*Biological Forum – An International Journal* **15(5a): 108-115(2023)**

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Effect of Biological Soil Amendments on Plant Growth and Soil Microbial Population in Peach Replant Sick Soil

***Johnson Lakra[1]*, Dharam Paul Sharma[1], Kuruva Mallikarjuna[1] and Shashi Kant Ekka[2]***
*[1]Department of Fruit Science,*
*Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh), India.*
*[2]Department of Horticulture, Allahabad School of Agriculture,*
*Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad (Uttar Pradesh), India.*

*(Corresponding author: Johnson Lakra*)*
*(Received: 29 March 2023; Revised: 21 April 2023; Accepted: 01 May 2023; Published: 15 May 2023)*
*(Published by Research Trend)*

**ABSTRACT: Agriculture is demanding more environmentally safe, sustainable production practices due to the adverse effect of conventional practices on soil biological activity and diversity. Soil rehabilitation and root growth stimulation is also of prime importance in orchards suffering from peach replant disease (PRD). Present study hypothesized that the fumigation, biofumigation, soil microbial inoculants can improve soil microbial activity and feeder root development, thereby having a positive impact on tree growth in newly established orchards, especially PRD sites. Furthermore, the effect of the various treatments on soil microbial community activity was examined, using soil enzyme assays and conventional microbial plate counts. The biofumigaton that performed the best in terms of growth increase were *Brassica* seed meal combination with PGPR. Soil enzyme assays indicated significant changes in soil microbial activity, with fumigated soil showing lower activity. Soils amended with PGPR had higher microbial activity.**

**Keywords:** peach replant disease, biofumigation, PGPR, plant growth, soil viable microorganism, enzyme activity.

## INTRODUCTION

There is a general shift in horticulture towards more environmentally friendly, sustainable production practices. This is mainly due to the adverse effect on soil biological activity and diversity through conventional management practices such as low organic matter input (Grace *et al*., 1994) and routine use of chemicals in pest and weed control. Soil microorganisms are largely responsible for soil properties such as structure and fertility, in terms of nutrient cycling (Lee and Pankhurst 1992). Chemical fumigants applied disturbed the apple replant soil microecological environment to different degrees, including inhibiting the content of the pathogen *Fusarium* sp., reducing soil microbial diversity and the total number of OTUs, and altering the physical and chemical properties of soil (Jiang *et al*., 2022).

In addition, these organisms play a role in pathogen suppression as a result of competition and antagonistic action. Soil microorganisms also synthesize various plant growth regulating compounds (Brown, 1972). Nutrients supplied by BSMs, especially available phosphorus, in fumigated soils determined the post-restoration changes in bacterial community composition (Peng *et al*., 2023). A decrease in soil biodiversity and activity can therefore lead to poor root proliferation and nutrient uptake, ultimately having a negative effect on growth and yield. Therefore, there is now a need for the development of biological soil management systems that will help to rehabilitate agricultural soils. Soil rehabilitation and root growth stimulation is of prime importance in orchards suffering from peach replant disease (PRD). This disorder is associated with poor growth of young apple trees planted on previous peach sites. Although the etiology is still not fully understood, it seems to be mainly a problem of biological origin involving a complex of various soil fungi and bacteria, as well as nematodes in certain areas. Recent studies show that there is a possible shift in the microbial community composition towards pathogens dominating the soil microbial profile (Mazzola, 1998, 1999). PRD has been controlled successfully in most cases by the application of a broad spectrum fumigant, with methyl bromide being the most effective and extensively used;

however, due to its impeding phase-out, alternatives are needed.

Present studies hypothesised that the fumigation and soil microbial inoculants can improve soil microbial activity and feeder root development, thereby having a positive effect on tree growth in newly established orchards, especially PRD site. The benefits derived from adding organic matter to soil are well documented and include increased cation exchange capacity (CEC), nutrient availability and water holding capacity, improved soil structure and nutrient uptake and pathogen suppressive effects (Rabie, 2001). The first objective of this study was to evaluate different fumigants and bio-agents on plant growth parameters of peach in replant orchard. Since the causal factors of PRD are mainly biotic, mechanisms of control can be associated with soil microbial community modification. Therefore, we examined the effect of treatments on soil microbial community activity, using soil enzyme activity assays and conventional microbial plate counts. An attempt was also made to establish whether changes in microbial activity could be used as indicators of tree performance, in terms of growth of young plants.

## MATERIAL AND METHODS

*A. Orchard study sites and experimental design*

Experiments were conducted in newly established commercial orchards in district Sirmaur (30°53.388'N; 77°20.477'E), during the year 2018 to 2019. The orchard site was located at an elevation of 1868 m above mean sea level one of the main peach fruit production regions in the Himachal Pradesh, India; where, summer is moderately hot during May-June while, winter is quite severe during December-February. The experiment was laid out using randomization block design (RBD), comprising of 16 treatments including 3 variants viz. soil fumigation, PGPR, different soil amendments and a control (without any treatment); each with three replications, during the first week of January, 2018.

*B. Treatment application and planting*

At the experimental site, the pits (filled with soil) were drenched with 5 liters of formaldehyde solution (1:9), $H_2O_2$ with silver as well as 3 kg of *Brassica* seed meal. Thereafter, the pits were immediately covered with 25 micron polythene sheet exposed to the sunlight for a period of four weeks prior to planting to avoid leakage of fumes and thereby ensuring the complete, uniform and effective fumigation of pits. After 30 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 fumes from the basin area. After two weeks seedlings were transplanted in the treated basin along with soil ball adhering to the roots. Neem based granular formulation (Azadirachtin 0.15 %), Cow urine formulation were applied one weeks before planting and PGPR (*Bacillus licheniformis*) at the time of planting.

In particular (Table 1), the data on tree growth and vigour characteristics were recorded to study the effect of different replant soil amendments. Observations regarding growth parameters, viz. increase plant height, increase 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 Calipers 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 plant. 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 Israeistam (1979). Microbial count was performed by standard plate count technique (Wollum, 1982) 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). The method used for estimating urease enzyme activity was given by Tabatabai and Bremner (1972), phosphatase enzyme estimation was carried out by method given by Tabatabai and Bremner (1969) and 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). 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 $p<0.05$.

**Table 1: Details of the treatments.**

| Treatment | Treatment details | Time of application |
|---|---|---|
| $T_1$ | Formaldehyde 37% (1:9) | 5-weeks before planting (WBP) |
| $T_2$ | Hydrogen peroxide with silver | One weeks before planting (OWBP) |
| $T_3$ | *Brassica* seed meal (*Brassica juncea*) | 4-weeks before planting (WBP) |
| $T_4$ | Neem based granular formulation (Azadirachtin 0.15 %) | One weeks before planting |
| $T_5$ | Cow urine formulation | One weeks before planting |
| $T_6$ | PGPR (*Bacillus licheniformis*) | At the time of planting |
| $T_7$ | Formaldehyde + Cow urine formulation | 5-WBP + OWBP |
| $T_8$ | Hydrogen peroxide with silver + Cow urine formulation | One weeks before planting |
| $T_9$ | *Brassica* seed meal + Cow urine formulation | 4-WBP + OWBP |
| $T_{10}$ | Neemgranuals + Cow urine formulation | OWBP |
| $T_{11}$ | PGPR (*Bacillus licheniformis*) + Cow urine formulation | At the time of planting + OWBP |
| $T_{12}$ | Formaldehyde + PGPR (*Bacillus licheniformis*) | 5-WBP + At the time of planting |
| $T_{13}$ | Hydrogen peroxide with silver + PGPR (*Bacillus licheniformis*) | OWBP + At the time of planting |
| $T_{14}$ | *Brassica* seed meal + PGPR (*Bacillus licheniformis*) | 4-WBP + At the time of planting |
| $T_{15}$ | Neemgranuals + PGPR (*Bacillus licheniformis*) | OWBP + At the time of planting |
| $T_{16}$ | Control | No treatment |

## RESULTS AND DISCUSSION

*A. Growth traits*

**(i) Plant height.** The reconnaissance of data enumerated in Table 2, reveal that plant height was significantly affected ($p<0.05$) by the different rhizosphere soil treatments during both the years. It is apparent from the table that maximum (19.67 %) increase in plant height was recorded in seedlings raised on replant soil with treatment $T_{14}$ (*Brassica* seed meal + PGPR), which was found on par with $T_9$ (18.56 %), $T_{13}$ (18.36 %) and $T_8$ (17.43 %) treatments. While, significantly minimum with $T_{16}$ (8.52 %) treatment. In the year 2019, the percent increase in plant height $T_9$ (20.41 %), $T_{13}$ (19.93 %) and $T_8$ (19.47 %) treatments, stand on par with maximum (20.99 %) increase in plant height recorded with treatment $T_{14}$ (*Brassica* seed meal + PGPR). Whereas, minimal increase in plant height (8.88 %) were recorded with $T_{16}$ (control). Pooled analysis of data showed similar trend and significantly higher (20.04 %) increased plant height was recorded with $T_{14}$, that was found to be at par with $T_9$ (19.78 %), $T_{13}$ (19.15 %) and $T_{11}$ (17.65 %) treatments. The minimum (8.70 %) was recorded in plants under $T_{16}$ treatment.

**(ii) Stem diameter.** From the close examination of the data presented in Table 2, it is evident that increase in stem diameter of peach seedlings was significantly ($p<0.05$) affected by different soil treatments during both the years of study. During 2018, the maximum (25.83 %) increase in stem diameter was recorded in treatment $T_{14}$ (*Brassica* seed meal + PGPR), which was found at par with $T_9$ (23.28 %) and $T_{13}$ (22.68 %) treatments. While, the minimum (10.94 %) stem diameter was recorded under $T_{16}$ (control). However, in 2019, the maximum (26.36 %) increase in stem diameter was recorded with treatment $T_{14}$, which was found on par with $T_9$ (23.78 %), $T_{13}$ (23.39 %) and $T_5$ (21.99 %) treatment. Whereas, the minimum (11.02 %) increase in stem diameter was recorded with $T_{16}$ (control) treatment. Pooled analysis also showed that the different soil replant treatments had significant effects on the increase stem diameter. Significantly higher (26.09 %) stem diameter was recorded with $T_{14}$, which was found to be at par (23.53 % and 23.04 %) with $T_9$ and $T_{13}$ treatments, respectively. The significantly lower (10.98 %) stem diameter was observed with $T_{16}$ (control), which was on par with $T_1$ (11.37 %) and $T_4$ (13.99 %) treatments.

**(iii) Leaf area.** The perusal of data pertaining to leaf area provides substantiation that plants exhibited great variation ($p<0.05$) in response to different replant treatments during the period of observation as presented in Table 2. During 2018, maximum (51.46 $cm^2$) leaf area was recorded in peach plants raised on replant soil with treatment $T_{14}$ (*Brassica* seed meal + PGPR), which was statistically on par (49.35 $cm^2$) with $T_9$ treatment. While, the minimum (31.23 $cm^2$) leaf area was observed in peach seedling with treatment $T_{16}$ (control) treatment. However, in the year 2019, the leaf area (51.38 $cm^2$) was recorded in $T_{14}$ (*Brassica* seed meal + PGPR) which was statistically on par with replant soil treatment $T_{13}$ (50.04 $cm^2$). Meanwhile, the minimal (36.44 $cm^2$) leaf area was noticed with $T_1$ (Formaldehyde) treatment. Pooled analysis of the data also indicated the significant effects on leaf area of peach plants. The maximum (51.42 $cm^2$) leaf area was observed with $T_{14}$, which was statistically on par (49.28 $cm^2$) with $T_9$ treatment and minimum (34.52 $cm^2$) with $T_{16}$ treatment.

**(iv) Total chlorophyll content.** The scrutiny of data given in Table 2, indicate that different treatments exerted significant ($p$<0.05) influence on the leaf chlorophyll content during course of investigation. During 2018, significantly maximum (3.49 mg $g^{-1}$) leaf chlorophyll content was recorded in replant soil with treatment $T_{14}$ (*Brassica* seed meal + PGPR), which was found on par with leaf chlorophyll content obtained in $T_9$ (3.45 mg $g^{-1}$), $T_8$ (3.44 mg $g^{-1}$) and $T_{11}$ and $T_{13}$ (3.43 mg $g^{-1}$) treatments. While, the minimum leaf chlorophyll content was recorded (3.13 mg $g^{-1}$) in peach seedling with replant treatment $T_{16}$ (control) treatment. However, in the year 2019, the leaf chlorophyll contents with $T_{14}$ (3.51 mg $g^{-1}$), $T_{13}$ (3.45 mg $g^{-1}$), $T_{11}$ (3.39 mg $g^{-1}$) and $T_8$ (3.37 mg $g^{-1}$) were observed, which stood on par with maximum leaf chlorophyll (3.52 mg $g^{-1}$) recorded in $T_9$ (*Brassica* seed meal + Cow urine formulation) treatment. Whereas, minimal (3.14 mg $g^{-1}$) leaf chlorophyll were recorded with $T_{16}$ (control) treatment. Pooled analysis of data showed that maximum (3.50 mg $g^{-1}$) leaf chlorophyll was recorded with $T_{14}$ treatment, which was statistically on par with leaf chlorophyll content obtained with $T_9$ (3.49 mg $g^{-1}$), $T_{13}$ (3.44 mg $g^{-1}$),$T_{11}$ (3.41 mg $g^{-1}$) and $T_8$ (3.41 mg $g^{-1}$) treatments. Whereas, minimum (3.13 mg $g^{-1}$) leaf chlorophyll content was recorded with $T_{16}$ treatment.

**Table 2: Effect of different soil management amendments on plant height, stem diameter, leaf area and total chlorophyll content of replanted peach orchard.**

| Treatments | Plant height (% increase) | | | Stem diameter (% increase) | | | Leaf area ($cm^2$) | | | Total chlorophyll content (mg $g^{-1}$ FW) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | POOLED | 2018 | 2019 | POOLED | 2018 | 2019 | POOLED | 2018 | 2019 | POOLED |
| $T_1$ | 10.84 | 11.34 | 11.09 | 11.82 | 11.92 | 11.87 | 32.66 | 36.44 | 34.55 | 3.17 | 3.19 | 3.18 |
| $T_2$ | 12.12 | 13.19 | 12.65 | 16.04 | 17.99 | 17.01 | 41.22 | 43.68 | 42.45 | 3.24 | 3.29 | 3.27 |
| $T_3$ | 14.38 | 15.36 | 14.87 | 16.27 | 18.12 | 17.20 | 40.96 | 45.21 | 43.08 | 3.31 | 3.35 | 3.33 |
| $T_4$ | 12.12 | 13.03 | 12.58 | 13.23 | 14.75 | 13.99 | 35.97 | 38.68 | 37.32 | 3.21 | 3.16 | 3.18 |
| $T_5$ | 11.56 | 12.59 | 12.07 | 13.86 | 21.99 | 17.93 | 37.11 | 43.83 | 40.47 | 3.29 | 3.25 | 3.27 |
| $T_6$ | 13.67 | 15.01 | 14.34 | 15.68 | 15.02 | 15.35 | 43.03 | 48.61 | 45.82 | 3.30 | 3.33 | 3.32 |
| $T_7$ | 14.32 | 15.22 | 14.77 | 16.74 | 15.67 | 16.21 | 42.31 | 46.04 | 44.18 | 3.25 | 3.29 | 3.27 |
| $T_8$ | 17.43 | 19.47 | 18.45 | 20.79 | 21.56 | 21.18 | 45.86 | 49.10 | 47.48 | 3.44 | 3.37 | 3.41 |
| $T_9$ | 18.56 | 20.41 | 19.78 | 23.28 | 23.78 | 23.53 | 49.35 | 49.21 | 49.28 | 3.45 | 3.52 | 3.49 |
| $T_{10}$ | 9.72 | 10.60 | 10.16 | 18.99 | 19.51 | 19.25 | 42.09 | 46.69 | 44.39 | 3.32 | 3.31 | 3.31 |
| $T_{11}$ | 16.92 | 18.39 | 17.65 | 19.43 | 19.93 | 19.68 | 45.98 | 48.57 | 47.28 | 3.43 | 3.39 | 3.41 |
| $T_{12}$ | 15.07 | 17.68 | 16.38 | 17.91 | 18.81 | 18.36 | 43.78 | 45.70 | 44.74 | 3.33 | 3.31 | 3.32 |
| $T_{13}$ | 18.36 | 19.93 | 19.15 | 22.68 | 23.39 | 23.04 | 46.45 | 50.04 | 48.25 | 3.43 | 3.45 | 3.44 |
| $T_{14}$ | 19.67 | 20.99 | 20.04 | 25.83 | 26.36 | 26.09 | 51.46 | 51.38 | 51.42 | 3.49 | 3.51 | 3.50 |
| $T_{15}$ | 16.68 | 16.72 | 16.70 | 18.18 | 18.62 | 18.40 | 40.47 | 44.33 | 42.40 | 3.30 | 3.34 | 3.32 |
| $T_{16}$ | 8.52 | 8.88 | 8.70 | 10.94 | 11.02 | 10.98 | 31.23 | 37.81 | 34.52 | 3.13 | 3.14 | 3.13 |
| **$CD_{(0.05)}$** | **5.02** | **4.59** | **3.33** | **5.27** | **4.50** | **3.39** | **3.14** | **1.34** | **2.95** | **0.16** | **0.15** | **0.11** |

*B. Soil viable microbial count*

**(i) Bacterial count.** It is evident from the data presented in Table 3, that soil bacteria was significantly affected (p<0.05) by the different rhizosphere soil treatments during the course of analysis. During both the years of study, significantly maximum bacterial count ($118.00\times10^5$ cfu $g^{-1}$ soil and $121.67\times10^5$cfu $g^{-1}$ soil in 2018 and 2019, respectively) was recorded in rhizosphere soil with $T_{14}$ (*Brassica* seed meal + PGPR), which was statistically on a level of equality with $T_9$, $T_{13}$ and $T_{11}$ treatments, during both the years of investigation. However, the minimum count ($90.33\times10^5$cfu $g^{-1}$ soil and $93.33\times10^5$cfu $g^{-1}$ soil) was observed in $T_1$ (Formaldehyde) treatment, during 2018 and 2019, respectively. Pooled data reveal that the maximum ($119.83\times10^5$cfu $g^{-1}$ soil) bacterial count was recorded with $T_{14}$ which was statistically on par with $T_9$ ($116.83\times10^5$cfu $g^{-1}$ soil) and $T_{11}$ ($116.50\times10^5$cfu $g^{-1}$ soil). However, the minimum ($91.83\times10^5$cfu $g^{-1}$ soil) bacterial count was recorded with $T_1$ treatment.

**(ii) Fungal count.** From the perusal of the data enumerated in Table 3, it is clear that different replant treatments had a significant effect (p<0.05) on the accountability of soil fungal during both the years of study. During the year 2018, notably maximum ($20.00\times10^3$cfu $g^{-1}$ soil) fungal count was recorded in rhizosphere soils with treatment $T_{14}$ (*Brassica* seed meal + PGPR), which was found on par with $T_{11}$ (18.33 $\times10^3$cfu $g^{-1}$ soil) and $T_9$ ($17.33\times10^3$cfu $g^{-1}$ soil) treatments. However, the minimum ($8.67\times10^3$cfu $g^{-1}$ soil) fungal count was recorded with $T_1$ (Formaldehyde) treatment. Similar trend was observed during the year 2019, as maximum fungal count ($23.00\times10^3$cfu $g^{-1}$ soil) was recorded in rhizosphere of plants with treatment $T_{14}$, which was found on par with $T_{11}$ ($22.33\times10^3$cfu $g^{-1}$ soil) and $T_9$ ($21.67\times10^3$cfu $g^{-1}$ soil) treatments in count observed. However, the minimum ($10.33\times10^3$cfu $g^{-1}$ soil) count was obtained from plants rhizosphere soil with $T_1$ (Formaldehyde) treatment. Pooled data revealed that the maximum ($21.50\times10^3$cfu $g^{-1}$ soil) fungal count was recorded with $T_{14}$, which was on par with $T_{11}$ ($20.33\times10^3$cfu $g^{-1}$ soil). The minimum ($9.50\times10^3$cfu $g^{-1}$ soil) fungal count was recorded with $T_1$ treatment.

**(iii) Actinomycetes count.** Different peach replant treatments influenced soil actinomycetes count significantly (p<0.05) as evident from the data given in Table 3, during both the years of investigation. In the

year 2018, markedly maximum ($17.33\times10^2$cfu $g^{-1}$ soil) actinomycetes count was recorded in treatment $T_{14}$ (*Brassica* seed meal + PGPR), which stands on a same level of significance to the actinomycetes count recorded under $T_9$ ($16.67\times10^2$cfu $g^{-1}$ soil) and $T_{11}$ ($16.33\times10^2$cfu $g^{-1}$ soil) treatments. Similar trend, in 2019, was recorded where significantly maximum ($20.67\times10^2$cfu $g^{-1}$ soil) actinomycetes count was recorded with $T_{14}$ treatment, that was statistically on a par with $T_{11}$ ($19.33\times10^2$cfu $g^{-1}$ soil) and $T_9$ ($18.33\times10^2$ cfu $g^{-1}$ soil) treatments. However, the minimum count ($4.00\times10^2$cfu $g^{-1}$ soil and $5.00\times10^2$cfu $g^{-1}$ soil in 2018 and 2019, respectively) was recorded with $T_1$ treatment. Almost similar trend was also followed in pooled data where the maximum ($19.00\times10^2$cfu $g^{-1}$ soil) actinomycetes count was recorded with $T_{14,}$ which was on par with $T_{11}$ ($17.83\times10^2$cfu $g^{-1}$ soil) and $T_9$ ($17.50\times10^2$cfu $g^{-1}$ soil) treatments. The minimum ($4.50\times10^2$cfu $g^{-1}$ soil) count was recorded with $T_1$ treatment.

**Table 3: Effect of different soil management amendments on total viable microbial count in replanted peach rhizosphere.**

| Treatments | Bacterial count ($10^5$cfug$^{-1}$ soil) | | | Fungal count ($10^3$cfu g$^{-1}$ soil) | | | Actinomycetes count ($10^2$cfu g$^{-1}$ soil) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2018 | 2019 | Pooled | 2018 | 2019 | Pooled | 2018 | 2019 | Pooled |
| $T_1$ | 90.33 | 93.33 | 91.83 | 8.67 | 10.33 | 9.50 | 4.00 | 5.00 | 4.50 |
| $T_2$ | 95.33 | 99.33 | 97.33 | 12.00 | 14.00 | 13.00 | 7.00 | 9.00 | 8.00 |
| $T_3$ | 94.67 | 99.00 | 96.83 | 13.00 | 15.00 | 14.00 | 8.00 | 9.33 | 8.67 |
| $T_4$ | 91.33 | 95.33 | 93.33 | 10.67 | 12.00 | 11.33 | 5.00 | 6.67 | 5.83 |
| $T_5$ | 93.00 | 95.33 | 94.17 | 11.00 | 13.00 | 12.00 | 6.00 | 7.00 | 6.50 |
| $T_6$ | 92.33 | 98.67 | 95.50 | 11.67 | 12.00 | 11.83 | 6.33 | 8.33 | 7.33 |
| $T_7$ | 96.00 | 101.00 | 98.50 | 12.67 | 13.33 | 13.00 | 9.00 | 11.67 | 10.33 |
| $T_8$ | 97.67 | 101.67 | 99.67 | 16.33 | 17.33 | 16.83 | 11.33 | 13.33 | 12.33 |
| $T_9$ | 115.00 | 118.67 | 116.83 | 17.33 | 21.67 | 19.50 | 16.67 | 18.33 | 17.50 |
| $T_{10}$ | 99.00 | 107.00 | 103.00 | 14.00 | 17.67 | 15.83 | 12.00 | 13.67 | 12.83 |
| $T_{11}$ | 113.33 | 119.67 | 116.50 | 18.33 | 22.33 | 20.33 | 16.33 | 19.33 | 17.83 |
| $T_{12}$ | 91.67 | 97.67 | 94.67 | 13.00 | 17.33 | 15.17 | 10.33 | 11.67 | 11.00 |
| $T_{13}$ | 114.00 | 118.00 | 116.00 | 15.33 | 20.00 | 17.67 | 14.33 | 15.67 | 15.00 |
| $T_{14}$ | 118.00 | 121.67 | 119.83 | 20.00 | 23.00 | 21.50 | 17.33 | 20.67 | 19.00 |
| $T_{15}$ | 97.33 | 103.33 | 100.33 | 13.00 | 15.00 | 14.00 | 11.00 | 11.67 | 11.33 |
| $T_{16}$ | 91.33 | 95.00 | 93.17 | 9.00 | 11.33 | 10.17 | 5.00 | 5.67 | 5.33 |
| **$CD_{(0.05)}$** | **5.25** | **5.78** | **3.82** | **2.99** | **2.36** | **1.86** | **2.64** | **2.71** | **1.85** |

*C. Soil enzymatic activities*

**(i) Urease activity.** Different peach replant treatments influenced soil urease activity significantly ($p<0.05$) as evident from the data given in Table 4, during both the years of investigation. In the year 2018, markedly maximum (28.38 µg urea $g^{-1}$ soil $h^{-1}$) urease activity was recorded in treatment $T_{14}$ (*Brassica* seed meal + PGPR) which was closely (27.56 µg urea $g^{-1}$ soil $h^{-1}$) followed by $T_9$ treatment. On the contrary, least urease activity (13.91 µg urea $g^{-1}$ soil $h^{-1}$) was obtained in rhizosphere soil with treatment $T_{16}$ (control) treatment. Similar trend was observed during the year 2019, as treatment $T_{14}$, resulted in maximum (28.32 µg urea $g^{-1}$ soil $h^{-1}$) urease activity, which stood at an equality in value (27.55 µg urea $g^{-1}$ soil $h^{-1}$) obtained with $T_9$ treatment. The minimum (13.68 µg urea $g^{-1}$ soil $h^{-1}$) urease activity was recorded in $T_{16}$ (control) treatment. Pooled data revealed that maximum (28.35 µg urea $g^{-1}$ soil $h^{-1}$) urease activity in soil was recorded with $T_{14}$ treatment, statistically superior to all other replant soil treatments. The minimum (13.79 µg urea $g^{-1}$ soil $h^{-1}$) urease activity was recorded with $T_{16}$ treatment.

**(ii) Dehydrogenase activity.** It is evident from the data presented in Table 4, that dehydrogenase activity was significantly affected ($p<0.05$) by the different rhizosphere soil treatments during both the years of study. During the year 2018, significantly maximum dehydrogenase activity (22.30 µg TPF $g^{-1}$ soil $h^{-1}$) was recorded in rhizosphere soil with treatment $T_{14}$ (*Brassica* seed meal + PGPR), which was found on par (21.33 and 21.06 µg TPF $g^{-1}$ soil $h^{-1}$) with dehydrogenase activity observed with $T_9$ and $T_{13}$ treatments, respectively. However, the minimum dehydrogenase activity (14.18 µg TPF $g^{-1}$ soil $h^{-1}$) was observed in $T_{16}$ (control) treatment. Similarly, in the year 2019, maximum dehydrogenase activity (23.17 µg TPF $g^{-1}$ soil $h^{-1}$) was recorded in rhizosphere of replanted peach plants with treatment $T_{14}$, which was found statistically similar with $T_9$ (22.72 µg TPF $g^{-1}$ soil $h^{-1}$) treatment. Whereas, minimum (14.91 µg TPF $g^{-1}$ soil $h^{-1}$) in rhizosphere of plants raised on replant soil with $T_1$ (Formaldehyde) treatment. Pooled data reveal that the maximum (22.73 µg TPF $g^{-1}$ soil $h^{-1}$) dehydrogenase activity was recorded with $T_{14}$, which was found on par with $T_9$ (22.03 µg TPF $g^{-1}$ soil $h^{-1}$) and the minimum (14.57 µg TPF $g^{-1}$ soil $h^{-1}$) dehydrogenase activity was recorded with $T_{16}$ treatment.

**(iii) Phosphatase activity.** The data enumerated in Table 4, unveil that different replant treatments had a significant effect ($p<0.05$) on the activity of soil

phosphatase enzyme during both the years of study. During the year 2018, notably maximum (90.92 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) phosphatase activity was recorded in rhizosphere soil with treatment $T_{14}$ (*Brassica* seed meal + PGPR) which was statistically on par (90.77 and 89.74 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) with $T_{13}$ and $T_9$ treatments, respectively. However, the minimum (65.44 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) phosphatase activity was recorded in $T_{16}$ (control) treatment. In the year 2019, maximum (91.09 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) phosphatase activity was recorded in rhizosphere of plants with treatment $T_{14}$, which was statistically on par (90.79 and 90.49 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) with phosphatase activity observed under $T_9$ and $T_{13}$ treatments, respectively. However, the minimum (65.61 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) phosphatase activity was found in rhizosphere soil with $T_{16}$ (control) treatment. Pooled data revealed that maximum (91.01 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) phosphatase activity was recorded with $T_{14}$, which was on par with $T_{13}$ (90.63 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$). The minimum (65.53 µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) phosphatase activity was recorded with $T_{16}$ treatment.

**Table 4: Effect of different soil management amendments on enzymatic activity in rhizosphere of replanted peach.**

| Treatments | Urease activity (µg urea $g^{-1}$ soil $h^{-1}$) | | | Dehydrogenase activity (µg TPF $g^{-1}$ soil $h^{-1}$) | | | Phosphatase activity (µmole p-nitrophenol $g^{-1}$ soil $h^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **2018** | **2019** | **Pooled** | **2018** | **2019** | **Pooled** | **2018** | **2019** | **Pooled** |
| $T_1$ | 16.04 | 15.27 | 15.66 | 14.84 | 14.91 | 14.88 | 68.11 | 67.48 | 67.80 |
| $T_2$ | 20.93 | 20.91 | 20.92 | 15.96 | 16.67 | 16.32 | 76.10 | 75.62 | 75.86 |
| $T_3$ | 21.43 | 21.30 | 21.36 | 17.10 | 16.73 | 16.92 | 76.64 | 77.67 | 77.16 |
| $T_4$ | 16.68 | 16.60 | 16.64 | 15.18 | 15.15 | 15.17 | 69.58 | 69.58 | 69.58 |
| $T_5$ | 19.84 | 19.87 | 19.85 | 15.08 | 15.14 | 15.11 | 70.97 | 71.35 | 71.16 |
| $T_6$ | 21.62 | 20.59 | 21.11 | 16.60 | 16.00 | 16.30 | 75.58 | 75.18 | 75.38 |
| $T_7$ | 21.48 | 21.68 | 21.58 | 16.86 | 17.81 | 17.34 | 78.37 | 78.12 | 78.25 |
| $T_8$ | 25.94 | 26.17 | 26.06 | 19.80 | 19.84 | 19.82 | 84.96 | 85.24 | 85.10 |
| $T_9$ | 27.56 | 27.55 | 27.56 | 21.33 | 22.72 | 22.03 | 89.74 | 90.79 | 90.26 |
| $T_{10}$ | 23.57 | 23.65 | 23.61 | 18.31 | 18.91 | 18.61 | 82.38 | 82.27 | 82.33 |
| $T_{11}$ | 25.34 | 24.97 | 25.16 | 18.67 | 19.64 | 19.16 | 82.83 | 82.83 | 82.83 |
| $T_{12}$ | 21.72 | 22.30 | 22.01 | 18.33 | 18.75 | 18.54 | 80.18 | 80.18 | 80.18 |
| $T_{13}$ | 25.19 | 25.58 | 25.39 | 21.06 | 20.54 | 20.80 | 90.77 | 90.49 | 90.63 |
| $T_{14}$ | 28.38 | 28.32 | 28.35 | 22.30 | 23.17 | 22.73 | 90.92 | 91.09 | 91.01 |
| $T_{15}$ | 22.84 | 22.98 | 22.91 | 17.65 | 19.33 | 18.49 | 81.07 | 81.07 | 81.07 |
| $T_{16}$ | 13.91 | 13.68 | 13.79 | 14.18 | 14.96 | 14.57 | 65.44 | 65.61 | 65.53 |
| $CD_{(0.05)}$ | **0.94** | **1.22** | **0.75** | **1.26** | **0.87** | **0.75** | **1.24** | **0.67** | **0.69** |

In the present study, different replant soil management amendments were found to exert significant ($p$<0.05) influence on tree growth and vigour. Pre-plant fumigation in combination with PGPR as well as soil management practices resulted in increased vegetative growth in terms of plant height, stem diameter, leaf area and leaf chlorophyll content under open field conditions (Tables 2). The maximum growth and vigour in respect of all these parameters was observed with treatment $T_{14}$ (*Brassica* seed meal + PGPR), whereas, the minimal plant growth and vigour in control i.e. without any treatments, during both the years of study. Soil fumigation and PGPR were suggested to be the most efficient method of ensuring uniform, vigorous growth (Singh *et al.,* 2018 in apple and Thakur *et al.* (2018) in peach. Biofumigation significantly increased the growth of plants compared to all other treatments under field conditions. Confirmed reports of other studies with different horticultural and agricultural crops (Mattner *et al.,* 2008; Mazzola *et al.,* 2007, 2015). Similar, results have been reported by Catska *et al.* (1979) 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.

Further, the results are in line with Utkhede (1999) 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.

Seed meals of plants in the Brassicaceae contain high levels of glucosinolates, and reduce populations of replant-associated fungi and nematodes, and improved apple seedling growth (Mazzola *et al*., 2007; Mazzola and Mullinix, 2005). Mazzola *et al.,* (2007) demonstrated that suppressive effects of the seed meals did not correlate with glucosinolate levels, and effects can be variety and pathogen specific. In some cases it appears that *Brassica* seed meals can enhance certain populations of beneficial soil microbes and there by trigger pathogen suppression that is more durable than

that which would be expected by a biofumigant effect alone.

The application of the PGPR registered a significant increase in total microbial population (Table 3). Their abundance in rhizosphere gives an indication of their possible role in decomposition of organic matter, fixation of atmospheric nitrogen, phosphate solubilization and transformations of nutrient elements. The results are in accordance with the work of Cakmacki *et al.* (2007); Aseri *et al.* (2008); Raj and Sharma (2009); Seo *et al.* (2009); Pesakovic *et al.* (2013) who reported increased rhizobacterial population with PGPR inoculation.

Soil enzymes can be considered a key tool for assessing soil quality, involved in the main geochemical processes of plant nutrients. Therefore, their activity in soil can be attractive alone as a measure of soil health (Dick, 1994). The present investigation is in agreement with the findings of Singh and Sharma (2017); Thakur and Sharma (2018) who reported maximum enzymatic activity in combinations of Soil fumigation + PGPR + Biocontrol under replant situations on apple and peach seedlings. Soil application of seed meals, as soil organic amendments, could represent an alternative to mineral fertilizers and the presence of glucosinolates in the seed meals of Brassicaceae, an alternative to chemical pesticides and a source of organic matter capable of stimulating soil biological activity. Many studies have investigated the effects of classic organic amendments on nutrient availability and on soil enzymatic activities (Fernández *et al*., 2009; Lahkdar *et al*., 2010; Panda *et al.,* 2009).

## CONCLUSIONS

from present investigations it can be concluded that combined treatment (*Brassica* seed meal+PGPR) wasmost effective to record positive influence of plant growth traits, total viable microbial count and soil enzymatic activities on the peach plants grown under replant sick soil in open field conditions.

## FUTURE SCOPE

There are much more ecofriendly treatments were used for the sustained the environment and further there are much work remain to be completed and many reaction possibilities that can be used in improving this research.

**Acknowledgements.** Authors are highly thankful to the facilities and funds provided by Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan 173 230 (H.P.). This investigation was also financially supported (Fellowship - NFST) by Ministry of Tribal Affairs, Govt. of India.

**Conflict of Interest.** None.

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**How to cite this article:** Johnson Lakra, Dharam Paul Sharma, Kuruva Mallikarjuna and Shashi Kant Ekka (2023). Effect of Biological Soil Amendments on Plant Growth and Soil Microbial Population in Peach Replant Sick Soil. *Biological Forum – An International Journal*, *15*(5a): 108-115.