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Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of Pomegranate (*Punica granatum* L.) in Indian Thar Desert

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ABSTRACT

Production of horticultural crops has undergone enormous change in recent years due to development of innovative technologies including integrated nutrient management practices using biofertilizers. The present study represents the positive response of biofertilizers in nursery seedlings followed by their transplantation in harsh field conditions of Indian Thar Desert. Nursery and field experiments were carried out to assess the effectiveness of selected N_2-fixing bacteria and AM fungi alone or in combination, on the growth and biomass production of *Punica granatum*. In both experiments, the combined treatment of *Azotobacter chroococcum* and *Glomus mosseae* was found to be the most effective. Besides enhancing the rhizosphere microbial activity and concentration of various metabolites and nutrients, these bioinoculants helped in better establishment of pomegranate plants under field conditions. A significant improvement in the plant height, plant canopy, pruned material and fruit yield was evident in 5-year-old pomegranate plants in field conditions. In view of the above results, use of biofertilizer technology may be adopted for the establishment and development of other horticultural plant species in arid regions.

1. Introduction

Pomegranate (*Punica granatum* L.) is an economically important commercial fruit plant species belonging to family Punicaceae. The plant is drought tolerant, winter hardy and can thrive well under desert conditions. Pomegranate is a good source of protein, carbohydrate, minerals, antioxidants, vitamins A, B and C, also been used in controlling diarrhea, hyperacidity, tuberculosis, leprosy, abdominal pain and fever. Due to its multipurpose medicinal uses it is also known as "Dadima" in Ayurveda (Prakash Paranjpe, 2001) and as "Superfruit" in the global functional food industry (Martins et al., 2006). Pomegranate juice contains antioxidants such as soluble polyphenols, tannins, anthocyanins and may have antiatherosclerotic properties (Michel et al., 2005), and can be used in the treatment of cancer and chronic inflammation (Ephraim and Robert, 2007). Commercial pomegranate orchards are found in Indian Thar Desert, which is characterized by nutrient-deficient sandy soils, low organic matter, high wind velocity coupled with high evaporation rates, high temperature and solar radiation, low and irregular distribution of rainfall and generally experience water deficit during plant growth period (Panwar and Tarafdar, 2006). Vegetation of Thar Desert is often hindered by the lack of resident microflora, which acts as both source and sinks for essential plant nutrients and is fundamental to the transformation of various nutrients. Production of horticultural crops has undergone significant changes in recent years due to development of innovative technologies including integrated nutrient management practices involving biofertilizers, which include phosphate-solubilizing bacteria (PSBs), symbiotic and non-symbiotic N_2-fixing bacteria and arbuscular mycorrhizal (AM) fungi. The use of biofertilizers in enhancing plant growth and yield has gained momentum in recent years because of higher cost and hazardous effect of chemical fertilizers. Nitrogen-fixing bacteria and arbuscular mycorrhizal fungi were found to enhance the growth and production of various fruit plants significantly (Khanizadeh et al., 1995; Ghazi, 2006), besides improving the microbiological activity in the rhizosphere (Kohler et al., 2007). Though there are many reports on the effect of different biofertilizers on various fruit plants, no information is

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Table 1
Soil characteristics of the experimental area

Parameters	Quantity
Soil classification (USDA)	Hyperthermic typic haplocamborthid
Sand (%)	84.1
Silt (%)	6.5
Clay (%)	8.5
Field capacity	150 mm m^{-1} profile
pH (soil:water, 1:2.5)	8.1
Electrical conductivity (dS m^{-1})	0.2
Organic matter (%)	0.3
Total nitrogen (mg kg^{-1})	310
NO_3-N (mg kg^{-1})	1.87
Total P (mg kg^{-1})	702
Olsen P (mg kg^{-1})	3.9
Organic P (mg kg^{-1})	272
FDA activity (p kat g^{-1})	940
Dehydrogenase (p kat g^{-1})	2.0
N_2-ase activity (nmol C_2H_4 h^{-1})	52
AMF spores (50 g^{-1})	35

available on usefulness of these biofertilizers with respect to pomegranate. Our preliminary study has indicated that these soil microorganisms are very common in arid soils and form association with most of the arid fruit plants (Aseri and Rao, 2000). Hence, an attempt has been made to examine the effect of these biofertilizers namely *Azospirillum brasilense* (Ab), *Azotobacter chroococcum* (Ac), *Glomus fasciculatum* (Gf) and *Glomus mosseae* (Gm) on growth and nutrient uptake as well as rhizosphere microbial activity of pomegranate in field conditions.

2. Materials and methods

This study was conducted at Central Research Farm, Central Arid Zone Research Institute (CAZRI), Jodhpur, located at 26°18′N and 73°01′E. The farm soil is loamy sand (hyperthermic typic haplocamborthid, USDA classification). Some characteristics of the soil are presented in Table 1. Strains of *Azospirillum* (an associative symbiotic N_2-fixing bacterium) and *Azotobacter* (a free living non-symbiotic N_2-fixing bacterium) were isolated using N-free semisolid malate medium (Day and Dobereiner, 1976) and Jensen's N-free agar medium (Jensen, 1954), respectively, from the roots and rhizosphere soil of field-grown pomegranate. The identification of strains was carried out by Institute of Microbial Technology (IMTECH), Chandigarh (India) as *A. brasilense* and *A. chroococcum*. The pure cultures of these strains were maintained in Microbiology Section of CAZRI, Jodhpur using above-mentioned media. After 3 days of growth, the cells were centrifuged, washed twice in sterile distilled water and suspended in 0.15 M phosphate buffer at pH 7.0. Ten milliliters of cell suspension having 10^8 cell mL^{-1} was used as inoculums for both bacterial types at the time of planting pomegranate cuttings. Spores of AM fungi were extracted from the rhizosphere soils of field grown pomegranate plants by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Total number of spores was estimated by the method of Gaur and Adholeya (1994) and spore densities were expressed as the number of spores per 50 g of soil. Taxonomic identification of AM spores up to species level was based on spore size, spore colour, wall layers and hyphal attachment and was made using the identification manual of Schenck and Perez (1990) and the description provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.wvu.edu). The AM fungi were identified as *G. fasciculatum* and *G. mosseae*. The pure cultures were maintained on pearl millet/wheat plant roots under sterile condition. Ten grams of soil including root bits containing about 8–10 viable AM fungal propagules per gram soil were used as inoculum and spread as a thin layer 2 cm below soil surface in polythene bag (30 cm × 10 cm) containing 900 g substrate (soil:-farm yard manure:pond silt, in the proportion of 3:1:1 by volume). In case of control treatment, similar amount of inoculum was sterilized in autoclave and added to polythene bag in same manner. Semi-hard wood cuttings of pomegranate (6-month-old cuttings) were kept in 200 ppm indole butyric acid solution for 12 h and then planted in polybags of one each. The experiment was laid out in a complete randomized design consisting of six treatments (control, A.b., A.c. G.f., G.m. and A.c. + G.m.) all with 40 replications during kharif season (June–September) in the year 2000. The temperature during the experiment ranged from 25 °C (night) to 35 °C (day). Dual inoculation of *A. chroococcum* and *G. mosseae* was selected based on the data obtained on various combinations of AM fungi and nitrogen-fixing bacteria under sterile condition during our preliminary study (Aseri and Rao, 2000). The plants were watered on alternate days to field capacity.

After sampling rhizosphere soil along with root fragments of 4-month-old seedlings grown in polythene bags, 10 plants from each treatment were harvested and leaf area (leaf area meter, C-203, USA) and total chlorophyll (Arnon, 1949) were measured. Total phenols, reducing sugars and amino nitrogen in the leaves were analyzed from the alcohol extracts (Mahadeven et al., 1965). Shoot along with leaves were dried at 70 °C till constant weight and dry weight were recorded.

The percent root colonization was determined by root slide technique (Read et al., 1976) after clearing 1 cm root segment with KOH and staining with trypan blue. Dehydrogenase activity, a measure of microbial activity, was assayed by the method of Tabatabai (1982). The soil samples were incubated with 2,3,5-triphenyl tetrazolium chloride, and production of triphenyl formazone was determined spectrophotomitrically. Hydrolysis of fluorescence diacetate (FDA) was determined by the standard procedure of Schnurer and Rosswall (1982) and the fluorescein released was quantified spectrophotomitrically. For this, soil sample (0.1 g) was placed in plastic tubes and 10 mL sterile potassium buffer (pH 7.6, 60 mM) was added to it. The reaction started after adding fluorescein diacetate (1 mg mL^{-1} in acetone). Tubes were sealed and kept in an incubator at 37 °C four 4 h. After incubation, 10 mL acetone was added to stop the reaction and after centrifugation (3200 rpm), supernatant's optical density was determined at 490 nm. Total nitrogen-fixing potential (nitrogenase activity) of the soil was determined by incubating 50 mg of soil in 7 mL test tube containing 3 mL nitrogen-free semisolid malate medium for 48 h at 30 °C. The cotton plugs were replaced with suba seals and 10% of air was replaced with C_2H_2 and later ethylene (C_2H_4) produced was estimated by an AIMIL-Nucon Gas chromatograph fitted with Porapak-N column (2 × 0.003 m) using N_2 as carrier gas at a flow rate of 25 mL min^{-1}. Nitrogenase (N_2-ase) activity was expressed as n mol C_2H_4 produced per hour (Rao and Venkateswarlu, 1982). For activity of alkaline phosphatase in the rhizosphere soil, the *p*-nitrophenyl phosphate was used as a substrate in a borex–NaoH (Ph 9.4) buffer (Tabatabai and Bremner, 1969). Dry powder of shoot was used for analyzing total nitrogen (by microkjeldhal method), phosphorus (by vanado-molybdo phosphoric yellow colour method), potassium (by flame photometer), calcium and magnesium (by titrimetry employing disodium salt of EDTA) after di/tri acid digestion (Jakson, 1967). Estimates of coper, zinc, manganese and iron were made by using atomic absorption spectrophotometer (Varian AA1475).

Out of the remaining seedlings, 25 healthy plants of each treatment were transplanted at CR Farm, CAZRI during January 2001 in rows with 6.0 m inter and intra row spacing. For this purpose, pits with depth and diameter of 0.6 m were dug and filled each with 30 kg of the same soil mixture used for the plantlets

Table 2
Effect of inoculation with different biofertilizers on growth of pomegranate after 4 months

Treatments	Branches (number plant^{-1})	Leaf area (cm^2 plant^{-1})	Shoot dry wt. (g plant^{-1})
Control	5 ± 0.58	193.4 ± 0.64	1.39 ± 0.006
A. brasilense	8 ± 0.33	304.9 ± 0.55	1.82 ± 0.009
A. chroococcum	8 ± 0.58	289.0 ± 0.57	1.65 ± 0.012
G. fasciculatum	11 ± 0.58	246.4 ± 0.6	1.61 ± 0.009
G. mosseae	11 ± 0.33	262.7 ± 0.95	1.64 ± 0.012
A. chroococcum + *G. mosseae*	12 ± 0.58	292.9 ± 0.83	1.89 ± 0.006

± represents standard error of means.

production added with 100 g endosulfan dust (4%). The plants were irrigated fortnightly intervals. Immature fruits along with few leaves and young branches were removed during the first season (November–February, 2001). After every winter season pruning was done for which weak and dead limbs and basal suckers were removed to give proper shape to plants. Pruning is encouraged for the growth of new spurs in each season and it helps the plant to sustain more fruit weight (Panwar et al., 1994). Pruned materials (weak and dead shoot portion along with leaves) weights were recorded after drying in a hot-air oven to a constant weight and ground to a fine powder. Plant canopy was calculated using the formula πr^2 in which radius mean was measured from all the directions of crown. Plant height, canopy, pruned material and fruit yield were recorded yearly for next 5 years (2002–2003, 2003–2004, 2004–2005, 2005–2006, and 2006–2007). The means were compared using standard errors of the mean.

3. Results

Sprouting of pomegranate occurred in 7–10 days in the biofertilizers inoculated cuttings while it took 8–10 days longer (data not given) in non-inoculated controls, resulting, 4 months later, in a higher number of branches (Table 2). However, significant differences were not observed in number of branches between various biofertilizers used. A significant enhancement (27.4–57.6%) was observed in leaf area. The increase was maximum with *A. brasilense* followed by dual inoculation treatment. Biofertilizer inoculation had enhanced shoot dry weight by 16–36%. The inoculation effect varied among treatments with the maximum in dual inoculation treatment while minimum with *G. fasciculatum*. Inoculation had resulted in a significantly higher total chlorophyll content as well as accumulation of reducing sugars, total phenols and amino nitrogen in 4 months old inoculated plants (Table 3). Total chlorophyll was observed highest in dual inoculated seedlings followed by *G. mosseae* and *A. brasilense* alone. A similar trend was found in reducing sugars and amino nitrogen contents, whereas total phenols were found to be maximum with *A. brasilense* followed by dual inoculation treatment.

Soil inoculation with biofertilizers had significantly enhanced the activities of dehydrogenase, alkaline phosphatase and nitrogenase (N_2-ase) and hydrolysis of fluorescein diacetate in rhizosphere soils of pomegranate compared to that of uninoculated control plants (Table 4). Four months after inoculation, percent root colonization by AM fungi was enhanced by 15–380% over the control (Table 4). Uninoculated control also showed infection by mycorrhiza which could be attributed to the presence of native AM fungi in the soil. Dual inoculation resulted in maximum mycorrhizal root infection. A similar trend was noticed with AMF spore build up in the rhizosphere soil (Table 4). The maximum increase in spore number over the control (203 spores 50 g^{-1} soil) was observed with dual inoculation treatment.

Uptake of various nutrients was significantly higher in pomegranate upon inoculation with various beneficial microorganisms (Table 5). In general, dual inoculation led to maximum uptake of N, P, K, Ca, Mg and micronutrients in pomegranate seedlings. In case of N, dual inoculation treatment was followed by *A. brasilense* and *A. chroococcum*, whereas in case of P, dual inoculation treatment was followed by *G. mosseae* and *G. fasciculatum*, respectively.

K, Ca, Mg, Cu, Zn, Mn and Fe were found in significantly higher concentration in dual inoculation treatment. However, significant

Table 3
Effect on inoculation with different biofertilizers on chlorophyll content and various metabolites (mg g^{-1} fresh weight) in pomegranate after 4 months

Treatments	Total chlorophyll	Reducing sugars	Total phenols	Amino nitrogen
Control	5.45 ± 0.012	2.95 ± 0.012	1.81 ± 0.009	2.73 ± 0.015
A. brasilense	5.75 ± 0.012	3.35 ± 0.015	2.15 ± 0.012	3.35 ± 0.029
A. chroococcum	5.60 ± 0.017	3.15 ± 0.01	1.95 ± 0.009	3.02 ± 0.027
G. fasciculatum	5.77 ± 0.027	3.15 ± 0.012	2.05 ± 0.023	3.30 ± 0.021
G. mosseae	5.84 ± 0.026	3.45 ± 0.017	2.08 ± 0.018	3.43 ± 0.021
A. chroococcum + *G. mosseae*	6.0 ± 0.088	3.65 ± 0.012	2.14 ± 0.009	3.57 ± 0.02

± represents standard error of means.

Table 4
Effect of inoculation with biofertilizers on rhizosphere enzyme activity, hydrolysis of FDA, AM fungal spore density and percent mycorrhizal root colonization

Treatments	Dehydrogenase (p kat g^{-1} soil)	Nitrogenase (nmol C_2H_4 h^{-1})	Alkaline phosphatase (n kat 100 g^{-1} soil)	FDA-hydrolysis (p kat g^{-1} soil)	AM spores (50 g^{-1} soil)	Percentage root colonization (%)
Control	7.33 ± 0.015	211 ± 1.45	9.1 ± 0.115	1785 ± 2.60	104 ± 1.73	20 ± 0.57
A. brasilense	8.72 ± 0.015	392 ± 1.45	12.3 ± 0.115	2340 ± 3.84	129 ± 2.72	23 ± 0.57
A. chroococcum	8.26 ± 0.023	387 ± 1.15	12.2 ± 0.26	2135 ± 3.18	228 ± 2.72	65 ± 1.45
G. fasciculatum	8.06 ± 0.026	316 ± 1.73	13.2 ± 0.12	2149 ± 4.04	290 ± 5.78	83 ± 2.40
G. mosseae	8.26 ± 0.032	332 ± 0.88	13.8 ± 0.088	2230 ± 2.33	298 ± 4.63	91 ± 2.40
A. chroococcum + *G. mosseae*	9.05 ± 0.021	403.5 ± 2.40	15.5 ± 0.145	2670 ± 2.33	307 ± 6.08	96 ± 2.02

± represents standard error of means.

Table 5
Nutrient uptake (mg plant^{-1}) by Pomegranate as influenced by inoculation with various biofertilizers after 4 months

Treatments	N[a]	P[a]	K[a]	Ca[b]	Mg[b]	Cu[b]	Zn[b]	Mn[b]	Fe[b]
Control	18.9 ± 0.23 (1.36)	2.5 ± 0.09 (0.18)	13.2 ± 0.18 (0.95)	1.18 ± 0.01 (850)	0.26 ± 0.01 (186)	0.022 ± 0.001 (16.3)	0.12 ± 0.01 (86.9)	0.21 ± 0.02 (152.4)	0.15 ± 0.02 (106)
A. brasilense	25.6 ± 0.18 (1.41)	4.5 ± 0.12 (0.25)	22.0 ± 0.06 (1.21)	1.68 ± 0.01 (927)	0.46 ± 0.01 (258)	0.032 ± 0.001 (18.2)	0.17 ± 0.02 (94.5)	0.28 ± 0.01 (157.8)	0.26 ± 0.01 (146)
A. chroococcum	22.7 ± 0.12 (1.38)	3.4 ± 0.12 (0.21)	18.1 ± 0.06 (1.10)	1.46 ± 0.02 (886)	0.37 ± 0.01 (277)	0.026 ± 0.001 (16.5)	0.15 ± 0.02 (91.1)	0.26 ± 0.01 (157)	0.22 ± 0.01 (135)
G. fasciculatum	22.0 ± 0.06 (1.37)	4.83 ± 0.09 (0.30)	18.6 ± 0.09 (1.16)	1.50 ± 0.02 (935)	0.41 ± 0.01 (252)	0.030 ± 0.001 (19.1)	0.14 ± 0.02 (91.3)	0.25 ± 0.01 (158.4)	0.24 ± 0.01 (150)
G. mosseae	22.4 ± 0.12 (1.37)	5.57 ± 0.12 (0.34)	20.3 ± 0.09 (1.24)	1.61 ± 0.02 (986)	0.39 ± 0.01 (241)	0.027 ± 0.001 (17.4)	0.15 ± 0.02 (95.1)	0.26 ± 0.01 (160.6)	0.26 ± 0.01 (157)
A. chroococcum + *G. mosseae*	27.0 ± 0.29 (1.43)	6.4 ± 0.15 (0.34)	22.8 ± 0.06 (1.21)	1.69 ± 0.02 (899)	0.49 ± 0.01 (261)	0.037 ± 0.001 (19.6)	0.17 ± 0.01 (90.3)	0.29 ± 0.02 (158.6)	0.30 ± 0.02 (161)

± represents standard error of means.
[a] Figures in parenthesis indicate the percent concentration.
[b] Figures in parenthesis indicate the ppm concentration.

Fig. 1. Effect of inoculation with various biofertilizers on plant height of Pomegranate grown in field condition (C: control; Ab: *Azospirillum brasilense*; Ac: *Azotobacter chroococcum*, Gf: *Glomus fasciculatum*; Gm: *Glomus mosseae*). Vertical bars are standard errors of the means.

variations were noticed in efficiency of different biofertilizers. Among nitrogen fixers and AM fungi, *A. brasilense* and *G. mosseae*, respectively, were found to enhance maximum uptake of aforesaid nutrients as compared to control.

The results of our field study reveals that inoculated plants had resulted in a significant enhancement in plant height (Fig. 1), plant canopy (Fig. 2), pruned plant material (Fig. 3) and fruit yield (Fig. 4) with a maximum increase in dual inoculation treatment. Enhanced plant height, canopy, pruned plant material and fruit yield were recorded in first year (2002–2003) and almost similar trend afterwards (2003–2007). Further appraisal of field study data indicates that *A. brasilense* and *G. mosseae* enhanced all field study parameters among N_2-fixing bacteria and AM fungi, respectively. However, overall maximum increase in these parameters was observed in dual inoculation treatment followed by *A. brasilense*.

4. Discussion

During the present study, sprouting of pomegranate was occurred in 7–10 days in the biofertilizers inoculated cuttings while it took 8–10 days longer (data not given) in non-inoculated controls. It may be due to the action of plant growth regulators mainly indole butyric acid secreted by both nitrogen fixers and AM fungi (Luis et al., 2003). Occurrence of maximum branching in 2-year-old mulberry cuttings upon bacterial inoculation (Santhi and Ponnuswamy, 1995) and enhanced rooting in Prosopis cuttings by *Azospirillum* inoculation (Felker et al., 2005) has also been reported. Increased leaf area and shoot dry weight upon inoculation were

Fig. 2. Effect of inoculation with various biofertilizers on plant canopy of Pomegranate grown in field conditions (C: control; Ab: *A. brasilense*; Ac: *A. chroococcum*, Gf: *G. fasciculatum*; Gm: *G. mosseae*). Vertical bars are standard errors of the means.

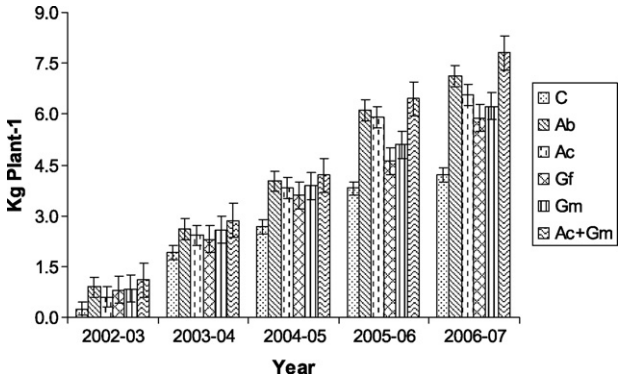

Fig. 3. Effect of inoculation with various biofertilizers on pruned material of Pomegranate grown in field conditions (C: control; Ab: *A. brasilense*; Ac: *A. chroococcum*, Gf: *G. fasciculatum*; Gm: *G. mosseae*). Vertical bars are standard errors of the means.

observed in different fruit plants. Similar observations of enhanced leaf area and shoot dry weight were made in different fruit plants upon inoculation with AM fungi (Khanizadeh et al., 1995), *Azospirillum* (Paramaguru and Natarajan, 1993) and *Azotobacter* (Dibut Alverz et al., 1996). During the present study it was observed that dual inoculation with *A. brasilense* and *G. mosseae* had resulted in a higher biomass production compared to that of uninoculated or single inoculation treatment. Ashokan et al. (2000) reported a significant enhancement in the growth of banana and custard apple plants after dual inoculation with AM fungi and *Azotobacter*. A significantly higher total chlorophyll content as well as higher accumulation of various metabolites (reducing sugar, total phenol and amino nitrogen) might have resulted from enhanced plant growth and biomass production (Kohler et al., 2007). These observations are in conformity with those from Tiwary et al. (1999) who reported higher chlorophyll content and so photosynthesis in banana upon inoculation with N_2-fixing bacteria. Mathur and Vyas (2000) reported enhanced synthesis of reducing sugars, free amino acids, soluble protein and chlorophyll in *Ziziphus mauritiana* seedlings upon inoculation with AM fungi.

The increases observed in dehydrogenase, alkaline phosphatase, nitrogenase and hydrolysis of fluorescein diacetate may be related mainly due to increase in the rhizosphere microbial population as a consequence of the inoculation treatments (Skujins, 1973; Aseri and Tarafdar, 2006). The enhanced phosphatase activity may help the plant to mobilize P and thereby increase the biomass production (Tarafdar and Gharu, 2006). A significant enhancement in total nitrogen-fixing potential as reflected by increased nitrogenase activity in rhizosphere soils was observed. The measurement of these enzymatic activities can provide an early indication of changes in soil fertility, since they are related to mineralization of such important nutrient elements as N, P and C (Ceccanti et al., 1994).

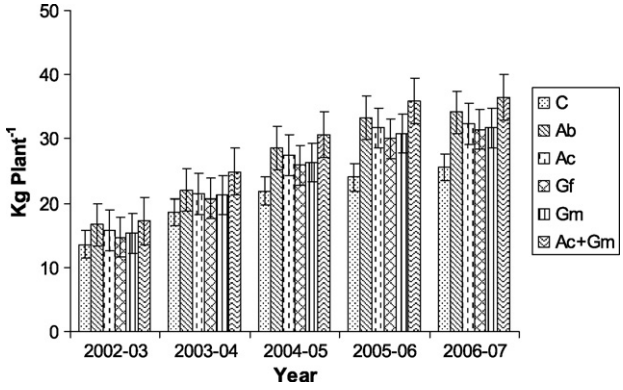

Fig. 4. Effect of inoculation with various biofertilizers on fruit yield of Pomegranate grown in field conditions (C: control; Ab: *A. brasilense*; Ac: *A. chroococcum*, Gf: *G. fasciculatum*; Gm: *G. mosseae*). Vertical bars are standard errors of the means.

The degree of root colonization and sporulation in rhizosphere soils varied significantly among different treatments. The degree of mycorrhizal colonization and sporulation was higher in dual inoculation as compared to single inoculation treatment. This suggests that plant growth promoting rhizobacteria act as a helping tool for AM fungi for better establishment and functioning of symbiosis (Kohler et al., 2007). *A. chroococcum* act as helper bacteria when associated with *G. mossae* and increase the production of tomato (Sushma Gupta Sood, 2003). There was a good agreement between AM fungal spore density and percent root colonization.

Higher uptake of nutrients was due to the synergistic effect of improved biomass and higher nutrient concentration in the inoculated plants (Table 5). Maximum N and P uptake was observed in dual inoculation treatment which may be due to the improved symbiotic N_2 fixation as well as due to improved phosphatase activity and thereby P mobilization and subsequent P uptake by mycorrhizal hyphae. These results confirmed the earlier findings of Singh and Sharma (1993) who reported enhanced N and P concentration in sweet orange when inoculated with biofertilizers.

Inoculated seedlings were found to contain significantly higher amount of other nutrients viz. K, Ca, Mg, Cu, Fe, Zn and Mn mainly in dual inoculation treatment. These observations are in conformity with those of Ghazi, 2006. This enhancement might be due to the production of nutrient-solublizing enzymes by microorganisms and ability of AM fungal hyphae towards uptake of immobile ions, besides increasing the surface area of roots by tapping larger soil volume (Kothari et al., 1991; Li. et al., 1991).

On behalf of enhanced accumulation of various metabolites, increased rhizosphere activity and higher nutrient uptake, transplanted pomegranate grew significantly better in field conditions and growth trend were almost the same between all the treatments as found during nursery period. Similar observations of increased plant growth and fruit production under field conditions were made in banana and tomato by Jeeva et al. (1988) and Kohler et al. (2007), respectively.

5. Conclusion

The present study represents the positive response of biofertilizers in nursery seedlings followed by their transplantation in harsh field conditions of Indian Thar Desert. In conclusion, our results showed that biofertilizer technology played a vital role in helping *P. granatum* to establish and thrive in Thar Desert soils. The pre-inoculation of nursery seedlings with selected N_2-fixing bacteria or AM fungi or combination of synergistically interacting species may be helpful to produce vigorous plants to survive and thrive under stressed soils.

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