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Managing apple replant disease: the effect of rootstocks and soil treatments on tree performance and biological activities

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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 data over the years 2015 and 2016 reveled that M.793 rootstock had significantly maximum plant growth and vigour parameters and microbial counts. Among the treatments, highest growth and vigour parameters and microbial counts were recorded maximum in combined treatment. The interaction between rootstocks and treatments revealed that combinations of M.793 × combined treatment recorded plant growth and vigour traits bacterial counts, fungal counts and actinomycetes counts compared to other rootstocks and treatment combinations under replant situations.

Keywords: apple, microbial, PGPR, replant soil, rootstocks, (biocontrol)

1. Introduction

Apple (*Malus* × *domestica* Borkh.) is mainly grown in north Western Himalayan region which include states of Jammu and Kashmir, Himachal Pradesh, Uttrakhand, 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, enzymatic activates 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]. 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 [5] and Leinfelder and Merwin, 2006) [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 Hinman, 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 (No treatment), soil fumigation (with formaldehyde), PGPR (*Bacillus licheniformis* CK-1), biocontrol (*Trichoderma viride*) 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: (*Bacillus licheniformis* CK-1 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 Detection of rhizosphere microbial counts

Microbial counts was performed by standard plate counts technique, Wollum, (1982) [9] 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) [10] and Pikovskaya's medium, Pikovskaya, (1948) [11] 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$\pm$2°C for 48 hours. After the incubation period, the microbial counts was expressed as colony forming unit per gram of soil (cfug^{-1} soil).

2.7 Plant Growth traits

2.7.1 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).

2.7.2 Number of feathers

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

2.7.3 Leaf area

Ten fully expanded mature leaves were taken from each replication 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 (cm^2).

2.7.4 Plant volume

The total above ground plant volume of each plant was calculated from the data on height and spread measurements according to the formulae suggested by Westwood, (1978) [13] and was expressed in cubic meters (m^3).

i) For a tree that was taller than wide (Prolate Spheroid),

Volume = $4/3\ \pi ab^2$

ii) For a tree that was wider than tall (Oblate Spheroid),

Volume = $4/3\ \pi a^2 b$

Where,

$\pi = 3.14$

a = ½ the major axis (height)

b = ½ the minor axis (spread)

2.8 Statistical analysis

Data on plant growth and soil microbial counts 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) [13]. In addition to show the interrelationships between rootstocks in combination with soil management treatments and mean values of each studied plant growth and soil microbial counts statistical analysis program (SPSS) was used.

3. Results and Discussion

3.1 Microbial counts

Among the treatments, bacterial (117.52 10^5cfu/g soil) counts, fungal counts (14.96 10^4cfu/g soil) were recorded maximum in combined treatment and actinomycetes counts (12.72 10^2cfu/g soil) in soil fumigation treatment during 2015. In 2016, highest bacterial counts (118.27 10^5cfu/g soil), fungal counts (15.46 10^4cfu/g soil) were recorded maximum in combined treatment, compared to other treatments however, minimum in control and highest actinomycetes counts (13.96 10^2cfu/g soil) in control and lowest in biocontrol (Table 1). Different rootstocks did not show consistent influence on rhizobacterial, fungal and actinomycetes counts during both the years of study (Table 3). The interaction between rootstocks and treatments was found to be non-significant in respect of rhizobacterial, fungal and actinomycetes counts during 2015 and 2016. Numerically, all other rootstocks registered higher rhizobacterial, fungal and actinomycetes counts with combined treatment combinations figures 5 to 7, respectively.

Present study indicates that the enzyme activates of apple soil was increased with the application of combined treatment (Soil fumigation + PGPR + *Trichoderma viride*) and M.793 rootstock. Kumar *et al.*, (2014) [14] 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) [15] 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) [16] 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) [17] who also reported that 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 [16] 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. 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) [17].

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

Treatment	Bacterial count (10^5cfu/g soil)		Fungal count (10^4cfu/g soil)		Actinomycetes count (10^2cfu/g soil)	
	2015	2016	2015	2016	2015	2016
Control	97.13^d	98.38^d	12.94^{cb}	13.46^{cb}	13.47^a	13.96^a
Soil fumigation	96.69^d	97.69^d	13.46^b	13.46^{cb}	13.72^a	12.96^c
PGPR	113.94^b	112.94^b	13.71^{ba}	13.46^{cb}	13.22^a	12.96^c
Biocontrol	104.44^c	107.44^c	14.46^a	14.21^b	12.71^{ba}	12.71c
Combined	117.52^a	118.27^a	14.96^a	15.46^a	12.96^{ba}	13.21^b

Degree of significance of $P \leq 0.05$

Table 3: Effect of rootstocks on soil biological activity in replanted apple

Rootstocks	Bacterial count (10^5cfu/g soil)		Fungal count (104cfu/g soil)		Actinomycetes count (10^2cfu/g soil)	
	2015	2016	2015	2016	2015	2016
Seedling	105.64	106.84	13.42	13.22	13.61	13.61
M.793	106.44	107.24	13.80	14.02	13.01	13.01
MM.111	105.30	106.70	14.00	14.60	13.04	13.02
M.7	106.40	107.00	14.40	14.20	13.21	13.02
LSD	NS	NS	NS	NS	NS	NS

Degree of significance of $P \leq 0.05$

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 (144.79 cm), number of feathers (1.87), leaf area (39.00 cm^2) and plant volume (1.40 m^3) in 2015. During 2016, highest plant height (197.09 cm), number of feathers (3.78), leaf area (39.06 cm^2) and plant volume (13.23 m^3) in M.793 rootstock however, minimum in seedling rootstock (Table 4). Among the treatments, plant height (160.37 cm), number of feathers (2.14), leaf area (45.41 cm^2) and plant volume (1.88 m^3) in 2015 and during 2016, plant height (242.24 cm), number of feathers (4.99), leaf area (43.96 cm^2) and plant volume (22.18 m^3) were recorded maximum in combined treatment, compared to other treatments however, minimum in control (Table 2). Among the rootstocks and treatment combinations of combined treatment with M.793 rootstock recorded maximum plant height (169.37 cm), number of feathers (2.52), leaf area (447.61 cm^2) and plant volume (2.15 m^3) in 2015 and during 2016, highest plant height (287.67 cm), number of feathers (5.60), leaf area (64.62 cm^2) and plant volume (28.21 m^3) compared to other rootstock and soil treatment combinations given in figures 1 to 4, respectively.

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 [18] and Kviklys *et al.*, 2007 [19]) 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 [20], Ferree and Warringon, 2003 [21], Rana and Chandel, 2003 [22], Karlidag *et al*., 2007 [23], Kirad *et al.*, 2009 [24], Tripathi *et al.*, 2014 [25], Kipkoriony and Fusao, 2006 [26]) who also recorded increased plant height and spread with the application of plant growth promoting rhizobacteria and *Trichoderma viride*.

Rumberger *et al.* (2004) [27] 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 [28], Miethling *et al.*, 2000 [29], Westover *et al.*, 1997 [30]) as have changes in rhizosphere microbial communities due to intra-specific variation (Carelli *et al.*, 2000 [31], Cattelan *et al.*, 1998 [32], Di Giovanni *et al.*, 1999 [33]). 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 and 2016. 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 *Pseudomonas* in ARD (Gu and Mazzola, 2003 [34], Mazzola, 1997 1998 [35]). 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) [36].

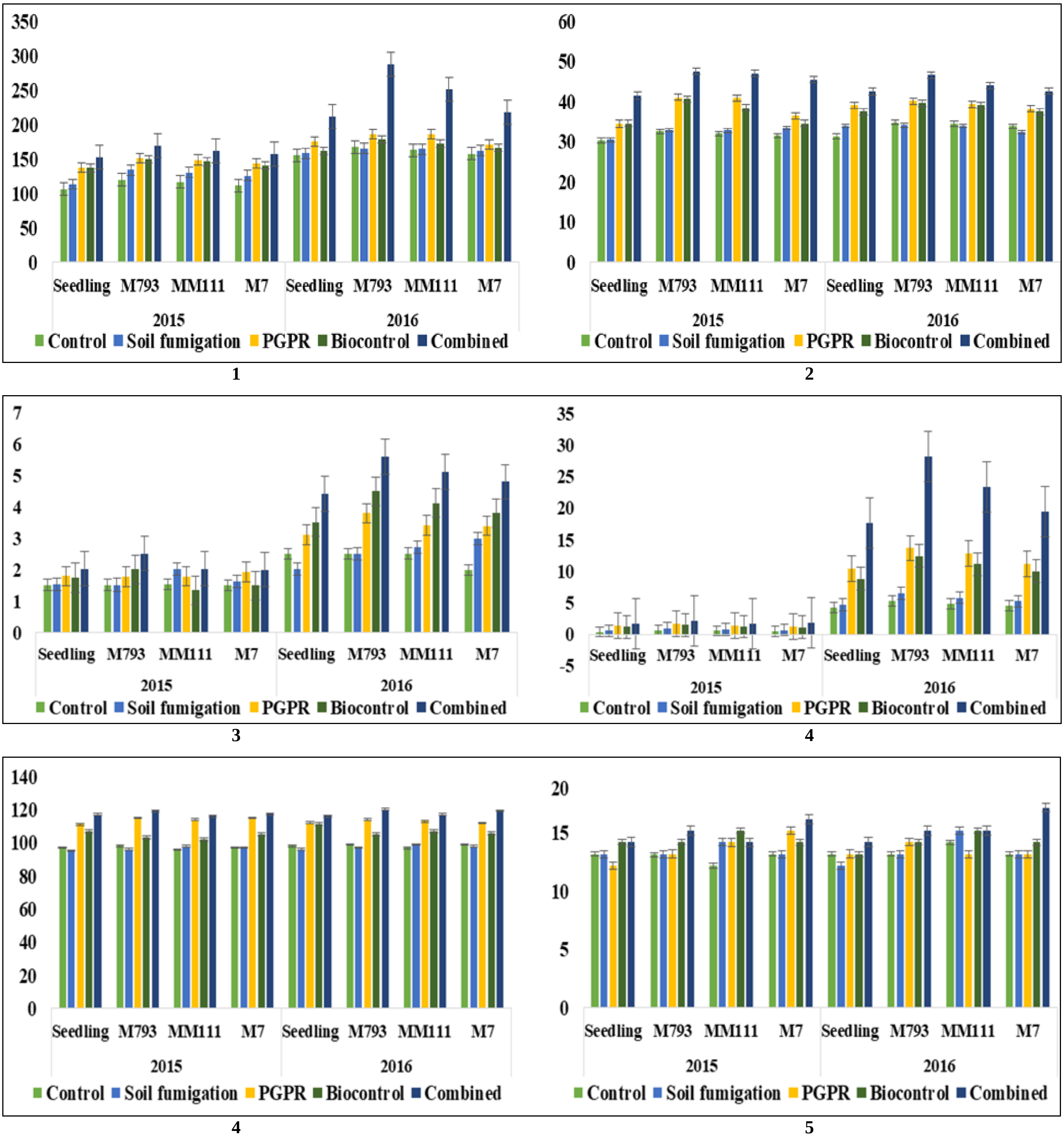

Fig 1: Effect of different rootstocks and treatments on plant height (1), leaf area (2), number of feathers (3), plant volume (4), bacterial count (5) and fungal count (6) of replanted apple. Vertical bar represent mean of three replication ± SE m and LSD ($p \leq 0.05$)

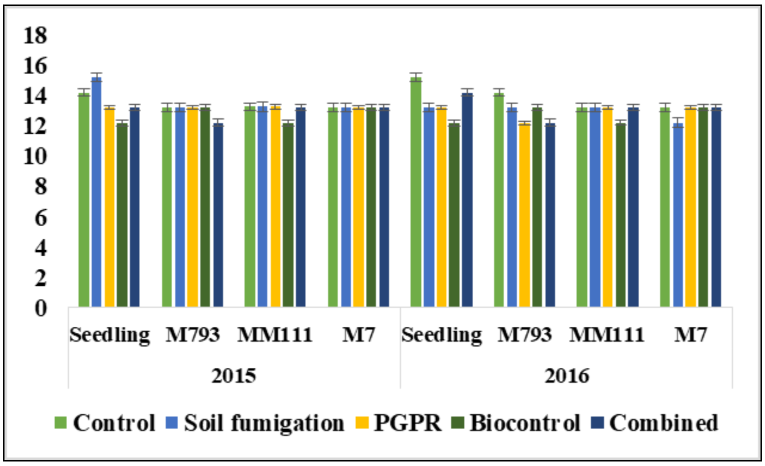

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Fig 2: Effect of different rootstocks and treatments on actinomycetes count (7) of replanted apple. Vertical bar represent mean of three replication ± SE m and LSD ($p \leq 0.05$)

Table 2: Effect of treatments on plant growth traits in replanted apple

Treatment	Plant Height		Leaf area		Number of feathers		Plant volume	
	2015	2016	2015	2016	2015	2016	2015	2016
Control	113.45[e]	160.77[d]	31.63[e]	33.65[e]	1.52[dc]	2.38[e]	0.54[e]	4.73[e]
Soil fumigation	125.87[d]	162.64[d]	32.44[d]	33.65[d]	1.68[b]	2.56[d]	0.76[d]	5.58[d]
PGPR	145.38[b]	179.94[b]	38.27[b]	39.22[b]	1.83[b]	3.44[c]	1.45[b]	12.05[b]
Biocontrol	143.24[c]	169.87[c]	37.03[c]	38.45[c]	1.66[cb]	3.99[b]	1.27[c]	10.61[c]
Combined	160.37[a]	242.24[a]	45.41[a]	43.96[a]	2.14[a]	4.99[a]	1.88[a]	22.18[a]

Degree of significance of $P \leq 0.05$

Table 4: Effect of rootstocks on plant growth traits in replanted apple

Rootstocks	Plant Height		Leaf area		Number of feathers		Plant volume	
	2015	2016	2015	2016	2015	2016	2015	2016
Seedling	129.29[d]	172.61[d]	34.26[d]	36.95[c]	1.72[c]	3.12[d]	1.07[d]	9.17[d]
M.793	144.79[a]	197.09[a]	39.00[a]	39.06[a]	1.87[a]	3.78[a]	1.40[a]	13.23[a]
MM.111	140.67[b]	187.57[b]	38.25[b]	38.17[b]	1.75[b]	3.58[b]	1.17[b]	11.59[b]
M.7	135.89[c]	175.11[c]	36.32[c]	36.96[c]	1.72[c]	3.40[c]	1.08[c]	10.12[c]

Degree of significance of $P \leq 0.05$

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 microbial counts in the apple under replanted site. Most of growth and soil biological activates had positive correlation with plant volume in present study.

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