International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 2726-2730

Received: 28-01-2018
Accepted: 29-02-2018

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Enzyme activities in peach rhizosphere as influenced by various soil management practices under replant situations: A pot culture study

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Abstract
Soil enzymes represent an important component of microbial communities present in the plant rhizosphere where they play a substantial role in maintaining soil health and its environment. A unique balance of microbial especially enzyme activities contribute to maintaining soil health. Evaluation of soil health therefore requires indicators of all these components. A pot-culture study in field condition was conducted to evaluate the effect of different soil management practices viz. soil fumigation, SSP, PGPR and biocontrol along with control (i.e. recommended package of practices) on microbial population (fungal, bacterial and actinomycetes) and enzymes activities in a replant soil. Different soil treatments showed significant ($p<0.05$) variations on enzymes activity over the control. Highest urease, dehydrogenase and phosphatase activity was recorded in peach rhizosphere with combined treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) and the lowest value was obtained in T_1 (recommended POP) treatment during the course of investigation. Likewise enzymatic activities, similar trend was noticed for microbial population where the combined treatment resulted in significantly ($p<0.05$) highest count of the aforementioned biological parameters, during both the years of study, compared to all other treatments. It can be concluded that different soil management regimes exerted a significant effect in terms of the measured soil biological parameters.

Keywords: peach, replant, soil fumigation, ssp, pgpr and biocontrol

Introduction
Peach [*Prunus persica* (L.) Batsch] is ranked third most important temperate fruit of Himachal Pradesh after apple and plum in respect of area and production with an area of about 5,076 ha and production of 8,045 MT (Anonymous 2016). 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.

Although the area and production under peach cultivation has increased comparatively; however, the productivity remain static and now a declining trend have been reported during last few years. In Himachal Pradesh, being a hilly region due to limited land resources and choice of crops for diversification in hill states, orchardists are compelled to replant same fruit crop in old orchard site, which lead to drastic economic loss not only due to uprooting of old trees but also because of poor establishment of new plantations on the same site. Repeated cultivation of the same plant species on the same field is the primary factor leading to replant problems. As a result, a general decline in the growth and productivity of replanted peach orchard is observed commonly, which is referred as PRD (Peach replant disease).

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) [5]. The reasons for low productivity could be many but one of the most important reasons is age of orchards. In general, orchards of stone fruits more than 20 years of age have shown much more unfruitfulness than the young orchards. Most of peach orchards in Himachal Pradesh planted during seventies and early eighties have either outlived their economic bearing life or declined due to the adverse effect of insect pests and diseases. This practice makes plants vulnerable to replant problem. There has been increasing concern

about poor growth of peach trees planted at sites where peach tree grew before. The situation resulting in this poor growth is generally known as replant problem (Thompson 1959) [27].

Plant rhizosphere represents soil-plant root system ecological niche where roots release large quantity of metabolites from living root hairs or fibrous root systems which serves as chemical signals for soil microbes to interact with the plant and the main nutrient sources available as a food to support their growth and persistence in the rhizosphere. Microbial communities particularly enzymes present in the rhizosphere play a pivotal role in ecological fitness and functioning of their plant host, both in relations to direct interactions with plants and with regard to decomposition of organic matter and nutrient cycling (Pavel *et al*., 2004) [19]. The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell-associated or free enzymes. Enzymes are the vital activators in most biochemical processes of soil; likewise they are considered good indicators to predict changes of soil quality and productivity thus play a substantial role in maintaining soil health and its environment (Nannipieri *et al*., 2012) [16]. Urease enzyme activity is important for the hydrolysis of urea fertilizers applied to the soil into NH_3 and CO_2. In soil ecosystems, phosphatase enzymes are believed to play critical roles in catalyzing the hydrolysis of P-ester bonds binding P to C in organic matter, thereby enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Pascual *et al*., 2002) [18]. Further, many previous studies have been demonstrated that that long-term fertilization, tillage, management regime, leaf litter, and root exudates were the major factors affecting the soil microbial activities both quantitatively and qualitatively. In recent years, studies on microbial biomass and enzymes activity have engaged the attention of any researchers. However, most of these studies are confined to agricultural cropping systems (Wright *et al*., 2005; Mandal *et al*., 2007; Jannoura *et al*., 2013) [29, 13, 10] and forest ecosystems (Barbhuiya *et al*., 2004; Feng *et al*., 2009) [4, 7] but, information regarding those under temperate fruit crops like peach, cherry, apricot, plum etc., are scarce.

The hypothesis we assume to carry out this study was that pre-plant soil fumigation being a primary measure employed for the control of replant disease due tothe perceived uncertainty regarding the etiology of replant disease kills all of the soil micro flora (both beneficial and harmful), therefore, in order to regain the soil health status as influenced by different soil management practices such as addition of fertilizers, organic amendments, use of bio-inoculants like PGPR, *Trichoderma* etc, could be attributed to soil microbial activity as a good indicator of changes to soil quality. Thus, the objectives of this experiment were: i) to determine soil microbial population ii) to assess soil enzyme activities, such as dehydrogenase, phosphatase (acid and alkaline) and urease in a fumigated soil after different replant treatments.

Materials and methods

The experiment was laid out at Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The experimental orchard 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.

Raising of peach seedlings

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.

Plant materials and experimental details

The suitable methodology was adopted to understand the response of peach seedlings to replant soil. One year old polybag raised peach seedlings 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 studies were conducted under pot-culture experimentation, 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, 2015. 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), T_2 (Insitu grafted plant + Soil fumigation (SF) + Recommended package of practices), 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)) and T_6 (Insitu grafted plant + Soil fumigation (SF) + PGPR + Bio-control (*Trichoderma* + Neem/oil cake) + SSP (25% more of recommended))

Soil fumigation and planting

Soil from replanted peach orchard site at Matnali was brought to the experimental field of Department of Fruit Science. There a heap of soil was sterilized with formalin (1:9) solution and covered under polythene sheet for three weeks. 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.

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.

Estimation of soil enzymes activity

Urease

The method used for estimating urease enzyme activity was given by Tabatabai and Bremner (1972) [26].

Phosphatase

The phosphatase enzyme estimation was carried out by method given by Tabatabai and Bremner (1969) [25].

Dehydrogenase

Dehydrogenase enzyme estimation in soil was carried out by using the reduction of 2, 3, 5-triphenyltetrazolium chloride (3%) method given by Casida *et al*. (1964) [6].

Detection of total microbial count

Microbial count was performed by standard plate count technique (Wollum, 1982) [28] by employing different media for different groups of microorganisms.Suspension of 0.1ml from dilution blank was spread over pre-poured solid media *viz.*, Nutrient Agar, Potato Dextrose Agar and Kenknight's Munaiers medium with the help of glass spreader under aseptic conditions for enumeration of bacteria, fungi and actinomycetes, respectively, as per the recommendation. Plates were incubated in inverted position at 28±2ºC for 48 hours. After the incubation period, the microbial count was expressed as colony forming unit per gram of soil (cfu g^{-1} soil).

Results

Urease activity

The reconnaissance of data enumerated in Table 1 reveal that urease activity was significantly affected ($p<0.05$) by the different rhizosphere soil treatments during both the years of pot-experimentation. During the year 2015, significantly highest urease activity (395.1 µg urea g $soil^{-1}$ h^{-1}) was recorded in rhizosphere of plants raised on replant soil with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par (393.3 and 391.7 µg urea g $soil^{-1}$ h^{-1}) with urease activity observed in T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. However, the least urease activity (383.4 µg urea g $soil^{-1}$ h^{-1}) was observed in T_1 (control), which was statistically on par (385.7 µg urea g $soil^{-1}$ h^{-1}) with T_2 (SF+ recommended POP) treatment. Similarly, in the year 2016, highest urease activity (395.0 µg urea g $soil^{-1}$ h^{-1}) was recorded in peach rhizosphere with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) on par with T_4 (393.5 µg urea g $soil^{-1}$ h^{-1}), whereas, lowest (387.7 µg urea g $soil^{-1}$ h^{-1}) in rhizosphere of plants raised on replant soil with treatment T_1 (Recommended POP), closely followed by T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP) treatments.

Table 1: Effect of different soil treatments on enzymes activity in peach rhizosphere grown in pots

Treatments	Urease activity (µg urea g $soil^{-1}$ h^{-1})		Dehydrogenase activity (µg TPF g $soil^{-1}$ h^{-1})		Phosphatase activity (µ mol L^{-1} g^{-1} h^{-1})	
	2015	2016	2015	2016	2015	2016
T_1	383.4	387.7	313.17	315.03	287.47	288.97
T_2	385.7	387.9	315.91	317.90	289.81	291.13
T_3	387.8	389.7	317.79	319.93	291.38	291.45
T_4	393.3	393.5	321.69	323.59	295.57	295.79
T_5	391.7	391.8	319.91	321.85	293.83	293.71
T_6	395.1	395.0	323.27	325.39	297.21	297.10
$LSD_{(0.05)}$	3.59	2.46	2.61	4.89	2.48	2.62

Table 2: Effect of different soil treatments on microbial count in peach rhizosphere grown in pots

Treatments	Bacterial count (10^5 cfu/ g soil)		Fungal count (10^3 cfu/ g soil)		Actinomycetes count (10^2 cfu/ g soil)	
	2015	2016	2015	2016	2015	2016
T_1	39.00	43.25	9.75	10.75	8.50	9.25
T_2	45.50	47.50	3.00	4.75	3.75	5.00
T_3	55.00	65.00	5.25	6.25	6.75	7.50
T_4	91.50	103.00	8.50	9.50	10.75	11.50
T_5	59.25	67.50	6.50	7.25	9.25	9.75
T_6	109.50	111.75	9.25	11.75	11.25	12.25
$LSD_{(0.05)}$	6.09	6.00	2.07	2.36	2.20	2.55

Dehydrogenase activity

Different pot replant treatments influenced soil dehydrogenase activity significantly ($p<0.05$) as evident from the data given in Table 1, during both the years of investigation. In the year 2015, markedly highest dehydrogenase activity (323.3 µg TPF g^{-1} h^{-1}) was recorded in treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) which was statistically on a par (321.7 µg TPF g^{-1} h^{-1}) noticed with T_4 (SF + PGPR + 25% more of recommended SSP). On the contrary, least dehydrogenase activity (313.2 µg TPF g^{-1} h^{-1}) was obtained in rhizosphere of plants grown in pot replant soil with treatment T_1 (Recommended POP), incomparably lower than all other treatments. Similar trend was observed during the year 2016, as treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) resulted in maximum (325.4 µg TPF g^{-1} h^{-1}) dehydrogenase activity, which stands at an equality in values (323.6 and 321.9 µg TPF g^{-1} h^{-1}) obtained with T_4 (SF + PGPR + 25% more of recommended SSP) and T_5 (SF + Biocontrol + 25% more of recommended SSP) treatments, respectively. The minimum (315.03 µg TPF g^{-1} h^{-1}) dehydrogenase activity was recorded in T_1 (Recommended POP), closely followed by T_2 (SF + recommended POP) and T_3 (SF + 25% more of recommended SSP) treatments in ascending order.

Phosphatase activity

The data procured in Table 1 demonstrate that different replant treatments had a significant effect on the activity of soil phosphatase enzyme ($p<0.05$) during both the years of study. During the year 2015, notably highest phosphatase activity (297.2 µg PNP g^{-1} h^{-1}) was recorded in rhizosphere of plants with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) which was statistically at par (295.6 µg PNP g^{-1} h^{-1}) with T_4 (SF + PGPR + 25% more of recommended SSP) treatment. However, the lowest phosphatase activity (287.5 µg PNP g^{-1} h^{-1}) was recorded in T_1 (control), standing close with T_2 (SF+ recommended POP) treatment. In the year 2016, maximum phosphatase activity (297.1 µg PNP g^{-1} h^{-1}) was recorded in rhizosphere of plants

with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par (295.8 µg PNP g^{-1} h^{-1}) with phosphatase activity observed in T_4 (SF + PGPR + 25% more of recommended SSP). However, the minimum (289.0 µg PNP g^{-1} h^{-1}) activity was found in peach rhizosphere soil with T_1 (control) treatment, closely followed by T_2 (SF+ recommended POP) and T_3 (SF+ 25% more of recommended SSP) treatments.

Soil microbial count

Bacterial count

It is evident from the data presented in Table 2 that soil bacteria was significantly affected ($p<0.05$) by the different replant soil treatments under pot-cultivation during the course of analysis. During both the years of study, significantly highest bacterial count (109.50 and 111.75×10^5 cfu/g soil in 2015 and 2016, respectively) was recorded in rhizosphere of plants raised on replant soil with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically superior to all other treatments. However, the lowest count in 2015 (39.00×10^5 cfu/g soil) and in 2016 (43.25×10^5 cfu/g soil) was observed in control i.e. T_1 (Recommended POP) treatment, which was found to be significantly lower than all other treatments.

Fungal count

Different pot replant treatments influenced soil fungal count significantly ($p<0.05$) as evident from the data given in Table 2 during both the years of investigation. In the year 2015, markedly highest fungal count (9.75×10^3 cfu/g soil) was recorded in treatment T_1 (Recommended POP) statistically on par (8.50 and 9.25×10^3 cfu/g soil) with T_4 (SF + PGPR + 25% more of recommended SSP) and T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatments, respectively. However, in 2016, significantly highest fungal count (11.75×10^3 cfu/g soil) was recorded with T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically on par (10.75 and 9.50×10^3 cfu/g soil) with T_1 (Recommended POP) and T_4 (SF + PGPR + 25% more of recommended SSP) treatments, respectively. On the contrary, during 2015 least fungal count (3.00×10^3 cfu/g soil) was obtained in rhizosphere of plants raised on replant soil with treatment T_2 (SF+ recommended POP), significantly lower than all other treatments. While, in the year 2016, minimum fungal (4.75×10^3 cfu/g soil) count was recorded with treatment T_2 (SF+ recommended POP) closely similar to T_3 (SF+ 25% more of recommended SSP).

Actinomycetes count

From the perusal of the data enumerated in Table 2, it is clear that different replant treatments had a significant effect ($p<0.05$) on the population of soil actinomycetes under pot-surveillance. Notably the highest actinomycetes (11.25 and 12.25×10^2 cfu/g soil in 2015 and 2016, respectively) count was recorded in rhizosphere of plants with treatment T_6 (SF + PGPR + Biocontrol + 25% more of recommended SSP) 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 during both the years of study. However, the lowest actinomycetes count (3.75×10^2 cfu/g soil in 2015) was recorded with T_2 (SF+ recommended POP) treatment, which was significantly lower than all other treatments. While, in the year 2016, treatment T_2 (SF+ recommended POP) recorded minimum (5×10^2 cfu/g soil) actinomycetes count that was on par with T_3 (SF+ 25% more of recommended SSP) treatment.

Discussion

The significant variations showed by different soil management practices apropos of enzymatic activities and increased microbial population in fumigated soil were expected. We hypothesized that soil microbial activity would be affected by the different soil management approaches in response to soil fumigation and ultimately, improving the health status of replant sick-soil. The observed results on soil biological parameters support the proposed hypothesis. The substantiation provided by the data analysis suggest that soil microbial activity were perhaps influenced by the inputs added in the form of nutrients i.e. recommended doses of N, P & K; particularly, the organic amendments like FYM and use of bio-inoculants such as PGPR and *Trichoderma viride* etc. Findings of Prakash *et al*., 2007 [21] showed that the addition of FYM along with NPK fertilizers increased SOC by 37.8%, over NPK-alone treatment. Furthermore, applying FYM along with N or NPK resulted in significantly higher microbial biomass carbon and populations of microorganisms.

The activities of the enzymes studied in this experiment may help in assessing the changes in soil organic carbon as these enzymes play a pivotal role in the decomposition of organic matter and nutrient cycling. Dehydrogenase activity in soil depends on the content of soluble organic carbon and, the increased organic matter in the surface soil enhances the soil enzyme activities (Nannipieri *et al*., 2012) [16]. The major reason for increased DHA in the surface soil could be attributed to the greater availability of soluble organic C, nutrients and, stimulated microbial activity by inoculation of bio-formulations. This result is in agreement with the observation made by Adak *et al*. (2014) [1] in mango orchard. Furthermore, our result is in consistent with the findings of Saha *et al*. (2008) [23] who indicated that organic manure in response to bio-inoculants had a positive effect on urease activity.

In addition to organic matter, some other factors also influenced organic carbon in soil. In this study, contrast analysis results indicated that in all pot-culture treatments; soils receiving nutrients and FYM in combination with the use of bio-inoculants i.e. PGPR and *Trichoderma* could be ascribed to variations exerted by different treatments to a great extent. These findings are also in conformity with those of Jarak *et al*., (2012) [11] who also reported the ability of *Trichoderma viride*, *Pseudomonas* sp., and *Bacillus* sp. and*Azotobacterchroococcum*straintoenhancemaizegrowth (*Zea mays*L.) under field conditions. These results are also in line with those obtained by Kaur and Reddy, (2015) [12] who found that the highest yield was obtained by bio-inoculation of treatments individually or in combination with bio-fertilizers in maize-wheat cropping system. The results are further supported by the findings of Gaind *et al*., (2006) [8] who also reported that incorporation of compost prepared from paddy straw and fungal inoculants in wheat improved enzymatic activities and phosphorous content of soil.

The greater microbial count could be attributed to the accumulation of organic matter and its breakdown into simpler compounds through PGPR activity. In other words, the increase in the size of microbial community was proportional to the increased organic matter content of the soil. The results of present study are in agreement with the observations of Seo *et al*., (2010), Jarak *et al*., (2012) [11] and Pesakovic *et al*., (2013) [20] who reported increased microbial

population with bacterial inoculation in strawberry.The findings are in line with the work of Aseri *et al*., (2008) [3]; Raj and Sharma, (2009) [22]; Mazzola and Gu, (2000) [14]; Mazzola, (1998) [15] who also reported increased rhizobacterial population with PGPR inoculation.Furthermore, the rhizosphere is known to be a zone of increased microbial activity and consequently enzyme activity.

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