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Stimulation Impact of Rhizospheric Microbe's Glomeromycota AM Fungi and Plant Growth Promoting Rhizobacteria on Growth, Productivity, Lycopene, B-Carotene, Antioxidant Activity and Mineral Contents of Tomato beneath Field Condition Cultivated in Western Ghats Covering Semi-Arid Region of Maharashtra, India

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Abstract

The rhizosphere is the slim region of soil that's directly influenced by root secretions and accompanying soil microorganisms known as root microbiome. The rhizosphere involving the soil pores comprises numerous beneficial bacterium and others different microorganisms. Microbial communities play a vital role within the functioning of plants by stimulating their morphology, physiology and development. Several species of the rhizosphere microorganism are helpful to plant growth and overall productivity. The useful plant-microbe associations within the rhizosphere are the principal determinants of plant and soil health (SH). Rhizobacteria comprise mycorrhization helper microorganism and plant growth promoting rhizobacteria (PGPR) are support arbuscular mycorrhizal fungi (AM fungi) to colonize the plant roots. Tomato is the second most common cultivated vegetable within the world for biological process and functions. Tomato has high values in soluble fat, vitamin A, B, C, lycopene, flavonoids, and β-carotene and is of course low in calories. Tomato consumption are extremely useful to human health (HH) because of several crucial nutrients are accessible. In current study, the impact of inoculating tomato with consortium AM fungi and PGPR on growth, fruit quality and productivity was estimated. The inoculated AM fungi are containing Aculospora logula-15%, Glomus fasciculatum-20%, Glomus intraradices-40%, Gigaspora margarita-15% and Scutellospora heterogama-10% infective propagules in inoculum. The consortium PGPR treatments were inoculated with Azotobacter chroococcum, Pseudomonas fluoresces and Fraturia aurantia (10-9CFU/g) and also the Control [100% Recommended Rate of fertilizers (RRF)] treatment was without microbial inoculated. Phyto-morpho-chemical factors, containing Lycopene, β-carotene, antioxidant activity, growth, fruit yield, fruit potassium (K) and macro and micro nutrients uptake in shoot were improved by AM fungi and PGPR mediated tomato as compared with control (100% RRF). Maximum lycopene, β-carotene, fruit K and antioxidant activity (AA) were recorded in plants treated with multiple biostimulants of AM fungi + PGPR treatment. Maximum height, biomass and marketable yield were observed in AM fungi + PGPR treated plants and minimum in control (100% RRF). A correlational statistics between lycopene, β-carotene, AA with fruit and shoot K ($P<0.05$) was ascertained. It was absolutely concluding that the utilization of AM fungi + PGPR treatment had the utmost impact on productivity, lycopene, β-carotene, AA, K contents on tomato to enhance its nutritious worth for HH.

Keywords: Rhizospheric Microbes, AM Fungi, PGPR, Lycopene, Minerals.

Introduction

Microbial communities (MC) play an important role within the functioning of plants by influencing their morphological, physiology and development. Several members of the beneficial rhizosphere microbiome (BRM) colonize the roots to protecting microbic defend through plant defense mechanisms (PDM) and are useful to plant growth (PG), nutritive fruit quality (NFQ) and productivity. The significance of the rhizosphere microbiome (RM) for PG and productivity has been well known for the overwhelming majority of RM. The rhizosphere soil (RS) is directly influenced by root secretions and accompanying soil microorganisms (SM) known as the root microbiome (RM). The RM plays a vital role among the functioning of plants by prompting their overall performance. Microbial species of the rhizosphere are much helpful to PG, productivity and increase SH. The helpful plant-microbe associations (PMA) within the rhizosphere are the major factors of plant development and SH. AM fungi and PGPR are very potential rhizosphere microorganism (PRM) to colonised and associated with plant roots and supportive to phosphate (P) and K solubilizing, free living symbiotic nitrogen fixating (SNF), antibiotic manufacturing and reducing plant pathogens (PP), predators and parasites in terrestrial plants in worldwide ecosystems. The foremost common RM within the mycorrhizosphere is genus *Glomus* and *Pseudomonas*.

Tomato (*Lycopersicum esculentum L.*) is momentous vegetable crops grown in worldwide as well as in India for local ingestion and exportation. Tomato is the second most well liked cultivated vegetable within the world once the potato which can be eaten raw in salad or cooked, peeled, or made into purees ketchup, soup or powdered or juice in any canning industry. Lycopene is the most significant and notorious phytobioactive compound of tomatoes. Lycopene molecular weight is 536. 89 and molecular formula is C40H56 (Figure 1).

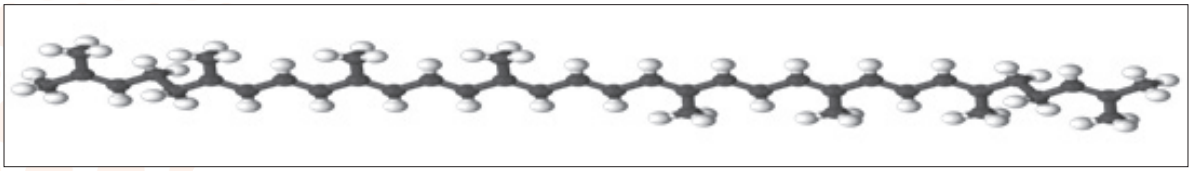

Figure1: Ball and stick model of the lycopene molecule; Source, Molecular mass = 536.89g.mol^{-1}, Molecular Formula= $C_{40}H_{56}$, Molecular Composition= C: 89.49%, H: 10.51%

In addition to lycopene, the tomato fruits contain high values in fat soluble vitamins A, B, C, flavonoids, B-carotenoids and is of course low in calories whose action interacted with those of polyphenols, resulting in an overall benefit on HH [1-4]. Tomato consumption are extremely beneficial to HH because of its several essential nutrients (SEN) are accessible. These composition has high antioxidant capability in both fresh and processed tomatoes [2, 5], associating the fruit with lower rates of bound varieties of cancer and disorder [2, 6]. Lycopene is additionally answerable for the reddening of the tomato, because of the differentiation of the chloroplasts; therefore this pigment is enormously vital with respect to the ultimate nutritionary and marketable quality of plant produce [4, 7]. Important tomato manufacturing and processing states in India are Uttar Pradesh, Karnataka, Maharashtra, Haryana, Punjab and Bihar.

According to Hart and Scott [8] the antioxidant content of tomato principally depends on genetic and environmental factors, and also the ripening stage. Lycopene content depended on cultivars, stage of maturity and growth conditions [2]. The adventagous role of tomato fruit has been associated with antioxidant constituents such as carotenoid and β-carotene that directly rely on tomato intake [9]. Studies show that K has a major impact on tomato growth and fruit quality (lycopene) [10]. The antioxidant content of contemporary tomato is often laid low with several pre and post-harvest factors [11]. The management of mineral nutrition could be a key pre harvest issue that determines the yield and fruit quality of tomato plants. The target of this study is to work out the results of inoculating tomato with consortium AM fungi and PGPR on the standard of tomato fruit under field conditions and compared with conventional agriculture (CA) during Rabi season. The results are helpful to farmers and societies to nutritionary values of tomato producing through organic nutrients.

Materials and Methods

Location, Climate and Soil of Experimental Site

A field experiment was performed at the farmer field of Junnar taluka of Pune district, Maharashtra, India during the Rabi season. The site Junnar taluka is positioned among 19°11'59" North and 73°52'47" East longitude and its elevation from sea level is around 689 meters. The study area is a part of the Western Ghats and represents undulating hilly terrain stretched approximately 60 km within side the North-South direction. It is a chain of hills, ranging with an elevation from 600 m to 1600 m above mean sea level. The weather is sub-tropical with a mean maximum temperature ranging among 35-40oC in summer time and a mean minimum between 7-11oC in winter. The mean annual rainfall is around 700 mm. The soil is alluvial, deep, properly drained, and of excessive fertility. The major portion of this location is underneath cultivation of different agricultural, horticultural, medicinal and aromatic crops.

Soil Sampling and Analysis

Soil samples (0-30cm) were collected randomly from each site using standard conning and quartering method before (Zero time) and after harvest. Air dried soil samples were used for different physico-chemical analysis. pH was determined in 1.25 (w/v) solutions of dried samples in water and the same was used for determination of electrical conductivity (EC). Air dried samples was processed (addition of 40% NaoH

and distillation) in a Kel Plus Nitrogen estimation system (Class DX, Pelican Equipment's) followed by determination of available nitrogen by titration with 0.02N H2SO4 [12]. Available phosphorus was determined by Olsen method using samples with high pH sodium bi-carbonate as extracting agent [13-14]. Available potassium was determined in a 1N ammonium extract using flame photometer [14]. Extractable micronutrient Cu, Fe, Zn, Mn in soil was analysis using DTPA extraction standard procedures [15].

Microorganisms Applications

AM fungi Inoculant

Density of AM fungi that were mixed with triple sterile talc powder adjusted with 3000 infected propagules (IP) per gram of inoculants containing growing subtract, infected roots bits and hyphal and mycelial mass. AM fungi inoculum contained Aculospora *logula*-15%, *Glomus fasciculatum*-20%, *Glomus intraradices*-40%, *Gigaspora margarita*-15%, and *Scutellospora heterogama*-10% infective propagules (IP). AM fungi @500 IP/ plant were applied as root dipping method at the time of planting.

PGPR Inoculant

PGPR microbial inoculants (*P. fluoresces, A. chroococcum and Fraturia aurantia*) have been proliferated in nutrient broth medium. Then every PGPR becomes eliminated on the top of logarithmic growth phase, and become aseptically transferred to plastic containers, which include triple sterile talc powder, and then were mixed well. PGPR concentration was adjusted to 1×10^9 CFU/g in all inoculants. PGPR mixture prepared and contained *Pseudomonas fluorescent; Azotobacter chroococcum* and *Fraturia aurantia* (1:1:1 CFU). PGPR @ 0.5 g/plant become inoculated across the seedling during the time of transplanting. PGPR have been additionally applied tomato growth stages in three equal splits dose.

Chemical Fertilizers (CF) Applications

Various level of chemical fertilizers were applied in control treatment in the form of DAP 1.08 q ha^{-1}, MoP 0.23 q ha^{-1} and Urea 1.75 q ha^{-1} as 100 % recommended rate of fertilizers (RRF) as farmer's practiced. Urea was also applied as top dressing in equal splits as per recommendations.

Field Preparation, Nursery Raising and Experimental Design

The experimental land was opened with a power tiller and kept exposed to the sun prior to next ploughing. It was prepared afterwards by ploughing and cross ploughing followed by laddering. The cropping pattern of the land was Paddy–wheat/ maize–vegetables. Tomato (*L. esculentum* L., F1 Hybrid, variety- *Arka Abha*) seedlings were prepared on experimental field site. After 25 days, uniform size of tomato seedlings were selected and treated with and without AM fungi/ PGPR and transplanted in experimental plots with spacing of 60X40cm. AM fungi and PGPR treatment were considered as AM fungi alone, PGPR alone and AM fungi + PGPR (consortium PGPR consist of P. *fluoresces*, A. *chroococcum and Fraturia aurantia*), and to compared to control treatment without microbial inoculant (100% RRF). Plantation was finished in the first week of November and experimental plots size was 10.0X10.0 meter with four replicate in each treatment in randomized complete block design (RCDB). 100% RRF was added to control treatments only. Recommended cultural operations (RCO) were carried out during entire cropping period to ensure a healthy crop. Field soil testing was estimated Zero time and at harvest. Lycopene, β–carotene, AA, and K in fruit and nutrients uptake in shoot were determined. The physicochemical properties of the soil were determined at harvest (Figure 2-8).

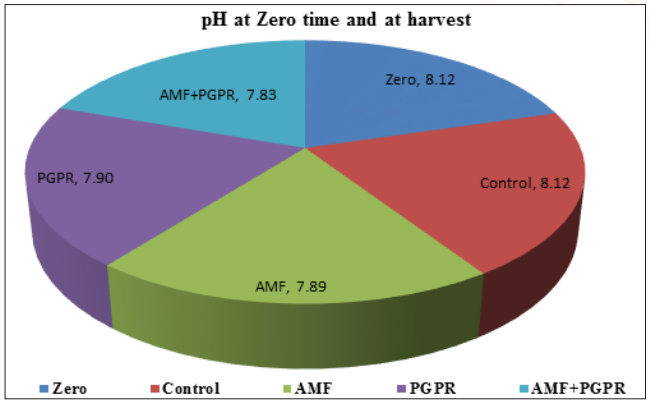

Figure 2: Soil pH at zero time and after crops harvest

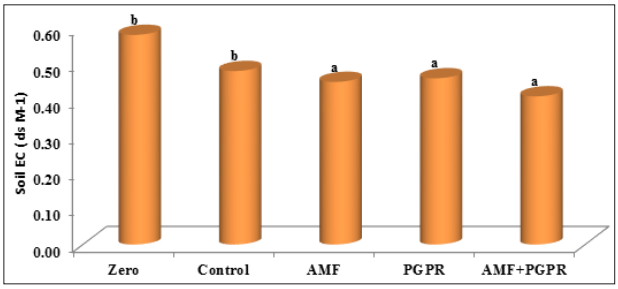

Figure 3: Soil electric conductivity at zero time and after crops harvest

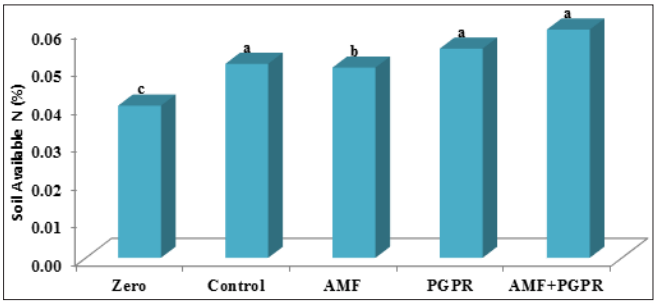

Figure 4: Soil available nitrogen concentration at zero time and after crops harvest

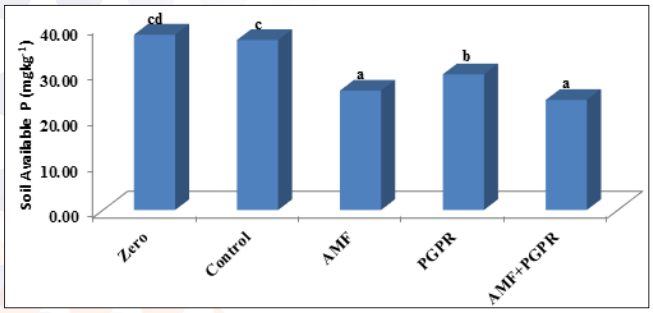

Figure 5: Soil Olsen's p concentration at zero time and after crops harvest

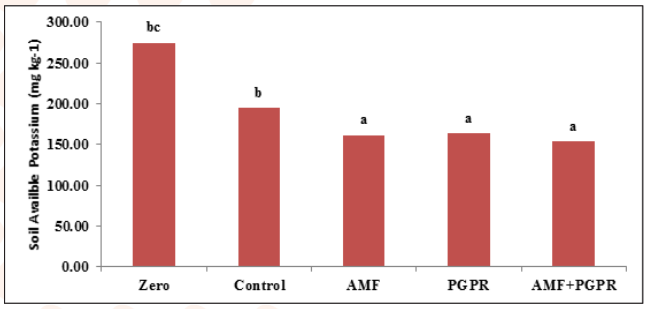

Figure 6: Soil available potassium concentration at zero time and after crops harvest

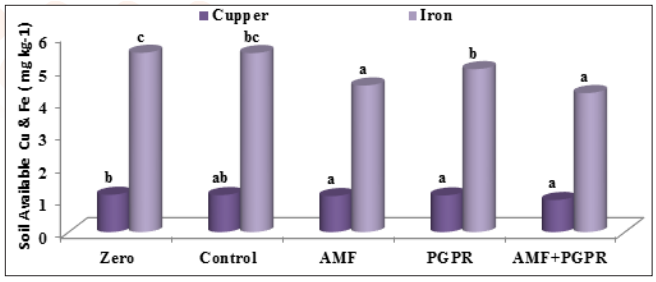

Figure 7: Soil available cupper and iron concentration at zero time and after crops harvest

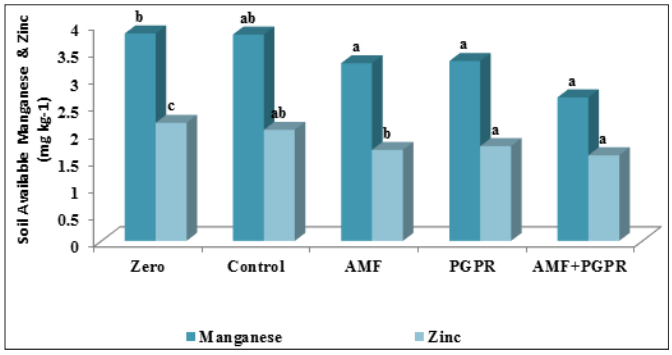

Figure 8: Soil available Manganese and Zinc concentration at Zero time and after crops harvest

Morphological and Yield Attributes of Trials

The observations were recorded at 45 days after transplanting (DAT) and at harvest on randomly twenty plants were selected after one meter of each plot boundary in each replicates for all the characters such as plant height (cm), number of branches/plant, leaf water potential (LWP) percentage, Leaf area (cm^2) and root length (cm). The observation of tomato fruits diameter (cm), biomass (t ha^{-1}) and gross yield (q ha^{-1}) was recorded after harvest of the crops.

Assessment of Relative Leaf Water Potential (RLWP) Percentage

Relative LWP percentage was measures as fresh and constant weight method.

Assessment of Shoot Nutrients (ASN)

Randomly four places were selected for plants samplings in all tomato plots. For nutrients analysis of the shoot systems, the oven-dried samples were finely ground. Nitrogen (N) in the shoots was determined using an elemental analyser (EA 3000, Eurovector, Italy). To estimate the phosphate (P) and potassium (K) level in the shoots, 1g of the finely ground sample was subjected to a wet oxidation treatment using tri-acid (HNO_3:H_2SO_4:$HClO_4$; 10:1:4) digestion in a digestion block (KELPUS, KES121; Pelican Equipment, Chennai, India) at 200°C. Following acid digestion, the samples were diluted and filtered for further nutrient analysis. Shoots P was determined by the vanado molybdophoshoric acid colorimetric method [16] using a spectrophotometer (Specord 200; Analytik Jena, Germany). K was measured by the ammonium acetate method of Hanway and Heidel [17] by using a flame photometer (Model FP114; Thermo Scientific, USA). To determination of iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) content in shoots samples were digested in a microwave (Mars 5, CEM). Following the US EPA 3051A method (US EPA 2007), the metal concentration in the acid digestive was determined using atomic absorption spectrophotometer (AAS) (SOLAAR, TJA Solution, UK).

Assessment of Fruit Lycopene and β-carotene Content

Tomato sample preparation

The mature, fresh and healthy pear type tomato was selected and washed with tap water, followed by washing with distilled water (DW). Tomato fruits were blended for one minute using manual home blender. Ground fruit tissues were kept on ice box in dark condition.

Extraction of lycopene and β –carotene contents

The extraction and determination of fresh fruit lycopene content was based on the method of Fish et al. [18]. Lycopene and β -carotene contents from fresh tomato was extracted with hexane, ethanol, acetone (1:1:1), containing 2.5% BHT. One gram of the tomato fruit puree was put in 50 ml covered test tubes, then hexane, 0.05% (W/V) + butylated hydroxytoluene (BHT) in acetone 95%, ethanol (in a ratio 1:1:1) made lycopene extraction solution and was added to tubes. Then, tubes were shaken for 10 minute and 6 ml cold double distilled water were added to each tube and agitated for several minutes. Then the tubes were released for 15 minutes in room temperature. The absorbance of supernatant (hexane layer) containing lycopene was red with spectrophotometer at the wavelength of 503 nm and β-carotene was calculated at 487nm. Values of lycopene (mg kg^{-1}) and β-carotene (mg kg^{-1}) were estimated as (x/y) X A503 X 3.12 and (x/y) X A487 X 3.12 respectively.

Assessment of Antioxidant activity (Free radical scavenging effect test)

Assessment of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging impact was performed in keeping with Hsu et al. [19] with some modifications by Rosales et al. [20]. Aliquots of 0.5 ml of methanolic tomato extract and a couple of 2.5 ml of freshly prepared 0.1mmol L−1 DPPH methanolic solutions were completely mixed and unbroken for 60 min within the dark and cold. The absorbance of the reaction mixture at 517 nm was estimated with a spectrophotometer. 0.5 ml Methanol, substitution the extract, was used as the blank. The free radical scavenging impact was calculated as follows:
Scavenging effect (%) = [1 − (Absorbance of samples/Absorbance of blank)] × 100.

Assessment of Fruit Potassium content

Fresh tomato fruit was washed with de-ionized water to remove any contamination from them. Then they were dried in a thermo ventilated oven at 70°C for 3 days. To obtain extract, 0.3 gm of dried sample with 2.5 ml of digestion mixture acids (Salicylic acid, sulfuric acid with selenium) was heated on 100°C. When temperature of sample decreased, 3 ml of H2O2 added and agitated well. Again extract heated on 330°C. K concentrations in extracts of fruit were assayed by flame photometry methods (flame emission spectrometer).

Assessment of mycorrhizal root colonization (MRC) percentage in root system

Approximately 1-2 g freshly collected fine roots were used for staining and the assessment of MRC percentage. Root were washed in fresh water, cleared with 10% KOH, acidified with 1N HCl, and stained with 0.05% Trypan blue [21. Quantification of root colonization for AM fungi was conducted using the gridline intersection method [22] and 100 segments of each sample were observed under a compound microscope (Leica DM750). The presence or absence of AM fungal structure in root system such as vesicles, arbuscules and hyphae at particular fixed points was recorded, and the results were expressed as a percentage MRC of observations.

Statistical Analysis

Observations on growth, productivity and alternation in physico- chemical properties in soil and nutrient uptake were analysed using SPSS (SPSS Inc. version 17.0). Results were subjected to one way analysis of variance and the significant difference was determined according to Duncan's Multiple Range Test at significant level of $P<0.05$.

Result

Physico-chemical Properties of Soil (Zero time and at Harvest)

Comparing the physico-chemical properties of the soil before (Zero time) and after the harvest of experimental crops was presented in figure 2-8. A slightly alternation of pH was recorded in treatments (Figure 2). Microbial mediated tomato showed a significant ($P< 0.05$) decrease in electrical conductivity (Figure 3). The factors responsible for the change in pH and EC in the continuous alterations of equilibrium between cations and anions present in the soil. Plant uptake of soluble salts by crops and or leaching of cations such as calcium, magnesium can decrease the pH and at the same time chloride accumulation in the surface due to capillary action can be responsible for the decrease in EC [4, 23-25]. Available N in the soil is directly associated with soil organic matter (SOM). The gradual increase in N is due to an increase in SOM and the microbial activities (MA) which make N available from organic matter (OM) to microbial inoculants treated plots. A maximum significant increase ($P<0.05$) in available N ((0.06%) was recorded from AM fungi + PGPR treated plot (Figure 4) and minimum in control (0.04%). Available P content was noticed significantly lower ($P<0.05$) in AM fungi (25.29 mg kg-1) and AM fungi + PGPR (23.93 mg kg^{-1}) treatments compared to zero time (38.1 mg kg^{-1}), PGPR (29.48 mg kg^{-1}) and control (36.94 mg kg^{-1}) soil and it may be due to P mobilizing activity of mycorrhiza, added during plantation activities (Figure 5). The sharp decrease in K content in soil was noticed in all microbial treated tomato compared to control (Figure 6). Maximum decrease of K was recorded in AM fungi + PGPR (154.00 mg kg^{-1}) treatment and minimum in control (195.12mg kg^{-1}) as compared to Zero time (275.15 mg kg^{-1}). Various factors including weathering, upward translocation of soluble ions through capillary action, involvement from the degradation of plants litters may be responsible for such variation of K content in different treatments of tomato [4, 26].

Among the zero time micro-nutrients analyzed from experimental plot, the deficiency is highest for AB-DTPA extractable Fe (5.48 mg kg^{-1}) followed by Mn (3.83 mg kg^{-1}), Zn (2.18 mg kg^{-1}) and Cu (1.14 mg kg^{-1}). The perusal of data presented in the figure 7 reveals that the maximum amount of Cu (1.13 mg kg^{-1}) was recorded under control (100% RRF) tomato cultivated soil and minimum amount of available Cu (0.98 mg kg^{-1}) was recorded in AM fungi + PGPR treated soil as compared to zero time (1.14 mg kg^{-1}) soil sample analysis. The sharp decrease of Cu content in soil was indicate that it is reduce due to microbial treatment of tomato. Tomato plants were uptake soil Cu to need of survival and growth. An inquisition of data in figure 7 indicates that Fe content in soil of 5.48 mg kg^{-1} was found to be the maximum in zero time soil sampling and minimum in AM fungi + PGPR treatment (Figure 7). By comparing the Mn and Zn properties of soil before and after the experiment is presented in figure 8. Decrease response of micro-nutrients concentration in soil was observed in all treatment as compared to initial soil samples (zero time) of Mn and Zn contents. Maximum Mn (3.81 mg kg^{-1}) and Zn (2.05 mg kg^{-1}) contents in soil was recorded in control and minimum Mn (2.65 mg kg^{-1}) and Zn (1.58 mg kg^{-1}) in AM fungi + PGPR treatments as compared to zero time Mn (3.83 mg kg^{-1}), Zn (2.81 mg kg^{-1}) soil analysis.

Measurement of Plant Growth Parameters (MPGP) Morpho- agronomic Characters (MAC)

MAC observations were recorded at 45 DAT and the maturity of the crops. The AM fungi, PGPR along and with combination treated tomato performed better than untreated control (100%

RRF). No significant differences were recorded at 45 DAT of MAC. Plant height, number of branches/plant, leaf moisture, leaf area, and root length were significantly influenced by AM fungi + PGPR application in all the treatments after harvest of the crops (Tables 1). The AM fungi and PGPR alone and with combination (AM fungi + PGPR) treated *L. esculentum L.* performed better than untreated control (100% RRF). Significant differences were recorded between the treatments. Consortium AM fungi + PGPR treated tomato showed a significant increase in plant height, number of branches, LWP, leaf area, and root length as compared to non-microbial control (NMC) (100% RRF, Farmer's practiced). AM fungi and PGPR association has also positively correlated with plant growth. It is assumed that vegetable crops benefit positively to AM fungal symbiosis [4, 27] and it makes little growth without mycorrhiza unless heavily fertilized [4, 25, 28-32]. At the time of harvest, AM fungi, PGPR and AM fungi + PGPR tomato plants showed significant ($P<0.05$) increased growth parameters as compare to NMC plants (Table 1).

Treatment	Plant Height (cm)		No. of Branches/ Plant		Leaf Area (cm^2)	
	45 DAT	120 DAT	45 DAT	120 DAT	45 DAT	120 DAT
Control	21.38b ± 0.57	61.43a ± 3.93	3.35d ± 0.12	7.95b ± 0.30	9.92d ± 0.05	17.15c ± 0.19
AM fungi	26.73b±1.47	65.51a±4.10	4.28ab±0.07	9.42ab±0.46	13.12bc±0.07	20.25bc±0.18
PGPR	25.75b±1.27	62.18ab±2.93	3.98c±1.45	8.68b±2.12	12.78c±2.14	19.65bc±1.21
AM fungi + PGPR	27.78a±1.12	82.45a±1.93	5.15a±2.83	10.46a±1.98	15.12a±1.12	25.36a±1.43

Table1: Effect of AM fungi and PGPR on tomato growth characters plant height, number of branches/ plant and leaf area ±SE-Std error; Values in a column followed by the same letter are not significantly different at $P< 0.05$ according to DMRT

Maximum plant height (82.45±1.93cm), number of branches per plant (10.46±1.9), percentage LWP) (30.20±9.97), leaf area (25.36±1.43 cm2) and root length (26.65±0.38 cm) was recorded in AM fungi + PGPR treated tomato as compared to control (100% RRF), height (61.43±3.93cm), number of branches per plant (10.46 ±1.9), percentage LWP (35.62±1.48), leaf area (17.15±0.19 cm2) and root length (12.33±0.42 cm) at the time of harvesting. AM fungi and PGPR association has also positively correlated with plant growth and biomass. It is expected that, tomato plant benefited positively to AM fungi and PGPR symbiosis in early application.

Yield and its Attributes Characters

The tomato fruit diameter, dry biomass, and marketable gross yield increased significantly in the plants receiving treatment of AM fungi + PGPR in comparison to the control (Table 2).

Treatment	Leaf Water Potential (%)		Root Length (cm)	
	45 DAT	120 DAT	45 DAT	120 DAT
Control	83.65a±0.62	30.20b±9.97	8.23bc±0.42	12.33cd±0.42
AM fungi	84.30a±0.76	34.95b±1.57	16.88b±0.49	24.65a±0.38
PGPR	83.12b±2.45	34.19ab±1.98	15.58b±0.49	23.65ab±0.38
AM fungi +PGPR	84.15a±1.96	35.62a±1.48	17.68a±0.49	26.65a±0.38

Table 2: Effect of AM fungi and PGPR on tomato leaf moisture and root length at 45 DAT 120 DAT ±SE-Std error; Values in a column followed by the same letter are not significantly different at $P< 0.05$ according to DMRT

Maximum fruit diameter (6.89±0.12 cm), dry biomass (7.22±0.04 t ha^{-1}) and gross yield (270.43±0.80 t ha^{-1}) were recorded in AM fungi + PGPR treatment followed by AM fungi [fruit diameter (5.89±0.12cm), dry biomass (6.42±0.04 t ha^{-1}) and gross yield (269.93±0.80 t ha^{-1})], PGPR [(fruit diameter 5.49±0.12cm), dry biomass (6.02±0.04 t ha^{-1}) and gross yield (268.23±0.80 t ha^{-1})] and control [(fruit diameter 4.94±0.18cm), dry biomass (5.26±0.10 t ha^{-1}) and grass yield (247.29±1.98 t ha^{-1})] at final harvest. Significant differences were recorded between the treatments and control. Prasad [4] reported that when *S. tuberosum* cultivars (Kufri pukhraj, Kufri sindhuri and Kufri laukar) and tomato were inoculated with consortium AM fungal inoculant (*Aculospora logula, Glomus fasciculatum, Glomus intraradices, Gigaspora margarita* and *Scutellospora heterogama*), the percent of edible tubers and tomato increased due to improvement of root volume and macro and micro nutrients absorption by plants. The yield of tomato can be affected by the interaction of mycorrhizae, PGPR and fertilizers (Table 2). Mean assessments indicated that in mycorrhiza and PGPR inoculated tomato with increasing of K, P and other minerals rate than tomato yield increased. The highest yield was noticed in AM fungi + PGPR treated tomato followed by AM fungi, PGPR and lowest in NMC (Table 3).

Treatment	Fruit Diameter (cm)	Dry Biomass (tha^{-1})	Gross Yield (qha^{-1})
	At harvest	At harvest	At harvest
Control	**4.94b±0.18**	**5.26b±0.10**	**247.29bc±1.98**
AM fungi	5.89a±0.12	6.42a±0.04	269.93a±0.80
PGPR	5.49ab±0.12	6.02ab±0.04	6.02ab±0.04
AM fungi + PGPR	6.89a±0.12	7.22a±0.04	270.43a±0.80

Table 3: Effect of AM fungi and PGPR on tomato fruit diameter, dry biomass and grass yield at harvest
±SE-Std error; Values in a column followed by the same letter are not significantly different at P< 0.05 according to DMRT

It has been shown that tomato yield increased by AM fungi + PGPR treatments. Mycorrhizae fungi improve plant growth by facilitating mineral nutrition and progressing water relations which led to larger plant size and healthier yield quality. It is assumed that a consortium of AM fungal and PGPR inoculum having the potential to reduce the high application rate of fertilizers needed to produce high yield [4, 6]. Tomato [4, 33], soybean [34-37], allium spices [25], pulses [35], peppermint [38], *Terminali arjuna* [39] and *Azadirachta indica* [40] plants treated with mycorrhizal strains had higher colonization in the rhizosphere, greater nutrients absorption, and yield.

Biochemical Analysis of Fresh Tomato Fruit

The outcomes have showed that the AM fungi and PGPR treatments have the capability to change tomato fruit quality such as lycopene, β-carotene, AA and fruit K contents (Figure 9-11).

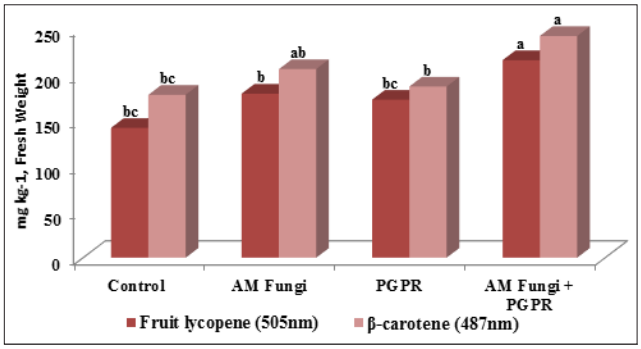

Figure 9: Influence of AM fungi and PGPR on lycopene (505nm) and β-carotene (487nm) contents of fresh mature tomato fruit

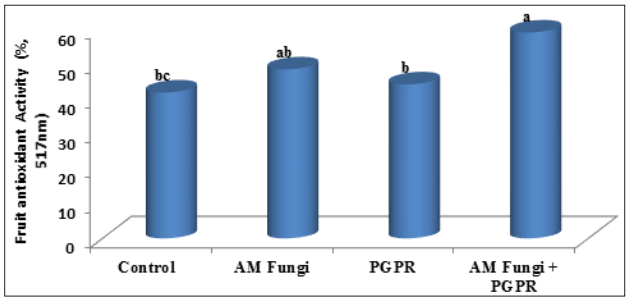

Figure 10: Effect of AM fungi and PGPR on mature and fresh tomato fruit antioxidant activity

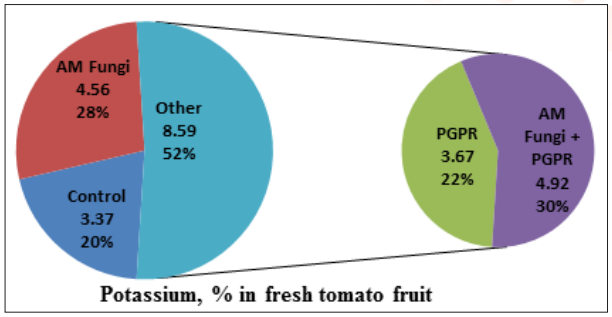

Figure11: Impact of microbes on percentage potassium content in mature fresh tomato fruit

Evidence explained considerably distinction between applied AM fungi and PGPR treatments on tomato fruit as lycopene, β-carotene, AA, fruit K contents and shoot macro and micro nutrients uptake. Fruit lycopene, β-carotene, AA and fruit K content increased in all treatment of AM fungi, PGPR and AM fungi +PGPR treatments, compared to the NMC (100% RRF) treatment. Maximum lycopene content was remarked in the AM fungi + PGPR treatment (212.28 mg kg^{-1} fresh fruit) followed by AM fungi alone (178.83± 1.51 mg kg^{-1} fresh fruit), PGPR alone (172.22± 1.87 mg kg^{-1} fresh fruit) and control (147.54± 2.11 mg kg^{-1} fresh fruit). An evinced from the figure 9 shows the maximum amount of β-carotene (241.7± 1.11 mg kg^{-1} fresh fruit) by tomato fruits was recorded under treatment AM fungi + PGPR whereas the minimum amount of β-carotene (177.6±2.1 mg kg^{-1} fresh fruit) was recorded NMC (100% RRF) treatment. Data revel that maximum AA was also recorded in the AM fungi + PGPR (*Pseudomonas* + *Azotobacter* + *Fraturia*) treatment (59.06± 1.08 %) and minimum in control (41.85±1.51%), which had differed significantly from other treatments (Figure 10).

The bioavailability of lycopene is also affected by the microbial dosage and the presence of other carotenoids such as β-carotene. The concentrations of β-carotene were also determined (Figure 10). Jonson et al. [41] found that the bioavailability of lycopene was significantly higher when it was ingested along with β-carotene than when ingested alone. On this basis determined the concentration of β-carotene alongside that of lycopene. The lycopene/β-carotene ratios of the tomato are less than unity, showing that there is higher amount of β-carotene level than lycopene, which may further enhance the bioavailability of lycopene in these products, whereas, it is greater than unity in fresh tomato fruits. This is in agreement with the findings of Johnson et al. [41] and this ratio may also be an additional parameter for determining the bioavailability of lycopene in

tomatoes. K content in tomato fresh fruit presented in figure 11 revealed that K content in fresh fruit showed in significant influence of all the treatments over the control. An inquisition of data indicates that maximum K content in fresh tomato fruit (4.92%) was under treatment AM fungi + PGPR, whereas the minimum amount of K in fresh fruit (3.37%) was absorbed under treatment NMC (100% RRF). K contents increased in fresh tomato fruit may be due to improved absorption and utilization of K through AM fungi and PGPR application.

Macro-nutrient Uptake by Shoot System

Nitrogen Uptake

An examination of data indicates that N uptake (Figure 12) by tomato shoot systems shows that all the microbial treatments had a significant influence by N uptake as compared to NMC (100% RRF) treatment. The maximum N uptake (4.95%) was obtained under treatment AM fungi + PGPR where the two microbial inoculant were applied. However, the lowest value of N uptake (3.18%) by tomato shoot was recorded under NMC treatment. The uptake of N by the tomato shoot systems went on increasing with the successive microbial application. Since the uptake is a resultant of concentration and biological yield.

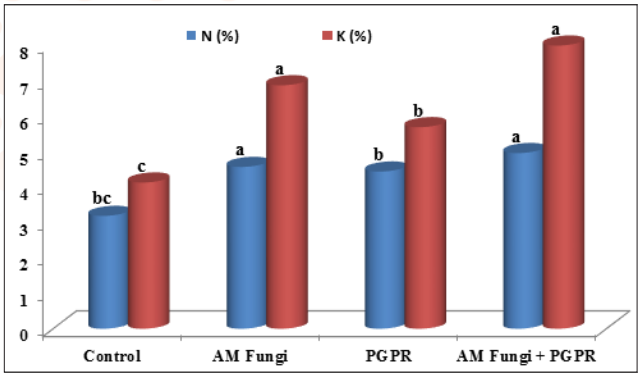

Figure 12: Percentage nitrogen and potassium uptake in tomato shoot system at harvest

Phosphorus Uptake

A glance on data in figure 13 shows the highest uptake of P (0.26%) by the tomato shoot under treatment AM fungi + PGPR. The minimum P uptake was recorded under NMC (0.17%). The effect of microbial (AM fungi+ PGPR) inoculation on P uptake was significant. P uptake increased may be due to improved absorption and utilization available soil P at higher rates.

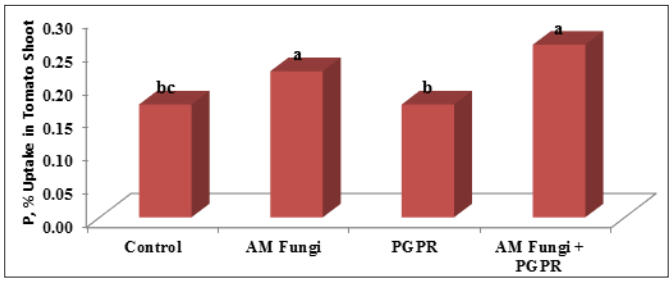

Figure 13: Percentage uptake of phosphorus contents in tomato shoot system at harvest

Potassium Uptake

K translocation in tomato shoot has been presented in figure 12. An inquisition of data indicates that maximum K uptake (7.98%) by tomato shoot recorded in AM fungi + PGPR treatment, where the two consortium microbial stimulants were applied. The minimum K uptake of 4.12% was observed in treatment NMC where 100% RRF applied. K uptake was increasing may be due to improved absorption and utilization of K at higher rates of available soil K.

Micronutrient nutrient uptake by shoot

Copper Uptake

The perusal of data presented in the figure 14 reveals the highest uptake of Cu (0.0017%) by the tomato shoot was recorded in treatment AM fungi + PGPR. The effect of AM fungi + PGPR in treatments noticed to exert a significant effect on the Cu removal by tomato shoot. The minimum Cu (0.001%) uptake was recorded under NMC (100% RRF) treatment.

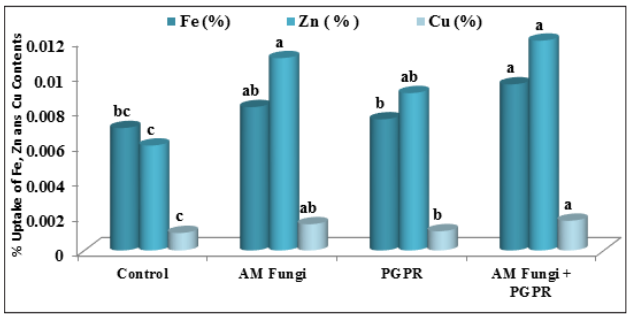

Figure 14: Percentage uptake of iron, zinc and copper contents in tomato shoot system at harvest

Iron Uptake

An inquisition of data in figure 14 indicates that maximum Fe uptake (0.0095%) by tomato shoot recorded under treatment AM fungi + PGPR and the lowest amount of Fe removal by tomato shoot was obtained under NMC treatment (0.007%).

Zinc Uptake

The data presented in the figure 14 reveals that maximum Zn uptake (0.012%) by tomato shoot recorded under treatment AM fungi + PGPR. The AM fungi and PGPR mediated tomato plant were found to exert a significant effect on the Zn uptake by tomato shoot. The minimum Zn uptake of 0.006% was observed under treatment NMC (100% RRF).

Mycorrhizal Root Colonization (MRC) Percentage

MRC percentage in tomato root system was observed in all treatments including NMC (100% RRF) plants (Figure 15). Maximum percentage of MRC was observed in AM fungi + PGPR mediated tomato crop followed by AM fungi alone (75.45%), PGPR alone (55.33%) and NMC (45.33%) plants at harvest. Statistically significant ($P<0.05$) differences were observed between the treatments. MRC was increased with additionally added PGPR with mycorrhizae. In general, AM fungal strains mediated plants have been encouraging by higher water and mineral nutrients uptake from the soils because they increased the total root surface [4, 30-31, 36]. The colonization potential of AM fungi decreases in control treatment

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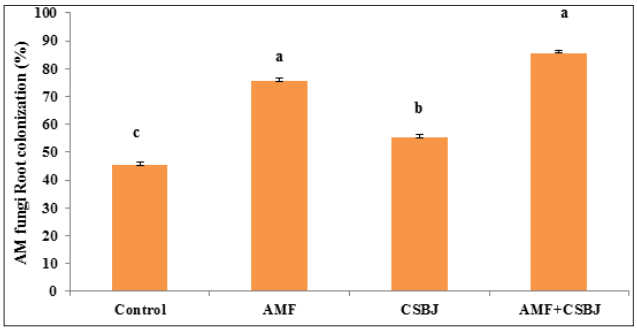

Figure15: AM fungal root colonization percentage at the time of harvest

Discussion

Numerous studies have showed, the impact of plant treated with suitable RM on tomato fruit quality parameters [4, 7, 11, 42, 43], however results indicate that AM fungi and PGPR changes the fruit quality and quantity. Supported the results of this study, K uptake by shoot content within the fruit increased altogether treatments, compared to NMC treatment (Figure 1). These results showed a synergistic impact of AM fungi and PGPR on K uptake in tomato crop. Several microorganisms within the soil are capable to solubilize unavailable forms K bearing minerals, such as micas, illite and orthoclases, by excretion organic acids that either directly dissolves rock K or chelate silicon ions to bring the K into solution [44-45]. PGPR inoculation additionally considerably improved nutrient contents by plants. Increased nutrients uptake by plants inoculated with AM fungi and PGPR has been attributed to the manufacture of plant growth regulators at the root interface, which inspired root development and resulted in higher absorption of water and nutrients from the soil [34, 37, 40, 46-48]. Data indicate that AM fungi and PGPR were increase plant growth, plant nutrition, LWP, root growth pattern, plant competitiveness and responses to external stress factors. PGPR have additionally been shown to induce systematic resistance to fungal, bacterial, and viral pathogens in varied crops such as bean, tomato, radish, and tobacco [49]. Different PGPR together with associative bacterial microorganism (BM) such as *Azospirillum, Bacillus, Pseudomonas, Enterobacter* have been used their helpful effects on PG [50]. Benefits of mycorrhizae to plants comprise enhanced mineral nutrition [4, 25, 28-30, 39, 48] protection against pathogens [35, 47, 51, 53] and increased resistance or tolerance to stress [29, 53]. Shoot and fruit K increased after AM fungi and PGPR were used together (Table 4), instead of, AM fungi or PGPR were used alone.

These results showed the optimistic synergistic interactions between AM fungi and PGPR on tomato fruit and shoot contents. AM fungi are well known to effect plant growth and health by improving mineral nutrition [4, 6, 25, 36, 39, 40, 46, 54] and by increasing resistance to tolerance of biotic [55] and abiotic stress [53]. Synergistic interactions between AM fungi and asymbiotic N2 fixing bacteria such as *Azotobacter chroococcum, Acetobacter diazotrophicus, Bradyrhizobium japanicum* and K mobilizer (*Fraturia aurantia*) have been designated by numerous researchers [34-35,56]. Both AM fungi and PGPR complement one another in their role in N fixation, plant hormone production, P and K solubilization, and increasing surface absorption. The positive synergistic interactions among mycorrhizosphere AM fungi and numerous N fixing, P and K solubilizing bacteria is that the basis of application of those microbes as biofertilizer and bioprotectant agents [35, 57]. These microbes are regulated by AM fungi for their own advantage that successively benefit the host plant. All these studies suggest that colonization of plant roots by AM fungi significantly influences the mycorrhizosphere microorganisms, including PGPR. The results indicated, the maximum fruit K content and shoot mineral contents uptake were observed in AM fungi + PGPR treatment followed by AM fungi alone, PGPR alone and NMC (100% RRF) treatments, that declared a positive synergistic interactions between AM fungi and PGPR and tomato plant. Prasad et al. (35) showed that PGPR has positive impact on plant growth and productivity and reduced harmful diseases.

Based on the findings; maximum lycopene and β-carotene contents were noticed in AM fungi + PGPR treatment followed by AM fungi, PGPR and NMC treatment (Figure 9). On the other hand, these treatments had the maximum fruit K content and nutrients uptake by shoot. Significant difference ($P<0.05$) was noticed between lycopene with shoot K, and nutrients uptake by tomato shoots. Other works showed that K uptake has affected on the lycopene and carotenoid content in tomato plant. Taber [58] showed that fruit K and fruit lycopene concentration have a direct correlation. Perkins-veazie and Roberts [59] delineated fruit red colour and ripening disorders are correlated with fruit K content. The ripening of tomato fruit, from green to full red, involves the build-up of lycopene and carotenoids and therefore the disappearance of chlorophyll pigment in fruit, colourless precursor carotenoids, phytoene and phytofluene, lead to lycopene synthesis at the breaker stage (fruit blossom green end breaks with red streaks, <10% colour change) [60-64]. Fanasca et al. [65] delineated that a high proportion of K within the nutrient solution improved quality attributes such as lycopene and β-carotene content. Trudel and Ozbun [66] reported that the K impact on lycopene content is associated to the significance of that element in protein synthesis and therefore the activity of acetic thiokinase. It has been determined that the maximum utmost of AA was within the AM fungi + PGPR treatment and minimum was discovered within the PGPR treatment that was higher than NMC (100% RRF) treatment (Figure 3). One of the physiological processes which can markedly alter or cut back the nutritionary quality and also the AA of the various plant products consumed by humans is oxidative stress. The environmental factors that induce oxidative stress in plants include air pollution, herbicide/pesticide utilization, heavy metal contamination, drought, salinity, injuries, UV light, unfavourable temperatures and photo inhibition from excessive solar radiation [67-68]. The utilization of the AM fungi and PGPR could also be due to ability to

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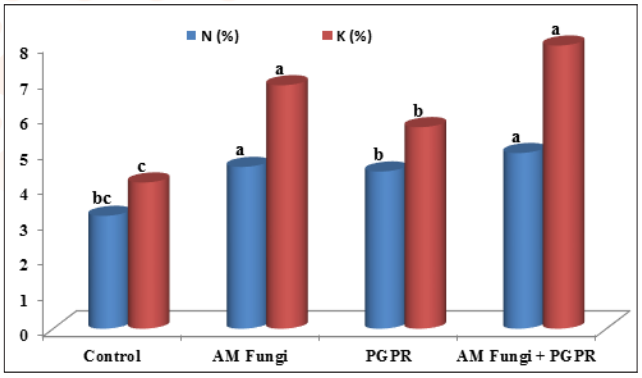

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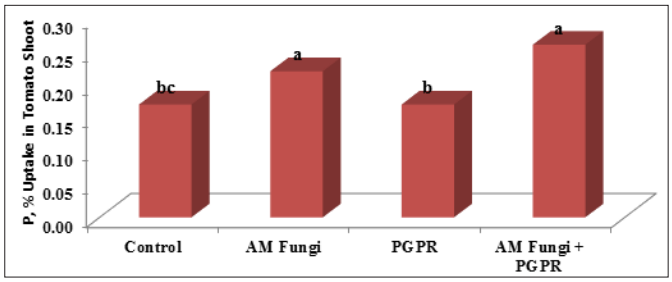

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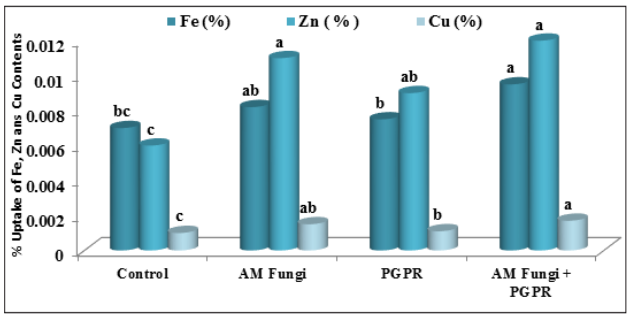

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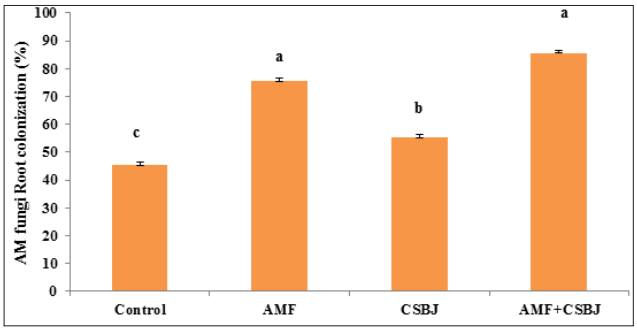

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reduce negative effects of environmental stress improved fruit AA. The production of plant growth regulators (PGR) by the microorganisms is another vital mechanism usually related to growth stimulation [69]. AM fungi are identified to have an effect on PG and health by increasing resistance to tolerance of biotic [55, 70] and abiotic stress [4, 30, 35, 53, 71].

Conclusion

The outcomes indicate that inoculation with consortium AM fungi and PGPR had positive effects on tomato growth and its attributes characters along with improve shoot nutrients uptake which controlled to producing superior gross yield without uses of CF. It is determined that the usage of AM fungi or PGPR alone can increase lycopene, β-carotene, AA, K content in fruit and nutrients uptake of tomato shoot compared with NMC (100% RRF), and once PGPR additional to the AM fungi treatment, these factors are greater, that show a optimistic interaction between AM fungi + PGPR. The promotion of mycorrhizal and bacterial biofertilizers has the advantage of permitting reduced CF inputs to save the environment. Economize on CF use in tomato crop production providing a sustainable and environmentally safer substitute and farmers should encourage the uses of RM such as AM fungi and PGPRs biofertilizers for field assessment. The present study revealed that plants mediated with AM fungi and PGPR can play a key role in reducing CF inputs in sustainable production systems (SPS) of tomato cash crop. AM fungi and PGPR biofertilizers inoculation influenced growth, productivity, lycopene, β-carotene, AA, and fruit K and nutrients uptake such as N, P, K, Cu, Fe, Mn and Zn as compared to the different doses of CF (NMC). From this study, it can be concluded that using AM fungi and PGPR inoculums could reduce the CF inputs needed to produce vegetables since increased plant growth parameters, LMP, nutrients concentration in fruit and shoots, and MRC percentage were obtained when AM fungi + PGPR was applied to tomato plants and this was comparable to NMC plants treated with 100% RRF.

Conflict of Interest

The author declares that he has no conflict of interest in the publication of this manuscript.

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