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Effects of bio-organics and chemical fertilizers on nutrient availability and biological properties of pomegranate orchard soil

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The study was carried out during 2009 to 2010 on 8 years old pomegranate (*Punica granatum* L.) trees cv. 'Kandhari Kabuli' to investigate the conjoint efficiency of bio-organics used along with chemical fertilizers on nutrient availability and physico-chemical and biological properties of soil. Conjoint application of bio-fertilizers 80 g/tree, vermicompost 20 kg/tree, FYM 20 kg/tree, green manure (GM) sunnhemp (*Crotalaria juncea* L.) and recommended dose of fertilizers (RDF) of Nitrogen, Phosphorus and Potassium (NPK), resulted in significantly maximum porosity (60.27%), water holding capacity (WHC) (60.31%), bulk density (0.97%), particle density (2.25%), organic carbon (1.90%), soil pH (6.89), soil N (405.56%), P (22.02%), K (419.00%), Iron (Fe) (66.92 ppm), Maganese (Mn) (61.95 ppm), Zinc (Zn) (2.33 ppm) and Cupper (Cu) (3.25 ppm). The microbial biomass pool in terms of *Pseudomonas* sp, soil fungi, *Azotobacter chroococcum*, *Actinomycetes* and Arbuscular mycorrhizal fungi increased by 385.57, 60.26, 134.19, 168.02 and 39.87%, respectively over the control.

Key words: Pomegranate (*Punica granatum* L.), biofertilisers, farm yard manure (FYM), vermicompost, green manuring.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the important fruit crop of the world. Use of various organic manures and fertilizers is a good practice to maintain physico-chemical and biological properties of the soil. To meet the recommended requirements of manure and fertilizers for different fruit crops, it could either be through inorganic or organic sources to meet 50% through organic and 50% through inorganic fertilizers for better yield. An excessive and indiscriminate use of chemical fertilizers has resulted in considerable deterioration of soil health. It also disturbs the soil microorganisms, reduce pH and build up of P₂O₅ and K₂O that cause reduction in soil health.

Addition of organic matter will not only provide needed nutrients including micronutrients but also improve physical condition of soil, improve aeration, provide better scope for root growth and production. It is a sound practice for sustainable horticulture base on low chemical input. Integrated nutrient management is a production system which favours the maximum use of organic material and discourages the use of synthetically produced agro-inputs for maintain soil productivity and fertility. Improvement in nutrient availability (Sudhakar et al., 2002), soil physical conditions and enzymatic activity (Maheswarappa et al., 1999) have been reported due to vermicompost application in fruit crops. The extensive

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use of chemical fertilizers which affect the soil health results in decreased soil productivity (Macit et al., 2007).

The use of bio-fertilizers is being sought to maintain and improve soil quality and productivity levels at low input costs. Use of organic sources of nutrients helps to conserve the soil health by maintaining the equilibrium of organic matter and soil microflora ultimately helping to improve physical, chemical and biological properties of the soil (Walia and Kler, 2009). Application of vermicompost along with mineral fertilizers has given encouraging results in terms of crop productivity and maintenance of soil health (Naik and Babu, 2007). Korwar et al. (2006) also reported that, the application of vermicompost, FYM, and sunnhemp (*Crotalaria juncea* L.) as green manure (GM) along with chemical fertilizers resulted in the maintenance of the physical, chemical and biological properties of soil. Therefore, sustained efforts are needed to improve and maintain this most important natural resource base- the soil through judicious integration of mineral fertilizers, organic and GM and bio-fertilizers so that, it nourishes intensive cropping without being irreversibly damaged in the process.

MATERIALS AND METHODS

The field experiment was conducted on pomegranate cv. 'Kandhari Kabuli' during 2008 to 2010. The experimental farm is located at 30°50' 45" latitude and 77°88' 33" longitude at an elevation of 1320 m above mean sea level, representing mid hill zone of state. The climate of the area is typically sub-temperate. The annual rainfall ranges between 800 to 1300 mm. The orchard soil was sandy in texture with pH 6.55, 0.59 dS/m electrical conductivity (EC) and 0.61% organic carbon content. Water holding capacity (WHC), bulk density and porosity of surface soil at 15 cm depth were 31.65, 1.30, and 48.50%, respectively. The initial available N, P, and K contents of the soil were 309.85, 11.75 and 342.82 kg/ha, respectively. DTPA extractable micro-nutrients viz, Zn, Mn, Fe, Cu, and boron (B) were 1.97, 45.72, 57.24, 2.47 and 0.70 ppm, respectively. The experimental soil also contained an initial viable microbial population of *Pseudomonas* sp (3×10^4 colony forming units (cfu)/g soil, *Azotobacter chroococcum* (2×10^6 cfu), soil fungi (0.13×10^6 cfu), *actinomycetes* sp (0.38×10^5 cfu) and 563 spores of arbuscular mycorrhizal (AM) fungi/kg of soil.

The experiment was laid out considering 3 levels of NPK fertilizers (50, 75, and 100% of recommended dose), bio-fertilizers, vermicompost, FYM and GM (sunnhemp) in different conjoint combinations. The treatments were replicated thrice, where the significant differences were analyzed statistically in randomized block design. Different inputs of bio-organic and inorganic nutrient sources, namely bio-fertilizers: 80 g/tree (B_{80}), vermicompost (20 kg/tree), FYM (20 kg/tree) and GM (sunnhemp) were applied in different conjoint combinations (T_1 - T_{12}) along with a control. The treatments comprised following combinations, that is, $VC_{20} + NPK_{50}(T_1)$; $FYM_{20} + NPK_{50}(T_2)$; $VC_{20} + B_{80} + FYM_{20} + GM + NPK_{50}(T_3)$; $VC_{20} + NPK_{75}(T_4)$; $FYM_{20} + NPK_{75}(T_5)$; $VC_{20} + B_{80} + FYM_{20} + GM + NPK_{75}(T_6)$; $VC_{20} + NPK_{100}(T_7)$; $FYM_{20} + NPK_{100}(T_8)$; $VC_{20} + B_{80} + FYM_{20} + GM + NPK_{100}(T_9)$; $VC_{20} + RDF(T_{10})$; $FYM_{20} + RDF(T_{11})$; $VC_{20} + B_{80} + FYM_{20} + GM + RDF(T_{12})$ and control (NPK only).

The bio-fertilizers consortia comprised of *A. chroococcum*, P solubilizing bacteria (*Pseudomonas* sp), *Actinomycetes* and AM fungal species namely, *Glomus fasciculatum* and *G. mossae*.

Microbial culture were applied through band application in the basin at 15 cm depth being followed by a light irrigation for the proliferation of the cultures. A range of agricultural residues, all dry wastes, dry leaves of crops and trees, mixed vegetables (organic waste) were inoculated along with adult epigeic earthworms (*Eisenia foetida*) for the purpose of vermicomposting. Vermicompost used in the experiment contained 2.12% N, 0.93% P_2O_5 , 1.11% K_2O , 174.8 ppm Fe, 96.31 ppm Mn, 24.23 ppm Zn, and 4.78 ppm Cu. Another input GM sunnhemp (*Crotalaria juncea* L.) was grown in the tree basin and was incorporated into the soil at the time of flowering during the last week of August. FYM contained 0.50% N, 0.25% P_2O_5 , 0.50% K, 0.004% Zn, 0.0003% Cu, 0.007% Mn and 0.45% Fe. The NPK fertilizers sources were urea (46% N), single super phosphate (16% P_2O_5) and muriate of potash (60% K_2O). Nitrogen was applied in two split doses. Half N was applied in the first fortnight of February. The remaining quantity was also applied in two split doses. First dose was dressed at the time of fruit set and second dose about 4 to 5 weeks after fruit set at 30 cm away from the tree trunk by broadcasting in the tree basin.

Composite soil samples (15 cm deep), weighing 1 kg were collected, taken to the laboratory in polythene bags and stored in refrigerator at 4°C. These samples were analyzed for various physical, chemical and biological properties using standard methods. Bulk density and particle density was determined according to the Specific Gravity method (Kanwar and Chopra, 1976). Soil porosity was calculated using the standard formula. WHC of soil was estimated using Keen-Raczowski Box method (Piper, 1966). Soil pH and EC were measurement in a 1:5 (w/v) aqueous solution, using a Crison GLP 21 pH meter and a Crison GLP 31 conductivity meter, respectively.

Soil organic carbon was determined according to wet oxidation method (Walkley and Black, 1934). Available N was estimated using alkaline K permanganate method (Subbiah and Asija, 1956), available P was estimated by Olsen's method (Olsen et al., 1954) and available K was extracted in 1 N neutral normal ammonium acetate using a flame photometer (Merwin and Peach, 1951). Available micro-nutrients, viz Fe, Cu, Mn, and Zn were estimated by 0.005 M DTPA extraction method (Lindsay and Norvell, 1969) on atomic absorption spectrophotometer model Analyst-400.

The isolation of pure and viable bacterial count was done by serial dilution technique on nutrient agar (*Pseudomonas* sp), Martin's Rose Bengal Medium (Fungi), Jensen's Media (*A. chroococcum*), Kenknight and Munaires Medium (*Actinomycetes*). 10 g of soil from each sample was drawn and serially diluted aseptically to 10^{-6} and 10^{-7} dilution. 1 ml of each sample dilution was spread on specified medium. Arbuscular mycorrhizal (AM) fungal spores present in the soil were recorded through wet sieving and decanting method (Gerdemann and Nicolson, 1963) and the spore count was done according to most probable number method described by Porter (1979).

RESULTS AND DISCUSSION

Soil physicochemical properties

Conjoint application of bio-organics and mineral fertilizers were effective in decreasing bulk density and particle density of the soil. However, $VC_{20} + B_{80} + FYM_{20} + GM + RDF(T_{20})$ was most effective with minimum (0.97 Mg/m^3) and decreased bulk density of 21.64% over control (Table 1). This treatment also resulted in maximum (60.31%) water holding capacity and increased porosity (45.82%) over control with a value of 60.27%. The lowering of bulk density in combined application of

Table 1. Effect of bio-organic and inorganic sources on physico-chemical properties of soil of pomegranate.

Treatment	Bulk density (Mg m ⁻³)	Particle density (Mg m ⁻³)	WHC (%)	Porosity (%)	pH	EC (dSm ⁻¹)	Organic carbon (%)
T ₁	1.14	2.44	47.47	46.48	6.82	0.29	1.84
T ₂	1.14	2.50	46.27	44.43	6.81	0.29	1.83
T ₃	0.98	2.26	59.33	59.55	6.89	0.28	1.89
T ₄	1.13	2.44	47.92	48.01	6.72	0.29	1.85
T ₅	1.14	2.47	46.88	45.91	6.69	0.30	1.84
T ₆	0.98	2.26	59.44	59.61	6.88	0.28	1.89
T ₇	1.12	2.44	48.92	48.81	6.64	0.30	1.86
T ₈	1.12	2.44	47.46	47.72	6.63	0.30	1.85
T ₉	0.98	2.26	59.37	59.76	6.86	0.29	1.89
T ₁₀	1.07	2.31	55.21	58.32	6.72	0.31	1.88
T ₁₁	1.07	2.34	55.15	57.88	6.71	0.30	1.87
T ₁₂	0.97	2.25	60.31	60.27	6.88	0.31	1.90
T ₁₃	1.18	2.48	41.19	41.33	6.54	0.31	1.75
LSD _{0.05}	0.01	0.01	1.05	0.95	0.02	NS	0.12

VC₂₀, Vermi-compost: 20 kg tree⁻¹; B₈₀, bio-fertilizer: 80 gtree⁻¹; FYM₂₀, 20 kg tree⁻¹; GM, green manure (Sun hemp); RDF, EC, WHC.

organic manure and mineral fertilizers resulted from high soil organic carbon, more pore space and better soil aggregation. These findings are in line with those of Kannan et al. (2005) who reported that, decrease in bulk density, increase in porosity and better WHC of soil were mainly due to the action of gum compounds, polysaccharides and fulvic acid compounds of organic matter on the soil structure and hence, an increased microbial and enzymatic activities accelerated by vermicompost application (Ghuman and Sur, 2006). Hashemimajid and Golchin (2009) reported that, application of vermicompost decreased particle density but increased WHC of soil. Variation in WHC of soil could be attributed to the addition of organic matter, difference in quantity and nature of colloidal materials present, pH and salt contents of the soil (Laxminarayana and Patiram, 2006). Different treatments of conjoint application changed soil pH towards neutral range. In general, organic sources have tendency towards neutral pH soil. There is slight decrease in pH with organic sources and that attributed to the production of organic acids, viz, oxalo-acetic acid, glutamic acid (Srikanth et al., 2000). The treatment combination VC₂₀ + B₈₀ + FYM₂₀ + GM + RDF (T₂₀) also exhibited a maximum of 1.90% of soil organic carbon content.

Nutrient availability

All treatment combinations improved the available N, P and K contents in soil (Table 3). However, the conjoint application of VC₂₀ + B₈₀ + FYM₂₀ + GM + RDF (T₂₀) recorded significantly higher available N, P, and K contents over control. In treatments with addition of bio-

organics, mycorrhizal infection in combination with *A. chroococcum* stimulated biological N₂-fixation. Increased availability of N with the application of bio-organics could be attributed to the greater multiplication of microbes which converted organically bound N to organic form (Archina, 2008). Subramaniam and Kumaraswamy (1989) reported an increase in available P content of soil which enhanced liable P by complex formation of Ca, Mg, and Al. The organic materials also formed a cover on sesquioxides, reduced P-solubilizing capacity and hence increased the availability in the soil solution (Bhardwaj and Omanwar, 1992). Kohler et al. (2007) reported that, availability of micro-nutrients in the soil which significantly resulted in highest build-up of 66.922.33, 3.25, 61.95 and 0.81 ppm of available Fe, Zn, Cu, Mn and B, respectively (Table 3). Marathe et al. (2009) reported that, significant increase in available Fe, Zn, Mn and Cu were observed with organic manure especially Vermicompost, either alone or in combination with inorganic or GM (*Crotalaria juncea* L.) or bio-fertilizers (*Azotobacter* + PSB). Moreover, the inoculation of microbial cultures, application of vermicompost and GM in conjugation with chemical fertilizers improved nutrient cycling process to increase the availability of micro-nutrient ions in the soil solution.

Soil biological properties

Application of conjoint combination of VC₂₀ + B₈₀ + FYM₂₀ + GM + RDF (T₂₀) resulted in maximum count (cfu/g soil) of *Pseudomonas* (73.03 × 10⁻⁶), soil fungi (43.80 × 10⁻⁶), *A. chroococcum* (51.92 × 10⁻⁶), *actinocyetes* sp (43.35 × 10⁻⁶), respectively tremendously higher than

Table 2. Effect of bio-organic and inorganic sources on soil macro and micronutrients of pomegranate.

Treatments	Macronutrients (kg ha ⁻¹)			Micronutrients (mg kg ⁻¹)				
	N	P	K	Fe	Cu	Zn	Mn	B
T ₁	329.27	16.65	329.61	51.87	2.52	2.06	46.20	0.69
T ₂	325.67	15.67	323.73	49.76	2.49	2.02	45.27	0.69
T ₃	401.51	21.10	414.60	64.29	3.23	2.16	60.57	0.80
T ₄	343.28	17.76	330.20	56.26	2.65	2.11	48.08	0.73
T ₅	340.25	16.78	327.07	52.99	2.66	2.08	47.51	0.66
T ₆	402.62	21.32	416.74	65.78	3.24	2.30	60.81	0.65
T ₇	389.09	19.72	347.04	59.36	2.95	2.15	50.94	0.75
T ₈	386.88	18.91	344.62	57.36	2.72	2.14	49.81	0.68
T ₉	403.72	21.36	417.18	65.91	3.24	2.31	60.69	0.67
T ₁₀	394.85	20.46	403.49	62.24	3.08	2.20	58.01	0.79
T ₁₁	391.87	19.67	402.56	63.49	3.07	2.18	56.99	0.77
T ₁₂	405.56	22.02	419.00	66.92	3.25	2.33	61.95	0.81
T ₁₃	313.17	14.83	345.28	46.74	2.61	2.02	45.46	0.73
LSD _{0.05}	2.60	1.02	2.30	4.65	0.31	0.07	1.41	NS

VC₂₀, vermi-compost: 20 kg tree⁻¹; B₈₀, bio-fertilizer: 80 g tree⁻¹; FYM₂₀ 20 kg tree⁻¹; GM, (Sun hemp); RDF, EC, WHC.

Table 3. Effect of bio-organic and inorganic sources on biological properties of soil in pomegranate.

Treatment	<i>Pseudomonas</i> (× 10 ⁻⁷ cfu g ⁻¹)	<i>A. Chroococcum</i> (× 10 ⁻⁷ cfu g ⁻¹)	<i>Actinomycetes</i> (× 10 ⁻⁷ cfu g ⁻¹)	Soil fungi (× 10 ⁻⁷ cfu g ⁻¹)	AM spore count (per 50 g soil)
T ₁	59.07	41.31	31.27	23.41	1358.33
T ₂	59.96	38.79	30.94	24.35	1321.65
T ₃	72.58	50.42	39.87	42.27	1402.15
T ₄	49.11	38.21	29.20	29.31	1264.30
T ₅	48.35	34.73	29.26	28.31	1254.80
T ₆	72.23	51.41	39.55	42.70	1409.12
T ₇	36.10	35.72	27.20	39.68	1160.28
T ₈	36.04	32.67	26.27	39.33	1159.24
T ₉	71.23	50.91	39.50	42.68	1404.12
T ₁₀	38.25	39.11	28.28	42.04	1215.77
T ₁₁	37.35	36.91	28.24	41.37	1214.19
T ₁₂	73.03	51.92	40.82	43.80	1412.16
T ₁₃	15.04	22.17	15.23	27.33	1033.75
LSD _{0.05}	2.15	1.81	1.28	1.74	20.18

VC₂₀, vermi-compost: 20 kg tree⁻¹; B₈₀, bio-fertilizer: 80 g tree⁻¹; FYM₂₀, 20 kg tree⁻¹; GM, (Sun hemp); RDF, cfu.

control (Table 2). The data presented also showed maximum AM fungal spore count (1412.16/50 g soil) recorded in treatment combination with an increase 39.87% over control. The increasing levels of microbial biomass of the soil might be due to increasing levels of bio-organics applicaton. Shashidhar et al. (2009) reported that, increased microbial population of bacteria, fungi and *Actinomycetes* were significantly recorded with the application of 100% recommended N through sunnhemp (*Crotalaria juncea* L.) GM + 10 kg bio-fertilizers + FYM + RDF of P and K.

The increased microbial population might be due to

application of different types of organic manures in turn provides adequate biomass as a feed for the microbes and helps in increasing microbial population in soil. Inoculation of soil with *Azotobacter* increased microflora population whereas; addition of AMF inoculum had synergistic effect on *Azotobacter*. The synergistic host response could mainly be due to the production of phytohormones or growth regulators produce by these microbes. It has been reported that, spore of AM fungi seems to give *Azotobacter* an operational base in the vicinity of roots and supply of carbon that increases the efficiency of both AM fungi and *Azotobacter* (Saxena and

Tilak, 1994). Furthermore, the inoculation of AM fungi increased root colonization attributed to higher sporulation and accounted for increased available P content of the soil rhizosphere. It is well established that, P sufficiency of soil provides enough accumulation of metabolites required to promote mycorrhizal formation and thereby roots exudates enough photosynthesis to sustain ongoing colonization process. The greatest increase in bacterial and fungal population occurred in the sesbania plots as GM (Shah et al., 2010). Increased microbial population in soil could be due to enhanced organic matter inputs from the GM legumes and cropping intensity.

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