Research Article

Physiological changes induced by Arbuscular Mycorrhizal Fungi (AMF) and plant growth promoting fungi (PGPF) in tomato (*Lycopersicum esculantum*)

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Abstract

Experiment was conducted to assess the effect of AMF and PGPF for physiological changes induced during the interaction of AMF and PGPF in tomato. The nutrient uptake was enhanced during the combined application of AMF along with *T. harzianum*. However the uptake efficiency was most reverse with respect to the combined delivery of AMF + *A. solani*. Assay on the mycorrhizal nutrient efficiency (MNE) in tomato indicated that combined application of AMF with *T. harzianum* enhanced the uptake of P, K, Mg, S, Fe,Cu, Mo and B than non-mycorrhizal control. Besides it also increased the uptake of Zn and N. Comparative evaluation between AMF and other indicated that the interactive nutrient efficiency (INE)in relation to the uptake of P, Mg, Fe and B was the maximum in AMF + *T. harzianum* treated plants rather than the mycorrhizal control.

Key words: *Lycopersicum esculantum*, Micro and Macronutrients, AMF, PGPF

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Among suitable practices for sustainable management of agricultural soils, the use of biofertilizers such as arbuscular mycorrhizal (AM) fungi and plant beneficial bacteria can lead to yield enhancement and fruit quality improvement (Bona et al 2015). Avis et al (2008) reviewed that Plant Growth Promoting Microorganisms (PGPM) like *Trichoderma, Pseudomonas, Rhizobium* and *Glomus* promote plant growth and productivity (primary effect) and equally play a role in reducing disease (secondary effect) to improve plant growth and nutritional status. The soil, AMF or other fungi and host plant are three important factors in investigation as these components interact with each other, the positive effect of native AMF is only better visualized when the plants are also grown in their native soil (Pankova et al 2011). AM fungi and beneficial saprophytic mycoflora in terms of plant growth promotion, root colonization and disease suppression are discussed and its implication to sustainable agriculture is considered (Saldajeno et al 2008). Phytohormones play an important role in plant growth and development, as well as in the systemic response induced by beneficial microorganisms (Elsharkawy et al 2012).AMF have been shown to cause changes in the rhizosphere that could affect microbial populations both qualitatively and quantitatively and to promote the activity of other micro-organisms that may compete with soilborne pathogens (Filion et al 1999; Barea et al 2002). It has also been demonstrated that some *Trichoderma* strains may influence AMF activity (Martınez et al 2004). Increase in uptake of different minerals may be due to increased surface area of soil contact by extraradical hyphae, increased movement of nutrients through hyphae and modification of the root environment (Bolan 1991). Hence, attempts were made to evaluate the effect of AMF and other PGPF to enhance the nutrient uptake pattern in tomato (*Lycopersicum esculantum* Mill.).

Materials and Methods

Experimental design and treatments. A pot culture experiment was carried out to study the interaction of AMF and other rhizospheric biocontrol fungi with respect to physiological changes on Tomato cv. TO1389 (Syngenta India Limited, Pune, Maharashtra). The experiment was conducted in the botanical garden of Botany department, Arts Science and Commerce College, Naldurg (15^0 26' North latitude and 75^0 7' East longitudes and an altitude of 678 m above mean sea level).

Application of AMF and PGPF. The experiment consisted of seven treatments with various inoculums combinations. The details of treatments were as given below:

T1: Control without using AMF & other PGPF

T2: Indigenous consortium of AMF (*Glomus, Acaulospora* and *Sclerocystis*)

T3: AMF + *Trichoderma viride(ASCNB/fungi /44)*

T4: AMF + *Trichoderma harzianum (ASCNB/fungi/43)*

T5: AMF + *Aspergillus niger (ASCNB/fungi/17)*

T6: AMF + *Alternaria solani (ASCNB/fungi/35)*

T7: s AMF + *Rhizopus stolonifera (ASCNB/fungi/25)*

Inoculum of indigenous AMF were maintained in green house with *maize* (*Zea mays* L.) containing 270-300 spores /100 g soil. It was applied @ 30 g per each plantat the time of sowing. The other isolated fungi were used in the experiments viz., *T.viride, T. harzianum, A.niger, A.solani* & *R.stolonifer* with 25 × 10^5 CFUs/g^{-1} spores of mycelial water suspension. It was inoculated @1g (mycelial disc)/per each plant.

Analyses of nutrients changes in tomato plant. Tomato plants were harvested after 90 days of treatment and dried. Whole dried plant material including root and shoot was ground in grinder and mixture. The ground material was collected in polythene bags and used for biochemical (Macro & Micro nutrients) analysis. Plant material was subjected tothe analyses of nutrients such as Nirogen, Phosphrous, Potassium, Calcium, Magnesium, Sulphur, Sodium, Zinc, Ferrous, Copper, Mangenese, Molybdenum and Boron. For Assessment of various nutrients, Subbiah and Asija (1956), Olsen et al (1954), Hanway and Heidel (1952), GOI (2011) and Jackson (1967) methods were used.

Mycorrhizal nutrient efficiency (MNE) was studied to describe the mycorrhizal effect on nutrient uptake by using the formula of Wu and Zou (2009).

Mycorrhiza 1 Nutrient Efficiency (MNE) (%) = (Ei – Eni)/(Eni) x 100

Where Ei and Eni were the values of nutrient elements of plants treated by AMF and non - AMF

Interactive Nutrient Effect of other fungi (INE) for nutrient efficiency over AMF was calculated by considering nutrient value of AMF treated plant as mycorrhizal control. Interactive effect (Excess nutrient content in the plant tissue than Mycorrhizal (AMF) plant due to interaction of both fungi) was calculated by using following formula

INE = (Nutrient elements of AMF and other fungi treated plant – Nutrient elements of AMF plant)/(Nutrient elements of AMF plant) x 100

Statistical analysis. The data collected from the experiment were analyzed statistically following the procedure given by Mungikar (1997).

Results and Discussion

Macronutrientschanges in tomato plant (Figures 1, 2&3)

Nitrogen (N). In tomato, Nitrogen content in the plant tissue was maximum in the plants treated with AMF + *Trichoderma viride* (3.53 %) and it was followed by plant treated with AMF + *T. harzianum* (3.14 %), AMF + *R. stolonifer* (3.07 %), AMF + *A.niger* (2.91 %) , AMF alone (2.63 %) and AMF + *A.solani* (2.18 %).Nitrogen was found minimum in the non-mycorrhized plants (1.96 %).

The mycorrhizal nutrient efficiency (MNE) for Nitrogen content in the plant tissue was 80.1 % increase over control.Interactive nutrient efficiency (INE) was studied, combination of AMF + *T. viride* (34.2 %) was better than mycorrhizal control plant whereas interaction of AMF + *A. solani* (-17.1 %) showed negative efficiency than mycorrhizal plant

Phosphorus (P). In tomato Phosphorus content in the plant tissue was maximum in plant treated with AMF + *T. harzianum* (0.16 %) and AMF + *A. niger* (0.16 %) it was followed by plant treated with AMF + *T. viride*(0.13 %). Phosphorus was found minimum in control thenon-mycorrhizedplants (0.083 %).

The mycorrhizal nutrient efficiency for Phosphorus content in the plant tissue was 92.8 % increase over control when AMF + *T. harzianum* and AMF + *A. niger* was supplied.

Potassium (K). In Tomato Potassium content in the plant tissue was maximum in the plants treated with AMF + *T. harzianum* (4.45 %) and followed by the

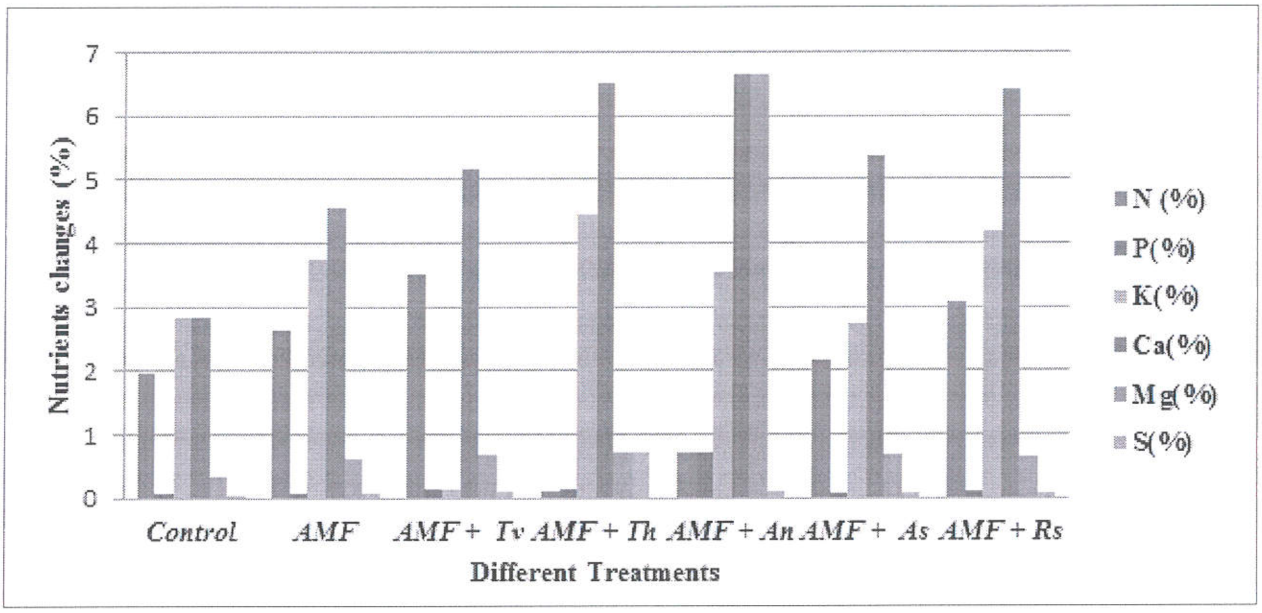

Figure 1. Macronutrients changes in tomato cv. TO1389 plant due to AMF and other PGPF after 90 days of treatmeents.

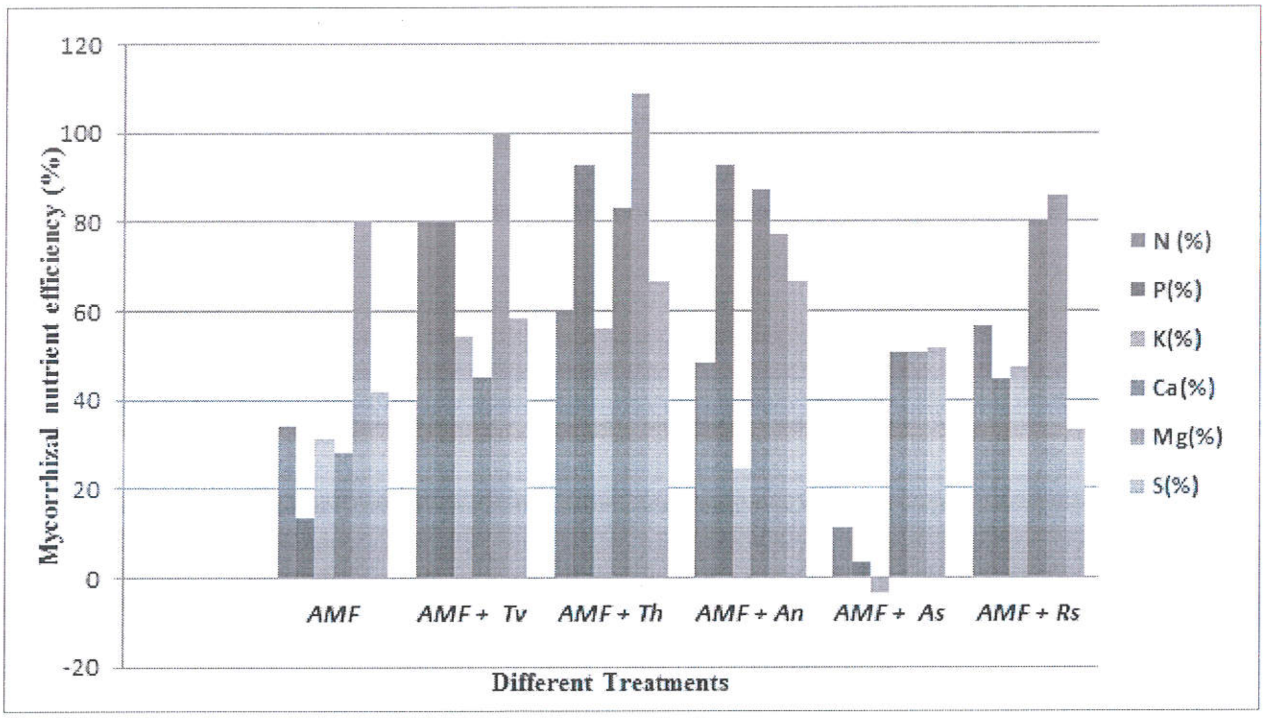

Figure 2. Mycorrhizal nutrient efficiency (MNE) of macronutrients in tomato due to interaction of AMF and other PGPF.

plant treated with AMF + *T. viride*(4.4 %). The MNE for Potassium content in the plant tissue was 56.1 % increase over control in the AMF co-inoculation with *T. harzianum*.

Calcium (Ca). In tomato Calcium content was maximum in plant treated with AMF + *A. niger* (6.65 %), and it was followed by plant treated with AMF + *T. harzianum* (6.5 %), AMF + *R. stolonifer*(6.4 %) and was minimum in the non-mycorrhized plants (3.55 %).*The* MNE forCalcium content indicated that the plant tissue had 87.3 % increases over control.

Magnesium (Mg). The Magnesium content was maximum in AMF + *T. harzianum* (0.73 %) treated plants as against untreated control. The MNE was 108 % increase over control when AMF + *T. harzianum* was supplied. Interactive nutrient efficiency (INE) was studied, combination of AMF + *T. harzianum* was 15.9 % better than mycorrhizal control plant whereas interaction of AMF + *T. viride* (-1.6 %) showed negative efficiency.

Sulphur (S). TheSulphur content in the plant tissues treated with AMF + *T. harzianum*was (0.1 %) and was minimum in control (non-mycorrhizal) plant (0.06 %).Mycorrhizal nutrient efficiency for Calcium content in the plant tissue was 66.7 % increased by the treatment of AMF + *T. harzianum* and AMF + *A. niger* over non-mycorrhizal control. Interactive Nutrient efficiency (INE) was studied, combination of AMF + *T. harzianum* and AMF + *A. niger* was 17.6 % more better than mycorrhizal control plant whereas interaction of AMF + *R. stolonifer* (- 5.9 %) showed negative efficiency than mycorrhizal plant.

Micronutrients changes in Tomato plant (Figures 4, 5 & 6)

Zinc (Zn). In tomato, Zinc content in the plant tissue was maximum in the plants treated with AMF + *T. viride* (3.73ppm) and was followed by plant treated with AMF + *T. harzianum* (3.64ppm) treated plants as against untreated control. Zinc was found minimum in the non-mycorrhized plants (1.84ppm). Mycorrhizal nutrient efficiency for Zinc content was 102.7% increase over control when AMF + *T. viride* was treated plants than the non mycorrhized plants.

Ferrous (Fe). The Ferrous content in the plant tissue was maximum in theplants treated with AMF + *T. harzianum* (1202.81ppm)and was followed by plant treated with AMF + *R. stolonifer* (1110.11ppm) and minimum in thenon-mycorrhized plants (495.23ppm).The MNE for Ferrous content in the plant tissue was 142.9 % increase over control in the AMF co-inoculated with *T. harzianum.* Interactive Nutrient efficiency (INE) was studiedwith combination of AMF + *A. solani(- 26.8%) sowed negative efficiency.*

Copper (Cu). The Copper content in the plant tissue was maximum in the plants treated with AMF + *T. harzianum* (43.03 ppm) and was followed by plant treated with AMF + *A. niger* (41ppm) and minimum in the non-mycorrhized plants (23.71 ppm). The MNE for Copper content in the plant tissue had 81.5 % increase over control in the AMF co-inoculated with *T. harzianum.* The INE was studied with the combination of AMF + *A. solani* (- 19.9 %) showed negative efficiency.

Manganese (Mn). The Manganese content in the plant tissue was maximum in the plants treated with AMF + *A. niger* (49.1 ppm) and was followed by plant treated with AMF + *T. viride* (42.3ppm).Manganese was found minimum in the non-mycorrhized plants (28.16ppm).The MNE for Manganese content indicated that the tissue had 74.4 % increase over control .The INE was studied with the interaction of AMF + *A. solani* (- 7.1 %) and AMF + *R. stolonifer*(-4.3 %) resulted in negative efficiency.

Molybdenum (Mo). The Molybdenum content in the plant tissue was maximum in the plants treated with AMF + *T. harzianum* (0.79 ppm) treated plants as against untreated control. Molybdenum was found minimum in control (non-mycorrhizal) plant (0.51ppm).The MNE content in the plant tissue had 54.9 % increase over control. The INE was studied with the combination of AMF + *R. stolonifer* (- 15.9 %) and AMF + *A. niger* (- 6.3 %) showed negative efficiency.

Boron (B). The Boron content in the plant tissue was maximum in the plants treated with AMF + *T. harzianum* (33.8ppm) and was followed by plant treated with AMF + *A. solani* (33.48ppm) and was minimum in control (non-mycorrhizal) plant (17.98 ppm). The MNE for Boron content in the plant tissue was 88 % increase over control. The INE was showed negative in AMF + *A. niger* (-12.2 %) than mycorrhizal plant.

During interaction, increase in the carbon supply to the VAM fungi by the host plant enhances the uptake and the transfer of phosphorus from fungi to host plant (Bucking and Shachar-Hill 2005). Biochemical changes due to inoculation of AMF species is supported by Sreenivasa (1992) where he found that the local isolate *G. macrocarpum* was the best for improving P, Zn, Cu, Mn and Fe nutrition in non-sterilized soil than other isolates (*G. fasciculatum*, *Gigaspora margarita*,

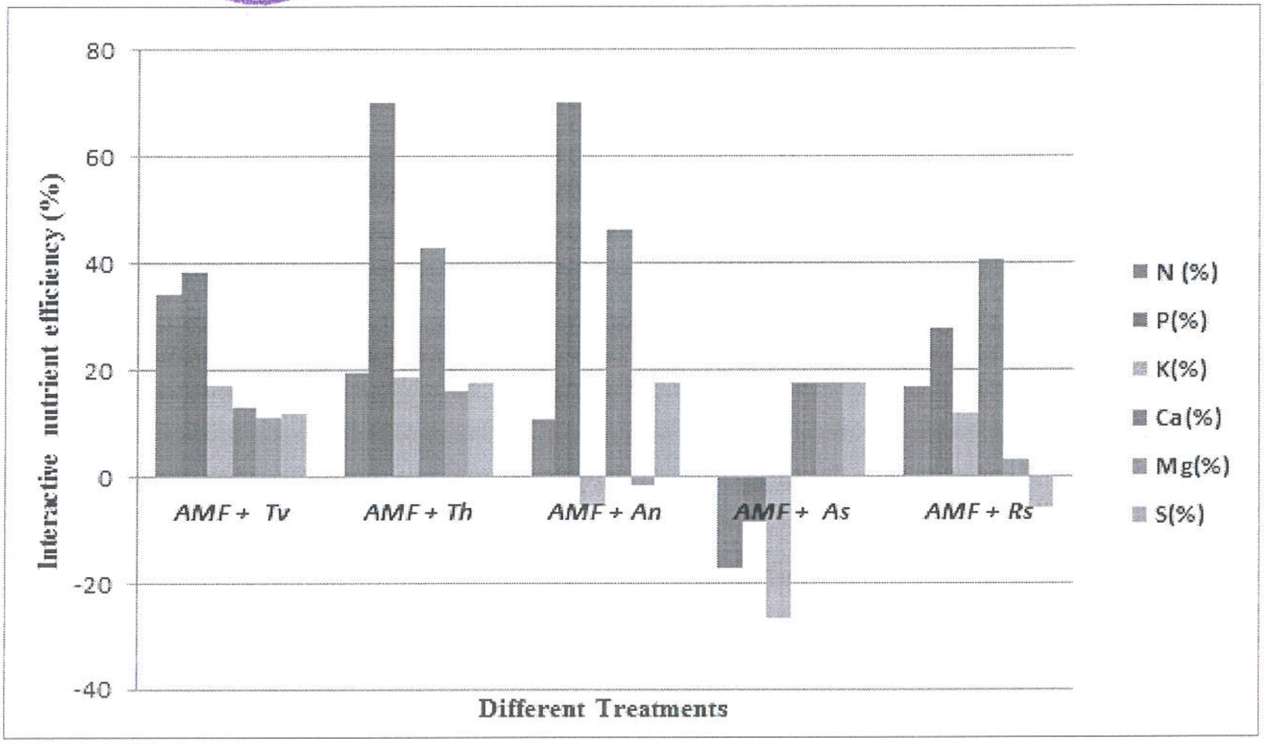

Figure 3. Interactive autrient efficiency (INE) of maronutrients in tomato due to interaction between AMF and other PGPF.

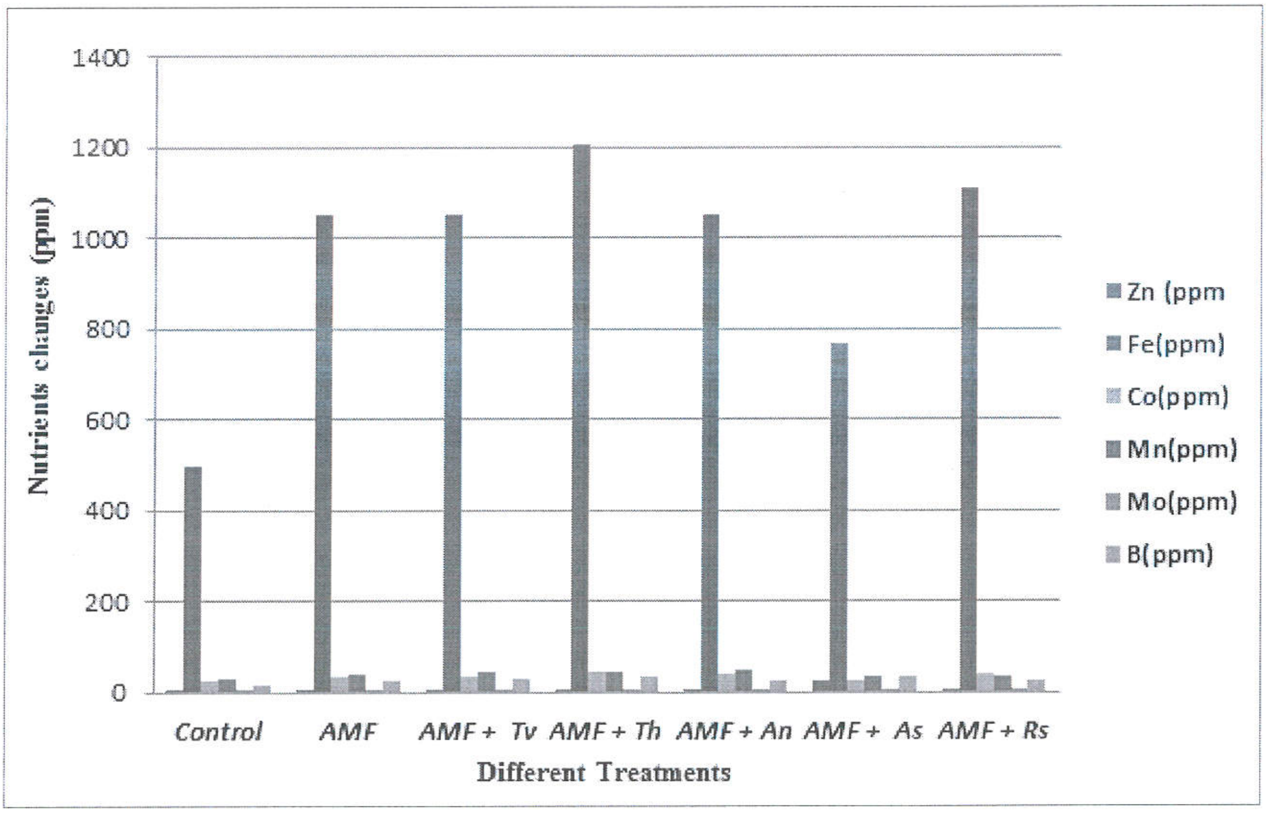

Figure 4. Micronutrients changes in tomato plant due to AMF and PGPF after 90 days of treatments.

Acaulospora laevis and *Sclerocystis dussii*) in chilli. Hadad et al (2012) tried indigenous inoculum collected from rhizosphere of Omani soil and alien inoculum of two AMF species (*Glomus mosseae*)on Tatoo cultivar and Donato cultivar of tomato where Omani mycorrhiza treatment greatly enhanced planttissue P which was reflected in the number and weight of the tomato fruits. Pulido et al (2003) noticed that when tomato plant was treated with combination of *Glomus clarum*(AMF) and *Azospirillum brasilense* (*Rhizobacterium*) maximum N and P extraction was achieved (47.6 kg/ha and 5.84 kg/ha) than *Glomus clarum* alone (39.2 kg/ha and 4.52 kg/ha) or control (39.2 kg/ha and 3.37 kg/ha). In saline soil, Hage-Ahmed et al (2009) proved that mycorrhizal tomato (*Solanum lycopersicum* L.) plants increase N, P, K and Ca uptake than non mycorrhizal plants.

In a greenhouse study (pot experiment) Chanie and Assefa (2006) evaluated positive responses in shoot phosphorus concentration of tomato plant at low P concentration levels (0.04 ppm) due to inoculation of AMF while high P concentration levels (0.92ppm) showed negative effect. Lenin et al (2010) applied *Glomus fasciculatum* grown on maize to the plants of *L. esculentum, S. melongena, C. annuum* and *Abelmoschus esculentus* and observed increased phosphorus and potassium in AM fungi treated seedlings as compared to non-mycorrhizal seedlings (controlled). The positive effect of AMF inoculation (*Glomus mosseae*) on Fe uptake (1398 ± 112 mg/pot) was found in Rice cv. Shafagh over Fe uptake (876 ± 152 mg/pot) of nonmycorrhizal plant in Phosphorus as well as Zink deficient soil (Hajiboland et al 2009). Selvaraj et al (2009) reported enhanced uptake of nutritional ingredients (N, P, K, Zn, Ca and Mn) by the application of *Acaulosporascro biculata, Gigaspora margarita, Glomu saggregatum, G. geosporum, G. mosseae, Sclerocystis pakistanika* and *Scutellospora heterogama* isolates of indigenous AM fungi. Bioinoculation of indigenous AMF consortium isolated from the field having the lowest level of fertilization and yield resulted in higher nutrient uptake [P (65.5 mg / kg) K (58.0), Fe (1.35)] in grains of Wheat than nonmycorrhizal plant [P (35.4 mg / kg) K (48.7), Fe (0.48)] (Roesti et al 2006). Tanwar et al (2013) assessed a remarkable increase in the plant phosphorus and nitrogen content by co-inoculation of *Funneliformis mosseae, Acaulosporalaevis* and *T. viride*. Among both the AMF species F. mosseae proved to be more effective strain compared to A. laevis for tomato. Hemashenpagam and Selvaraj (2011) assessed nutrient content in root and leaf of

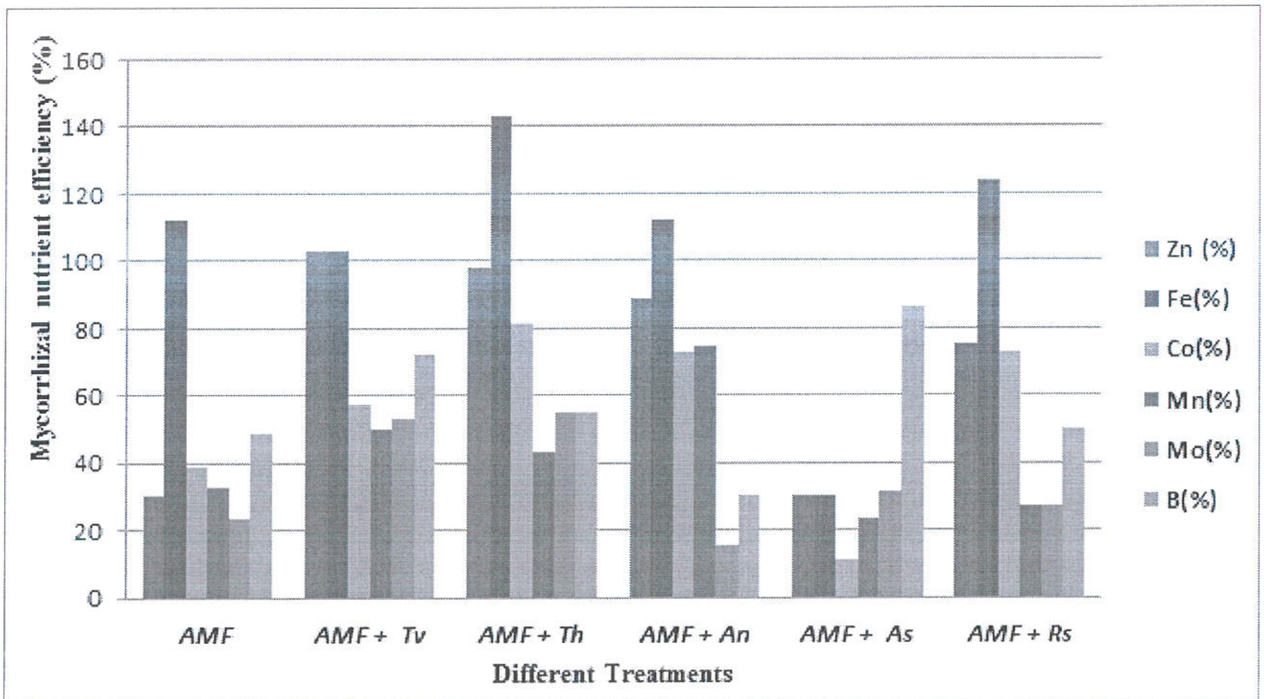

Figure 5. Mycorrhizal nutrient efficiency (MNE) of micronutrients in tomato due to interaction AMF and other PGPF.

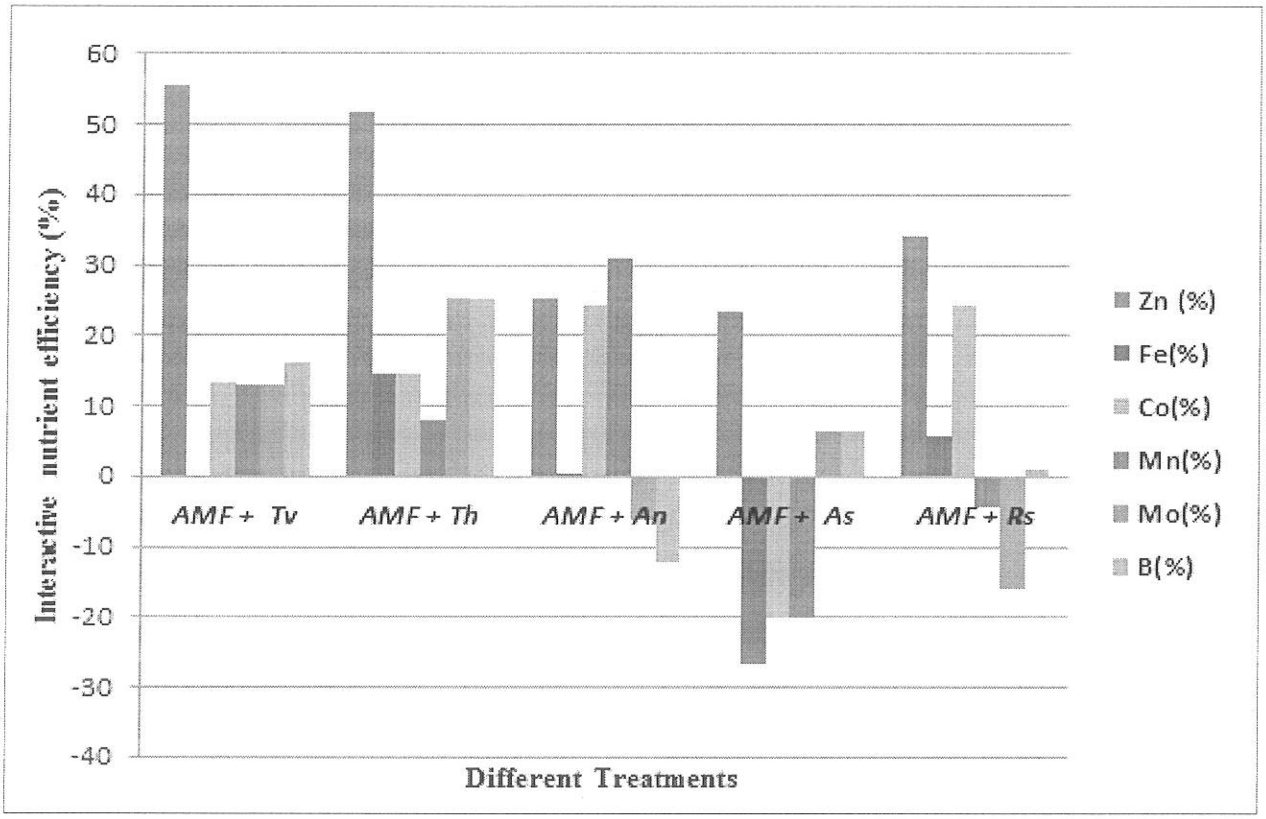

Figure 6. Interactive nutrient efficiency (INE) of microonutrients in tomato due to interaction between AMF and other PGPF.

Solanum viarum seedlings where they got 0.204 g /plant of P in leaves of non- mycorrhizal plant, 0.244 g /plant in mycorrhizal plant (*G. aggregatum*) while 0.248 g /plant in AMF + *T. harzianum* treated plant, further results of K, Zn, Cu, Mn and Fe showed that treatment of AMF or AMF + *T. harzianum* was effective than nonmycorrhizal control.

It is valid & no error u must add Babu and Reddy (2011) https:// www. researchgate. net /.../ 227300051_Dual_Inoc. and Error! Hyperlink reference not valid. (2011) observed significantly increased P (150%), K (67%), Ca (106%) and Mg (180%) in shoot tissues of bamboo (*Dendrocalamus strictus*) by co-inoculation of (AM) fungi and *Aspergillus tubingensis* grown in fly ash as compared to control plants. Souchie et al (2010) assessed shoot P content of clover after inoculation with AMF + *Aspergillus niger* which was higher than AMF alone treatment while mono-inoculation of A. niger resulted in less P than AMF alone treatment. In *Triticum aestivum* copper and Zinc content in shoot was only enhanced by AMF and not by *A. niger*, but P in soil was depleted in order of A. niger < AMF <AMF + A. niger (Tarafdar and Marschner 1995).

Conclusion

It is concluded that the extent of absorbing nutrients from the soil depends on different combinations of AMF and other PGPF. Arbuscular mycorrhizal association is the most widespread and common root-fungus association. AM fungi improve growth of the plants through increased uptake of available soil phosphorus and other non-labile mineral nutrients essential for plant growth. Minerals may be increased due to surface area of soil contact by extra & intra radical hyphae & increased movement of nutrients through hyphae and roots.

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