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Pot-culture studies on soil and leaf nutrients status of peach in response to different soil management techniques for preventing replant disease

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Abstract

Peach [*Prunus persica* (L.) Batsch] is the most important temperate and deciduous fruit tree grown in India. Replant disease is a major constrain in establishing the new plantations of stone fruits such as peach on old orchard sites and towards their improved productivity. Peach replant disease (PRD) is characterized by stunted growth and reduced yields; attributed to several biotic and abiotic causes; its symptoms are vague and highly inconsistent; making it difficult to diagnose and overcome. Since, several previous studies showed that the practices for controlling replant disease have included soil profile modification coupled with soil fumigation. Therefore, the present study was conducted with the aim of analyzing the response of peach plants to soil fumigation and organic and inorganic amendments made in a replant soil under pot-cultivation. In this experiment; we studied the effect of different soil management techniques for controlling PRD in soil from replanted peach orchard cultivated for >30 years under the same crop. Six replant soil treatments including 3 variants viz., soil fumigation, PGPR and biocontrol in 5 different combos and a control (i.e. recommended package of practices); were employed to each of four plants in each treatment. Different treatments significantly ($p<0.05$) affected the available soil and total leaf nutrients content of peach plants grown in a replant soil. The results from this study provide evidence that combined treatment (Soil fumigation +PGPR +Biocontrol+25% more of recommended SSP) helped improving the soil and plant nutrient status and thus may alleviate replant problem in peach.

Keywords: Soil, leaf nutrients, peach, PGPR, soil management techniques and replant disease

1. Introduction

Repeated cultivation of the same plant species on the same field that previously cropped with the similar or closely related species resulting in poor growth of fruit trees is termed as “replant disease”. The crops most affected include apple, peach, cherry and citrus. Replant disease of *Prunus* is characterized by poor growth, stunting, and delayed crop production, and in severe cases, tree death. Symptoms’ of peach replant disease include retarded growth, severe stunting, drying of a portion of new growing plant and various degrees of discoloration and interveinal chlorosis; however in severe cases, the diseased plants die (Koch, 1955) [35]. Affected trees have uneven and poor growth with fewer healthy feeder roots that were small, necrotic and feeble along with very few branches. When diseased plants are transferred to fresh soil, which had no prior evidence of causing the same disease nor had been used to plant closely related crops, they exhibit a recovery in vigour (Savory, 1966) [55].

Peach tree replant disease, though reported in the literature for more than two centuries, has yet to have its causes clearly defined. Decline in peach productivity has been attributed to fungi, bacteria, nematodes, toxic agents, insect-pests, nutritional disturbances and spray residues (Benizri *et al*. 2005) [6]. Since, it is a complex syndrome of various problems so it’s comparatively easy to prevent the replant problem than to control it because of the fact that its exact etiology is difficult to understand as symptoms are vague and inconsistent and may vary from country to country and even region to region under different environmental conditions. As a result, pre-plant soil fumigation is the primary measure employed for the control of replant diseases due to the perceived uncertainty regarding the etiology of replant disease (Mai and Abawi, 1981; Willet *et al*. 1994) [37, 69]. Replant problems have been attributed to physical, chemical and, particularly, biological disorders of the soil (Traquair, 1984) [65], with a range of successful treatments in various fruit-growing areas reflecting this diversity. For example, preplant soil disinfection with biocides, including formalin (Covey *et al*., 1984) [34], chloropicrin (Hoestra, 1968) and methyl bromide (Koch *et al*, 1980) [34], improved the growth of seedlings in greenhouse tests and young trees in orchard trials, despite a concern that broad-spectrum biocides may destroy potentially beneficial mycorrhizal fungi (Traquair, 1984) [65]. Chemical agents often eliminate replanting disease, but they can also reduce the biological activity of the soil, which in turn reduces growth and yield (Gur *et al*., 1998) [23]. In British Columbia, Slykhuis and Li (1985) [59] demonstrated in greenhouse tests that the growth of apple seedlings in soil from old orchards was generally increased by preplanning treatments with specific fumigants, fungicides, or MAP fertilizer. The greatest benefits resulted from the use of both MAP and a fumigant or fungicide.

For this reason, agro-technical measures, such as fertilization or addition of bio-inoculants, can also play a role in controlling replant disease. Mineral fertilization, especially with specific nutrients, also directly effects plant growth and pathogen development in exhausted soils (Gullino and Mezzalama, 1993) [21]. Phosphorus, especially in the form of mono-ammonium phosphate, plays an important role (Utkhede and Smith, 1994) [67]. Organic fertilizers, such as bio-humus, peat or farmyard manure, are also useful (Tagliavini *et al*., 1993) [63]. Organic amendments are often applied to increase soil fertility, crop quality, or both (Edmeades, 2003; Mohammadi *et al*., 2013) [13, 45]. Furthermore, organic manure enhances the environmental sustainability of agricultural systems by increasing the organic matter of the soil and decreasing chemical inputs (Mohamed and Abdu, 2004) [44]. In addition to providing necessary nutrients for crops and improving soil physico-chemical properties, organic fertilizer is able to enhance soil microbial activity of soil, such as improving activity of soil enzymes and increasing soil microbial biomass (Sun *et al*., 2003; Mao *et al*., 2008) [61, 39]. Co-application of organic manure and chemical fertilizers is a significant approach to maintain and improve soil fertility, and increase fertilizer use efficiency (Ming-gang *et al*., 2008) [42].

Thus the aim of this study was to determine the effect of different soil agro-techniques on the content of minerals in soil and total nutrients in the leaves of replanted peach cv. July Elberta'.

Materials and methods

Soil collection and preparation

Soil was collected from a private orchard at village Matnali, Tehsil Rajgarh, District Sirmour, a replanted peach orchard site and brought to the experimental field of Department of Fruit Science for pot-experimentation. There a portion of the soil mixture was used in the control treatment as untreated (i.e. no fumigation) while the remaining heap of soil was sterilized with formalin (1:9) solution and covered under 25 micron transparent polythene sheet exposed to the sunlight for a period of three weeks so as to avoid leakage of formaldehyde fumes and thereby ensuring the complete, uniform and effective fumigation. Afterward the soil heap was opened and worked in such a way to exclude formaldehyde fumes from soil, completely. After two weeks the manures and fumigated-soil were mixed together and re-filled in plastic containers each of 50 Kg capacity along with peach seedlings raised in polythene bags for carrying out pot-culture studies.

Plant material

One year old uniform seedlings were planted in black polythene bags (18″ × 9″ size) containing a mixture of soil, FYM and sand (2:1:1). The optimum level of moisture was maintained in the growing media of polybags by regular irrigation. Planting was done in plastic containers under open field conditions, in first week of February, 2014**.**

Experimental details

A pot-culture study was conducted from 2014 to 2016 at an elevation of 1250 m above mean sea level at 30° 51’N latitude and 76° 11’E longitude in the Nando block of the Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The experimental area lies under the sub-temperate, sub-humid mid-hill agro climatic zone II of Himachal Pradesh where, summer is moderately hot during May-June while, winter is quite severe during December-January. The annual rainfall ranges between 110-120 cm and the major amount of which is received during June to September. The soil, brought from replanted peach orchard site, was mountainous sandy loamy soil and having pH 6.5, organic carbon 1.05%, available N, P and K were 145, 34.9 and 153.2 kg/ha, respectively. The present investigations were conducted on 1-year old polybag raised peach seedlings that were planted in 50 liters plastic container and filled with soil and FYM (3:1) along with soil ball adhering to the plants and then applied with different replant soil treatments. The experiment was laid out using completely randomization design (CRD), comprising of 6 treatments including 3 variants viz., soil fumigation, PGPR and biocontrol in 5 different combos and a control (i.e. Recommended package of practices); each with four replications, during the first week of January, 2014. Those seedlings were then grafted with scion variety ‘July Elberta’ in February 2015. The details of experimental treatments are given as under

T_1 = Insitu grafted plant + Recommended package of practices (POP)
T_2 = Insitu grafted plant + Soil fumigation (SF) + Recommended package of practices (POP)
T_3 = Insitu grafted plant + Soil fumigation (SF) + SSP (25% more of recommended)
T_4 = Insitu grafted plant + Soil fumigation (SF) + PGPR + SSP (25% more of recommended)
T_5 = Insitu grafted plant + Soil fumigation (SF) + Bio-control (*Trichoderma* + Neem/oil cake) + SSP (25% more of recommended)
T_6 = Insitu grafted plant + Soil fumigation (SF) + PGPR + Bio-control (*Trichoderma* + Neem/oil cake) + SSP (25% more of recommended)

Time of application: (PGPR and *Trichoderma viride*)

Plant Growth Promoting Rhizobacteria (PGPR 250ml) and Bio control (*Trichoderma viride* 100g) were applied at the time of planting in pit/pots and then repeated after every three months up to December 2016.

Pre and Post analysis of replanted orchard soil samples were done in 2014 and 2015-16, respectively, to compute the physico-chemical characteristics of soil to verify the effect of different treatments. Soil samples representing 0-45 cm depth were collected from four sites of each peach tree basin from replant orchard site before the start of experiment and in the fall each year during the course of investigation. The samples were dried in shade, grounded, passed through 2 mm sieve and stored in cloth bags. The soil samples were analysed for bulk density and field capacity by standard procedure lay out by Kanwar and Chopra (1976) [31]. The chemical analysis of soil pH and electrical conductivity was determined by 1:2 or 1:5 Soil water suspension methods using digital pH meter and conductivity meter, respectively, as described by Jackson (1973) [30, 29]. Organic carbon was extracted according to the Chromic acid titration method, given by Walkely and Black (1934) [68]. The available macronutrient status i.e., N, P and K content of orchard soils were determined according to the standard methods laid down by Subbiah and Asija (1956) [60]; Olsen *et al*., (1954) [47]; and Merwin and Peech (1951), accordingly.

For plant nutrients analysis, leaf samples were collected from the middle of current season's growth all around the periphery of the tree, as recommended by Kenworthy (1964) [33], in the month of July each year during the course of investigation. Samples were washed first under tap water followed by 0.1 N HCL, distilled water and finally with double glass distilled water, dried in an oven at 60±5°C and ground to fine powder (Chapman, 1964) [9]. The digestion of leaf samples for various nutrient elements was done in diacid mixture (nitric acid: perchloric acid in the ratio of

4:1). A separate digestion was carried for nitrogen estimation using concentrated sulphuric acid and digestion mixture as suggested by Jackson (1967) [30, 29]. Analysis for various nutrient elements viz., 'N' was done by micro Kjeldahl method (A.O.A.C., 1980); 'P' by phospho-molybdo-vanadate method (Jackson 1973) [30, 29] and 'K', 'Zn', 'Fe', and 'Mn' were determined on Atomic Absorption Spectrophotometer Element AS AAS4141 whereas for 'Ca' and 'Mg' procedure layout by David (1960) [11] and Fishman (1966) [16] was followed. Data were subjected to one-way analysis of variance (ANOVA) as suggested by [Gomez and Gomez, 1984] [18]; and to mean separation with the Fisher's Least Significant Differences (LSD) test with $P<0.05$, using the statistical analysis program (SPSS).

Results

Soil parameters

Available nitrogen

Data on pot-trials pertaining to effects of different replant peach rhizosphere soil treatments on available nitrogen content are presented in Table 1. It is clearly evident from data that available soil nitrogen content was significantly affected by different replant soil treatments during both the years of study. During the year 2015, significantly highest available soil nitrogen (157.89 ppm) was recorded in the rhizosphere of peach seedlings grown on replant soil with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par with soil N (153.59 and 151.91 ppm) obtained in T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. However, the lowest soil N (145.97 ppm) was registered in control. Similarly, in the year 2016, the highest soil N (161.51 ppm) was obtained in peach rhizosphere pot-soil with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par with T_4 (157.39 ppm) and T_5 (155.58) treatments. However, the lowest soil N (147.53 ppm) was observed in T_1 (control), which was found to be statistically at par with soil N obtained in treatments T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP), during both the years under pot-culture study.

Available phosphorus

The perusal of data presented in Table 1 reveal that there were significant differences among different pot replant soil treatments with respect to available soil P during both the years of analysis. The maximum available P content (51.39 ppm and 55.58 ppm in 2015 and 2016, respectively) was recorded in T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment. However, the minimum soil P (35.27 ppm and 45.51 ppm in 2015 and 2016, respectively) was observed in control, closely followed by T_2 (SF+ recommended POP) treatment. During both the years of pot-culture experiment, the treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) was found to be statistically on a level of equality with T_3 (SF+ 25% more of recommended SSP), T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments.

Available potassium

Different replant treatments had significant effect on available soil K content during both the years of pot-culture studies (Table 1). The significantly higher (165.03 and 165.95 ppm) soil K was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment during 2015 and 2016, respectively. However, the lowest (155.45 ppm in 2015 and 157.00 ppm in 2016) soil K content was observed with control. The replant soil treatments T_3 (SF+ 25% more of recommended SSP), T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments were found to be on par (159.15, 163.35 and 161.83 ppm, respectively) with soil K in treatment T_6 (165.03 ppm) in 2015, while during 2016, similar trend was observed among different peach replant treatments exclusive of T_3 (SF+ 25% more of recommended SSP) treatment.

Table 1: Effect of different soil treatments on available macronutrients concentration in peach rhizosphere grown in pots

Treatments	Macronutrients concentration (ppm)					
	Available N		Available P		Available K	
	2015	2016	2015	2016	2015	2016
T_1	145.97	147.53	35.27	45.51	155.45	157.00
T_2	147.83	151.67	40.63	47.65	157.91	159.63
T_3	149.87	153.35	45.51	49.83	159.15	161.19
T_4	153.59	157.39	49.29	53.89	163.35	164.29
T_5	151.91	155.58	47.25	51.77	161.83	163.43
T_6	157.89	161.51	51.39	55.58	165.03	165.95
$LSD_{(0.05)}$	7.54	7.02	7.40	6.25	5.98	3.76

Leaf nutrients status

Nitrogen (%)

The perusal of the data given in Table 2 reveal that different replant treatments influenced leaf N content significantly under pot-culture surveillance. During both the years of analysis, highest leaf N (3.37 and 3.51 % in 2015 and 2016, respectively) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically on par with T_3 (SF+ 25% more of recommended SSP), T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments. Meanwhile, minimal leaf N (3.09

and 3.13 in 2015 and 2016, respectively) was observed with T_1 (Recommended POP), closely followed by T_2 (SF+ recommended POP) treatment.

Phosphorus (%)

The data presented in Table 2 shows that different peach replant treatments had a significant effect on per cent P content of the leaves in pot-cultivation. During 2015, treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) recorded maximum leaf P (0.32 %) content, which was statistically on par (0.27 %) with T_4 (SF + PGPR + 25% more of recommended SSP) treatment. While, the minimal leaf P (0.18 %) content was recorded with T_1 (Recommended POP), closely followed by T_2 (SF+ recommended POP), T_3 (SF+ 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments. In 2016, maximum leaf P (0.35 %) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) statistically on par with T_4 (0.29 %) and T_5 (0.27 %) treatments. However, minimum leaf P (0.21 %) was observed in T_1 (Recommended POP), statistically similar with all other treatments except T_6 treatment

Potassium (%)

Different peach replant soil treatments had a significant effect on per cent K content of leaves during 2016 only as depicted in Table 2. Among different treatments, T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) recorded highest (2.57 %) leaf K in 2016, which was statistically at par (2.49 and 2.45 %) with T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. The lowest Leaf K (2.13 %) was recorded with T_1 (Recommended POP), closely followed by T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP) treatments.

Calcium (%)

Different replant treatments influenced the leaf Ca content significantly as evident from the data presented in Table 2. During both the years of investigation a similar trend was noticed with maximum leaf Ca (2.55 and 2.73 % in 2015 and 2016, respectively), which was statistically on par with leaf Ca content obtained in T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments. The lowest leaf Ca (2.21 and 2.39

% during 2015 and 2016, accordingly) was recorded with T_1 (Recommended POP), closely followed by T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP) treatments.

Magnesium (%)

The data presented in Table 2 show that different replant treatments had a significant effect on per cent Mg content of the leaves during 2015; however, in 2016 no consistent change was noticed with respect to pot-culture studies. Significantly, highest leaf Mg (0.67 %) content in 2015 was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment. However, the minimum leaf Mg content (0.57 %) was recorded in T_1 (Recommended POP without fumigation), closely followed by all other treatments except T_4 (SF + PGPR + 25% more of recommended SSP) and T_6 treatments.

Iron (ppm)

A perusal of data presented in Table 3 reveal the significant influence exerted by different replant treatments in relation to leaf Fe content of replanted peach under pot-culture studies during both the years of analysis. The highest leaf Fe content (235.9 and 237.0 ppm in 2015 and 2016, respectively) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment, which was statistically on par with T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments, for both the years. The lowest leaf Fe content (229.3 and 230.4 ppm during 2015 and 2016, respectively) was recorded T_1 (Recommended POP), stands on a level of equality with treatments T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP) in descending order.

Manganese (ppm)

Different replant treatments didn’t showed any consistent effect on the foliar Mn (Table 3) content during 2015. However, the highest leaf Mn (159.7 ppm) content in 2016 was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), closely followed by T_4 (155.2 ppm) and T_5 (157.1) treatments. The lowest leaf Mn (139.5 ppm) content was recorded

with T_1 (Recommended POP) treatment, statistically at par with T_2 (143.6 ppm) and T_3 (145.0 ppm) treatments.

Zinc (ppm)

The perusal of data presented in Table 3 reveal that leaf Zn content was significantly influenced by different replant treatments during the entire study period under pot-cultivation. The highest leaf Zn (35.31 and 37.01 ppm in 2015 and 2016, respectively) was recorded in treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par with T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments. The lowest leaf Zn (29.23 and 30.34 ppm during 2015 and 2016, accordingly) was recorded with T_1 (Recommended POP), closely followed by T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP) treatments.

Table 2: Effect of different soil treatments on leaf macro nutrients concentration of peach plants grown in pots

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Calcium (%)		Magnesium (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
T_1	3.09	3.13	0.18	0.21	1.93	2.13	2.21	2.39	0.57	0.55
T_2	3.11	3.19	0.19	0.23	1.95	2.17	2.29	2.45	0.59	0.57
T_3	3.25	3.39	0.23	0.25	2.11	2.23	2.32	2.48	0.61	0.59
T_4	3.34	3.43	0.27	0.29	2.23	2.49	2.51	2.67	0.65	0.63
T_5	3.31	3.41	0.21	0.27	2.19	2.45	2.45	2.63	0.63	0.60
T_6	3.37	3.51	0.32	0.35	2.27	2.57	2.55	2.73	0.67	0.65
$LSD_{(0.05)}$	0.12	0.17	0.06	0.08	NS	0.26	0.15	0.12	0.07	NS

Table 3: Effect of different soil treatments on leaf micronutrients concentration of peach plants grown in pots

Treatments	Iron (ppm)		Manganese(ppm)		Zinc (ppm)	
	2015	2016	2015	2016	2015	2016
T_1	229.27	230.35	139.15	139.48	29.23	30.34
T_2	230.07	231.65	145.45	143.57	30.03	31.64
T_3	231.78	232.13	146.61	145.03	31.68	32.10
T_4	233.51	235.25	150.07	155.23	33.39	35.23
T_5	232.74	234.21	148.50	157.08	32.75	34.15
T_6	235.97	237.03	153.63	159.69	35.31	37.01
LSD $_{(0.05)}$	3.69	3.48	NS	10.89	3.35	3.48

Discussion

Soil nutrient status

In present study, every recorded parameter for soil nutrient status except soil pH and organic carbon was significantly influenced by inoculation of plant growth promoting rhizobacteria (PGPR). The increased available nitrogen, phosphorus and potassium content were recorded in combined treatment (Soil fumigation + PGPR + Biocontrol) as evident from Table 1 under pot-cultivation. The increase in available nitrogen and phosphorus content may be due to increased biological nitrogen fixation and phosphate solubilization by micro-organisms. Organic compounds containing phosphorus are decomposed and mineralized by bacteria. Bacterial strains belonging to genera *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aerobacter, Flavobacterium* and *Erwinia* have the ability to solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite and rock phosphate (Rodríguez and Fraga, 1999; Rodríguez *et al*., 2006). Strains from genera *Pseudomonas, Bacillus* and *Rhizobium* are reported among the most powerful phosphate solubilizers (Illmer and Schinner, 1992; Halder and Chakrabarty, 1993; Banerjee *et al*., 2010). Many bacteria isolated from the rhizosphere (rhizobacteria) are capable of increasing the availability of soil phosphorus to plants either by mineralization of organic phosphate or solubilization of insoluble inorganic phosphates with production of acids.

Phosphate solubilization is highly dependent on pH. Some bacteria have the ability to solubilizing inorganic P due to chelation, exchange reaction, phosphate production and excretion of organic acids which decreases soil pH and render the insoluble phosphate into soluble form. Generally, the solubility of calcium phosphates and magnesium also increases with decreasing pH. Phosphate dissolving bacteria are known to reduce the pH by secretion of a number of organic acids such as formic acid, acetic acid, succinic acid, etc. As a result, some of these acids may form chelators with the cations such as Ca and Fe, and such chelation results in effective solubilization of phosphates (Taalab and Badr 2007). Furthermore, they are able to solubilizing organic P compounds through the action of phosphatase, phytases, phosphonatases and C-P lyases enzymes (Lugtenberg and Kamilova 2009). The results of present study are in agreement with number of workers who have reported phosphate solubilization by *Bacillus* as dominant inorganic phosphorus compound solubilizing microbes (Gupta *et al*. 2002; Jana 2007). The increase in soil potassium content upon bacterial inoculation may be due to increase in dissolution rate of silicates and minerals which releases K, production of enzymes like chitinase and cellulase that causes breakdown of minerals and, increased root exudation accompanied by accelerated microbial proliferation and respiration that may lead to O_2 depletion in the rhizosphere and facilitate dinitrification specifically as reported by Mishustin *et al.* (1981) or Barker *et al.* (1997). These findings are also in agreement with the work of Dwivedi *et al.* (1999), Singh *et al.* (2010) and Esitken *et al.* (2010). Therefore, the increase in soil nutrient status might be due to the fact that the micro-organisms help in formation of enzyme complex, mobilizing the unavailable forms of nutrient elements especially through increased biological nitrogen fixation, phosphate solubilization, iron chelating siderophore production, increased EC and cation exchange capacity by which nutrients become available for absorption by roots.

Leaf nutrients status

The present study indicates that higher foliar macro and micro nutrients was recorded with combined treatment i.e., T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) while the lowest concentration of these essential nutrient elements was obtained in T_1 (Recommended POP) treatment. The results supported by Hudska (1977); who reported that soil fumigation or steam sterilization greatly improved top and root growth. Further, the results on the effects of soil solarization and fumigation on soil microbial suppression and the demography of peach tree

fine roots (<1 mm in diameter) indicated that peach root longevity may be significantly affected by interactions with the soil microbial community, and these interactions may also influence the rate of both physical and anatomical root development (Tanner *et al*. 2006). Thus, changes in root architecture and physiology affect water and nutrient absorption, therefore the activity of the root system plays a central role in adaptation to environmental conditions and ultimately, plant performance. Furthermore, soil inoculants such as PGPR and AM fungi were shown to cause changes in root morphology and architecture (Glick, 1995; Zahir *et al*., 2004; Gravel *et al*., 2007). Generally, the enhancement in essential plant nutrients are more pronounced in plant growth promoting rhizobacterial inoculation which resulted in significant increase in foliar macronutrients (Table 2) and micronutrients (Table 3). The results are in conformity with Pirlak *et al.* (2007) who reported that bacterial spraying with strains of *Bacillus* and *Pseudomonas* alone or in combination significantly affected leaf nutrient elements' content (N, P, K, Mg, Ca, Fe, Mn, Cu and Zn) investigated in "Granny Smith and Starkrimson" cultivars of apple, except Mg in "Starkrimson" when compared with the control. Further, the application of plant growth promoting rhizobacteria resulted in increased nutrient element (N, P, K, Ca, Mg, Zn, Fe, Cu and Mn) content in strawberry leaf (Ipek *et al.* 2014). PGPR enhances plant nutrition via associative nitrogen fixation, phosphate solubilization, or phytosiderophore production (Richardson *et al*., 2009). It is very difficult to understand and quantify the impact of PGPR on roots and, thereby, on the plant as a whole. However, two types of mechanisms are, generally, considered to be involved. On the one hand, some PGPR can enhance nutrient availability/uptake for plant roots. On the other hand, certain PGPR trigger specific systemic responses, mostly by unknown signaling mechanisms. The impact of PGPR on plant nutrition may result from effects on plant nutrient uptake and/or on plant growth rate (Mantelin and Touraine, 2004).

The increase in leaf nitrogen as depicted in Table 2 may be due to enhanced nitrogen supply due to nitrogen fixation by rhizobacterial strains. This increased nitrogen supply can be attributed to better metabolic activities and high protein synthesis. These observations are supported by Rathi and Bist (2004) and Esitken *et al*. (2006). The increase in leaf macronutrients may be due to phosphate solubilizing, mineralizing other organic or inorganic nutrients and phytohormone producing capacity of plant growth promoting rhizobacterial strains which stimulated leaf nutrient content. The results are in line with the findings of Gryndler *et al*. (2002) [20], Aseri *et al*. (2008) [3], Singh and Singh (2009), Karlidag *et al*.

(2013) and Osman and El-rhman (2010). Further, bacterial inoculation of soil decreased the pH of the soil. Decreased soil pH stimulated the availability of plant nutrient element (PNE) such as P and Ca (Orhan *et al*. 2006). Moreover bacterial inoculation could prevent formulation of Ca-P complex and resulted into higher uptake of Ca-P to the plant. Growth responses to soil fumigation were usually greater in acidic than in alkaline soils for any given level of P. Sewell *et al*. (1988) reported that soil P content and pH had large effects on growth in fumigated soils; that had large effects on the growth response to soil fumigation. In fumigated soils, the height of seedlings was generally greater, the greater the soil P content.

The micronutrients like Fe, Mn, Cu and Zn were highest in T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment in *Insitu* grafted plants grown in a replant soil. The increase in micronutrient content may be due to increase in improved nutrient uptake by plants, plant growth, root surface area or the general root architecture, production of siderophores and plant growth regulators. The results are in line with the findings of Esitken *et al*. (2006), Aseri *et al*. (2008), Singh and Singh (2009), Bhattacharyya and Jha (2012) [3], Karlidag *et al*. (2013) and Ipek *et al*. (2014). Plant growth promoting rhizobacteria reported to produce many organic and inorganic acid and decrease the soil pH which play a crucial role in nutrient acquisition (Zn, Fe, Cu and Mn) by plants growing in low nutrient soils and their release in response to nutrient starvation differs between plant species (Ae *et al*., 1990 and Fox and Comeford, 1990). The concentrations of fumaric, malic and citric acids can also chelate Zn, Fe, Cu and Mn in Zinc, iron, copper and manganese oxides, thus making them available for uptake by the plant (Ohwaki and Hirata, 1992; Marschner, 1995).

Thus, plants take up most of their mineral nutrients through the rhizosphere, where microorganisms interact with plant products in root exudates. Plant root exudates consist of a complex mixture of organic acid anions, phytosiderophores, sugars, vitamins, amino acids, purines, nucleosides, inorganic ions, enzymes and root border cells which have major direct or indirect effect on the acquisition of mineral nutrients required for plant growth and development (Bottini *et al*. 2004; Turan *et al*. 2012)

Reference

1. AOAC Official methods of analysis of Association of Analytical Chemists, 13th ed. (ed. W. Horowitz). Benjamin Franklin Station, Washington, DC, 1980, 1018.
2. Ae N, Arihara J, Okada K, Yoshihara T, Johansen C. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science. 1990; 248:477-480.
3. Aseri GK, Jain N, Panwar J, Rao AV, Meghwal PR. Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in Indian Thar Desert. Scientia Horticulture. 2008 117:130-135.
4. Banerjee S, Palit R, Sengupta C Standing D. Stress induced phosphate solubilization by Arthrobacter sp. and Bacillus sp. isolated from tomato rhizosphere. Australian Journal of Crop Sciences. 2010; 4:378-383.
5. Barker WW, Welch SA, Banfield JF. Geomicrobiology of silicate minerals weathering. Rev. Mineral. Geochem. 1997; 35:391-428.
6. Benizri E, Piutti S, Verger S, Pages L, Vercambre G, Poessel JL *et al*.. Replant diseases: bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. Soil Biology and Biochemistry. 2005; 37:1738-1746.
7. Bhattacharyya PN, Jha DK. Plant growth promoting rhizobacteria (PGPR): emergence in agriculture. World J Microb. Biot. 2012; 28:1327-1350.
8. Bottini R, Cassan F, Piccoli P. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. App. Microb. Biot. 2004; 65:497-503.
9. Chapman HD. Suggested foliar sampling and handling techniques for determining the nutrient status of some field horticultural and plantation crops. Indian Journal of Horticulture. 1964; 21:97-119.
10. Covey RP, Koch BL, Haglund WA. Control of apple replant disease with formaldehyde in Washington. Plant Dis. 1964; 68:981-983.
11. David DJ. The determination of exchangeable sodium, potassium, calcium and magnesium in soils by atomic-absorption spectrophotometry. Analyst. 1960; 85:495-503.
12. Dwivedi MP, Negi KS, Jindal KK, Rana HS. Effect of bioregulators on vegetative growth of strawberry. Scientific Horticulture. 1999; 6:79-84.
13. Edmeades DC. The long-term effects of manures and fertilizers on soil productivity and quality: a review. Nutr. Cycl. Agroecosys. 2003; 66:165-180.
14. Esitken A, Lutfi P, Metin T, Fikrettin S. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. Scientia Horticulture. 2006; 110:324-27.
15. Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Scientia Horticulturae. 2010; 124:62-66.
16. Fishman MJ. The use of atomic absorption for analysis of natural waters. At. Abs. News. 1966; 1(5):102-106.
17. Glick BR. The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology. 1995; 41:109-117.
18. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research (2nd ed.), John Willey and Sons Inc, New York, 1984, 680.
19. Gravel V, Antoun H, Tweddell RJ. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: Possible role of indole acetic acid (IAA). Soil Biology and Biochemistry. 2007; 39:1968-1977.
20. Gryndler M, Vosátka M, Hrselova H, Catska V, Chvátalova I, Jansa J Effect of dual inoculation with arbuscular mycorrhizal fungi and bacteria on growth and mineral nutrition of strawberry. Journal of Plant Nutrition. 2002; 25:1342-1358.

21. Gullino ML, Mezzalama M. Influence of cultural practices and chemical treatments on soilborne diseases of fruit crops. Acta Hort. 1993; 324:35-46.
22. Gupta A, Meyer JM, Goel R. Development of heavy metal resistant mutants of phosphate solubilizing Pseudomonas sp. NBR14014 and their characterization. Current Microbiology. 2002; 45:323-327.
23. Gur A, Luzzati J, Katan J. Alternatives for soil fumigation in combating apple replant disease. Acta Hort. 1998; 477:107-114.
24. Halder AK, Chakrabarty PK. Solubilization of inorganic phosphate by Rhizobium. Folia Microbiology. 1993; 38:325-330.
25. Hoestra H. Replant diseases of apple in the Netherlands. Med. Lanb. Wageningen, 1968, 68-13.
26. Hudska G. Trials on testing and disinfecting soils affected by stone fruit replant disease. Vedecke Prace Ovocnarske. 1997; 6:235-246.
27. Illmer P, Schinner F. Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biology and Biochemistry. 1992; 24:389-395.
28. Ipek M, Pirlak L, Esitken A, Dönmez MF, Turan M, Sahin F. Plant growth promoting rhizobacteria (PGPR) increase yield, growth and nutrition of strawberry under high calcareous soil conditions. Journal of Plant Nutrition. 2014; 37:990-1001.
29. Jackson ML. Soil Chemical Analysis. Asia Publising House, Bombay, 1967, 498.
30. Jackson ML. Soil Chemical Analysis. Prentice hall, New Delhi, 1973, 120.
31. Kanwar JS, Chopra SL. Agricultural chemistry. S Chand and Co., Delhi, 1976, 130-131.
32. Karlidag H, Yildirim E, Turan M, Pehluvan M. Plant growth promoting rhizobacteria mitigate deleterious effects of salt stress on strawberry plants (Fragaria × ananassa). Hort. Science, 2003; 48:563-567.
33. Kenworthy AL. Fruit, nut and plantation crops, deciduous and evergreen: a guide for collecting foliar samples for nutrient elements analysis. Horticulture Department of Michigan State University, Mieneo, 1964, 1-39.
34. Koch BL, Covey RP, Haglund W. Effect of soil fumigation on the early growth and production of ‘Delicious’ apple trees. J Amer. Soc. Hort. Sci.1980; 105:887-890.
35. Koch LW. The peach replant problem in Ontario. I. Symptomology and distribution. Canadian Journal of botany. 1955; 33:450-460.
36. Lugtenberg BJ, Kamilova F. Plant growth promoting rhizobacteria. Annu. Rev. Microbiol. 2009; 63:541-556.
37. Mai WF, Abawi GS. Controlling replant diseases of pome and stone fruits in Northeastern United States by preplant fumigation. Plant Diseases. 1981; 65:859-864.
38. Mantelin S and Touraine B. Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp. Bot. 2004; 55:27-34.
39. Mao J, Olk DC, Fang X, He Z, Schmidt-Rohr K. Influence of animal manure application on the chemical structures of soil organic matter as investigated by advanced solid-state NMR and FT-IR spectroscopy. Geoderma. 2008; 146:353-362.
40. Marschner H. Mineral nutrition of higher plants, 2nd ed. Academic Press Ltd., London, UK. 1995, 13:889.
41. Merwin HD, Peech M. Exchangeability of soil potassium in the sand, silt and clay fractions as influenced by the nature of the complimentary exchangeable cation. Soil Sci. Soc. Amer. Proc. 1951; 15:125-128.
42. Ming-gang X, Dong-chu L, Ju-mei L, Dao-zhu Q, Yagi K Hosen Y. Effect of organic manure application with chemical fertilizers on nutrient absorption and yield of rice in Hunan Southern China. Agr. Sci. China. 2008; 7:1245-1252.
43. Mishustin EN, Sinironova GA, Lokmacheva RR. The decomposition of silicates by microorganisms and the use of silicate bacteria as bacterial fertilizers. Biol. Bull. 1981; 8:400-409.
44. Mohamed MAH, Abdu M. Growth and oil production of fennel (Foeniculum vulgare Mill): effect of irrigation and organic fertilization. Biol. Agric. Hort. 2004; 22:31-39.
45. Mohammadi K, Heidari GR, Javaheri M, Karimi Nezhad MT. Soil microbial response to tillage systems and fertilization in sunflower rhizosphere. Arch. Agron. Soil Sci. 2013; 59:899-910.
46. Ohwaki Y, Hirata H. Differences in carboxylic acid exudation among P starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid level in roots. Soil Sci. Plant Nutr. 1992; 38:235-243.
47. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus by extraction with sodium bicarbonate. U. S. Dept Agri. Circ, 1954, 939.
48. Orhan E, Esitken A, Sezai Ercisli, Turan M, Sahin F. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Scientia Horticulturae, 2006; 111:38-43.
49. Osman SM, El-rhman Abd IE. Effect of organic and Bio N-fertilization on growth, productivity of Fig tree (*Ficus Carica*, L.). Research Journal of Agriculture and Biological Sciences. 2010; 6:319-328.
50. Pirlak L, Turan M, Sahin F and Esitken A. Floral and foliar application of plant growth promoting rhizobacteria (PGPR) to apples increases yield, growth, and nutrient element contents of leaves. Journal of Sustainable Agriculture. 2007; 30:145-155.
51. Rathi DS and Bist LD. Inorganic fertilization through the use of organic supplements in low chill pear cv. Pant pear-18. Indian Journal of Horticulture. 2004; 61:223-225.
52. Richardson AE, Baréa JM, McNeill AM Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009; 321:305-339.
53. Rodriguez H and Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology. 1999; 17:319-339.
54. Rodríguez H, Fraga R, Gonzalez T and Bashan T. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil. 2006; 287:15-21.
55. Savory BM. Specific replant diseases, causing root necrosis and growth depression in perennial fruit and plantation crops. Research Review 1. Commonw. Bur. Hort. Plant. Crops, East Malling, Maidstone, Kent, England, 1966.
56. Sewell GWF, Preece DA and Elsey RF. Apple replant disease: the influence of soil phosphorus and other

factors on the growth responses of apple seedlings to soil fumigation with chloropicrin. Annals of Applied Biology. 1988; 113:605-615.

57. Singh A, Singh JN. Effect of biofertilizers and bioregulators on growth, yield and nutrient status of strawberry cv. Sweet Charlie. Indian Journal of Horticulture. 2009; 66: 220-224.
58. Singh SR, Zargar MY, Singh U, Ishaq M. Influence of bio-inoculants and inorganic fertilizers on yield, nutrient balance, microbial dynamics and quality of strawberry (Fragaria × ananassa) under rainfed conditions of Kashmir valley. Indian Journal of Agricultural Sciences. 2010; 80:275-281.
59. Slykhuis JT, Li TSC. Responses of apple seedlings to biocides and phosphate fertilizers in orchard soils in British Columbia. Can. J Plant Pathol. 1985; 7:294-301.
60. Subbaiah BV, Asija GL. A rapid procedure for the determination of available nitrogen in soils. Current Science 1956; 25:259-260.
61. Sun RL, Zhao BQ, Zhu LS. Effects of long-term fertilization on soil enzyme activities and its role in adjusting-controlling soil fertility. J Plant Nutr. Fert. Sci. 2003; 9:406-410.
62. Taalab AS, Badr MA. Phosphorus availability from compacted rock phosphate with nitrogen to sorghum inoculated with phospho-bacterium. J Appl Sci Res. 2007; 3:195-201.
63. Tagliavini M, Hogue EJ, Neilsen GH. Phosphate and peat additions affect growth, P and N nutrition of peach seedlings in virgin and replant peach soil. Acta Horticulturae. 1993; 324:97-106.
64. Tanner SC, Reighard GL, Wells CE. Soil treatments differentially affect peach tree root development and demography in a replant site. Acta Horticulturae. 2006 713:381-390.
65. Traquair J. Etiology, control of orchard replant problems: a review. Canadian Journal of Plant Pathology. 1984; 6:54-62.
66. Turan M, Gulluce M, Von Wiren N, Sahin F. Yield promotion and phosphorus solubilization by plant promoting rhizobacteria in extensive wheat production. Journal of Plant Nutrition and Soil Science. 2012; 175:18-826.
67. Utkhede RS, Smith EM. Biotic and abiotic causes of replant problems of fruit trees. Acta Hort. 1994; 63:25-32.
68. Walkely A, Black CA. An examination of the Degtjareff method for determining soil organic matter and proposed modification of chromic valid titration method. Soil Science. 1934; 37:29-38.
69. Willet M, Smith TJ, Peterson AB, Hinman H, Stevas RG. Growing profitable apple orchards in replant sites: an interdisciplinary team approaching Washington State. Horticulture Technology. 1994; 4:175-181.
70. Zahir AZ, Muhammad A, William TFJ. Plant growth promoting rhizobacteria applications and pererpectives in agriculture. Advances in agronomy. 2004; 1:97-149.