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Effect of different microbial inoculants on soil properties, nutrient acquisition and growth of pomegranate (*Punica granatum*)

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ABSTRACT

An experiment was conducted during 2007 and 2008 to evaluate the effectiveness of various microbial inoculants in pomegranate saplings prepared from air layers. The present study demonstrates the beneficial effect of seven microbial inoculants, viz. *Gluconaetobacter diazotrophicus*, *Pseudomonas striata*, *Trichoderma viride*, *P. fluorescens*, *Azospirillum brasilense*, pink pigmented facultative methylotrophs (PPFM) isolated from pomegranate and cotton, as a sole inoculant or in combination, on nutrient acquisition and plant growth under semi-arid agro-climatic condition. Inoculation with *T. viride* recorded highest content of available N, P and DTPA extractable Zn content of the soil. This was followed by *A. brasilense* and *G. diazotrophicus* inoculation for nitrogen content while *P. fluorescence* and *P. striata* for phosphorus content. Among various microbial inoculants, *P. fluorescence* was found efficient in promoting photosynthesis and transpiration rate of the plant. Dual inoculation with N_2-fixing *A. brasilense* and P-solubilizing *P. striata* was most effective in promoting uptake of most of the major and micronutrients through beneficial interaction. A significant improvement in plant height and shoot biomass was also recorded in this treatment while root biomass was highest with *P. striata* inoculation. Microbial inoculants under study benefited the growth of saplings by improving the soil properties, fertility status, physiological parameters and nutrient uptake. Hence using these microbial inoculants in potting mixture for air layers may facilitate their better establishment and growth under field condition

Key words: Microbial inoculants, Nutrient uptake, Plant growth, Pomegranate, Soil properties

Pomegranate *(Punica granatum* L.), an economically important fruit crop is gaining popularity in arid and semi-arid regions of India. This region is characterized by nutrient deficient shallow gravelly soils, high temperature, low and irregular distribution of rainfall and low organic matter. Plant growth in this region is often hindered owing to insufficient resident microflora which acts as both source and sinks for essential plant nutrients and is fundamental to the transformation of various nutrients either inherited from soil or applied through anthropogenic sources. The utility of microbes in maintenance and built up of soil fertility, thereby, enhancing plant growth and yield is indispensable. They enrich the soil by addition of 25–40 kg N/ha, solubilize / mobilize 30–50 kg P_2O_5/ha, liberate growth promoting substances and vitamins, improve soil tilth and fertility and suppress the incidence of pathogens (Motsara *et al*. 1995) thereby leading to increased vigour and fruit yield of various fruit plants of arid to semi-arid regions like citrus (Marathe *et al*. 2009), grapes, *Ziziphus mauritiana* Lam. (Mathur and Vyas 2000) and other fruit crops. In addition, such bio-fertilizers are cheaper, eco-friendly and based on renewable energy sources. The use of bio-fertilizers through organic sources has gained momentum in recent years, especially in pomegranate which is globally considered to be a foreign exchange earning crop due to its antioxidant and nutraceutical value (Newman and Lansky 2007). Though there are many reports on the effect of different bio-fertilizers on various fruit plants, very little information is available on usefulness of such bio-inoculantas in pomegranate cultivation. Hence, an attempt has been made to assess the effectiveness of various plant growth-promoting micro-organisms, occurring in the region, on growth and nutrient uptake by pomegranate.

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MATERIALS AND METHODS

The experiments were conducted during 2007 and 2008 using 120-day-old air-layered saplings of pomegranate *cv*. Ganesh planted in pots (40 cm × 30 cm) containing 8 kg substrate (soil : sand : farmyard manure in the proportion of 2:1:1 by volume) during March and September 2007 at Research Farm of National Research Centre on Pomegranate,

Solapur, located at 17°68´ N and 75°91´ E. The soil used for experiment was calcareous ($CaCO_3$ 19.0%), alkaline (*p*H 8.06) in reaction with low electrical conductivity (0.22 dS/m). It was high in available K content (581.9 kg/ha), medium in available P (20.5 kg/ha) and organic carbon (0.60%) and low in available N (204.2 kg/ha). DTPA extractable micronutrients content of the soil were in high to sufficiency range (Fe 7.0 ppm, Cu 0.30 ppm, Mn 8.2 ppm and Zn 1.20 ppm). The trial was laid out in completely randomized block design consisting of 10 treatments with three replications having four plants in each replication. The treatments were T_1, *Gluconacetobacter diazotrophicus*; T_2, *Pseudomonas striata*; T_3, *Trichoderma viride*; T_4, *Pseudomonas fluorescens*; T_5, *Azospirillum brasilense* + *P. striata*; T_6, *G. diazotrophicus* + *P. striata*; T_7, *A. brasilense*; T_8, pink pigmented facultative methylotrophs (PPFM) isolate from pomegranate; T_9, PPFM isolate from cotton and T_{10}, control.

Strains of *G. diazotrophicus*, *P. striata*, *P. fluorescens*, *T. viride*, *A. brasilense* and PPFM culture were obtained from the Department of Microbiology, Marathwada Agricultural University, Parbhani and were multiplied in YPM (consisting of 1% yeast extract, 0.5% peptone, 1.5% mannitol and distilled water 1000 ml), King's B, Potato dextrose, Yeast extract (Mannitol 10 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.2 g, NaCl 0.1 g, $CaCO_3$ 4.0 g, yeast extract 1.0 g, congo red 1% solution 2.5 ml, distilled water 1000 ml, *p*H adjusted to 6.8–7.0 and glycerol peptone (glycerol 10 ml, peptone 10 g, distilled water 1000 ml, *p*H of the medium 7.0) broth, respectively following standard techniques. After three days of growth, the cells were centrifuged, washed twice in sterile distilled water and suspended in 0.15 M phosphate buffered at *p*H 7. Fifty milliliters of cell suspension having $1.0 \times 10^7 - 1.5 \times 10^8$ cells/ml was used as inoculums for above mentioned bacteria and were inoculated with the substrate used for growing pomegranate saplings as per above mentioned treatments at the time of planting. All the plants were watered on alternate days and maintained adopting similar cultivation practices.

Photosynthesis and transpiration rate were measured using LICOR INC., USA make LI-6400 portable photosynthesis system. After six months, plants were uprooted and various growth parameters like plant height, plant spread, number of branches, number of roots and average root length were recorded. The uprooted plants were separated into leaf, stem and root portions, washed thoroughly in sequence with water, liquid soap, acidic water and glass redistilled water and dried in shade for four days, followed by oven drying at 70 °C till constant weight, finally the dry matter weight of different parts were recorded. The oven-dried plant samples were grounded, mixed well and used for analyzing total N by microkjeldhal method, P by Vanadomolybdo phosphoric acid method, K using Elico make CL 361 flame photometer, Ca and Mg by titrimetric method employing disodium salt of EDTA and micronutrients Fe, Zn, Mn and Cu using Perkin Elmer, USA make AAnalyst 400 atomic absorption spectrophotometer (Jackson 1967).

Total nutrient uptake in the plant parts were calculated individually on the basis of nutrient content in leaf, stem and root and their respective dry mass, which was added together to find out total uptake. The means of two years pooled data were compared using standard errors of the mean.

Soil samples were collected at the end of experiment during both the years, dried and analyzed to determine chemical properties and fertility status. The soil *p*H, electrical conductivity, calcium carbonate, organic carbon and available N were determined following standard procedures (Jackson 1967), while available P was estimated according to method suggested by Olsen *et al.* (1954) and available K flame photometrically. Available micronutrients, viz Fe, Mn, Zn and Cu were determined using DTPA extractant and atomic absorption spectrophotometer. Data were analysed statistically using analysis of variance. Significancez of difference among the treatments effect was tested through 'F' test and critical difference was calculated, wherever the results were significant.

RESULTS AND DISCUSSION

Vegetative growth and physiological parameters

Data presented in Table 1 show that microbial inoculation of pomegranate saplings resulted in significant increase of total dry matter production over control in all the treatments. Among the inoculants *A. brasilense* + *P. striata* produced highest stem as well as total dry matter. Similarly, significant increase in leaf dry matter was also recorded with all inoculants. It was the maximum with *A. brasilense* + *P. striata* which was at par with *A. brasilense* and *T. viridi*. All inoculants except *G. diazotrophicus* resulted in significant increase in plant height which was maximum with *A. brasilense* + *P. striata* which was statistically at par with *P. fluorescens*. Significant increase in plant spread and production of number of branches was observed in *A. brasilense*. Over and above, inoculation with *A. brasilense* + *P. striata* and *P. fluorescens* resulted in highest above ground biomass production as compared to other inoculants. This promotion of plant growth might have been affected either through production of plant growth-promoting substances like indole acetic acid, indole buteric acid and cytokinin etc by *A. brasilense*, *P. fluorescens*, *P. striata* and PPFM (Thakuria *et al*. 2004, Torres-Rubio *et al*. 2000, Madhiyan *et al.* 2005). The effect of *T. viride* can be explained by its root colonizing capacity which might have eliminated stressors that hamper plant growth and enhanced the beneficial microbial activity in the rhizosphere (Harman *et al*. 2004). Similar observations of enhanced vegetative growth were made in different fruit plants upon inoculation with *Azospirillum*, *Trichoderma*, *Pseudomonas* (Attia *et al*. 2009) and PPFM (Keshvachandran *et al.* 2007). A significant increase in root biomass was also

Table 1 Effect of different microbial inoculants on vegetative growth, dry mass production, root and physiological parameters of pomegranate (pooled data of two seasons)

Treatment	Dry matter production (g / plant)				Plant height (m)	No of branches/ plant	Plant spread (cm)	No of roots/ plant	Root length (cm)	Shoot root biomass ratio	Photosynthesis (µ mol H_2O/ m^2/s)	Transpiration (m mol H_2O/ m^2/s)
	Leaves	Stems	Roots	Total								
G. diazotrophicus	12.22	17.31	8.79	38.67	0.77	13.83	43.00	9.17	26.22	0.31	10.23	6.76
P. striata	12.92	17.29	10.67	42.04	0.85	16.33	42.00	11.50	28.91	0.34	9.15	5.01
T. viride	14.78	19.58	9.97	44.33	0.87	14.00	45.99	9.50	27.86	0.31	8.40	5.38
P. fluorescence	14.19	21.64	10.50	46.34	0.91	19.50	42.83	10.00	28.89	0.32	11.09	7.71
A. brasilense + *P. striata*	15.90	22.67	10.15	48.72	0.96	22.67	47.95	11.83	25.87	0.28	9.22	6.84
G. diazotrophicus + *P. striata*	12.96	19.55	8.77	41.27	0.85	17.00	44.38	9.83	24.62	0.27	8.88	6.06
A. brasilense	15.32	19.31	8.63	43.25	0.84	13.33	51.33	10.67	29.34	0.27	8.36	5.32
PPFM (pomegranate)	13.48	17.85	9.42	40.21	0.83	16.33	46.63	9.00	28.28	0.31	7.53	4.85
PPFM (cotton)	13.89	16.11	8.71	38.70	0.82	14.50	46.67	10.33	30.48	0.31	7.09	4.97
Control	10.58	13.69	6.68	30.95	0.73	17.83	41.71	6.50	39.53	0.28	6.68	4.01
SE (m)±	0.87	1.67	0.44	2.90	0.04	1.78	2.57	0.83	1.77	0.02	0.62	0.47
CD (*P*=0.05)	1.82*	3.51*	0.93*	6.08*	0.087*	3.74*	5.39	1.75*	3.72*	0.032	1.30*	0.99*

**P*=0.01; *A, Azospirillum*; *G.,Gluconacetobacter;* PPFM, pink pigmented facultative methylotrophs; *P. Pseudomonas; T, Trichoderma*

noted with all inoculants which was maximum with *P. striata,* followed by *P. fluorescens*. Similarly, *A. brasilense* + *P. striata* and *P. striata* recorded significantly highest number of roots. *P. fluorescens* and *P. striata* are known to solubilize insoluble soil P and make available to plant, which in turn, might have promoted growth and number of the plant roots in these treatments. These observations are in conformity with those from Katiyar and Goel (2003) who reported increased root biomass upon inoculation with *Pseudomonas* sp. Treatments involving dual inoculation were highly beneficial as compared to that of non-inoculated or single inoculation treatments to increase vegetative growth parameters.

A significant increase in photosynthesis and transpiration rates has been noted with all inoculants except PPFM. Notably higher photosynthesis rate was observed with *P. fluorescens*, *G. dizotrophicus*, *A. brasilense* + *P. striata* and *P. striata* inoculation. Higher photosynthesis rate with *P. fluorescens* treatment may be due to increased uptake of Mg^{2+} and Fe^{2+} (Table 2) which are reported to have important role in chlorophyll formation (Shaahan *et al*. 1999), while with *A. brasilense* + *P. striata* treatment, the effect may be attributed to higher uptake of K^+ and Mn^{2+} which have important role to play in photosynthesis. Similarly, maximum transpiration rate was also recorded in these treatments which may be attributed to higher above ground biomass, ie more leaf area with more number of leaves. Tiwary *et al*. (1999) reported higher chlorophyll content and so photosynthesis

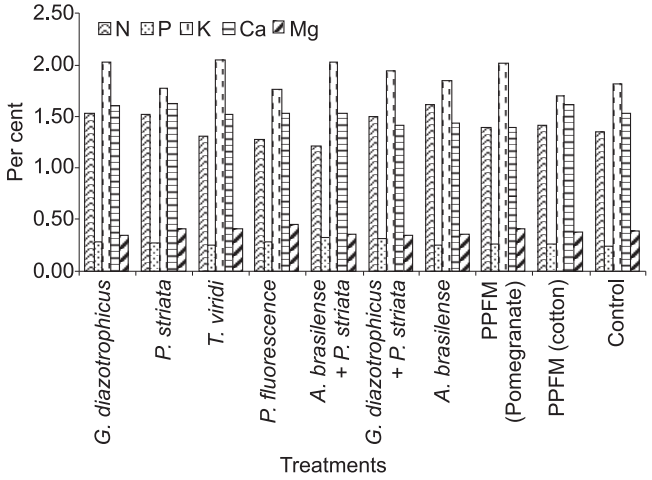

Fig 1 Effect of different inoculants on macro-nutrient content in the leaf of pomegranate

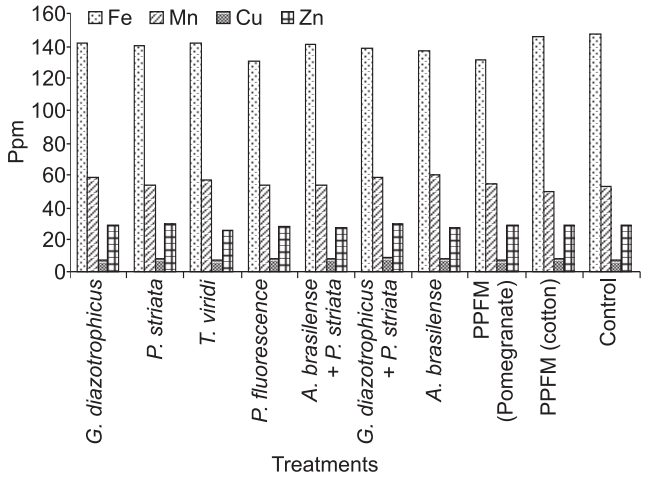

Fig 2 Effect of different inoculants on micro-nutrient content in the leaf of pomegranate

Table 2 Effect of different microbial inoculants on leaf, stem, root and total nutrient content (mg / plant) (pooled)

Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Leaf uptake									
G. diazotrophicus	187.5	35.0	257.2	196.8	42.2	1.719	0.696	0.099	0.384
P. striata	197.8	35.1	239.3	212.3	52.5	1.763	0.684	0.112	0.368
T. viride	195.2	38.5	295.2	222.0	61.2	2.108	0.840	0.105	0.396
P. fluorescence	180.6	40.3	246.5	216.7	64.3	1.864	0.774	0.116	0.405
A. brasilense + P. striata	193.9	51.7	323.1	243.0	57.1	2.230	0.856	0.130	0.426
G. diazotrophicus + P. striata	192.5	39.9	258.1	181.9	45.8	1.761	0.734	0.124	0.375
A. brasilense	249.2	39.5	275.2	217.5	56.3	2.143	0.938	0.123	0.425
PPFM (pomegranate)	187.7	36.4	277.0	188.6	56.1	1.734	0.735	0.105	0.382
PPFM (cotton)	195.4	37.2	238.6	223.1	52.8	1.984	0.684	0.117	0.396
Control	142.7	25.8	192.9	162.4	41.8	1.563	0.561	0.085	0.306
SE (m)±	19.7	3.34	23.0	20.8		0.14	0.05	0.01	
CD (*P*=0.05)	41.3	7.02*	48.2*	43.7	NS	0.297*	0.104*	0.025*	NS
Stem uptake									
G. diazotrophicus	119.0	30.8	203.4	220.4	59.0	2.86	0.845	0.170	0.565
P. striata	98.6	31.4	192.5	220.6	68.2	2.86	0.788	0.168	0.546
T. viride	134.6	29.9	213.6	238.5	93.0	3.08	0.935	0.182	0.675
P. fluorescence	110.8	32.1	252.6	253.2	93.5	3.07	1.071	0.202	0.699
A. brasilense + P. striata	136.3	32.7	279.0	285.8	84.2	3.46	1.102	0.197	0.808
G. diazotrophicus + P. striata	121.3	29.4	213.5	276.3	68.8	2.83	0.954	0.180	0.693
A. brasilense	115.7	27.5	218.0	259.8	70.8	3.46	0.984	0.181	0.652
PPFM (pomegranate)	106.1	27.5	190.0	235.7	73.5	2.81	0.831	0.154	0.606
PPFM (cotton)	95.9	21.6	161.9	230.5	64.3	2.21	0.791	0.146	0.543
Control	77.9	18.1	132.9	181.2	54.6	1.85	0.707	0.112	0.434
SE (m)±	9.76	3.48	25.1		9.64	0.08	0.08	0.02	0.06
CD (*P*=0.05)	20.5*	7.3*	52.6*	NS	20.25*	0.162*	0.162*	0.035*	0.123*
Root uptake									
G. diazotrophicus	52.0	9.5	126.5	134.5	34.9	2.482	0.449	0.167	0.278
P. striata	64.7	12.6	136.7	164.1	53.3	3.190	0.622	0.217	0.365
T. viride	55.6	11.2	133.2	135.9	49.7	2.938	0.466	0.190	0.336
P. fluorescence	62.4	12.9	150.0	143.9	48.9	2.937	0.535	0.205	0.331
A. brasilense + P. striata	61.9	12.7	149.5	159.0	52.2	2.894	0.543	0.200	0.375
G. diazotrophicus + P. striata	56.2	10.9	130.0	120.2	39.4	2.408	0.407	0.175	0.304
A. brasilense	53.4	10.8	139.9	117.3	28.7	2.433	0.456	0.175	0.276
PPFM (pomegranate)	65.1	10.5	149.1	126.1	43.0	2.886	0.518	0.183	0.281
PPFM (cotton)	55.0	9.6	135.9	127.5	35.6	2.354	0.396	0.168	0.255
Control	43.7	6.8	102.2	92.9	27.9	1.842	0.301	0.124	0.195
SE (m)±	4.57	0.93	10.3	13.6	4.82	0.05	0.05	0.01	0.02
CD (*P*=0.05)	9.59*	1.95*	21.58*	28.59*	10.12*	0.103*	0.102*	0.025*	0.051*
Total uptake									
G. diazotrophicus	358.6	75.3	587.2	551.6	136.1	7.06	1.99	0.44	1.19
P. striata	361.1	79.1	568.6	596.9	174.0	7.81	2.09	0.50	1.28
T. viride	385.4	79.6	642.0	596.5	203.8	8.13	2.24	0.48	1.41
P. fluorescence	353.8	85.3	649.1	613.7	206.7	7.88	2.38	0.52	1.43
A. brasilense + P. striata	392.1	97.1	751.6	687.7	193.6	8.58	2.50	0.53	1.61
G. diazotrophicus + P. striata	370.0	80.1	601.6	578.4	154.0	7.00	2.10	0.48	1.37
A. brasilense	418.3	77.7	633.1	594.6	155.8	8.03	2.38	0.48	1.35
PPFM (pomegranate)	358.9	74.3	616.1	550.4	172.6	7.43	2.08	0.44	1.27
PPFM (cotton)	346.3	68.3	536.5	581.1	152.7	6.55	1.87	0.43	1.19
Control	264.3	50.8	428.0	436.4	124.3	5.25	1.57	0.32	0.94
SE (m)±	24.5	6.3	44.5	40.9	16.5	0.12	0.12	0.02	0.10
CD (*P*=0.05)	51.4*	13.3*	93.4*	85.8*	34.6*	0.25*	0.25*	0.05*	0.20*

**P*=0.01; *A, Azospirillum*; *G.,Gluconacetobacter;* PPFM, pink pigmented facultative methylotrophs; *P., Pseudomonas; T, Trichoderma*

in banana upon inoculation with N_2-fixing bacteria.

Nutrient content in plants

The pooled analysis data of nutrient content in leaf, stem and roots showed little variation (Fig 1) and the results were non-significant for most of the nutrients. N (1.62%) and Mn (60.1 ppm) content in the leaf were significantly highest with *A. brasilense,* followed by *G. diazotrophicus* + *P. striata.* While P (0.323%) content in the leaf was highest with dual inoculation of *A. brasilense* + *P. striata,* followed by *G. diazotrophicus* + *P. striata* (Figs 1, 2). N (0.48 – 0.65%), P (0.125 – 0.173%), Fe (136.3 – 186.9 ppm), Mn (45.9 – 52.7 ppm) and Zn (30.6 – 35.2 ppm) content in stem and K (1.28 – 1.56%) and Zn (30.7 – 37.9 ppm) content in root showed significant variation with different microbial inoculation. This is attributed to variation in demand and uptake by the plants owing to varied vegetative growth under different treatments.

Plant nutrient uptake

Uptake of various nutrients by pomegranate air layers was significantly higher upon inoculation with various beneficial micro-organisms (Table 2). As N, P and K are mobile within the plant system, their maximum accumulation was recorded in leaf, followed by stem and root. Higher uptake of nutrient was due to the synergistic effect of improved biomass and higher nutrient concentration (Fig 1) in the inoculated plants. Higher N uptake was noted with *A. brasilense*, *A. brasilense* + *P. striata*, *T. viride* and *G. diazotrophicus* + *P. striata*, while it was lowest with PPFM isolate from cotton. Higher N uptake with *T. viride* might have resulted from enhanced decomposition of soil organic material while *A. brasilense* and *G. diazotrophicus* microorganisms might have improved N-nutrition in plant by fixing atmospheric nitrogen. An increased uptake of P and K were also recorded in pomegranate upon inoculation and the increase was maximum with *A. brasilense* + *P. striata,* followed by *P. fluorescens*. Although, the effect of *P. fluorescens* on P uptake was at par with *G. diazotrophicus* + *P. striata*, *T. viride*, *P. striata* and *A. brasilense*. Higher uptake of P was observed in all the treatment involving inoculation with *P. fluorescens* and *P. striata*. This may be due to solubilization of insoluble soil phosphate by these micro-organisms which subsequently become available to plant. Maximum N and P uptake was noted in dual inoculation treatment which may be due to improved N_2-fixation, improved phosphatase activity, thereby mobilization and subsequent P uptake by plant. Interestingly, *P. fluorescens* was found to promote plant growth as well as nutrient uptake to a higher degree as compared to other inoculants. But the mechanism behind this finding needs to be explored through in-depth study about the above mentioned microorganisms. Similarly, likewise effect on K uptake was noted with *P. fluorescens*, *T. viridi*, *A. brasilense* and PPFM isolate from pomegranate. A significantly higher uptake of Ca and Mg were observed with *A. brasilense* + *P. striata* and *P. fluorescens*. Ca and Mg being relatively less mobile within the plant system, their uptake was maximum in stem. There was significant increase in uptake of micronutrient cations like Fe, Mn, Cu and Zn upon inoculation with various microorganisms and maximum uptake was with *A. brasilense* + *P. striata*, while minimum uptake was with PPFM isolate from cotton. Inoculated air layers were found to take up significantly higher amount of other nutrients, viz K, Ca, Mg, Fe, Mn, Cu and Zn mainly in dual inoculation treatment, i e *A. brasilense* + *P. striata.* These observations are in conformity with those of Ghazi (2006). This enhancement might be due to the production of nutrient-solubilizing enzyme by various micro-organisms. Overall,

Table 3 Effect of different microbial inoculants on soil properties and fertility status (pooled averages of two seasons)

Treatment	*p*H	Electrical conductivity (dS/m)	organic carbon (%)	calcium carbonate (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)	Available Fe (ppm)	Available Cu (ppm)	Available Mn (ppm)	Available Zn (ppm)
G. diazotrophicus	8.27	0.20	0.63	18.9	226.6	19.4	581.9	7.2	0.33	9.7	1.40
P. striata	8.14	0.24	0.75	18.9	219.9	21.3	595.5	7.4	0.34	9.8	1.47
T. viride	8.18	0.25	0.68	18.8	242.9	22.6	634.5	7.9	0.33	10.1	1.74
P. fluorescence	8.17	0.25	0.69	18.7	219.1	22.3	614.1	8.6	0.36	10.2	1.60
A. brasilense + *P. striata*	8.23	0.24	0.66	19.3	216.4	16.8	624.2	6.8	0.39	9.0	1.49
G. diazotrophicus + *P. striata*	8.21	0.24	0.64	19.4	213.6	21.0	637.5	7.1	0.39	9.7	1.38
A. brasilense	8.18	0.22	0.59	19.4	227.7	18.2	632.8	8.6	0.35	8.7	1.50
PPFM (pomegranate)	8.15	0.24	0.66	19.4	208.2	16.6	617.8	7.6	0.32	9.1	1.43
PPFM (cotton)	8.12	0.24	0.67	19.5	219.9	20.5	643.8	7.5	0.32	8.6	1.33
Control	8.08	0.23	0.63	19.3	207.6	21.5	594.7	7.1	0.32	8.6	1.28
SE (m)±	0.04	0.01		0.21	9.24	1.32					0.20
CD (*P*=0.05)	0.084	0.021	NS	0.43*	19.4	2.78*	NS	NS	NS	NS	0.42*

**P*=0.01; *A, Azospirillum*; *G.,Gluconacetobacter;* PPFM, pink pigmented facultative methylotrophs; *P., Pseudomonas; T, Trichoderma*

higher stem uptake of Fe, Mn and Zn were noted as these elements are relatively less mobile within the plant system, whereas root uptake of Cu was higher as compared to stem and leaf uptake as Cu is able to displace most other ions from root exchange sites and is very strongly bound in the root-free space.

Soil chemical properties and fertility status

Microbial inoculation resulted in significant variation in soil reaction and electrical conductivity but the variation did not follow definite pattern (Table 3). There was significant reduction in $CaCO_3$ content with *P. fluorescens* which might have implication in increasing Fe uptake by plant. Increase in available N was observed with *A. brasilense* and *T. viridi* which may be attributed to associative N-fixation by former and increased decomposition of organic matter by the later. Considerable increase in available P was recorded with *T. viridi*, followed by *P. Fluorescence* and *P. Striata*. There was significant increase in DTPA extractable Zn with *T. viridi*, *P. Fluorescence*, *A. brasilense* and *A. brasilense* + *P. Striata*. Gyneshwar *et al.* (2002) also reported increased availability of trace elements by micro-organisms through production of growth promoting substances. All other nutrients and organic carbon content recorded non-significant variation.

The present study demonstrated the beneficial effect of various microbial inoculants on pomegranate plant growth and survival under semi-arid climatic condition. Among various microbial inoculants, *P. fluorescens* was found more efficient in promoting nutrient acquisition and plant growth in pomegranate. Furthermore, dual inoculation with N_2-fixing and phosphate solubilizing bacteria proved superior in enhancing plant growth through synergistic interaction which may be helpful to produce vigorous plants to survive and thrive under stressed soil condition.

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