Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 3139-3144
Received: 15-01-2018
Accepted: 16-02-2018

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Effect of different soil agro-techniques on leaf nutrient status of peach grown in a replant sick soil

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Abstract
Peach replant disease (PRD) is a complex syndrome typified by stunted growth and reduced yields in repeated cultivation of peach in old orchard sites. PRD is associated with several biotic and abiotic causes; its symptoms are vague and highly variable by sites under different environmental conditions; making it difficult to diagnose and overcome. In this experiment; we studied the effect of different replant soil treatments for controlling PRD in site preciously planted to peach for >30 years. We compared 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); each with four replications. The different soil management practices significantly ($p<0.05$) affected leaf nutrients content of peach plants grown in a replanted site. The results from this study provide evidence that combined treatment (Soil fumigation +PGPR +Biocontrol+25% more of recommended SSP) helped improving the total mineral nutrient status and thus may alleviate replant problem in peach.

Keywords: triclosan, TCS, determination, detection, sensor

Introduction
Peach [*Prunus persica* (L.) Batsch] is the most important temperate and deciduous fruit tree grown in India. Its cultivation is being undertaken in the mid-hill zone of Himalayas extending from Jammu and Kashmir to Khasi hills in North East (NE) at an altitude of (1000-2000) m above mean sea level. Low chilling peach cultivars are grown in sub-mountainous and plains of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Punjab, Haryana and Western Uttar Pradesh. It is also being grown to a limited scale in the hills of South India and in the NE region of the country. The total area under peach cultivation in India is about 19.24 thousand hectare with a total production around 97.44 MT (NHB, 2015) [30]. Peach is ranked third most important temperate fruit of Himachal Pradesh after apple and plum in respect of area and production.

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) [26]. 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) [37].

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) [4]. 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) [27, 46]. Replant problems have been attributed to physical, chemical and, particularly, biological disorders of the soil (Traquair, 1984) [43], 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),

chloropicrin (Hoestra, 1968) and methyl bromide (Koch *et al*., 1980) [25], 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) [43]. 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) [17]. In British Columbia, Slykhuis and Li (1985) [40] 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) [16]. Phosphorus, especially in the form of mono-ammonium phosphate, plays an important role (Utkhede and Smith, 1994) [45]. Organic fertilizers, such as bio-humus, peat or farmyard manure, are also useful (Tagliavini *et al*., 1993) [41].

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

Materials and Methods

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 under open field conditions, in first week of February, 2014**.**

Soil fumigation and planting

At the experimental site, the pits (filled with soil) were drenched with 5 liters of formaldehyde solution (1:9). Thereafter, the pits were immediately covered with 25 micron transparent polythene sheet exposed to the sunlight for a period of three weeks prior to planting avoids leakage of formaldehyde fumes and thereby ensuring the complete, uniform and effective fumigation of pits. After 21 days the polythene sheet was removed and basin soil was worked out or racked in such a way so as to ensure complete evolution of formaldehyde fumes from the basin area. After two weeks peach seedlings raised in polythene bags were planted in the treated basin along with soil ball.

Experimental details

A field trial was conducted during 2015-2016 in a private orchard at village Matnali, Tehsil Rajgarh, District Sirmour on replanted peach orchard site. The experiment was laid out using randomization block design (RBD), 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, 2015. The experimental site was located at an elevation of 1475 m above mean sea level at 30.85° N latitude and 77.3° E longitude under the 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-February. The annual rainfall ranges between 110-120 cm and the major amount of which is received during June to September.

The present investigations were conducted on 1-year old polybag raised peach seedlings transplanted to pits drenched with formaldehyde along with soil ball adhering to the plants and then applied with different replant soil treatments. Planting was done under open field conditions, in first week of February, 2014. The peach 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.

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) [24], in the month of July each year during the course of investigation. The handling and preparation of samples were done in accordance with procedure given by Chapman (1964) [7]. 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) [21]. Analysis for various nutrient elements viz., 'N' was done by micro Kjeldahl method (A.O.A.C., 1980) [1]; 'P' by phosphor-molybdo-vanadate method (Jackson 1973) [22] and 'K', 'Zn', 'Fe', 'Mn' and 'Cu' were determined on Atomic Absorption Spectrophotometer Element AS AAS4141 whereas for 'Ca' and 'Mg' procedure layout by David (1960) [9] and Fishman (1966) [11] was followed. Data were subjected to one-way analysis of variance (ANOVA) as suggested by [Gomez and Gomez, 1984] [13]; and to mean separation with the Fisher's Least Significant Differences (LSD) test with $P<0.05$, using the statistical analysis program (SPSS).

Results

Nitrogen (%)

The perusal of the data given in Table 1 reveal that different replant treatments influenced leaf N content significantly ($p<0.05$) during course of investigation. During both the years of study, highest leaf N (3.65 and 3.59 % in 2015 and 2016, respectively) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was significantly superior to all other replant treatments in 2015 but statistically at par (3.39 %) with leaf N recorded in treatment T_4 (SF +

PGPR + 25% more of recommended SSP) in 2016. Lowest leaf N (2.99 and 2.95 % during 2015 and 2016, respectively) was recorded in T_1 (Recommended POP) which didn't differ statistically from all other treatments except T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) in 2015 and T_4 and T_6 in 2016.

Phosphorus (%)

The data presented in Table 1 shows that different peach replant treatments had non-significant influence on per cent P content of the leaves during the year 2015. However, in 2016, significantly highest P (0.23 %) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), statistically on par (0.19 %) with T_4 (SF + PGPR + 25% more of recommended SSP) treatment. Meanwhile, leaf P content recorded with all other replant treatments was statistically at par with one another except T_6.

Potassium (%)

Different peach replant soil treatments had a significant ($p<0.05$) effect on per cent K content of leaves during both the years of study (Table1). In 2015, among different treatments, T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) recorded highest leaf K (2.27 %), which was statistically similar (2.21 and 2.17 %) with T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments, respectively while in 2016 at par (2.53 %) with T_4 (2.39 %) treatment. The lowest leaf K (1.74 and 1.71 % during 2015 and 2016, respectively) was recorded with T_1 (Recommended POP), which was statistically lower than all other treatments.

Calcium (%)

Different replant treatments influenced the leaf Ca content significantly ($p<0.05$) as evident from the data presented in Table 1 during both the years of study. In the year 2015, leaf Ca (2.45, 2.47, 2.53 and 2.47 % respectively) obtained in T_2 (SF+ recommended POP), 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, respectively, was statistically at par (2.58 %) with the maximum leaf Ca recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment. The lowest Leaf Ca (2.27 %) was recorded with T_1 (Recommended POP without fumigation), which was significantly lower than the leaf Ca obtained in all other treatments. During 2016, significantly highest leaf Ca (2.67 %) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment, statistically at par (2.59 %) with T_4 (SF + PGPR + 25% more of recommended SSP). However, least leaf Ca (2.39 %) content recorded with T_1 (Recommended POP) was found to be statistically at par with T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP).

Magnesium (%)

The data presented in Table 1 show that different replant treatments didn't show any consistent influence on per cent Mg content of the leaves during both the years of investigation. However, the Mg content varied from 0.59 % to 0.65 % during 2015 and 0.53 % to 0.64 % in the year 2016.

Iron (ppm)

Perusals of data given in Table 2 reveal the significant ($p<0.05$) effect exerted by different replant treatments in relation to leaf Fe content during both the years of analysis. The highest leaf Fe content (243.4 and 245.6 ppm in 2015 and 2016, respectively) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment, which was statistically on a 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 (227.2 and 230.9 ppm during 2015 and 2016, respectively) was recorded in T_1 (Recommended POP) that stands on a level of equality (228.53 and 235.4 ppm, correspondingly) with T_2 (SF+ recommended POP) treatment.

Manganese (ppm)

Different replant treatments didn't show any consistent effect on the foliar Mn content (Table 2) during the course of investigation However, the Mn content varied from 139.50 to 163.70 ppm during 2015 and 140.30 to 165.73 ppm in the year 2016.

Zinc (ppm)

The perusal of data presented in Table 2 reveal that foliar Zn content was influenced significantly ($p<0.05$) by different replant treatments during 2015 only, however, in 2016, the results were found to be non-significant. Treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) recorded highest leaf Zn (37.30 ppm) in 2015, which was statistically at par (35.70 ppm) with T_4 (SF + PGPR + 25% more of recommended SSP) treatment. The lowest leaf Zn (30.50 ppm) 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, which were statistically at par.

Table 1: Effect of different soil treatments on leaf macro nutrients concentration of peach

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Calcium (%)		Magnesium (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
T_1	2.99	2.95	0.16	0.18	1.74	1.71	2.27	2.39	0.59	0.53
T_2	3.02	3.01	0.18	0.17	1.95	1.93	2.45	2.43	0.60	0.62
T_3	3.09	3.05	0.18	0.17	2.00	1.97	2.47	2.50	0.62	0.64
T_4	3.29	3.39	0.21	0.19	2.21	2.39	2.53	2.59	0.63	0.59
T_5	3.13	3.20	0.19	0.18	2.17	2.25	2.47	2.53	0.62	0.64
T_6	3.65	3.59	0.23	0.23	2.27	2.53	2.58	2.67	0.65	0.62
$LSD_{(0.05)}$	0.33	0.31	NS	0.04	0.15	0.18	0.17	0.13	NS	NS

Table 2: Effect of different soil treatments on leaf micronutrients concentration of peach

Treatments	Iron (ppm)		Manganese (ppm)		Zinc (ppm)	
	2015	2016	2015	2016	2015	2016
T_1	227.21	230.90	139.50	140.30	30.50	32.70
T_2	228.53	235.35	140.35	148.80	32.10	33.00
T_3	237.95	239.50	145.39	145.50	32.90	33.50
T_4	241.32	243.26	150.35	159.82	35.70	33.90
T_5	239.45	241.98	148.70	147.50	33.50	35.20
T_6	243.35	245.63	163.70	165.73	37.30	37.50
$LSD_{(0.05)}$	4.73	5.91	NS	NS	3.28	NS

Discussion

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) [19]; 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) [42]. 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) [12, 47, 14]. 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 1) and micronutrients (Table 2). The results are in conformity with Pirlak *et al*. (2007) [34] 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) [20]. PGPR enhances plant nutrition via associative nitrogen fixation, phosphate solubilization, or phytosiderophore production (Richardson *et al*., 2009) [36]. 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) [28].

The increase in leaf nitrogen as depicted in Table 1 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) [35] and Esitken *et al*. (2006) [10]. 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) [15], Aseri *et al*. (2008) [3], Singh and Singh (2009) [39], Karlidag *et al*. (2013) [23] and Osman and El-rhman (2010) [33]. 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) [32]. 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) [38] 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 both types of planting viz. grafted and *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) [10], Aseri *et al*. (2008) [3], Singh and Singh (2009) [39], Bhattacharyya and Jha (2012), Karlidag *et al*. (2013) [23] and Ipek *et al*. (2014) [20]. 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) [2]. 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) [31, 29]. 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) [6, 44].

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