Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 4717-4721
Received: 29-03-2019
Accepted: 30-04-2019

Sivakumar K
Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

G Kumaresan
Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

N Sugapriya
Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Correspondence
G Kumaresan
Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Arbuscular Mycorrhizal Fungi (Am Fungi) and phosphate solubilizing bacteria (PSB) on tolerance of tomato under salt stress

Sivakumar K, G Kumaresan and N Sugapriya

Abstract
The enhanced effect of arbuscular mycorrhizal fungus (AM fungi) and phosphate-solubilizing bacteria (PSB) on the salt tolerance of tomato (*Lycopersicon esculentum* L.) grown in pots was explored. Pot experiments were carried out in tomato inoculated with AM fungi (*Glomus fasciculatum*) and PSB (*Bacillus megaterium* var *phosphaticum*) under 1% NaCl stress. Salinity increases Na^+ concentrations, but decreases K^+ contents and K^+/ Na^+ in shoot and root significantly. Meanwhile, salinity reduces growth parameters such as root dry weight, shoot dry weight, total phosphate contents, chlorophyll contents and biological soil quality (phosphatase enzyme activities). Dual inoculation of AM fungi and PSB showed significant higher variation of the ion concentration (K^+, Na^+), plant growth parameters, available phosphate contents, phosphatase enzyme activities and the reduction of pH than other treatments. Under salt stress, the percentage of mycorrhizal root colonization of plants co-inoculated with AM fungi and PSB were higher than those plants inoculated with AM fungi alone. It is concluded that AM fungi inoculation with PSB application could synergistically enhance salt tolerance of plants.

Keywords: AM Fungi, Phosphobacteria, tomato, salinity

Introduction
Salt stress is one of the most serious agricultural problems in arid and semiarid regions, where salt accumulates on the soil surface and make them unproductive. Globally, almost 1,000 million ha (7% of all land area) are affected by soil salinity (Giri *et al.*, 2007) [8]. In India, nearly 9.38 million ha area is occupied by salt affected soils out of which 5.5 million ha are saline soils and 3.88 million ha alkali soil. Exploitation of these soils by cultivating crops or planting fruit trees adaptive to saline soils has a promising future. But the situation is complicated due to the fact that the soils in these regions generally contain little organic matter and bioavailable mineral nutrients. Thus, the establishment of salt tolerant crops or fruit trees is difficult without the use of fertilizers which are usually expensive to farmers of low incomes. In this respect, biological processes such as mycorrhizal application and phosphate solubilizing microorganisms to alleviate salt stress are better options (Shi *et al.*, 2013) [28].
Arbuscular mycorrhiza (AM) is a symbiosis between soil fungi and plants and occurs naturally in saline soils. Salinity affects the formation and function of AM symbiosis, and AM symbiosis could improve plant growth and productivity under salt stress (Jahromi *et al.*, 2008; Zai *et al.*, 2012) [11, 34]. Thus, AM fungi under salt stress conditions have been considered as bioameliorators of saline soils (Shi *et al.*, 2013) [28]. Arbuscular mycorrhizal fungi are used to inoculate many crop plants to improve the nutrition and development of host plants (Jeffries, 1987) [12].
Phosphorus is one of the major essential macronutrients required for biological growth and development of plants. Phosphorous is associated with many vital functions and is responsible for several physiological and biochemical plant activities such as utilization of sugar and starch, photosynthesis and transporting of genetic traits. It promotes early root formation, plant growth and it improves the quality of fruits, vegetables and grains and is vital to seed formation (Deepika Divya Kadiri *et al.*, 2013) [3]. The improvement in the plant phosphorus (P) status has been recommended as the most important strategy of salinity stress tolerance in AM fungi colonized plants (Evelin *et al.*, 2009; Manchanda and Garg, 2011) [5, 17].
Phosphate Solubilizing Bacteria (PSB) are capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds and PSB produce phosphatase like phytase that hydrolyse organic forms of phosphate compounds efficiently (Sivakumar and Sugapriya, 2019) [30]. The inoculation of P-solubilizing microorganisms is also a promising technique because it can increase phosphorus availability in soils fertilized with rock phosphates

(Vassilev *et al.*, 2012; Sivakumar and Divakaran, 2019) [29].
According to Manchanda and Garg (2007) [16], plant roots are exposed to a range of soil microorganisms with which they have a variety of interactions. However, the effects of combined inoculation with AM fungi and PSB on salinity stress tolerance in colonized plants under saline conditions remain unclear.
Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable crops grown widely all over the world. It is a self-pollinated crop and is a member of Solanaceous family with 2n = 24 chromosomes (Rick 1969) [22].
Tomato universally treated as 'Protective Food', is being extensively grown as annual plant. Tomato is a rich source of minerals, vitamins, & organic acids (healthy acid). Tomatoes are important source of Lycopene, minerals, Vitamin-A, B and also excellent source Of Vitamin-C. Tomato is also rich in medicinal value. The pulp and juice are digestible and blood purifier. Frasher *et al.,* (1991) [6] reported decreased cancer risk with the intake of tomatoes. This neutraceutical effect of tomato is attributed to 'lycopene' a major carotenoids present in tomatoes.
The present study were to evaluate the effects of an AM fungi (*Glomus fasciculatum*), and a PSB (*Bacillus megaterium* var *phosphaticum*) which was isolated from salt-affected coastal soil samples, on tolerance of tomato (*Lycopersicon esculentum* L.) seedlings under 1% NaCl stress.

Materials and Methods

Plant materials

The experiment was carried out during Rabi season on Tomato (*Lycopersicum esculentum* L.) of 2018-19, Department of Agricultural Microbiology, Annamalai nagar. The experiment was conducted in RBD plot design consisting of 7 treatment combinations with 3 replications and was laid out with the different treatments allocated randomly in each replication.

Fungal inoculum

The mycorrhizal fungus was *Glomus fasciculatum*, the inoculum produced consists of a mixture of bedding material, spores, pieces of hyphae and infected root pieces.

Isolation of PSB

Rhizosphere soil samples of tomato were collected using sterile techniques from tomato plant in pot culture yard in the department of microbiology, Annamalai University and used for experimental analyses. The Soil samples were serially diluted upto 10^{-6} and spread plated on Pikovskayas agar media and incubated at 32 ºC for 3–5 days. Colonies were selected on the basis of phosphate solubilization as indicated by clear halo zone around the bacterial colonies.

Experimental design and biological treatments

To study the effects of AM fungi and PSB on tomato under NaCl stress, we used a full factorial experimental design, with seven treatments.
T1 - Control
T2 - *Glomus fasciculatum* alone
T3 - *Bacillus megaterium* var *phosphaticum* alone
T4 - 1% NaCl
T5 - *Glomus fasciculatum* 10 g and 1% NaCl
T6 - *Bacillus megaterium* var *phosphaticum* and 1% NaCl
T7 - *Glomus fasciculatum* 10 g and *Bacillus megaterium* var *phosphaticum* and 1% NaCl

Each treatment was replicated three times in a randomized block design and each treatment was comprised of 21 pots comprising 2 plants per pot. After 60 days, the plants were removed and the following morphological growth characters like plant height (cm), root length (cm), shoot dry weight (g), root dry weight (g), mycorrhizal colonization (%) and total phosphate were analysed.

Plant analyses

Root and shoot tissues were analysed for Na and K content. Dried shoot tissues were digested in Kjeldahl flask with 1 ml $HClO_4$ (8.1 M), 5 ml HNO_3 (13.2 M) and 0.50 ml H_2SO_4 (17.8 M).P concentrations of the root and shoot tissues were determined by an ammonium molybdate blue method (Jones *et al.,* 1991) [13], and those of K^+ and Na^+ by flame photometry (Sengupta and Chaudhuri, 2002) [26]. The leaves of tomato were tested for Chlorophyll content.

Shoot length and root length (cm)

After 90 days, two plants of each control and experimental pots the plants were removed gently from the soil without disturbing the root system. The roots were washed with tap water to remove the soil and debris particles. The shoot height was measured and expressed in cm scale. The length of the root was also measured in cm scale (both treated and control).

Fresh Weight of Roots and Shoots (g)

The roots and shoots of the plants were weighed in an electrical balance and the fresh weight of roots and shoots were expressed in grams.

Dry Weight of Shoot and Root (g)

The plants were uprooted gently from the soil without disturbing the root system. The roots were washed with tap water in order to remove the soil particles. The fresh shoot and root from each treatment and control were cut into pieces and kept in a hot air oven at 82 °C for 24-72 hours. The dried samples were weighed in an electrical balance and then shoot and root dry weight was recorded.

Mycorrhizal colonization (%)

The fresh root mass of two plants were used for determining AM fungi colonization. To assess AM fungi colonization, roots of two plants were washed with 10% KOH and then stained with 0.05% trypan blue (Phillips and Hayman 1970) [21]. The percentage of root colonized by AM fungi was estimated according to McGonigle *et al.,* (1990) [18]. The fungal roots were cut into small pieces of 1cm length and 20 bits were examined per sample for their AM fungi colonization under a compound microscope (100X magnification). Positive counts for AM fungi colonization included the presence of vesicles or arbuscules within the roots. The percentage of AM fungi colonization was calculated by using the following equation:

Percentage of AMF colonization= positive count of root segments /No. of root segments observed ×100%.

Soil samples collection

After 90 days, according to Riley and Barber (1969, 1970) [23, 24], the whole plants were removed from pots. The soil obtained by gently shaking the roots and collected in a sterilized petri plate. The rhizosphere soil was collected in another sterile petri plate. One part of the soil sample was stored at 4 °C for biological and biochemical analyses and the

other part of the soil sample was air-dried at room temperature for physical-chemical analysis.

Soil chemical analysis and enzymatic activity determination

The available phosphorus content in the soil was determined using sodium bicarbonate-extractable phosphorus colorimetric method (Olsen *et al.*, 1954) [19]. Phosphatase enzyme activity was determined according to the improved method of Hoffman (Kandeler *et al.,* 1999) [15], and phosphatase was represented to a phenol number of milligrams per gram of soil. Ten grams of dry soil from each sample were diluted with 50 ml of deionized water and measured by pH meter for pH.

Results and Discussion

Effects of *Glomus fasciculatum* and *Bacillus megaterium* on contents of Na^+, K^+ in tomato under stress.

Glomus fasciculatum and *Bacillus megaterium* on contents of Na^+, K^+ in tomato under NaCl stress K^+/ Na^+ decreased significantly in both shoots and roots in response to salinity ($p \leq 0.05$, Table 1). In treatments with T_5, T_6 and T_7, the accumulations of Na^+ significantly decreased compared with the treatment of NaCl in the shoots and roots. Among the above three treatments, the treatment of $T_7$was the most effective ($p \leq 0.05$, Table 1).

Table 1: Effects of *Glomus fasciculatum* and *Bacillus megaterium* on contents of Na^+, K^+ in tomato under stress.

Treatments	Shoots			Roots		
	Na^+ mg g^{-1}	K^+ mg g^{-1}	K^+ / Na^+	Na^+ mg g^{-1}	K^+ mg g^{-1}	K^+ / Na^+
T_1	0.14	28.58	213.08	0.15	14.13	94.72
T_2	0.10	22.33	237.90	0.14	15.05	109.01
T_3	0.11	22.17	212.61	0.15	14.62	98.22
T_4	0.50	16.02	31.64	0.47	8.23	16.71
T_5	0.32	17.58	54.46	0.29	12.11	40.65
T_6	0.36	18.42	50.95	0.36	13.30	36.12
T_7	0.25	18.54	74.05	0.28	13.56	46.38

Mean separation with each column was by Duncan's New Multiple Range Test ($p \leq 0.05$).

Effects of *Glomus fasciculatum* and *Bacillus megaterium* on growth of tomato under NaCl stress.

NaCl induced a significant reduction of the plant height, root length, shoot dry weight, root dry weight, chlorophyll and total phosphate contents in plants, while AM fungi, PSB, or dual inoculation AM fungi and PSB counteracted such reductions significantly. Among the three inoculations used, dual inoculation of AM fungi and PSB was the most effective. Dual inoculation of AM fungi and PSB showed significantly higher effects on plant growth parameters than those of individual inoculation with AM fungi or PSB ($p \leq 0.05$, Table 2). Structures characteristic of AM fungi were not observed in roots of controls and plants inoculated with PSB alone (data not shown). Under NaCl stress, the mycorrhizal colonizations of both AM fungi and combined inoculation AM fungi and PSB were decreased significantly, and the percentages root colonization of plants co-inoculated with AM fungi and PSB were significantly higher than those of plants inoculated with AM fungi alone ($p \leq 0.05$, Table 2).

Table 2: Effects of *Glomus fasciculatum* and *Bacillus megaterium* on growth of tomato under NaCl stress

Treatments	Plant height (cm)	Root length (cm)	Shoot dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Mycorrhizal colonization (%)	Chlorophyll content (g $plant^{-1}$)	Total phosphate (mg kg^{-1})
T_1	48.8	21.2	6.64	0.38	0	5.24	0.238
T_2	51.3	23.5	7.32	0.41	48.6	5.59	0.259
T_3	50.7	22.4	7.18	0.37	0	5.32	0.246
T_4	35.6	12.9	4.77	0.16	0	2.74	0.105
T_5	40.8	17.5	5.20	0.24	24.8	4.25	0.187
T_6	40.3	17.7	4.00	0.22	0	4.32	0.192
T_7	44.4	19.0	5.88	0.30	37.8	4.58	0.214

Mean separation with each column was by Duncan's New Multiple Range Test ($p \leq 0.05$).

Effect of *Glomus fasciculatum* and *Bacillus megaterium* on available phosphate concentration, phosphatase enzyme activities and pH in the rhizosphere of tomato under NaCl stress

Salinization induced a significant reduction of the available soil phosphate concentration and phosphatase enzyme activities, while AM fungi, PSB, or combined inoculation of AM fungi and PSB counteracted such reductions significantly. Among the three inoculations used, dual inoculation of AM fungi and PSB was the most effective for both available soil phosphate concentration and acid phosphatase enzyme activities ($p \leq 0.05$, Table 3). AM fungi, PSB, or combined inoculation of AM fungi and PSB induced a significant reduction of the pH in rhizosphere soil of tomato, while dual inoculation of AM fungi and PSB was the most effective ($p \leq 0.05$, Table 3).

Table 3: Effects of *Glomus fasciculatum* and *Bacillus megaterium* on available phosphate contents, phosphatase enzyme activities and pH in the rhizosphere of tomato under NaCl stress.

Treatments	Alkaline phosphate (mg kg^{-1})	Phosphatase enzyme activities (mg Phenol g^{-1} d^{-1})			Ph
		Alkaline	Neutral	Acid	
T_1	8.65	1.855	1.296	0.492	7.14
T_2	10.33	1.833	1.488	0.588	6.72
T_3	10.30	1.900	1.492	0.584	6.54
T_4	5.78	1.612	1.946	0.267	7.16
T_5	6.14	1.803	1.185	0.378	6.58
T_6	6.09	1.785	1.156	0.364	6.45
T_7	7.00	1.813	1.164	0.473	6.12

Mean separation with each column was by Duncan's New Multiple Range Test ($p \leq 0.05$).

Discussion

The deleterious effects of salinity on plant growth are associated with osmotic stress, ion toxicity or indirect effects of saline ions that cause plant imbalance (Ashraf and Harris, 2004; Zhang *et al.,* 2013) [2, 35]. High concentrations of Na^+ can disrupt various enzymatic processes in the cytoplasm (Zhang *et al.,* 2013) [35]. AM fungi may diminish such deleterious effects of osmotic stress, ion toxicity and enzymatic activities (Guo *et al.,* 2010; Zai *et al.,* 2012; Estrada *et al.,* 2013) [9, 34, 4]. Higher K^+ accumulation by mycorrhizal plants under salt stress conditions may help in maintaining a high K^+/Na^+ ratio, thus preventing the disruption of various enzymatic process, inhibition of protein synthesis and beneficial in influencing the ionic balance of the cytoplasm (Giri *et al*., 2003; Zhang *et al.,* 2013) [7, 35]. This study showed the beneficial effects of inoculation with either AM fungi and PSB, especially the dual inoculation of AM fungi and PSB, resulted in enhanced higher K^+ accumulation and K^+/Na^+ ratio of tomato. Increased phosphorus may result in decreased Na, which is indirectly related to K uptake (Allen and Cunningham, 1983; Giri *et al.,* 2003) [1, 7]. In the group of NaCl + AM fungi + PSB, a synergism of AM fungi and PSB in combination with NaCl led to the highest content of phosphorus in tomato compared with the other groups (Table 1, Table 2). Mycorrhizal symbiosis is a key component in helping plants to survive under adverse environmental conditions (Estrada *et al.,* 2013) [4]. Our results showed that plant height, root length, shoot and root dry weights and total phosphate contents of tomato of the treatments of T5, T6 and T7 were significantly higher than those of NaCl group in this study. Particularly, the dual inoculation with AM fungi and PSB had synergic effects on plant height, root length, shoot and root dry weights. Similar effects were found in *Leucaena leucoce*phala (Osorio and Habte, 2001) [20], clover (Souchie *et al.,* 2006) [32], *Kostelelzkya virginica* (Zhang *et al.,* 2011) [36], etc.

In this paper, PSB contribution on growth promotion may be related to the improvement of phosphorus solubilization ability (Table 1, Table 3). Mycorrhizal fungi enhanced chlorophyll content in tomato leaves, a result in congruence with other studies (Sannazzaro *et al.,* 2006; Sheng *et al.,* 2008) [25, 27]. This study showed that combined inoculation of AM fungi and PSB under NaCl stress could promote the chlorophyll content in tomato leaves. This may be due to saline soil inoculated with PSB increased the content of available phosphorus in soil (Table 3), and stimulating the production of plant hormones (Jacobsen et al., 1992) [10]. In this study it was clearly demonstrated that salt stress significantly inhibited mycorrhizal colonization. In the presence of NaCl, the germination of spores of AM fungi tested was delayed, and the specific rate of hyphal extension of AM fungi was reduced, with a subsequent decrease in the spread of mycorrhizal colonization (Juniper and Abbott, 2006) [14].

Conclusion

This study confirms that the dual inoculation of AM fungi and PSB increases tolerance of tomato and growth under 1% NaCl. In non-inoculated plants, 1% NaCl induced lower K^+/Na^+ ratios in the roots and shoots, available phosphate contents and phosphatase enzyme activities in rhizosphere soils, resulting in an important growth reduction in the roots and shoots. The combined inoculation of AM fungi and PSB alleviated the deleterious effects of 1% NaCl on tomato and stimulated plant growth principally by increasing K^+ accumulation and maintaining higher K^+/Na^+ ratios in root and shoot tissue and phosphatase enzyme activities in rhizosphere soils. It is concluded that AM fungi inoculation and PSB inoculation could enhance salt tolerance of plants.

References

1. Allen EB, Cunningham GL. Effects of vesicular arbuscular mycorrhizae on *Distichlis spicata* under three salinity levels. New Phytol. 1983; 93:227-236.
2. Ashraf M, Harris JC. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 2004; 166:3-16.
3. Deepika Divya Kadiri, Naresh Gorle, Krishnakanth Varada Raju Peetala, Sujatha Peela. Isolation, screening and identification of phosphate solubilising bacteria from different regions of Visakhapatnam and Araku Valley. Int. J. Adv. Biotechnol. Res., 2013; 4(4):518-526.
4. Estrada B, Arroca R, Maathuis FJM. Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. Plant Cell Environment. 2013; 36(10):1771-1782.
5. Evelin H, Kapoor R, Giri B. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot-London. 2009; 104(7):1263-1280.
6. Frasher GE, Beeson L, Phillips RL. Diet and lung cancer in California, Seventh-Day Adventists, Amer. J. epidemiology. 1991; 133:683-693.
7. Giri B, Kapoor R, Mukerji KG. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol Fertil Soils. 2003; 38(3):170-175.
8. Giri C, Pengra B, Zhu Z, Singh A, Tieszen L. Monitoring mangrove forest dynamics of the Sundarbans in Bangladesh and India using multi-temporal satellite data from 1973-2000. Estuar Coast Shelf Sci. 2007; 73:91-100.
9. Guo SX, Chen DM, Liu RJ. Effects of arbuscular mycorrhizal fungi on antioxidant enzyme activity in peony seedlings under salt stress. Acta Hortic Sin. 2010; 37(11):1796-1802.
10. Jacobsen I, Abbott LK, Robson AD. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. New Phytol. 1992; 120:371-38.
11. Jahromi F, Aroca R, Porcel R. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. Microbial Ecol**.** 2008; 55(1):45-53.
12. Jeffries ML. Use of mycorrhiza in agriculture. CRC, Critical Review of Biotechnology. 1987; 58:319-348.
13. Jones JR, Wolf JB, Mills HA. Plant analysis handbook. Micro-macro Publishing, Athens, CA, USA. 1991, 195-203.
14. Juniper S, Abbott LK. Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi. Mycorrhiza. 2006; 16(5):371-379.
15. Kandeler E, Tscherko D, Spiegel H. Long-term monitoring of microbial biomass, N mineralization and enzyme activities of a chernozem under different tillage management. Biol Fertil Soils. 1999; 28:343-351.
16. Manchanda G, Garg N. Endomycorrhizal and rhizobial symbiosis: how much do they share? J Plant Interact. 2007; 2:79-88.

17. Manchanda G, Garg N. Alleviation of salt-induced ionic, osmotic and oxidative stresses in *Cajanus cajan* nodules by AM inoculation. Plant Biosyst. 2011; 145(1):88-97.
18. Mcgonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 1990; 115:495-501.
19. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available-phosphorus in soils by extraction with sodium bicarbonate. USDA Circulation No.939. US Government Printing Office, Washington, DC, 1954, 19-27.
20. Osorio NW, Habte M. Synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of *Leucaena leucocephala* in an Oxisol. Arid Land Res Manag. 2001; 15(3):263-274.
21. Phillips JM, Hayman DS. Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 1970; 55:158-161.
22. Rick CM. Origin of cultivated tomato, current status of the problems. Abstract XI International Botanical Congress, 1969, 180.
23. Riley D, Barber SA. Bicarbonate accumulation and pH changes at the soybean root-soil interface. Soil Sci Soc Am Proc. 1969; 33:905-908.
24. Riley D, Barber SA. Salt accumulation at the soybean root-soil interface. Soil Sci Soc Am Proc. 1970; 34:154-155.
25. Sannazzaro AI, Ruiz OA, AlbertÓ EO, Menendez AB. Alleviation of salt stress in Lotus glaber by Glomus intraradices. Plant Soil. 2006; 285:279-287.
26. Sengupta A, Chaudhuri S. Arbuscular mycorrhizal relations of mangrove plant community at the Ganga river estuary in India. Mycorrhiza. 2002; 12:169-174.
27. Sheng M, Tang M, Chen H, Yang BW, Zhang FF, Huang YH. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza. 2008; 18:287-296.
28. Shi SL, Huo PH, Li JF. Phosphate solubilizing microorganisms and phosphate solubilizing rhizobium. Appl Mech Mater. 2013; 295:2328-2332.
29. Sivakumar K, Divakaran J. Synergistic effect between Phosphate Solubilising Bacteria and Arbuscular Mycorrhizal Fungi on Growth and P uptake in *zea mays*. Journal of Pharmacognosy and Phytochemistry.SP2: 2019, 590-593.
30. Sivakumar K, Sugapriya N. Effect of AM fungi and PSB inoculation on the per cent root colonization, am fungal spore number and PSB population in the rhizosphere soils of brinjal (*Solanum melongena* L.). Journal of Emerging Technologies and Innovative Research. 2019; 6(5):466-471.
31. Sivakumar K, Tholkappian P. Co-inoculation effect of AM fungi and phosphobacteria on the growth and yield of rhizosphere of Bhendi (*Abelmoschus esculentus* L.) as influenced by chemicals and biopesticides. International Journal of Pharmaceutical & Biological Archives. 2013; 4(2):375-378.
32. Souchie EL, AzcÓn R, Barea JM. Phosphate solubilization and synergism between P-solubilizing and arbuscular mycorrhizal fungi. Pesq Agrop Bras. 2006; 41(9):1405-1411.
33. Vassilev N, Eichler-Löbermann B, Vassileva M. Stress-tolerant P-solubilizing microorganisms. Appl Microbiol Biot. 2012; 95(4):851-859.
34. Zai XM, Zhu SN, Qin P, Wang XY, Che L, Luo FX. Effect of *Glomus mosseae* on chlorophyll content, chlorophyll fluorescence parameters, and chloroplast ultrastructure of tomato (*Prunus maritima*) under NaCl stress. Photosynthetica. 2012; 50(3):323-328.
35. Zhang HS, Qin CQ, Qin P. Effects of inoculation of arbuscular mycorrhizal fungi and phosphate-solubilizing fungus with different proportion on P-uptake of Castor Bean (*Ricinus communis* L.) and rhizosphere soil enzyme activities in coastal saline soil. Chin Agric Sci Bull. 2013; 29(12):101-108.
36. Zhang HS, Wu XH, Li G, Qin P. Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kostelelzkya virginica* growth and soil enzyme activities of rhizosphere and bulk soils at different salinities. Biol Fertil Soils. 2011; 47:543-554.