Soil agro-techniques and rootstocks for controlling replant problem in apple orchard

Niranjan Singh and DP Sharma

Abstract

Apple orchards planted in late sixties in Himanchal Pradesh and North Western Himalayan region have shown symptoms of declining productivity as these plants have completed their economic life span. Due to limited land and choice of crops for smaller micro climatic niches and incomparable economic equivalence of other fruits with apple, orchardists are compelled to replant old apple orchard sites. There has been substantial increase in the proportion of declining orchards which need to be changed. Therefore, standardization of suitable agro-techniques to combat replant problem in apple for better field survival rate and productivity under replant conditions for sustainability of apple industry in the state. In present study there were 20 treatments comprising of four apple rootstocks i.e. Seedling, M.793, MM.111 and M.7 and five different treatments i.e. control, soil fumigation, PGPR, biocontrol and combined (Soil fumigation + PGPR + Biocontrol) with three replications. The pooled data over the years 2015 and 2016 revealed that M.793 rootstock had significantly maximum plant growth and vigour parameters, soil enzymatic activities and microbial counts. Among the treatments, highest growth and vigour parameters, soil enzymatic activities and microbial counts were recorded maximum in combined treatment. The interaction between rootstocks and treatments revealed that combinations of M.793 × combined treatment recorded maximum growth and vigour traits dehydrogenase activity, urease activity, phosphatase activity, phytase activity, bacterial counts, fungal counts and actinomycetes counts compared to other rootstocks and treatment combinations under replant situations, which can be exploited for the management of replant problem in apple.

Keywords: apple, biomass production, enzyme, microbial, pgpr, replant soil, rootstocks, Trichoderma (biocontrol)

1. Introduction

Apple (Malus × domestica Borkh.) is mainly grown in north Western Himalayan region which include states of Jammu and Kashmir, Himachal Pradesh, Utrakhand, North Eastern hilly states and south Nilgiri hills in India. It is grown over an area of 277 (in ‘000 ha) with annual production of 2242 (in ‘000 MT) and productivity of 8.0 MT (NHB, 2016) [1]. Apple orchards planted in early sixties have shown symptoms of declining productivity as these plants have completed their economic life span. With increasing population and adverse environmental factors the land resource is shirking. Due to limited land resources and choice of crops for diversification in hill states, orchardists are compelled to replant old apple orchard sites with apple, 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 apple orchards is commonly observed.

Apple replant disease (ARD) is a complex syndrome that occurs in young apple trees in replanted orchard sites (Mai and Abawi, 1981) [2]. Apple replant problem, though reported in the literature for more than century, has yet to have its causes clearly defined. Decline in apple productivity has been attributed to fungi, bacteria, nematodes, toxic agents, insect-pests, nutritional disturbances and chemical residues (Benizri et al., 2005) [3]. The reasons for low productivity could be many but one of the most important reasons is age of orchards. In general, apple orchards of more than 40-50 years age have shown much more unfruitfulness than the young orchards. Most of apple orchards in Himachal Pradesh planted during sixties 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 apple trees planted at sites where apple tree grew before. The situation resulting in this poor growth is generally known as replant problem (Utkhede and Smith, 1994) [4].

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Therefore standardization of agro-techniques with integration of various management tools such as rootstocks, soil sterilization, biocontrol and PGPR is important to combat or reduce apple replant problem in old apple orchards (Haas and Defago, 2005 and Leinfelder and Merwin, 2006) [5, 6]. After several years, trees may recover from the initial growth depression and eventually reach the size and annual yields of unaffected trees (Foy et al., 1996) [7]. Despite this partial recovery, cumulative yields and profitability in ARD-affected orchards usually remain lower than in unaffected orchards (Peterson and Himan, 1994) [8]. There has been substantial increase in the proportion of declining orchards which need to be changed. Therefore, standardization of suitable agro-techniques to combat replant problem in apple for better field survival rate and productivity under replant conditions for sustainability of apple industry in the state.

2. Materials and methods

2.1 Location and Climate

The present investigation was carried out on farmer field at an elevation of 2040 m above mean sea level at with location 30° 54'N latitude and 77°19' E longitude near village Habban district of Sirmaur (Himanchal Pradesh) on replanted apple orchard site under rainfed conditions during the year 2015 and 2016. The experimental orchard lies under the temperate, sub-humid mid-hill agro climatic zone III 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.

2.2 Rootstocks establishment

One year old uniform seedling and clonal rootstocks namely seedling, M.793, MM.111 and M.7 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 natural condition, in first week of February, 2014.

2.3 Materials and Treatments

The suitable methodology has been used to understand the response of apple seedlings and clonal rootstocks to replant soil. One year polybag raised four rootstocks i.e. M. 793, MM.111, M.7 and Seedling were planted in declining apple site and pit filled with soil and FYM (3:1) along with soil ball and application of five soil management treatments viz., control, soil fumigation, PGPR, biocontrol and combined (Soil fumigation + PGPR + Biocontrol) in Factorial Randomization Block Design with three replications in first week of January, 2015. These rootstocks were grafted with ‘Super chief’ in March 2015.

2.4 Soil Fumigation and Planting

The pits were drenched with 10 liters of formaldehyde solution (1:9) after filling the pits. The pits were covered with polythene sheet for three weeks to avoid leakage of formaldehyde fumes. After three weeks the polythene sheet was removed and basin soil was worked in such a way to exclude fumes of formaldehyde from the basins. After two weeks polybag raised seedling and different clonal rootstocks.

2.5 Time of application: (PGPR and Trichoderma viride)

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

2.6 Soil Enzyme

2.6.1 Urease

The urease enzyme estimation was carried out by method given by Tabatabai and Brenner, (1969) [9]. Five gram of moist soil was taken in a volumetric flask (50 ml), added 0.2 ml of toluene and 9 ml of urea solution and mixed again for a few seconds. Then stopper the flasks and incubated for 2 hours at 37 °C. After the incubation, added approximately 35 ml of KCl-Ag2SO4 solution, swirled the flask for a few seconds, add allowed the flask to stand unit contents have cooled to room temperature (above 5 minute). Brought up the contents to 50 ml by addition of KCl-Ag2SO4 solution and mixed the contents thoroughly. To perform controls, followed the procedure described for assay of urease activity, but made the addition of 1 ml of 0.2 M urea solution after the addition of 35 ml of KCl-Ag2SO4 solution It is recommended that at least three replications are carried out. The reaction shows a linear time course up to 5 hours.

2.6.2 Phosphatase

The phosphatase enzyme estimation was carried out by method given by Tabatabai and Brenner, (1969) [9]. One gram of soil taken in test tube was incubated with 1ml of 5mM buffered sodium p-nitrophenyl phosphate in acetate buffer (pH 5.2) and 0.5ml toluene at 37 °C for 1 hour. Determination of p-nitrophenol involved the colourimetric analysis of the extract obtained by treating the incubated soil sample with 4 ml water, 10 ml of 0.5 M NaOH and by filtering it through Whatman No. 42 filter paper. The suspension obtained by shaking the mixture for 1 minute and absorbance of yellow colour of p-nitrophenol released was determined spectrophotometrically at 420 µ wavelength. The standard curve was prepared by p-nitrophenol (10-100 ppm). The results were expressed as µ mole of p-nitrophenol released per gram soil per hour (µmole p-nitrophenol g⁻¹soil h⁻¹).

2.6.3 Dehydrogenase

The dehydrogenase enzyme estimation was carried out by method given by Casida et al., (1964) [10]. One gram of air-dried soil taken in air-tight screw capped test tube was incubated with 0.2 ml of 3% 2, 3, 5 – triphenyl tetrazolium chloride (TTC) and 0.5 ml of 1% glucose solution at 28±0.5 °C for 24 h. Added 10 ml of methanol was added and allowed to stand for 6 h. Clear pink coloured supernatant liquid was and readings taken with a spectrophotometer at a wavelength of 485 nm (blue filter). Extrapolated triphenyl formazan (TPF) formed from the standard curve drawn in the range of 10 to 90 mg TPF ml⁻¹. The results were expressed as mg TPF formed per h per g soil.

2.6.4 Phytase

2.6.4.1 Inorganic Phosphate Standard Curve

Phosphate standard solutions were prepared by initially making a 1 ml stock solution of KH2PO4 in water. This was then diluted to prepare a range of phosphate standard concentrations ranging from 200 to 1,000 nmol/ml phosphate. To 1.0 ml of each reference standard was added 1.0 ml of 0.5 M tri-chloroacetic acid (TCA) solution and 1 ml of reagent A (prepared fresh by dissolving 5.0 g of FeSO4.7H2O in 90 ml of distilled water and adding 10.0 ml of 8.0% (w/v) ammonium molybdate. The 8.0% ammonium molybdate solution was prepared by dissolving 8.0 g of ammonium
molybdate in 50 ml of distilled H₂O. Then, 27.0 ml of 10 M H₂SO₄ was added and the volume as made to 100 ml with distilled water. After standing at room temperature for 5 minute, the absorbance of each was determined at 660 nm.

2.7 Detection of rhizosphere microbial counts

Microbial counts was performed by standard plate counts technique. Wollum, (1982) [11] 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, Jensen medium, Jensen, (1987) [12] and Pikovskaya’s medium, Pikovskaya, (1948) [13] with the help of glass spreader under aseptic conditions for enumeration of bacteria, free nitrogen fixing bacteria and phosphate solubilizing bacteria, respectively. Plates were incubated in inverted position at 28±2°C for 48 hours. After the incubation period, the microbial counts was expressed as colony forming unit per gram of soil (cfu/g soil).

2.8 Plant Growth traits

2.8.1 Plant height

The 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).

2.8.2 Number of feathers

Total number of branches per plant were counted in each plant and treatment.

2.8.3 Leaf area

Ten fully expanded mature leaves were taken from each replications of each treatment. The leaf area was measured with the help of portable Laser (CI- 202), CID Bio-Science leaf area meter and average leaf area of each treatment was calculated and expressed as square centimeter (cm²).

2.8.4 Plant volume

The total above ground plant volume of each plant was calculated from the data on height and spread measurements according to the formule suggested by Westwood, (1978) [14] and was expressed in cubic meters (m³).

i)  For a tree that was taller than wide (Prolate Spheroid), 
\[ \text{Volume} = \frac{4}{3} \pi a b^2 \]

ii) For a tree that was wider than tall (Oblate Spheroid), 
\[ \text{Volume} = \frac{4}{3} \pi a^2 b \]

Where,
\[ \pi = 3.14 \]
\[ a = \frac{1}{2} \text{ the major axis (height)} \]
\[ b = \frac{1}{2} \text{ the minor axis (spread)} \]

2.9 Statistical analysis

Data on plant growth and soil biological activities of replanted apple to determine the significance of differences analyzed by using Randomized Block Design (RBD)-two way analysis of variance (ANOVA) as suggested by Gomez and Gomez, (1984) [15]. In addition to show the interrelationships between rootstocks in combination with soil management treatments and mean values of each studied plant growth and physiological parameters statistical analysis program (SPSS) was used.

3. Results and Discussion

3.1 Soil biological (Enzymatic activities and microbial counts)

Plants grafted onto M.793 rootstock had significantly highest dehydrogenase activity (9.61 μ TPF g⁻¹ h⁻¹) in MM.111 and urease activity (40.08 μ mol L⁻¹g⁻¹h⁻¹), phosphatase activity (424.25 μ mol L⁻¹g⁻¹h⁻¹) and phytase activity (5186.33 μ mol L⁻¹g⁻¹h⁻¹) while, minimum in seedling rootstock. Different rootstocks did not show consistent influence on rhizobacterial, fungal and actinomycetes counts during both the years of study (Table 3 and 4). Among the treatments, dehydrogenase activity (12.08 μ TPF g⁻¹ h⁻¹), urease activity (57.22 μ mol L⁻¹g⁻¹h⁻¹), phosphatase activity (451.52 μ mol L⁻¹g⁻¹h⁻¹), phytase activity (5547.96 μ mol L⁻¹g⁻¹h⁻¹), bacterial (117.89 10⁵cfu/g soil), fungal counts (15.21 10⁶cfu/g soil) and actinomycetes counts (57.22 10⁵cfu/g soil) were recorded maximum in combined treatment, compared to other treatments however, minimum in control (Table 1 and 2).

The interaction effect of rootstock and treatment combinations revealed that with M.793 rootstock × combined treatment recorded increased highest urease activity [58.75 μ mol L⁻¹g⁻¹h⁻¹ (Fig. 6)], phosphatase activity [461.57 μ mol L⁻¹g⁻¹h⁻¹ (Fig. 7)], phytase activity [5581.33 μ mol L⁻¹g⁻¹h⁻¹ (Fig. 8)] and dehydrogenase activity [12.53 μ TPF g⁻¹ h⁻¹ (Fig.5)] in MM.111x combined however, minimum in seedling × control. The interaction between rootstocks and treatments was found to be non-significant in respect of rhizobacterial, fungal and actinomycetes counts. Numerically, all other rootstocks registered higher rhizobacterial, fungal and actinomycetes counts with combined treatment combinations figures 9 to 11, respectively.

Present study indicates that the enzyme activities of apple soil was increased with the application of combined treatment (Soil fumigation + PGPR + Trichoderma viride) and M.793 rootstock. Kumar et al., (2014) [16] also reported that the combined application of indigenous PGPR (B. megaterium, A. chlorophenolicus and Enterobacter) significantly increased 17.5%, 79.8%, 78.6% and 26.7% plant height, grain yield, straw yield and test weight under pot condition and also 29.4%, 27.5%, 29.5% and 17.6% under field conditions which supported our results. These findings are also in conformity with those of Jarak et al., (2012) [17] who also reported the ability of Trichoderma viride, Pseudomonas sp., Bacillus sp. and Azotobacter chroococcum strain to enhance maize growth (Zea mays L.) under field conditions. These results are also in line with those obtained by Kaur and Reddy, (2015) [18] who found that the highest yield was obtained by bio-inoculation of treatments singly or together with biofertilizer in maize-wheat cropping system. The results are further supported by the findings of Gaind et al., (2006) [19] who also reported that inoculation of PSB together with rock phosphate fertilizer increased the crop growth parameters (shoot height, shoot and root dry biomass) and grain yield of wheat. Improved enzymatic activities and improved soil phosphorous due to incorporation of compost prepared from paddy straw and fungal inoculants in wheat improved enzymatic activities and phosphorous content of soil. These findings are also in conformity with those of Kaur and Reddy, (2015) [19] who also reported that inoculation of PSB together with rock phosphate fertilizer increased the crop growth parameters (shoot height, shoot and root dry biomass) and grain yield of wheat. Improved enzymatic activities and improved soil phosphorous due to incorporation of compost prepared from paddy straw and fungal inoculants under wheat have been reported by Gaind et al., (2006) [19] which supported our findings. Higher enzyme activities in soil indicate the potential of soil to effect
the biochemical transformations necessary for the maintenance of soil fertility. Rao et al., (1990) [20]. The results of present study are in agreement with the observations of Seo et al., (2010) [21], Jarak et al., (2012) [17] and Pesakovic et al., (2013) [22] who reported increased microbial population with bacterial inoculation in strawberry. The findings are in line with the work of Aseri et al., (2008) [23] and Raj and Sharma, (2009) [24] 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.

There are positive correlations between soil enzyme activity and soil microbe quantity, microbe diversity, microbe biomass, and soil animal numbers (Groffman et al., 2001 [25], Taylor et al., 2002 [26] and Bandick and Dick, 1999) [27]. The soil enzymes are important components of soil, and soil enzymatic activities are correlated significantly with the soil fertility and efficiency of nutrition to plants. They are important indexes for determining the biological activity and productivity of soil (Tuyler, 1974) [28]. Generally, Pb²⁺ can directly interact with the active functional sites of the enzymes, and change their spatial conformation. The activities of urease appears to be more sensitive to pollution than that of other soil enzymes. The soil enzymatic activities in the planted group increased significantly than those of the control group. When a heavy metal replace the active functional sites of an enzyme by combining with their mercapto, amino, or carboxyl, the enzymatic activity inhibition would occur, called enzymatic passivation (Zhou, 1995) [29]. The oligotrophic microbes are very sensitive to metals, which indicated that the growth of microbial community is considerably limited in soils, poor in organic matter and nutrient content (Brookes, 1995) [30]. Further, it is considered that heavy metals mainly inhibit enzymatic reactions through either their complexing with substrate or blocking the functional groups of enzymes or reacting with complex enzyme-substrate (Speir et al., 1995) [31]. Inoculation with AM fungi enrich soil microbe quantities, equilibrate proportion of various microbes, maintain a stabilization of proper proportion of the microbes, enhance soil carbon, nitrogen, and phosphorous cycling power, thus improve the soil enzyme activity (Zhao et al., 2010) [32].

Table 1: Effect of treatments on soil enzyme activity and soil biological activity in replanted apple

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dehydrogenase activity (µ TPF g⁻¹ h⁻¹)</th>
<th>Urease activity (µ mol L⁻¹ g⁻¹ h⁻¹)</th>
<th>Phosphatase activity (µ mol L⁻¹ g⁻¹ h⁻¹)</th>
<th>Phytase activity (µ mol L⁻¹ g⁻¹ h⁻¹)</th>
<th>Bacterial count (10⁶ cfu/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.23⁺</td>
<td>24.29⁺</td>
<td>374.79⁺</td>
<td>4837.29⁺</td>
<td>97.76⁺</td>
</tr>
<tr>
<td>Soil fumigation</td>
<td>7.49⁺</td>
<td>24.61⁺</td>
<td>387.77⁺</td>
<td>4874.33⁺</td>
<td>97.19⁺</td>
</tr>
<tr>
<td>PGPR</td>
<td>9.98⁺</td>
<td>44.82⁺</td>
<td>444.79⁺</td>
<td>5187.96⁺</td>
<td>113.44⁺</td>
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<tr>
<td>Biocntrol</td>
<td>9.72⁺</td>
<td>42.39⁺</td>
<td>402.02⁺</td>
<td>5099.08⁺</td>
<td>105.94⁺</td>
</tr>
<tr>
<td>Combined</td>
<td>12.08⁺</td>
<td>57.22⁺</td>
<td>451.52⁺</td>
<td>5547.96⁺</td>
<td>117.89⁺</td>
</tr>
</tbody>
</table>

Degree of significance of P≤0.05

Table 2: Effect of treatments on soil biological activity and plant growth traits in replanted apple

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fungal count (10⁶ cfu/g soil)</th>
<th>Actinomycetes count (10⁶ cfu/g soil)</th>
<th>Plant height</th>
<th>Leaf area</th>
<th>Number of feathers</th>
<th>Plant volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.20⁺</td>
<td>24.29⁺</td>
<td>137.11⁺</td>
<td>32.64⁺</td>
<td>1.95⁺</td>
<td>2.63⁺</td>
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<tr>
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<td>24.61⁺</td>
<td>144.25⁺</td>
<td>33.05⁺</td>
<td>2.12⁺</td>
<td>3.17⁺</td>
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<tr>
<td>PGPR</td>
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<td>44.82⁺</td>
<td>162.66⁺</td>
<td>38.74⁺</td>
<td>2.63⁺</td>
<td>6.75⁺</td>
</tr>
<tr>
<td>Biocntrol</td>
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<td>42.39⁺</td>
<td>156.55⁺</td>
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<td>2.82⁺</td>
<td>5.94⁺</td>
</tr>
<tr>
<td>Combined</td>
<td>15.21⁺</td>
<td>57.22⁺</td>
<td>201.30⁺</td>
<td>44.69⁺</td>
<td>3.57⁺</td>
<td>12.03⁺</td>
</tr>
</tbody>
</table>

Degree of significance of P ≤ 0.05

3.2 Plant Growth traits

In the present study, different rootstocks and soil management treatments recorded significant increase in plant growth and physiological parameters. Among the rootstocks, M.793 rootstock had significantly higher increase in plant height (170.94 cm), number of feathers (2.83), leaf area (39.03 cm²) and plant volume (7.31 m³) however, minimum in seedling rootstock (Table 3). Among the treatments, plant height (201.30 cm), number of feathers (3.57), leaf area (44.69 cm²) and plant volume (12.03 m³) were recorded maximum in combined treatment, compared to other treatments however, minimum in control (Table 1). Among the rootstocks and treatment combinations of combined treatment with M.793 rootstock recorded maximum plant height (288.52 cm), number of feathers (4.06), leaf area (47.12 cm²) and plant volume (15.18 m³) compared to other rootstock and soil treatment combinations given in figures 1 to 4, respectively. Similar results were obtained by (Thakur, 2017 [33], Misratta et al., 2013 [34], Tukey et al., 1962 [35], Fallahi et al., 2002 [36], Chandel and Chauhan, 1992 [37]) which supported the results obtained in the present study. Seedling rootstock was found to be more sensitive to replant problem because of their susceptibility to soil borne disease in particular. In general, replant sites have more pathogens, thereby, directly affecting the plant growth and development of new saplings. Comparatively, the clonal rootstocks (M.793, MM.111 and M.7) have been reported to be more tolerant to soil borne diseases (Andreev, 1984 [38] and Kviklys et al., 2007 [39]) and have more biomass of adventitious roots. Production of plant growth regulators such as auxin, gibberellins and cytokinins by the plant growth promoting rhizobacteria has been suggested as possible mechanisms of action affecting plant growth. The findings are in line with reports of (Thakur, 2017 [33], Ferree and Warrington, 2003 [40], Rana and Chandel, 2003 [41], Karlidag et al., 2007 [42], Kirad et al., 2009 [43], Tripathi et al., 2014 [44], Kipkoriony and Fusao, 2006 [45]) who also recorded increased plant height and spread with the application of plant growth promoting rhizobacteria and Trichoderma viride.

Rumberger et al., (2004) [46] reported that apple rootstock genotype had a stronger effect on the rhizosphere soil microbial community composition than did the pre-plant soil treatments in soils. We found that 2 years later, rhizosphere communities of bacteria, fungi, and actinomycetes still clustered roughly together by rootstock genotype (Fig. 8 to 10, respectively). Plant species specific rhizosphere microbial communities have been reported widely (Marschner et al., 2001 [47], Miething et al., 2000 [48], Westover et al., 1997 [49])
as have changes in rhizosphere microbial communities due to intra-specific variation (Carelli et al., 2000 [50], Cattelan et al., 1998 [51], Di Giovanni et al., 1999 [52]). In our experiment, the same scion variety (‘Super chief’) was grafted onto four different apple rootstocks. The rhizosphere of M.793 had the highest culture able soil bacteria counts compared with the other rootstocks, and this rootstock also produced the highest plant growth during 2015 however, 2016 in M.7 least plant growth.

In our experiment, rootstocks strongly affected rhizosphere microbial community composition (Fig. 8 to 10, respectively). This suggests that rhizosphere fungi and bacteria communities may be more influential in the promulgation or suppression of ARD than bacteria and oomycetes at this site. These findings are similar to those of Mazzola that also implicated the involvement of fungi and pseudomonads in ARD (Gu and Mazzola, 2003 [53], Mazzola, 1997 1998 [54]). Rootstocks were not only a main factor contributing to observed changes microbial composition in the rhizosphere, but were also a dominant factor for tree growth and yield. Rootstock genotype selection is thus a promising alternative for managing ARD (Shengrui et al., 2006) [55].

Fig 1: Effect of different rootstocks and treatments on plant height (1), leaf area (2), number of feathers (3), plant volume (4), dehydrogenase activity (5) and urease activity (6) of replanted apple. Vertical bar represent mean of three replication ± SE m and LSD (p ≤ 0.05)
Fig 2: Effect of different rootstocks and treatments phosphatase activity (7), phytase activity (8), bacterial count (9), fungal count (10) and actinomycetes count (11) of replanted apple. Vertical bar represent mean of three replication ± SE m and LSD (p≤0.05)

Fig 3: (a) Old orchard (b) Uprooting of old plants (c) After soil fumigation (d) Planting of rootstock at Habban
Fig 4: Two year old plants after treatment in apple replanted field, (a) M.793 (b) MM.111 (c) M.7 (d) Seedling
Table 3: Effect of rootstocks on soil enzyme activity and soil biological activity in replanted apple

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dehydrogenase activity (µ TPF +h⁻¹)</th>
<th>Urease activity (µ mol L⁻¹·g⁻¹·h⁻¹)</th>
<th>Phosphatase activity (µ mol L⁻¹·g⁻¹·h⁻¹)</th>
<th>Phytase activity (µ mol L⁻¹·g⁻¹·h⁻¹)</th>
<th>Bacterial count (10⁵·cfu/g soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling</td>
<td>9.15⁺</td>
<td>36.96²</td>
<td>401.13²</td>
<td>3509.03²</td>
<td>106.24</td>
</tr>
<tr>
<td>M.793</td>
<td>9.38ᵇ</td>
<td>40.08ᵃ</td>
<td>424.25ᵃ</td>
<td>3186.3³</td>
<td>106.84</td>
</tr>
<tr>
<td>M.111</td>
<td>9.61ᵃ</td>
<td>39.31ᵇ</td>
<td>414.23ᵇ</td>
<td>3176.7⁰</td>
<td>106.00</td>
</tr>
<tr>
<td>M.7</td>
<td>9.06ᵇ</td>
<td>38.32ᵃ</td>
<td>409.1⁰</td>
<td>3075.2³</td>
<td>106.70</td>
</tr>
</tbody>
</table>

Degree of significance of P ≤ 0.05

Table 4: Effect of rootstocks soil biological activity and plant growth traits on in replanted apple

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fungal count (10⁵·cfu/g soil)</th>
<th>Actinomycetes count (10⁵·cfu/g soil)</th>
<th>Plant Height</th>
<th>Leaf area</th>
<th>Number of feathers</th>
<th>Plant volume</th>
</tr>
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<tbody>
<tr>
<td>Seedling</td>
<td>13.32</td>
<td>13.61</td>
<td>150.95⁰</td>
<td>35.60⁰</td>
<td>2.4²⁰</td>
<td>5.1²⁰</td>
</tr>
<tr>
<td>M.793</td>
<td>13.91</td>
<td>13.01</td>
<td>170.94⁰</td>
<td>39.03⁰</td>
<td>2.8³⁰</td>
<td>7.3¹⁰</td>
</tr>
<tr>
<td>M.111</td>
<td>14.30</td>
<td>13.03</td>
<td>164.12³</td>
<td>38.21²</td>
<td>2.6⁶⁰</td>
<td>6.3⁸⁰</td>
</tr>
<tr>
<td>M.7</td>
<td>14.30</td>
<td>13.11</td>
<td>155.5⁰</td>
<td>36.6⁴⁰</td>
<td>2.5⁶⁰</td>
<td>5.6⁶⁰</td>
</tr>
</tbody>
</table>

Degree of significance of P ≤ 0.05

4. Conclusion
From the present investigation it can be concluded that M.793 is most suited apple rootstock for replantation. Further, it is also inferred that combined treatment (Soil fumigation +PGPR +Biocontrol) is an appropriate soil agro-technique to mitigate replantation problem in apple. The consortium of M.793 rootstock and combined treatment recorded significant increase in plant growth and soil biological activates in the apple under replanted site.

5. Acknowledgements
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