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## Efficacy of different soil agro-techniques against peach replant disease as determined by vegetative growth of the plants under surveillance in a replanting site

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### Abstract

The aim of this experimental study was to evaluate the vegetative performance of the July Elberta' peach plants grafted and *Insitu* grafted raised on seedling rootstocks in a replant orchard soil. The experiment was conducted in an area known for intensive peach cultivation for more than 30 years and now the successive monoculture in the same soil has resulted in the problem of soil-sickness. Different soil management practices viz. soil fumigation, SSP, PGPR and biocontrol along with control (i.e. recommended package of practices) were employed as the practices for controlling replant disease and to trigger the growth of plants in a replant soil. The replant soil treatments showed significant ( $p < 0.05$ ) variations apropos of growth and vigour characteristics over the control. The results have indicated that growth in terms of plant height, stem diameter, number of feathers and leaves, leaf area and chlorophyll content were observed in combined treatment i.e. Soil fumigation + PGPR + Biocontrol + 25% more of recommended SSP. Thus, it can be inferred that the soil profile modification coupled with soil fumigation prove to be a potential agro-technique to combat peach replant disease.

**Keywords:** Replant disease, *Prunus persica*, Insitu, Soil fumigation, Replant problem, Tree vigour

### Introduction

Replant diseases are common in many regions of the world, known for centuries, related to the practice of replanting of fruit tree species successively in the same soil. Replant disease is a debilitating problem of soil sickness affecting most of the fruit crops including both pome and stone fruit when repeated cultivation of the same plant species is done on the same field that previously supported the similar or closely related species. Due to the 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. 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 disorder). This disorder is prevalent in orchards previously planted with peach and manifests itself as a decline in tree vigour and performance without 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) [1]. The reasons for low productivity could be many but one of the most important reasons is age of orchards. Peach trees attain precocity than most fruit tree species but have much shorter productive lives. 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. Consequently, frequent orchard replanting is necessity but is complicated by the occurrence of 'replant failure'. 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) [37].

The problem is specific to a plant genus or species, it is resident for the place where it appeared, immobile, and persists even for 20 to 30 years (Klaus, 1939) [18]. Replant problems have been classified as either nonspecific or specific.

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It is assumed that nonspecific replant suppression can affect several unrelated tree species, has a patchy distribution in the orchard and is often correlated with high number of plant parasitic nematodes, regardless of the previous fruit crop. In contrast, specific replant disease has a more uniform distribution throughout the orchard, affects only one or several closely related tree species and the cause is frequently attributed to biotic, generally fungal activity. Specific replant disease of *Prunus* results in poor growth, stunting, and delayed crop production, and in severe cases, tree death. The root system of the diseased trees are small, dark, compact and feeble (Savory, 1966) <sup>[35]</sup>, and show varying degrees of discoloration and necrosis (Koch, 1955) <sup>[20]</sup>.

The etiology of replant problems is unclear; however, the possible causes might include both biotic and abiotic factors (Politycka and Adamska, 2003) <sup>[31]</sup>. Since, *prunus* replant disease is a complex soil borne disease so it's comparatively easy to prevent the 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. Hence, precautionary measures from the previous crop should be followed such as proper nutrition and pathogen management, which otherwise interact with the allelochemicals and make the plant more susceptible to root disease. In plants, the most characteristic effects of replant diseases are the reduction of vigor and yield, besides the orchard's low longevity (Rutto & Mizutani, 2006; Bent *et al.* 2009) <sup>[34, 2]</sup>. Soil fumigation with chemical compounds has been used as a control technique, resulting in an increase in plant growth and yield, but it still presents low efficiency levels, high implementation costs, and a risk to human health and to the environment (Leinfelder & Merwin, 2006) <sup>[22]</sup>.

Since 1960s, the practices for controlling replant disease have included soil profile modification coupled with soil fumigation (McKenry, 1999) <sup>[29]</sup>. Crop rotation and soil sterilization with fungicides are potential control measures. Furthermore, 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) <sup>[23, 40]</sup>. Keeping in view the points gauged from previous replant studies, a study was proposed based on hypothesis that the use of soil organic amendments alongside with fumigation could be beneficial in optimizing the soil health status through improved organic matter content which can enhance soil fertility and ultimately the plant growth. Thus, the objective of this study was to evaluate the effect of different soil agro-techniques on vegetative performance of the July Elberta peach tree cultivar, in a replanting area.

## Materials and methods

### Plant material

The planting material was composed of grafted and *In situ* grafted peach plants both raised on the seedling rootstocks. For raising the peach seedlings stratified seeds 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. The grafted peach plants with scion cultivar July Elberta' were procured from the nursery of Department of Fruit Science, UHF, Nauni, Solan (HP).

### 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 raked in such a way so as to ensure complete evolution of formaldehyde fumes from the basin area. After two weeks grafted peach plants and seedlings raised in polythene bags were transplanted in the treated basin along with soil ball adhering to the roots.

### Experimental details

The experiment was conducted in a private orchard at village Matnali, Tehsil Rajgarh, District Sirmour on replanted peach orchard site, during the year 2014 to 2016. The orchard 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 replanting site had been previously cultivated with 'July Elberta' peach trees for more than 30 years, with a two-year period between the uprooting of the old trees and the setup of the field experiment.

The experiment was laid out using randomization block design (RBD), comprising of 6 treatments including 3 variants viz. soil fumigation, PGPR and biocontrol along with increased dose of SSP (Single Super Phosphate) 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 present investigations were conducted on grafted and *In situ* grafted peach plants planted in pits drenched with formaldehyde (except control) and then applied with different replant soil treatments. The homogeneity of the experimental area was maintained by transplanting of 1-year old polybag raised peach seedlings in a former peach orchard under open field conditions, in first week of February, 2014 i. e. a year prior to the plantation of peach grafted plants. The peach seedlings were then grafted with scion variety 'July Elberta' by the inverted "T" method, during the month of February 2015. The details of experimental treatments are given as under

T<sub>1</sub> = Grafted/*In situ* grafted plant + Recommended package of practices (POP)

T<sub>2</sub> = Grafted/*In situ* grafted plant + Soil fumigation (SF) + Recommended package of practices (POP)

T<sub>3</sub> = Grafted/*In situ* grafted plant + Soil fumigation (SF) + SSP (25% more of recommended)

T<sub>4</sub> = Grafted/*In situ* grafted plant + Soil fumigation (SF) + PGPR + SSP (25% more of recommended)

T<sub>5</sub> = Grafted/*In situ* grafted plant + Soil fumigation (SF) + Bio-control (*Trichoderma* + Neem/oil cake) + SSP (25% more of recommended)

T<sub>6</sub> = Grafted/*In situ* grafted plant + Soil fumigation (SF) + PGPR + Bio-control (*Trichoderma* + Neem/oil cake) + SSP (25% more of recommended) Standard recommended doses of FYM, N and K were applied in all the experimental trees

**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.

In particular, the data on tree growth and vigour characteristics were recorded to study the effect of different replant soil treatments. Observations regarding growth parameters, viz. plant height, stem diameter, number of feathers, leaf number, leaf area and chlorophyll content were recorded as per standard procedures during both the years of study. Plant height was measured from the ground level to the top with the help of a graduated scale and mean was worked out and expressed in centimeters (cm). Stem diameter of each replication of experimental plants was determined using Digimatic Vernier Caliper and results were expressed in millimeters (mm). Fully developed 20 leaves per tree were sampled in early August of each year from all around the periphery of the tree. The leaf area was determined using a portable Laser (CI-202), CID Bio-Science automated leaf area meter and expressed as square centimeters. Chlorophyll content was estimated with DMSO (Dimethyl Sulphoxide) method as suggested by Hiscox and Israeastam (1979) [15]. The data were subjected to one-way analysis of variance (ANOVA). The averages were separated by means of tests of the Least Significant Difference (LSD) at  $p < 0.05$ .

**Results****Plant height**

The reconnaissance of data enumerated in Table 1 reveal considerable ( $p < 0.05$ ) variation among different treatments apropos of plant height in case of *In situ* grafted plants rose on peach seedlings in a replant soil. Nevertheless, grafted plants didn't produce any consistent change. During the course of study in 2015, *In situ* grafted plants exhibited maximum (78.00 cm) plant height in a peach replant soil with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically on a par (57.50, 76.50 and 63.50 cm) with plant height recorded in T<sub>3</sub> (SF+ 25% more of recommended SSP), T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. While, the minimum (42.75 cm) plant height was observed in plants

raised onto peach seedling with replant treatment T<sub>1</sub> (Recommended package of practices), which was closely followed (48.50, 57.50 and 63.50 cm) by T<sub>2</sub> (SF+ recommended POP), T<sub>3</sub> (SF+ 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. Whereas, in the year 2016, the plant height (152.50 and 149.00 cm) recorded with T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively, stands on a level of equality with maximum plant height (173.75 cm) as noticed in treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP). However, the least plant height (91.50 cm) was recorded in T<sub>1</sub> (Recommended package of practices), which was statistically at par (93.00 cm) with T<sub>2</sub> (SF+ recommended POP) treatment.

**Stem diameter**

From the perusal of data presented in Table 1, it is apparent that different replant treatments had no significant effect on stem diameter of grafted and *In situ* grafted peach seedlings grown in a replant soil during 2015; however, in the year 2016 both types of plantings manifested considerable ( $p < 0.05$ ) variation in respect of stem diameter. During 2016, significantly maximum stem diameter (17.39 mm) was recorded in *In situ* grafted peach seedlings raised on replant soil with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par (16.84 and 16.19 mm) with T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. While, the minimal stem diameter (10.33 mm) was observed in plants raised onto peach seedling with replant treatment T<sub>1</sub> (Recommended Package of practices), which was significantly lower than all other treatments. However, in peach grafted plants, the stem diameter (22.46 mm) was observed in T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) that stands on a par (22.67 mm) with maximal stem diameter recorded in treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP). The minimum stem diameter (12.55 mm) was recorded with T<sub>1</sub> (Recommended package of practices), which was found to be statistically at par (14.67 mm) with T<sub>2</sub> (SF+ recommended POP) treatment.

**Table 1:** Effect of different soil treatments on plant height and stem diameter of peach

| Treatments            | Plant height (cm)            |        |               |        | Stem diameter (mm)           |       |               |       |
|-----------------------|------------------------------|--------|---------------|--------|------------------------------|-------|---------------|-------|
|                       | <i>In situ</i> grafted plant |        | Grafted plant |        | <i>In situ</i> grafted plant |       | Grafted plant |       |
|                       | 2015                         | 2016   | 2015          | 2016   | 2015                         | 2016  | 2015          | 2016  |
| T <sub>1</sub>        | 42.75                        | 91.50  | 71.25         | 127.50 | 7.76                         | 10.33 | 10.94         | 12.55 |
| T <sub>2</sub>        | 48.50                        | 93.00  | 82.00         | 130.00 | 8.10                         | 13.40 | 11.19         | 14.67 |
| T <sub>3</sub>        | 57.50                        | 120.00 | 83.50         | 136.00 | 8.21                         | 14.00 | 12.22         | 17.04 |
| T <sub>4</sub>        | 76.50                        | 152.50 | 91.50         | 150.00 | 9.48                         | 16.84 | 12.89         | 22.46 |
| T <sub>5</sub>        | 63.50                        | 149.00 | 87.50         | 145.50 | 8.94                         | 16.19 | 12.79         | 17.25 |
| T <sub>6</sub>        | 78.00                        | 173.75 | 92.75         | 155.25 | 9.87                         | 17.39 | 13.55         | 22.67 |
| LSD <sub>(0.05)</sub> | 21.69                        | 24.81  | NS            | NS     | NS                           | 2.89  | NS            | 2.83  |

**Number of feathers**

From the close examination of the data presented in Table 2, it is evident that number of feathers in grafted and *In situ* grafted peach seedlings was significantly ( $p < 0.05$ ) affected by different replant treatments during both the years of study. During 2015, significantly maximum number of feathers (8.00) was recorded in *In situ* grafted peach seedlings raised on replant soil with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically

superior to all other treatments. While, the minimum (1.25) in plants rose onto peach seedling with replant treatment T<sub>1</sub> (Recommended package of practices), that was closely followed by the values (2.50, 3.25 and 3.25) recorded with T<sub>2</sub> (SF+ recommended POP), T<sub>3</sub> (SF+ 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. However, in the year 2016, the number of feathers (6.25, 7.75 and 7.50) were observed in T<sub>3</sub> (SF+ 25% more of recommended SSP), T<sub>4</sub> (SF

+ PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively, stands on a par with maximum number of feathers (9.00) recorded in treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP). Whereas, minimal number of feathers (2.50) were recorded with T<sub>1</sub> (Recommended package of practices), statistically at par (4.75 and 6.25) with the values noticed in T<sub>2</sub> (SF+ recommended POP) and T<sub>3</sub> (SF+ 25% more of recommended SSP), respectively.

In grafted peach plants, significantly highest number of feathers (7.00) were recorded with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP) during 2015, which was statistically at par (4.25, 4.75, 6.50 and 6.75) with number of feathers observed in T<sub>2</sub> (SF+ recommended POP), T<sub>3</sub> (SF+ 25% more of recommended SSP), T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. While, the minimal number of feathers (2.75) were observed in plants raised onto peach seedling with replant treatment T<sub>1</sub> (Recommended package of practices), which statistically stands at an equality with T<sub>2</sub> (SF+ recommended POP) and T<sub>3</sub> (SF+ 25% more of recommended SSP) treatments. However, in the year 2016, the number of feathers (8.00, 11.00 and 9.00) were observed in T<sub>3</sub> (SF+ 25% more of recommended SSP), T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively, that stands on a par with maximum number of feathers (11.75) recorded in treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP). Whereas, minimal number of feathers (4.25) were recorded with T<sub>1</sub> (Recommended package of practices), closely followed by the values (6.75 and 8.00)

noticed in T<sub>2</sub> (SF+ recommended POP) and T<sub>3</sub> (SF+ 25% more of recommended SSP), respectively.

#### Number of leaves

Regarding number of leaves, both grafted and *In situ* grafted plants unfolded great ( $p<0.05$ ) variation in response to different replant treatments in present study as depicted in Table 2. The maximum number of leaves (165.50 and 169.25 in 2015 and 2016, accordingly) was recorded in *In situ* grafted peach seedlings raised on replant soil with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par (139.25 and 157.50 during 2015 and 2016, respectively) with number of leaves observed in T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) treatment. The minimum number of leaves (62.50 and 97.50 in 2015 and 2016, respectively) was observed in plants raised onto peach seedling with replant treatment T<sub>1</sub> (Recommended package of practices).

In the present study conducted during 2015 and 2016, number of leaves in grafted plants raised on a replant soil was also greatly affected ( $p<0.05$ ) by different treatments. The values with regard to number of leaves was markedly highest in 2015 (178.50) and 2016 (219.25) with T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment, which was statistically on a par (155.00 and 197.50 during 2015 and 2016, respectively) in T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) treatment. Among different replant treatments, T<sub>1</sub> (Recommended package of practices) recorded significantly lowest number of leaves in 2015 (129.00), which was statistically on a level of equality with other treatments except T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP) and in 2016 (135.75) followed by T<sub>2</sub> (SF+ recommended POP).

**Table 2:** Effect of different soil treatments on number of feathers and leaves of replanted peach

| Treatments            | Number of feathers           |      |               |       | Number of leaves             |        |               |        |
|-----------------------|------------------------------|------|---------------|-------|------------------------------|--------|---------------|--------|
|                       | <i>In situ</i> grafted plant |      | Grafted plant |       | <i>In situ</i> grafted plant |        | Grafted plant |        |
|                       | 2015                         | 2016 | 2015          | 2016  | 2015                         | 2016   | 2015          | 2016   |
| T <sub>1</sub>        | 1.25                         | 2.50 | 2.75          | 4.25  | 62.50                        | 97.50  | 129.00        | 135.75 |
| T <sub>2</sub>        | 2.50                         | 4.75 | 4.25          | 6.75  | 73.50                        | 107.00 | 134.00        | 154.00 |
| T <sub>3</sub>        | 3.25                         | 6.25 | 4.75          | 8.00  | 85.50                        | 132.25 | 141.00        | 167.25 |
| T <sub>4</sub>        | 4.75                         | 7.75 | 6.50          | 11.00 | 139.25                       | 157.50 | 155.00        | 197.50 |
| T <sub>5</sub>        | 3.25                         | 7.50 | 6.75          | 9.00  | 113.50                       | 133.50 | 148.00        | 185.50 |
| T <sub>6</sub>        | 8.00                         | 9.00 | 7.00          | 11.75 | 165.50                       | 169.25 | 178.50        | 219.25 |
| LSD <sub>(0.05)</sub> | 2.33                         | 3.84 | 2.93          | 4.39  | 29.28                        | 34.27  | 29.53         | 25.61  |

#### Leaf area

The perusal of data pertaining to leaf area provides substantiation that both grafted and *In situ* grafted plants exhibited significant ( $p<0.05$ ) variation in response of different replant treatments during the period of observation as depicted in Table 3. During 2015, maximum leaf area (43.55 cm<sup>2</sup>) was recorded in *In situ* grafted peach seedlings raised on replant soil with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par (42.41 and 41.85 cm<sup>2</sup>) with T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) treatment and T<sub>5</sub> (SF + Biocontrol + 25% more of recommended SSP) treatments, respectively. While, the minimum leaf area (38.42 cm<sup>2</sup>) was observed in plants raised onto peach seedling with replant treatment T<sub>1</sub> (Recommended package of practices), closely followed by the leaf area values of 39.18 and 39.78 cm<sup>2</sup> recorded with T<sub>2</sub> (SF+ recommended POP) and T<sub>3</sub> (SF+ 25% more of recommended SSP), correspondingly. However, in the year 2016, the leaf area (49.37 cm<sup>2</sup>) was recorded in T<sub>4</sub>

(SF + PGPR + 25% more of recommended SSP) which stands on a par with maximum leaf area (50.43 cm<sup>2</sup>) recorded in treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP). Meanwhile, the minimal leaf area (41.87 cm<sup>2</sup>) was noticed with T<sub>1</sub> (Recommended package of practices), statistically lower than all other replant treatments. Relevant to leaf area, grafted plants raised on a replant soil showed great ( $p<0.05$ ) variation amongst different replant treatments as elucidated by the data given in Table 3. The values with regard to leaf area in respect of grafted plants was markedly highest (47.71 cm<sup>2</sup> and 51.09 cm<sup>2</sup> in 2015 and 2016, respectively) was observed with T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP) treatment, which was statistically on par (45.97 and 45.80 cm<sup>2</sup>) during 2015 while (50.67 and 50.09 cm<sup>2</sup>) in 2016, with T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF+Biocontrol+25% more of recommended SSP) treatments, respectively. The minimum leaf area (39.93 and 43.93 cm<sup>2</sup> during 2015 and 2016, respectively) was recorded in T<sub>1</sub>

(Recommended package of practices), which was notably lower than all other treatments.

### Chlorophyll content

The scrutiny of data given in Table 3 indicates that different treatments exerted significant ( $p < 0.05$ ) influence on the leaf chlorophyll content of both types of planting viz., grafted and *In situ* grafted seedlings during course of investigation. *In situ* grafted peach seedlings raised on replant sick soil recorded highest leaf chlorophyll content (3.78 and 3.83 mg g<sup>-1</sup> during 2015 and 2016, respectively) with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), which was statistically at par (3.57 and 3.51 mg g<sup>-1</sup>) with leaf chlorophyll content obtained in T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF + Biocontrol + 25% more of recommended SSP) treatments, respectively in the year 2015. However, during 2016 the chlorophyll values of 3.53, 3.79 and 3.61 mg g<sup>-1</sup> was obtained in T<sub>3</sub> (SF+ 25% more of recommended SSP), T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) and T<sub>5</sub> (SF + Biocontrol + 25% more of recommended SSP) treatments, respectively, that stands on a

level of equality with T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP). Whereas, the lowest chlorophyll content (3.21 mg g<sup>-1</sup>) was recorded with T<sub>1</sub> (Recommended package of practices) during 2015 and 2016, that was statistically at par (3.27 and 3.35 mg g<sup>-1</sup>) with T<sub>2</sub> (SF + recommended POP) and T<sub>3</sub> (SF + 25% more of recommended SSP), respectively, in both the years of investigation.

Chlorophyll content in the leaves of grafted plants grown in a peach sick soil was also greatly altered by different replant treatments during both the years of study. The highest chlorophyll content of 3.97 and 4.05 mg g<sup>-1</sup> was observed with T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP) during 2015 and 2016, respectively, which was closely followed by T<sub>4</sub> (SF + PGPR + 25% more of recommended SSP) during both the years of study. The lowest leaf chlorophyll content (3.23 and 3.27 mg g<sup>-1</sup> during 2015 and 2016, respectively) was recorded in T<sub>1</sub> (control), which was statistically on par (3.30 and 3.33 mg g<sup>-1</sup>) with T<sub>2</sub> (SF+ recommended POP) and T<sub>3</sub> (SF + 25% more of recommended SSP) treatments, respectively, during 2015.

**Table 3:** Effect of different soil treatments on leaf area and chlorophyll content of peach

| Treatments            | Leaf area (cm <sup>2</sup> ) |       |               |       | Chlorophyll content (mg g <sup>-1</sup> fresh wt) |      |               |      |
|-----------------------|------------------------------|-------|---------------|-------|---|------|---------------|------|
|                       | <i>In situ</i> grafted plant |       | Grafted plant |       | <i>In situ</i> grafted plant                      |      | Grafted plant |      |
|                       | 2015                         | 2016  | 2015          | 2016  | 2015  | 2016 | 2015          | 2016 |
| T <sub>1</sub>        | 38.42                        | 41.87 | 39.93         | 43.93 | 3.21  | 3.21 | 3.23          | 3.27 |
| T <sub>2</sub>        | 39.18                        | 45.05 | 41.88         | 47.05 | 3.27  | 3.43 | 3.30          | 3.39 |
| T <sub>3</sub>        | 39.78                        | 46.79 | 42.57         | 48.39 | 3.35  | 3.53 | 3.33          | 3.59 |
| T <sub>4</sub>        | 42.41                        | 49.37 | 45.97         | 50.67 | 3.57  | 3.79 | 3.65          | 4.00 |
| T <sub>5</sub>        | 41.85                        | 48.51 | 45.80         | 50.09 | 3.51  | 3.61 | 3.52          | 3.95 |
| T <sub>6</sub>        | 43.55                        | 50.43 | 47.71         | 51.09 | 3.78  | 3.83 | 3.97          | 4.05 |
| LSD <sub>(0.05)</sub> | 1.77                         | 1.57  | 2.05          | 1.09  | 0.34  | 0.30 | 0.39          | 0.22 |

### Discussion

In the present study, different replant soil treatments were found to exert significant ( $p < 0.05$ ) influence on tree growth and vigour. Pre-plant fumigation in combination with soil management practices resulted in increased vegetative growth in terms of plant height, stem diameter, number of feathers, number of leaves and leaf area under open field conditions (Tables 1 to 3). The maximum growth and vigour in respect of all these parameters was observed with treatment T<sub>6</sub> (SF + PGPR + Biocontrol + 25% more of recommended SSP), whereas, the minimal plant growth and vigour in control i. e. recommended package of practices, during both the years of study. In general, poor growth under replant situations has been attributed to a variety of microorganisms including nematodes (Mai and Abawi, 1981) [23], bacteria (Doll *et al.* 2008) [12], complexes of fungi (Browne *et al.* 2006) [4] and oomycetes (Mazzola, 1998) belonging to the well-known root rot complex, *Rhizoctonia solani*, *Phytophthora* spp, *Cylindrocarpon* spp. and *Pythium* spp. were also shown to be an important biotic factors of replant problem (Manici *et al.* 2003; Manici and Caputo, 2010; Kelderer *et al.* 2012) [25, 26, 17]. Soil fumigation was reported to be the most effective method of ensuring uniform, vigorous growth (Ross and Meyer, 1975) [32]. Moreover, soil treatment with formaldehyde has been found to be a promising fumigant in overcoming the replant problems of peach and apple (Xue and Yao, 1998; Covey, 1984) [41, 10]. In most cases, soil fumigation increases the growth of fruit trees by 10-100% (Mai *et al.* 1981) [23]. Whatever causal factors have attributed to the replant problem, the soil fumigation could improve the growth of fruit trees and control the replant problem. The

results depicted in Tables 1, 2 & 3 clearly show the positive growth responses to soil fumigation in case of both grafted and *In situ* grafted plants during the course of study. Further, pot-trials on testing and disinfecting soils from both young and old orchards, affected by stone fruit replant disease revealed that soil fumigation or steam sterilization greatly improved top and root growth. Fumigation reduced the number of soil bacteria and increased that of actinomycetes (Hudska, 1977) [16]. Similar, results have been reported by Catska *et al.* (1979) [6] who recorded that soil fumigation or steam sterilization improved the micro flora composition and produced longer and heavier roots in apple and peach seedlings grown in treated soil from their respective orchards than in untreated soil.

Pre-plant 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) [23, 40]. But this method has many drawbacks like inhibition of beneficial soil micro flora including *Arbuscular mycorrhizal* fungi and increasing environmental pollution (Mazzola and Manici, 2012) [27]. As a result, stunting and poor growth of seedlings have been observed in some experiments with soil fumigation (Kleinschidt, 1972; Lambert, 1979) [19, 21], thereby, plant growth promoting rhizobacteria and *Trichoderma* treatments may contribute to increased plant growth through improvement of soil physical, chemical and biological properties which provide better environment for nutrient uptake and translocation by the plants as reported by Yadav *et al.* (2010) [42]. The results also signify that combination of soil fumigation with formaldehyde together with inoculation of

PGPR (*Bacillus sp*) and Biocontrol (*Trichoderma viride*) supplemented with increased dosage of SSP was found to be an effective measure to control replant problem of peach. Further, the results are in line with Utkhede (1999) [39] who carried out a research work pertaining to potential biological control agents against apple replant problems in soil of the Okanagan valley of British Columbia, Canada. Studies revealed that soil drenching with *Bacillus subtilis* strains significantly increased trunk cross-sectional area of apple seedlings in the ARD soil. Catska and Taube (1994) [5] reported that inoculation of apple seedlings with *Agrobacterium radiobacter* improved the growth of apple plants grown in soil with ARD. Studies on biological control of apple replant disease in British Columbia, Canada, established that the application of strain EBW4 of *Bacillus subtilis* alone was effective in promoting the growth of replanted apple trees. However, this treatment resulted in improved tree growth when applications were combined with fumigation, peat or NPK treatments (Utkhede, 1993) [38]. Similarly, the significant increase in seedling growth parameters of apple for several of the ARD soils in response to the addition of slow release fertilizers, compost and mulch extracts was reported by Schoor (2009) [36].

PGPR induced better root development and plant growth might be due to the production of phytohormones or enzymatic activities, as well as favour the establishment of rhizobial or mycorrhizal symbioses. *In vitro* studies on PGPR-inoculated plant roots have showed that many PGPR may reduce the growth rate of the primary root (Dobbelaere *et al.* 1999) [11], increase the number and/or length of lateral roots (Combes-Meynet *et al.* 2011; Chamam *et al.* 2013) [8, 7], and stimulate root hair elongation *in vitro* (Dobbelaere *et al.* 1999; Contesto *et al.* 2008) [11, 9]. Consequently, the improved uptake of minerals and water, thereby, the growth of the whole plant, increases.

Furthermore, many PGPR have the ability to produce peptide antibiotics. These are oligopeptides that inhibit synthesis of pathogens cell walls, influence membrane structures of cells, and hinders the formation of initiation complex on small subunit of ribosome's (Maksimov *et al.* 2011) [24]. Therefore, antibiosis against pathogenic soil bacteria and fungi appears to be another most obvious mode of growth promotion through PGPR inoculation, yet hormone production, solubilization of mineral organic material, and perhaps complex cell surface properties could play important role. The results are in conformity with Biro *et al.* (1998) [3] who reported reduction in the specific replant disease (SRD) by application of PGPR rhizobacteria on apple seedlings. Out of 65 isolates, 20 showed antagonism against the phytopathogenic microorganism. More than 100% plant growth promotion effect developed as a function of soil types with 12 - 40 strains that were also strongly influenced by the various environmental factors. The positive growth stimulation in the inoculated, steamed and also in the unsterilized 'sick' soils suggests that factors such as the persistence and/or hormone productions other than the antibiosis may be involved in the beneficial effects. Besides, species of *Pseudomonas* (Rumberger *et al.* 2007; McKenry, 1999) [33, 29] and *Bacillus* (Benizri *et al.* 2005) [1] have been reported to be positively associated with the apple and peach replant disease. These enzymes are supposed to degrade the cell wall of various bacterial and fungal plant pathogens and thus providing biological protection of crops from pathogens.

Moreover, the increase in leaf chlorophyll might be result of increased leaf area (Table 3), balanced nutritional

environment in the soil and thus kept iron physiologically active for chlorophyll synthesis in certain plants (El Morshedy, 1997) [13]. The results are in conformity with Godara (1993) [14] who also observed increased chlorophyll content in plants inoculated with *Azotobacter* as compared to un-inoculated peach plants. Plant growth hormones produced by rhizobacterial strains have also been found to increase the nitrogen (N) use efficiency and activities of nitrate reductase and carbonic anhydrase of plants. The higher N utilization by plants being a component of chlorophyll molecule also helps in increased photosynthesis. The increase in photosynthesis, stomatal conductance, transpiration rate and decreased stomatal resistance may be result of increased chlorophyll content, stomatal opening and CO<sub>2</sub> assimilation (Misratia *et al.* 2013) [30].

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