semi-arid and typically lacking in major and micronutrients are ideal for growing finger millet. Continuous cropping, low rates of applying organic matter, low use of mineral fertilizer, and inadequate recycling of crop residues are the main causes of this deficiency. Any one of these elements could have an impact on the crop's potential yield. Therefore, it is essential to optimize nutrient management practices and other related factors affecting finger millet cultivation in order to achieve higher yields under the relatively marginal local growing conditions.

In addition to offering empirical support to the customary wisdom possessed by farmers, a prudent blend of inorganic and organic fertilizers can enhance ragi yield. Additionally, this strengthens the soil's health and increases the productivity of ragi. Bacterial plant growth promotion requires good colonization of the promoting organism on the root surface. This can be achieved by either indirect (producing plant hormones, for example) or direct (nutrient competition and antagonistic interactions) mechanisms. Therefore, at a very early stage of plant development, it is crucial to enrich the strain that promotes plant growth (Waechter Kristensen *et al.*, 1997) [6]. Seasonal variations were observed in the quantity and quality of bacteria on the roots and in the nutrient solution in research conducted in commercial market gardens (Waechter-Kristensen *et al.*, 1997) [6].

In order to compete for simple substrates derived from plants, bacteria and fungi have evolved antagonistic strategies (Boer *et al.*, 2005) [7]. Aviles *et al.* (1996) [8] examined the evolution of fungi during the composting of cork industrial waste and postulated an increasing trend in the density of cellulolytic populations as a means of producing a growing medium. The development of biological control methods in soilless growing systems requires an understanding of the ecological and biological characteristics of the native microorganisms. Most bacteria on the surface of plants reside in their roots (Campbell and Greaves, 1990) [9]. In addition, they have the ability to grow more quickly than other microbes and make use of a variety of materials, including sources of nitrogen or carbon (Glick, 1995) [10]. Compant *et al.* (2005) [11] pointed out that additional rhizosphere microflora components that might support plant growth and suppress phytopathogens are PGPR and AM fungi. Their cooperative relationship with mycorrhizae is the main reason for this. Nitrogen fixers, fluorescent Pseudomonads, and sporulating bacilli are among the many studies that have documented the synergistic positive interactions between AM fungi and PGPR (Galleguillos *et al.*, 2000) [12]. On the other hand, certain neutral effects of the PGPR interaction and AM fungi have also been reported (Andrade *et al.*, 1997) [13].

The purpose of this study was to study the effects of applying a biofertilizer (*Bacillus* spp.) and determining the AM fungus inoculation (*G. intraradices*) on fruit weight, fruit quality, growth parameters, and SPAD values in young leaves of two strawberry cultivars grown in a soilless system.

Materials and Methods

Experimental design

The experiment consist of 8 treatments with three replication and the experiment was carried out in ARS, Dhadesguru, University of Agricultural sciences Raichur with Completely Randomized Block Design.

Preparation of Inoculum

Preparation of Mother Culture

100 ml sterilized nutrient broth were prepared in 250 ml conical flask and inoculated 24 hrs. old culture to conical flask containing sterile nutrient broth and allowed for incubation on a shaker for 6 to 7 days. After incubation the flask were taken out and used for the preparation of the broth culture.

Preparation of broth culture

Two litres of nutrient broth was prepared in big flask and sterilized. Small quantity of mother culture was taken and inoculated into flasks containing nutrient broth (Plate 6).

The flasks were incubated for the development of inoculum up to 10^7 CFU/ml. later it was confirmed by serial dilution and agar plating method.

Sterilization of carrier material

Talc powder was used as a carrier material. It was sterilized in the autoclave at 121ºC, 15 lb pressure for 30 minutes.

Mixing culture with carrier material

Bacillus culture was mixed properly with the sterilized carrier material in the ratio of 1:3 (100 ml broth with 300 gm Talc powder) and kept for shade drying to reduce the moisture levels to 30%.

Mass production of AM Fungi by open pot culture method

A standard culture of *Glomus intraradices* was obtained from Department of Agricultural Microbiology, University of Agricultural sciences, Bangalore.

Mass multiplication of *Glomus intraradices* inoculum by pot culture method

The sterilized soil: sand (1:1) mixture was filled in earthen pots at 5kg/pot. A small quantity of mixture was taken out at the centre with a sterilised spatula and the *Glomus intraradices* inoculum 20 g was added to potting mixture. Ragi were sown and crop is maintained for 30 days to mass produce mycorhizal propagules. After 30 days the shoot portion of the ragi plants were cut and removed. The roots were cut into small pieces and mixed with soil and sand mixture. The infected root bits, hyphae and chlamydospores of AMF from the pots were used as inoculum for further studies and also used for further screening for percent root colonization and spore count (Plate 1).

Per cent root colonization and spore count

The AMF per cent root colonization was assessed using the method described by Phillips and Hayman (1970). The roots were washed in tap water to remove the adhering soil particles and cut into bits of 1 cm length and then fixed in formalin: Acetic acid: Alcohol (FAA). The root bits fixed in FAA were washed thoroughly in water to remove fixative. The washed root bits were softened by simmering in 10 per cent KOH solution at 90 ℃ for 10-15 minutes in water bath. After cooling, the excess KOH was washed-off in tap water and then neutralised with two per cent HCl. The root bits were then stained with 0.05 per cent tryphan blue in lactophenol for two minutes. The excess stain from the root tissue was removed by cleaning in lactophenol. The root bits were examined under compound microscope (10x) for AMF colonisation. The per cent AMF colonisation was determined using the following formula.

$$\text{Percentage of root colonization} = \frac{\text{Number of infected root segments}}{\text{Total number of root segments observed}} \times 100$$

Isolation of *Glomus itraradices* spore

The AM fungal spores were isolated by wet sieving and decanting method. About 50 g of rhizosphere soil was suspended in water and stirred well. After settling of the heavier particles, the supernatant was filtered through a set of sieves of different size (1000, 300, 250, 105 and 45 µm). Finally, the soil suspension present in 45 µ sieve were transferred to 100 ml beaker by gentle washing. The spore suspension was filtered through Whatman No. 1 filter paper. The filter paper containing spores was placed on a petri dish and observed for *Glomus intraradices* spores and transferred to another moistened filter paper under stereomicroscope for further studies.

Seedling root dip

One kg of biofertilizer was mixed with five litres of water in a container and was mixed thoroughly. The roots of the seedling were dipped in the suspension for about 30 minutes and were taken out. The treated seedlings were transplanted immediately to the main field.

Field experiment

The main field was prepared and divided into plats as per the Randomized complete block design with three replication. Ragi seedlings were uprooted from nursery, treated with efficient native *Bacillus* isolates along with Mycorrhiza (*Glomus intraradices*) individually and combined form and transplanted

to the main plot and below are the growth parameters of field experiment.

Results and Discussion

Among the 10 isolates, two efficient *Bacillus* isolates were selected based on the performance under *in vitro* conditions. Mycorrhiza (*Glomus intraradices*) culture was also collected by using mass multiplication method. The interaction effect of *Bacillus* isolates and mycorrhiza on growth and yield of ragi was examined under field condition during Feb-April of 2019-20. The individual effect of *Bacillus* spp and *Glomus intraradices* along with RDF and interaction of *Bacillus* spp and *Glomus intraradices* along with RDF were studied under different sets of treatments along with reference strain and the results were compared with control, RDF. The uninoculated treatment served as a control. Following the standard procedure BCRC 2 and BCRS 3 biofertilizers were prepared, both mycorrhizal culture and biofertilizer were applied to the main plot according to the set of treatments. The two *Bacillus* isolates which were best suited under *in vitro* conditions *viz.*, BCRC 2 and BCRS 3 along with mycorrhiza (*Glomus intraradices*) were used individually and in combination with both along with RDF. The effect of co-inoculation of both *Bacillus* spp and mycorrhiza was assessed under Ragi field condition.

Growth parameters

On 25th DAT, the significant difference in the plant height was observed with the treatment T_7 (75% RDF + Isolate I +II + AM Fungi) with 32.5 cm over all the treatments, except treatment T_8 (75% RDF + Isolate I&II) with plant height of 30.1 cm. The treatment T_6 (75% RDF + Isolate II) with plant height of 28.6 cm was significantly higher than T_5 (75% RDF + Isolate I), T_4 (75% RDF + AM Fungi), T_3 (75% RDF + Reference strain) with plant height of 27.1 cm, 26.5 cm, 25.3 cm respectively. Lowest plant height was recorded with control *i.e.* 24.1 cm. and similar trend was followed in 50th and 75 DAT (Table 1, Fig 2, Plate 1).

Table 1: Effect of efficient *Bacillus* spp. and *Glomus intraradices* on plant height of ragi

Treatments	Plant Height (cm)			
	25 DAT	50 DAT	75 DAT	At harvest
T_1- Control	24.1	48.5	78.5	90.2
T_2- RDF	24.5	52.5	80.3	95.3
T_3- 75% RDF + Reference strain	25.3	54.2	82.5	98.5
T_4- 75%RDF + AM Fungi	26.5	55.3	83.2	98.8
T_5- 75% RDF + Isolate I	27.1	56.2	85.2	101.3
T_6- 75% RDF + Isolate II	28.6	58.6	86.5	102.1
T_7- 75% RDF + Isolate I + II + AM Fungi	32.5	62.5	90.2	108.3
T_8- 75% RDF + Isolate I + II	30.1	61.3	88.5	106.5
S.E.M±	1.07	0.95	0.88	1.60
C.D. at 5%	3.21	2.85	2.65	4.82

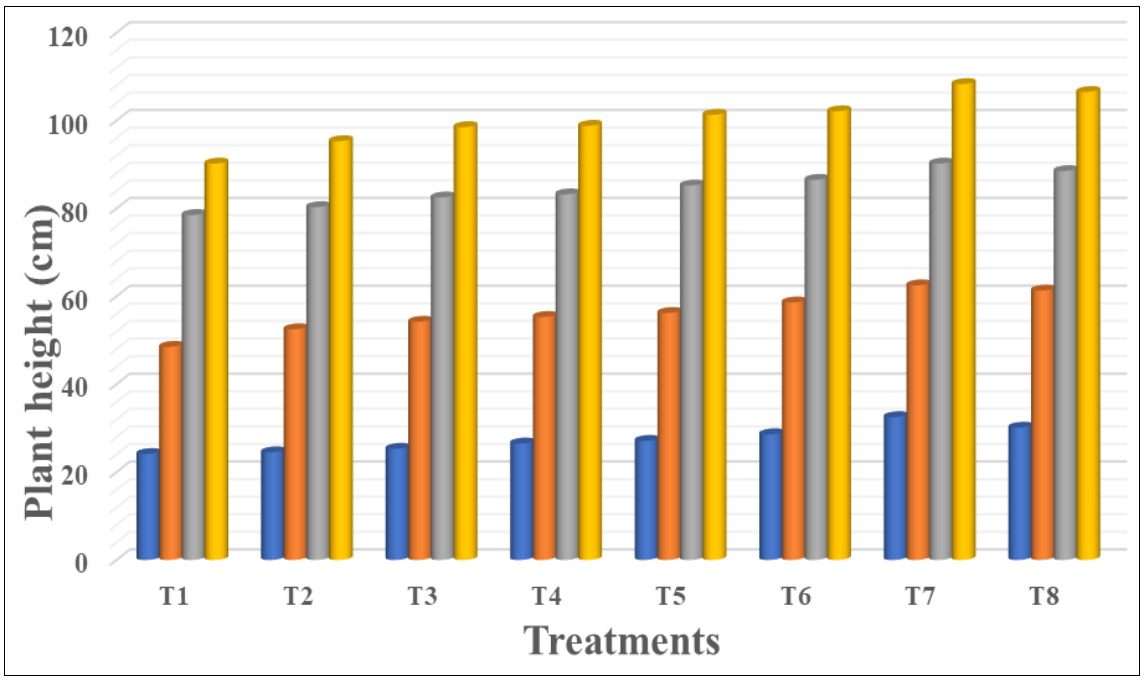

Fig 1: Effect of efficient *Bacillus* spp. and *Glomus intraradices* on plant hit of ragi.

Plate 1: Variation in the Plant height of Ragi with Treatments

Akinrinlola RJ (2018) [1] The result of the experiment conducted in the green house showed that four *Bacillus* strains *Bacillus simplex*, *Bacillus safensis*, *P.graminis*, *Bacillus megaterium* were effective in promoting the growth in soybean and wheat. The height of the inoculated plants higher than the uninoculated, this increase in the height is due to the increased nutrients uptakes and the PGPR activity in the rhizosphere (Sandeep C, *et al.*, 2011) [2].

Maximum number of tillers was recorded with treatment T_7 (75% RDF + Isolate I + II + AM Fungi) with combined inoculation of *Bacillus* spp and *Glomus intraradices* along with fertilizer recorded the maximum number of tillers (1.28) which is on par with treatment T_8 (75% RDF + Isolate I&II) with 1.25 number of tillers. These are significantly higher than all other treatments (Table 2, Fig 2).

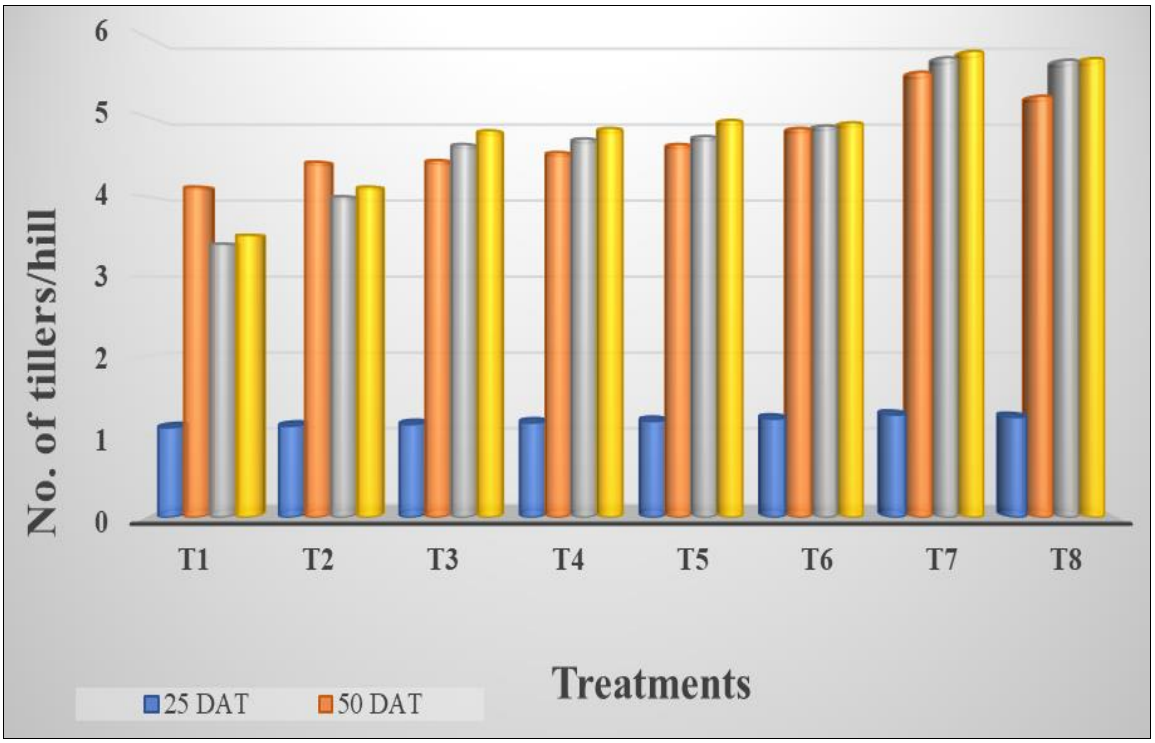

Fig 2: Effect of efficient *Bacillus* spp. and *Glomus intraradices* on plant hight of ragi

Table 2: Effect of efficient *Bacillus* spp and *Glomus intraradices* on number of tillers/hill

Treatment	No. of tillers/hill			
	25 DAT	50 DAT	75 DAT	At harvest
T_1- Control	1.12	4.12	3.41	3.52
T_2- RDF	1.14	4.43	4.00	4.12
T_3- 75% RDF + Reference strain	1.16	4.45	4.65	4.82
T_4- 75%RDF + AM Fungi	1.18	4.55	4.72	4.85
T_5- 75% RDF + Isolate I	1.20	4.65	4.75	4.95
T_6- 75% RDF + Isolate II	1.23	4.85	4.88	4.92
T_7- 75% RDF + Isolate I + II + AM Fungi	1.28	5.55	5.73	5.81
T_8- 75% RDF + Isolate I + II	1.25	5.25	5.70	5.72
S.E.M±	1.20	0.20	0.17	0.20
C.D. at 5%	NS	0.62	0.51	0.62

The highest chlorophyll a content was recorded in the treatment T_7 (75% RDF + Isolate I + II + AM Fungi) on par with T_8 (75% RDF + Isolate I + II) with 1.85 and 1.80 mg/g fresh weight respectively. Among the individual inoculants inoculation treatment T_6 (75% RDF + Isolate II) shows maximum chlorophyll a content followed by T_5 (75% RDF + Isolate I), T_4 (75% RDF + AM Fungi) and Reference strain showing chlorophyll a content of 1.79 mg/g, 1.71 mg/g, 1.66 mg/g and 1.59 mg/g respectively (Fig 3).

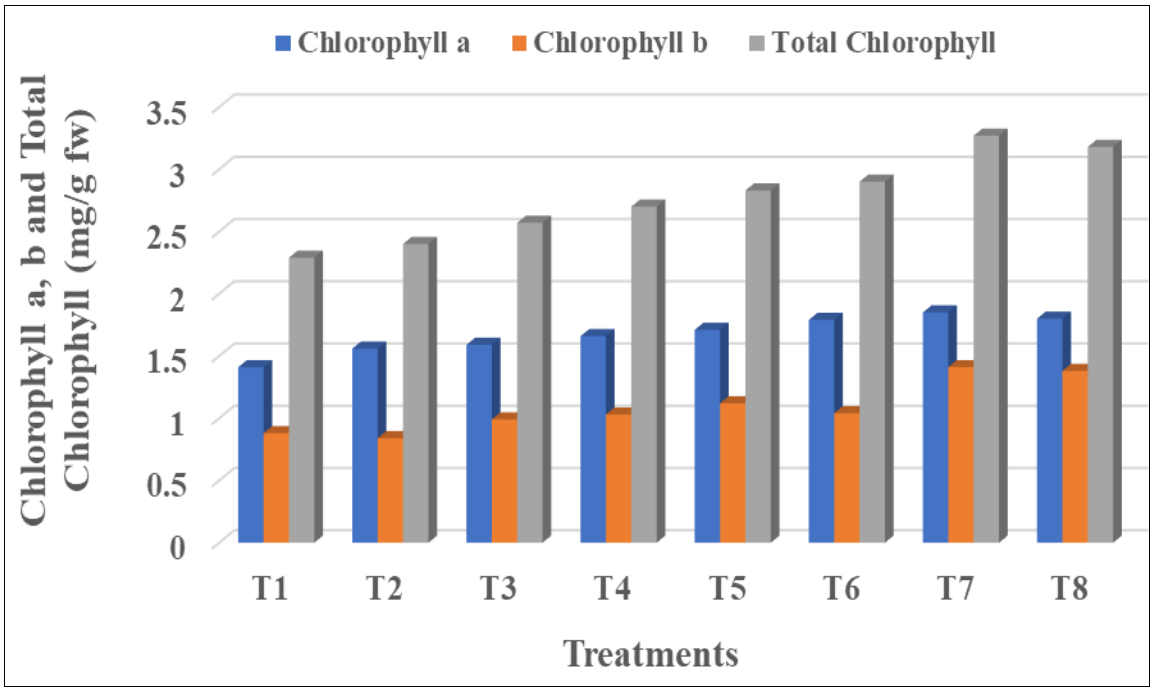

Fig 3: Effect of efficient *Bacillus* spp and *Glomus intraradices* on Chlorophyll content (mg/g fresh weight) of ragi

The data revealed that there was a significant difference between combined application of both bacterial inoculants and mycorrhizal fungi when compared to the treatments with individual inoculants with RDF and RDF alone inoculation. The combined inoculation in treatment T_7 (75% RDF + Isolate I + II + AM Fungi) showed maximum plant height over the other treatments. Compared to all the treatments the lowest plant height was recorded with the control. Similar trend was continued on 25th, 50th, 75th DAT and during harvest stage.

Both *Bacillus* isolates were characterised in the privies study, for P solubilization, siderophore production and IAA production and mycorrhiza were tested for the mass multiplication. Thus, these characters play major role in the growth and development of the plants. The increased plant height due to the root colonization of mycorrhiza and *Bacillus* isolates enhanced the availability of nutrients (nitrogen, phosphorous, potassium) for the growth of the plants by mobilization and solubilization and increase in the plant height is due to the increased nutrients uptake and PGPR activity in the rhizosphere.

Treatment with combined inoculation of both *Bacillus* spp and mycorrhiza with RDF T_7 (75% RDF + Isolate I + II + AM Fungi) shows longest root length of 23.7 cm followed by T_8 (75% RDF + Isolate I + II) with root length of 21.5 cm. The treatment with individual inoculation T_6 (75% RDF + Isolate II) on par with T_5 (75% RDF + Isolate I) with 20.6 cm and 20.1 cm respectively (Fig 4).

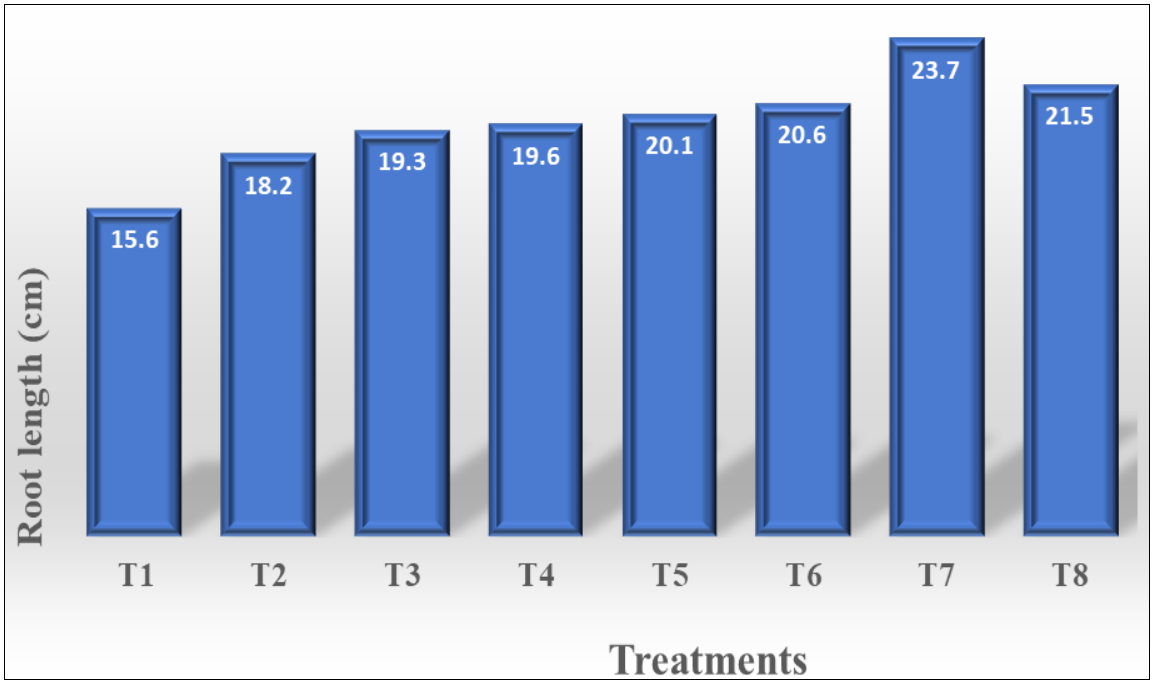

Fig 4: Effect of *Bacillus* spp and *Glomus intraradices* on root length of ragi

Aipova *et al.* (2010) [3] they studied and reported that inoculation with PSB significantly enhanced root length and radicle length of wheat as compared to the individuals. This could be due to the release of plant growth promoting substances by inoculants. Gomaa and Kholas (1999) [5] studied and reported that root length in mungbean was increased with combined inoculation of organic manure, biofertilizers and chemical fertilizers. Pot culture experiment was conducted by Bahadir P S *et al.* (2018) [4], s significant increase in the root length (cm) was observed for three test plants compare to the control plants.

The data in this experiment showed that there was a significant difference between combined application of both bacteria and mycorrhiza with RDF when compared to the individual inoculation with RDF and RDF alone inoculation. Highest plant growth parameters recorded with the combined inoculation T_7 (75% RDF + Isolate I + II + AM Fungi) compare all other treatments. Minimum plant growth parameters were recorded by the control treatments.

The enhanced plant growth is due to the activity of P solubilization and P mobilization, transformation of soil nutrients N, P_2O_5, K_2O and also due to the increased availability of plant growth promoting substances and other trace elements.

Arbuscular Mycorrhizal parameters

Per cent AM root colonization

Per cent root colonization was determined at 25, 50 and 75 DAT. At 25 DAT, the per cent root colonization was found to be increase with increase in the age of the crop and the maximum root colonization was recorded with treatment T_7 (75% RDF + Isolate I + II + AM Fungi) with 50.38% followed by the treatment T_4 (75% RDF + AM Fungi) with 45.41% root colonization. The lowest per cent root colonization was recorded with control treatment (9.96%). Similar trend was followed in 50th and 75 DAT (Fig 5) (Plate 2).

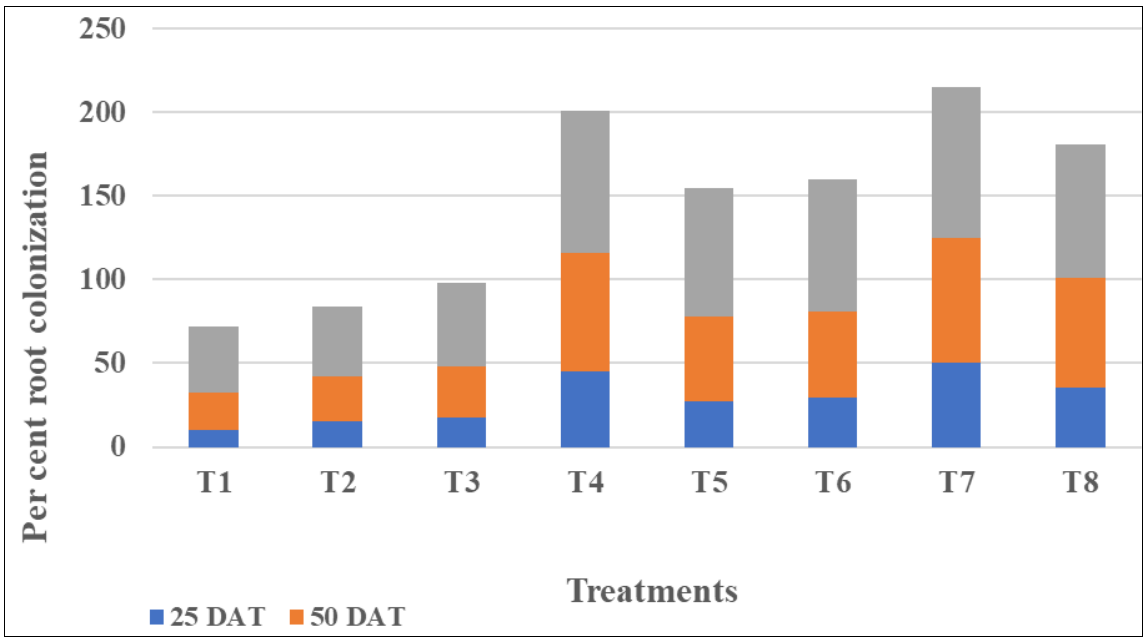

Fig 5: Effect of *Glomus intraradices* inoculation on per cent root colonization in roots of ragi

Plate 2: Root length of ragi plants of T_7 Treatment

Mycorrhizal spore count (Number of spores/50 g of soil): The mycorrhizal spore number was determined by wet sieving and decanting method on 25, 50 and 75 DAS. At 25 DAT, significantly highest spore count was recorded with the treatment T_7 (75% RDF + Isolate I + II + AM Fungi) with 130.47 spores per 50g followed by the treatment T_4 (75% RDF + AM Fungi) with 120.31 spores per 50g. The lowest spore count was recorded with control treatment (40.13 spores per gram) (Fig 6).

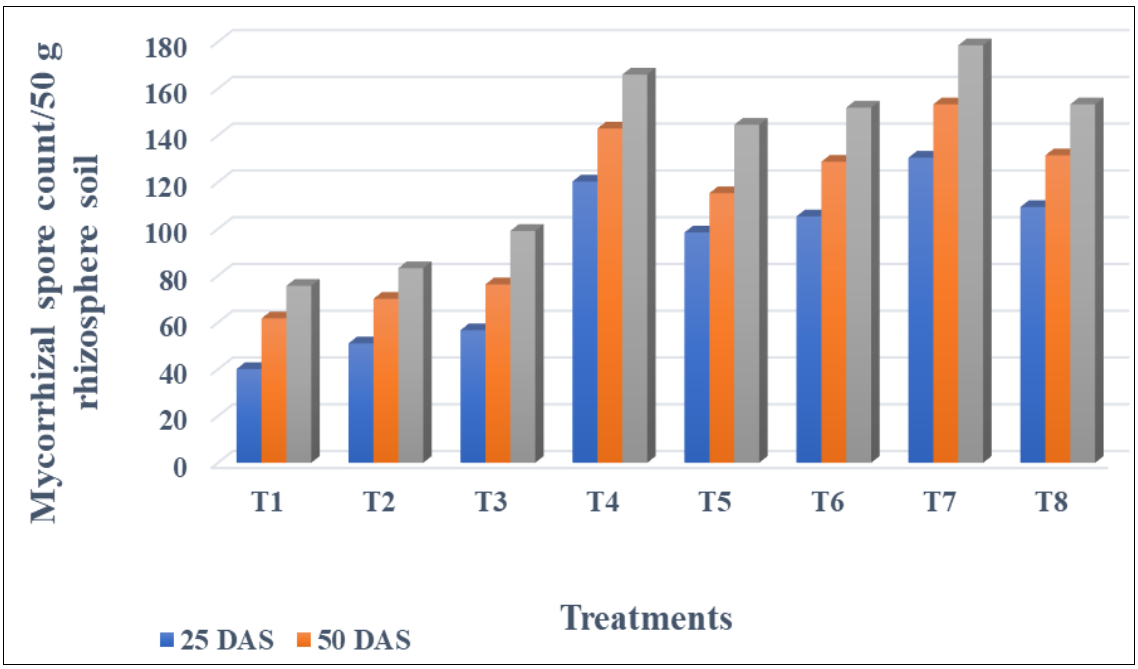

Fig 6: Mycorrhizal spore count per 50g of rhizosphere soils of ragi

Aboud MA, *et al.* (2014) [14] showed in their experiment that interaction of *B. subtilis*, *T. harzianum* with *G. mosseae* in AM fungi colonization, which reveals that increase in the root colonization, weight of mycorrhizal roots under field condition.

Conclusion

Bacillus spp. and *Glomus intraradices* acts synergistically with each other and they promote the plant growth parameters in a bigger way compare to the individual inoculation of both. This is

due to the combined inoculation helps in nutrient solubilization and uptake of the nutrients. Mycorrhiza also helps in the protecting the plants against the abiotic stress conditions like drought, salinity, flood *etc*. combined inoculation of *Bacillus* and *Glomus intraradices* helps to reduce the chemical fertilizers to the level of 40-50% in field condition with no effects on soil health, crop growth and nutrition.

References

1. Akinrinlola RJ, Yuen GY, Drijber RA, Adesemoye AO. Evaluation of Bacillus strains for plant growth promotion and predictability of efficacy by *in vitro* physiological traits. Int J Microbiol; c2018.
2. Sandeep C, Raman RV, Radhika M, Thejas MS, Patra S, Gowda T, Suresh CK, Mulla SR. Effect of inoculation of Bacillus megaterium isolates on growth, biomass, and nutrient content of Peppermint. J Phytolo; c2011.
3. Aipova R, Aitkeldiyeva SA, Kurmanbayev AA, Sadanov AK, Topalova OB. Assessments of biotechnological potential of phosphate-solubilizing bacteria isolated from soils of southern Kazakhstan. Natl Sci. 2010;2:841-845.
4. Bahadir PS, Liaqat F, Eltem R. Plant growth-promoting properties of phosphate-solubilizing Bacillus species isolated from the Aegean Region of Turkey. Turkish J Bot. 2018;42(2):183-196.
5. Gomaa AM, Kholas EMA. Partial replacement of chemical fertilizers by biofertilizers in mungbean (*Vigna radiata* L.) production. Ann Agric Sci. 1999;37(4):2419-34.
6. Kristensen WB, Sundin P, Gertsson UE, Hultberg M, Khalil S, Loehnertz BB, *et al*. Management of microbial factors in the rhizosphere and nutrient solution of hydroponically grown tomato. Acta Hort. 1997;450:335-340.
7. Boer W, Folman LB, Summerbell RC, Boddy L. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev. 2005;29:795-811.
8. Aviles M, Carmona E, Ordovas J, Ortega MC. Evolution de la carga fungica durante compostaje del residuo industrial del corcho para uso como sustrato de cultivar hortícola silvícola. Ministerio de Agricultura, Pesca y Alimentacion. Instituto Nacional de Investigation y Tecnologia Agrarian Alimentaria. Investigation Agrarian production Protection Vegetables. 1996;11:301-311.
9. Campbell R, Greaves MP. Anatomy and community structure of the rhizosphere. In: Lynch JM, editor. The Rhizosphere. Wiley; c1990. p. 11-34.
10. Glick BR. The enhancement of plant growth by free-living bacteria. Can J Microbiol. 1995;41:109-117.
11. Compant S, Duffy B, Nowak J, Clément C, Barka EA. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol. 2005;71:4951-4959.
12. Galleguillos C, Aguirre C, Barea JM, Azcón R. Growth-promoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. Plant Sci. 2000;159:57-63.
13. Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. Bacteria from rhizosphere and hyphosphere soils of different AM fungi. Plant Soil. 1997;192:71-79.
14. Aboud MA, Mosaye A, Abid RM. Interaction of Bacillus subtilis and *Trichoderma harzianum* on growth and yield of cucumber (*Cucumis sativus* L.). Int J Curr Res. 2014;6(8):7754-7758.