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Effect of biostimulant and biofertilizers on soil bio-chemical properties and plant growth of apple (*Malus x domestica* Borkh.) nursery

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Abstract

Aim: To assess the effect of biostimulant and biofertilizers on soil properties and plant growth of apple nursery under eco-friendly production system.

Methodology: The experiment was laid out in a randomized block design comprising 18 treatments of biostimulant, biofertilizers and their combinations. Each treatment was replicated thrice. Nursery beds of apple seedling rootstocks were prepared and Arbuscular mycorrhizal fungi, PGPR (*Bacillus* sp.) were applied by soil drenching method and commercial formulations of granular bio-stimulant containing humic acid (39%) + kelp extract (25%) + amino acids (9.0%) + ascorbic acid + thiamine + inositol + tocopherol (27%) were applied in two split doses by broadcasting in root zone. The effects of these various formulations were investigated during the course of studies.

Results: Linear growth, radial growth of scion (6.66 mm), number of leaves per plant, leaf area, success rate, saleable plants, total root length, fresh weight of shoots, dry weight of shoots, fresh weight of roots, dry weight of roots and total plant biomass of apple nursery plants were significantly increased with the combined application of biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant). This treatment also significantly improved the status of available soil N, P, K and population of fungi, bacteria as well as actinomycetes in the rhizosphere.

Interpretation: Application of biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant) proved to be the most effective combination of eco-friendly inputs for the production of good quality apple nursery plants with well developed shoot and root system and improved the soil bio-chemical properties.

Apple nursery beds
↓
Seedling rootstocks were transplanted and AM fungi was applied during the month of January
↓
PGPR (*Bacillius* sp.) was then applied 1 month after transplanting of stock during February
↓
Tongue grafting was performed during the month of March
↓
Recording of observations on plant growth parameters as per standard procedure
↓
Quantification of microbial flora (Fungi, Bacteria and Actinomycetes) by Serial Dilution Technique
↓
Estimation of nutrients in soil samples as per standard procedures

Key words: Apple, Biofertilizer, Biostimulant, Plant growth, Soil properties

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Introduction

Apple (*Malus* x *domestica* Borkh.) is the most widely grown temperate fruit and is the fourth most important fruit crop in the world. The predominance of apple area and production is in China, USA, Poland, Turkey, India, Iran and Italy (Glance, 2018). It belongs to family Rosaceace and sub-family Pomoideae. The economy of the growers of temperate region of India is largely dependent on the production of fruit crops where apple occupies the prime position. The total area under apple cultivation in India is 3,08,000 ha with annual production of 23,16,000 metric tons and productivity of 7.52 metric tons ha^{-1} (Glance, 2018). The productivity of apple is fluctuating every year and is very less as compared to 30-40 metric tons ha^{-1} in leading apple producing countries like USA, Italy and France etc. At present, apple industry in the country is confronted with problems of poor yield contributing to low productivity, wide annual fluctuations in production and poor quality of produce. Several factors are associated with these problems but use low grade and poor quality planting material for establishing of orchards is one of the major cause, since the ultimate success in fruit growing depends on the quality of planting material used for establishment of an orchard (Chauhan and Sharma, 2021).

Nursery plants are the foundation of orchards, thus the use of genuine and quality planting material is the basic pre-requisite for the success of an orchard enterprise. The introduction of new colouring strains, low chilling varieties and high yielding spur and semi spur varieties of apple in India has led to a substantial increase in demand for nursery plants (Chauhan and Sharma, 2021). Further, large number of apple orchards in the country has outlived their economic life and needs replantation. Thus, the demand of planting material of apple is increasing every year but quality planting material is the major hindrance in area expansion and replanting of apple orchards. In the last few years, organic cultivation has attracted many farmers across the country and henceforth, they are practicing organic farming. To provide organically produced good quality nursery plants for establishment of organic apple orchards, biostimulants and biofertilizers can be efficient alternative inputs for managing and maintaining the growth of nursery plants. Biostimulants are preparations of biological origin which can be used for the production of healthy and vigorous planting material under organic production system as these substances stimulate natural ecological processes to improve nutrient absorption and nutritional quality of plants (Yakhin *et al.,* 2017).

Biostimulant formulations containing sea weed extract, humic acid, ascorbic acid and amino acids etc., are eco-friendly inputs known to affect the growth and development of plants and are commercially available in the market. Biostimulants have been found active in promoting crop quality and many researchers have highlighted the relevance of biostimulant applications for selected functional quality traits. Soppelsa *et al.* (2018) had advocated the use of biostimulants for improving the tree growth, yield and fruit quality of apple under organic production system as biostimulants like seaweed extract has potential to increase the anthocyanin content and red colouration of apple skin by influencing the synthesis of secondary metabolites. Sea weed extract contain phytohormones like auxins, gibberellins, cytokinins, abscisic acid, ethylene, betaine, polyamines, vitamins, amino acids, antibiotics and micronutrients (Panda *et al.,* 2012), which helps in improving the plant growth, yield, nutrient uptake, resistance to stress conditions and reduce incidences of fungal and insect attack (Metting *et al.,* 1990). Humic acid is especially important because of its ability to chelate micronutrients to increase their bio-availability.

Humic substances are the most active component of organic matter and soil, responsible for several complex chemical reactions in the soil (Trevisan *et al.,* 2010). It comprises of 42-46% oxygen, 44-58% carbon, 0.5-4.0% nitrogen and 6-8% hydrogen (Larcher, 2004) and induce morphological, physiological, genetic and biochemical effects (Ferrara *et al.,* 2001; Varanini and Pinton, 2001; Nardi *et al.,* 2002; Chen *et al.,* 2004). Humic acid also have direct effect on plant growth due to increased chlorophyll content, respiration rate, solute penetration to plant membrane, thereby affecting dry matter, water and nutrient uptake by the plants (Baldotto *et al.,* 2009). The beneficial effects of humic substances include lateral root development and root hair formation in fruit crops (Ramos *et al.,* 2015). Hidayatullah *et al.* (2018) reported a significant increase in fruit set, fruit yield and leaf nutrients concentration of apple with potassium humate.

Ascorbic acid is an essential ingredient for metabolic reactions involved in regulating photosynthesis, hormone biosynthesis, regenerating other antioxidants, cell division and improving the nutritional quality (Gallie, 2012). Biofertilizers stimulate plant growth by fixing nitrogen in the soil and solubilize or mobilize nutrients and increase their availability to plants. AM fungi spore population in the rhizosphere soil and percentage of mycorrhizal infection in the roots have been found to be positively correlated with plant growth and leaf area of apple (Sharma and Sharma, 2006). AM fungi facilitates host plants to grow vigorously under stressful conditions by mediating complex communication events between the plant and the fungus leading to enhanced photosynthetic rate and other gaseous exchange related traits (Birhane *et al.,* 2012). Inoculation of apple rootstocks (M9, M7and MM106) with AM fungi has been reported to significantly increase the plant height, stem diameter, leaf size and total biomass (Hosseini and Gharaghani, 2015). PGPRs are bacteria that colonize the roots of plants and enhance plant growth.

Different strains of PGPRs were found to increase shoot length, number of leaves, leaf area, number of annual shoots and their diameter in nursery plants of apple (Karlidag *et al.,* 2007; Karakurt and Aslantas, 2010). These PGPR species regulate hormonal and nutritional balance and induce resistance against pathogens (Vejan *et al.,* 2016). Keeping in view the importance of bio-stimulants and bio-fertilizers in eco-friendly plant production system, the present study aimed to study the influence of biostimulant and biofertilizers on biochemical properties of soil and plant growth of apples in nursery for production of quality planting material.

Materials and Methods

The present study was carried out during the year 2018-2019 in the Department of Fruit Science, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan situated in the hilly regions of Western Himalayas. The experiment was laid out in a randomized block design with 18 treatments of biostimulant, biofertilizers and their combinations, which were replicated thrice. The treatments comprised of T_1: Biostimulant (100 g m^{-2} nursery bed), T_2: Biostimulant (200 g m^{-2} nursery bed), T_3: AM fungi (10 g per plant), T_4: AM fungi (20 g per plant), T_5: PGPR (50 ml per plant), T_6: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (10 g per plant), T_7: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (10 g per plant), T_8: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (20 g per plant), T_9: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant), T_{10}: Biostimulant (100 g m^{-2} nursery bed) + PGPR (50 ml per plant), T_{11}: Biostimulant (200 g m^{-2} nursery bed) + PGPR (50 ml per plant), T_{12}: AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{13}: AM fungi (20 g per plant) + PGPR (50 ml per plant), T_{14}: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{15}: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{16}: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant), T_{17}: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant) and T_{18}: Control (No bio-stimulant and bio-fertilizer). Soil of experimental block was slightly acidic in reaction and medium in organic carbon as well as available NPK contents.

The climate of experimental site is typically sub-temperate with moderately hot summers during May-June and severe winters during December-January. The annual rainfall of the area ranged between 800 and 1500 mm, the major amount of which is received during monsoon period from July to September. The nursery beds of 1.0 m^2 were prepared and FYM was added @ 8.0 kg m^{-2}. The seedling rootstocks were transplanted in the beds at a spacing of 25×20 cm in January. After proper establishment of rootstocks, tongue grafting was performed during 1st week of March at 15 cm height of stock using scion of Scarlet Spur-II variety. AM fungi was applied to the respective nursery beds as per treatments at the time of transplanting of stock, PGPR (*Bacillus* sp.) was applied one month after transplanting of stock by soil drenching method and commercial formulations of granular bio-stimulant containing humic acid (39%), kelp extract (25%), amino acids (9.0%), ascorbic acid + thiamine + inositol + tocopherol (27%) was applied in two split doses by broadcasting in root zone.

The first half dose was applied at the time of transplanting of stock while the remaining half dose was applied 45 days after first application. Uniform recommended cultural practices were given to all nursery plants under trial during the entire course of study. Linear and radial growth of the plant was measured with a measuring tape and digital vernier calipers, respectively, at the end of season. Leaf area was measured with the help of Leaf Area Meter (Licor Model–3100). To determine the fresh weight of shoots and roots, after uprooting the plants at the end of season, shoot and root portion was separated and cut into small pieces. The fresh weight of shoot and root segments was then recorded separately on electronic balance. To record the dry weight, the root and shoot segments were dried at 65 ± 5°C in an oven until a constant weight was obtained.

Total root length was measured with the help of root length scanner (Comair root length scanner). Total plant biomass was obtained by adding the dry weight of shoots and roots. Soil samples for chemical analysis were collected at the end of the experiment from each replicated bed with the help of screw type auger. The samples were dried in shade, grounded, sieved through 2 mm plastic sieve and analyzed for nitrogen by alkaline potassium permanganate method (Subbiah and Asija, 1956), phosphorus by stannous chloride reduced ammonium molybdate method (Olsen *et al.,* 1954) and potassium by neutral ammonium acetate extraction method (Merwin and Peech, 1951). Microbial flora (fungi, bacteria and actinomycetes) was quantified by serial dilution techniques. The data recorded on various parameters were appropriately computed, tabulated and analyzed by applying randomized block design using MS-Excel and OPSTAT.

Results and Discussion

The perusal of data presented in Table 1 reveals that the growth of apple nursery plants increased significantly with the increasing doses of biostimulant and biofertilizers. The growth of plants with combined application of biostimulant + AM fungi + PGPR was significantly higher as compared to plant growth under sole application of biostimulant, AM fungi or PGPR and combinations of biostimulant + AM fungi, biostimulant + PGPR or AM fungi + PGPR. The maximum linear growth (191.96 cm), increase in radial growth of rootstock (5.95 mm) and scion (6.66 mm), number of leaves per plant (59.00), leaf area (19.96 cm^2), fresh weight of shoots (115.71 g), dry weight of shoots (92.64 g), fresh weight of roots (100.49 g), dry weight of roots (85.61 g), total root length (17.33 m) and total plant biomass (178.25 g) was recorded in plants treated with T_{17} *i.e.,* biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant).

The success rate of plants was recorded cent-per cent under treatments T_9, T_{13}, T_{14}, T_{15}, T_{16} and T_{17}. Similarly, cent-per cent plants attained saleable growth under T_{16} and T_{17} whereas the minimum linear growth (142.76 cm), increase in radial growth of rootstock (3.27 mm) and scion (3.67 mm), number of leaves per plant (48.46), leaf area (16.45 cm^2), fresh weight of shoots (74.47 g), dry weight of shoots (47.31 g), fresh weight of roots (60.66 g), dry weight of roots (41.76 g), total root length (8.28 m) and total

Table 1: Effect of biostimulant and biofertilizers on linear and radial growth, leaf area, success rate, proportion of saleable plants and total plant biomass of apple nursery

Treatments	Linear growth (cm)	Increase in radial growth (mm)		Number of leaves/ plant	Leaf area (cm^2)	Success rate* (%)	Saleable plants** (%)	Total root length (m)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Total plant biomass (g)
		Rootstock	Scion										
T_1	156.83	5.29	5.57	50.33	18.39	86.67 (9.36)	70.00 (56.97)	9.67	82.86	57.61	62.33	42.05	99.66
T_2	166.23	5.82	5.98	54.40	19.59	96.67 (9.88)	76.67 (61.89)	10.26	89.06	59.20	73.46	49.12	108.32
T_3	162.33	4.14	5.00	52.16	17.42	86.67 (9.36)	76.67 (61.89)	9.28	82.85	50.57	66.02	45.70	96.27
T_4	166.66	4.42	5.21	56.13	18.28	93.33 (9.71)	80.00 (63.90)	10.47	106.55	74.97	73.35	53.96	128.93
T_5	166.33	3.93	4.66	50.46	18.96	90.00 (9.53)	76.67 (61.69)	10.87	104.18	73.50	83.95	55.28	128.78
T_6	169.83	5.39	5.51	55.86	18.15	90.00 (9.53)	83.33 (66.61)	11.86	83.54	58.28	67.91	44.27	102.56
T_7	174.73	5.99	5.72	57.20	19.05	96.67 (9.88)	86.67 (72.76)	12.26	93.21	64.69	74.33	56.06	120.75
T_8	170.26	5.69	5.95	56.26	18.58	96.67 (9.88)	86.67 (72.76)	11.20	94.38	63.73	79.32	60.22	123.95
T_9	177.46	6.14	6.23	58.06	19.86	100.00 (10.05)	90.00 (71.53)	12.48	98.41	76.66	98.06	75.44	152.10
T_{10}	176.00	5.05	5.40	51.53	19.00	90.00 (9.53)	83.33 (66.61)	11.29	92.10	60.09	86.93	64.81	124.90
T_{11}	180.40	5.42	5.77	55.53	19.66	93.33 (9.71)	86.67 (68.82)	12.58	104.57	75.47	88.03	67.55	143.02
T_{12}	183.53	4.51	5.22	52.01	18.34	96.67 (9.88)	90.00 (74.98)	13.34	95.12	73.73	85.69	61.19	134.92
T_{13}	185.66	4.73	5.38	54.20	18.79	100.00 (10.05)	93.33 (77.69)	13.67	110.04	83.15	90.81	69.27	152.43
T_{14}	186.83	5.19	5.52	53.13	18.26	100.00 (10.05)	93.33 (77.69)	15.15	104.61	83.53	89.92	65.55	149.08
T_{15}	187.50	5.66	5.97	55.40	19.22	100.00 (10.05)	96.67 (83.84)	15.37	108.42	86.34	92.75	73.37	159.71
T_{16}	188.43	5.39	5.64	54.86	18.56	100.00 (10.05)	100.00 (90.00)	16.59	106.49	87.96	96.55	74.94	162.90
T_{17}	191.96	5.95	6.66	59.00	19.96	100.00 (10.05)	100.00 (90.00)	17.33	115.71	92.64	100.49	85.61	178.25
T_{18}	142.76	3.27	3.67	48.46	16.45	80.00 (8.98)	66.67 (55.05)	8.28	74.47	47.31	60.66	41.76	89.07
$CD_{0.05}$	11.20	1.05	1.01	3.05	1.28	10.24	15.87	1.58	6.55	5.97	4.80	7.94	10.00

*Values in parenthesis are square root transformed values; ** Values in parenthesis are angular transformed values

T_1: Biostimulant (100 g m^{-2} nursery bed), T_2-Biostimulant (200 g m^{-2} nursery bed), T_3-AM fungi (10 g per plant), T_4-AM fungi (20 g per plant), T_5-PGPR (50 ml per plant), T_6-Biostimulant (100 g m^{-2} nursery bed) + AM fungi (10 g per plant), T_7-Biostimulant (200 g m^{-2} nursery bed) + AM fungi (10 g per plant), T_8-Biostimulant (100 g m^{-2} nursery bed) + AM fungi (20 g per plant), T_9-Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant), T_{10}-Biostimulant (100 g m^{-2} nursery bed) + PGPR (50 ml per plant), T_{11}-Biostimulant (200 g m^{-2} nursery bed) + PGPR (50 ml per plant), T_{12}- AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{13}- AM fungi (20 g per plant) + PGPR (50 ml per plant), T_{14}-Biostimulant (100 g m^{-2} nursery bed) + AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{15}-Biostimulant (200 g m^{-2} nursery bed) + AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{16}-Biostimulant (100 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant), T_{17}-Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant) and T_{18}-Control (No bio-stimulant and bio-fertilizer).

Table 2: Effect of biostimulant and biofertilizerson chemical and microbiological properties of apple nursery soil

Treatments	Available soil nutrients			Viable microbial count		
	Nitrogen ($kg\ ha^{-1}$)	**Phosphorus ($kg\ ha^{-1}$)**	**Potassium ($kg\ ha^{-1}$)**	**Fungi ($x10^4 cfu\ g^{-1}$)**	**Bacteria ($x10^6 cfu\ g^{-1}$)**	**Actinomycetes ($x10^4 cfu\ g^{-1}$)**
T_1	364.78	52.53	242.15	6.00	21.67	11.67
T_2	372.91	55.56	245.41	7.00	26.33	12.67
T_3	366.64	51.87	284.34	8.67	20.33	10.33
T_4	375.01	52.41	293.06	10.00	23.33	10.67
T_5	363.36	53.94	243.40	8.00	29.33	11.33
T_6	372.03	54.83	337.37	9.67	22.67	11.67
T_7	383.13	57.26	327.10	10.00	28.33	12.00
T_8	378.74	56.81	343.86	10.33	24.67	11.67
T_9	381.16	60.09	340.59	10.67	28.00	12.67
T_{10}	365.06	54.26	311.32	9.00	29.33	13.00
T_{11}	385.51	56.63	317.59	10.00	31.00	13.00
T_{12}	366.34	55.50	288.56	9.67	28.00	12.00
T_{13}	382.72	58.97	305.45	10.67	29.67	12.00
T_{14}	388.66	62.43	348.54	11.00	28.67	13.33
T_{15}	395.22	65.30	352.95	11.33	31.33	14.00
T_{16}	399.50	70.05	379.34	11.67	30.33	14.67
T_{17}	405.04	75.07	385.05	12.00	35.00	16.00
T_{18}	360.23	47.67	239.30	5.67	19.33	10.00
$CD_{0.05}$	13.74	5.02	8.09	1.09	3.61	2.71

T_1: Biostimulant (100 g m^{-2} nursery bed), T_2: Biostimulant (200 g m^{-2} nursery bed), T_3: AM fungi (10 g per plant), T_4: AM fungi (20 g per plant), T_5: PGPR (50 ml per plant), T_6: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (10 g per plant), T_7: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (10 g per plant), T_8: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (20 g per plant), T_9: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant), T_{10}: Biostimulant (100 g m^{-2} nursery bed) + PGPR (50 ml per plant), T_{11}: Biostimulant (200 g m^{-2} nursery bed) + PGPR (50 ml per plant), T_{12}: AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{13}: AM fungi (20 g per plant) + PGPR (50 ml per plant), T_{14}: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{15}: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (10 g per plant) + PGPR (50 ml per plant), T_{16}: Biostimulant (100 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant), T_{17}: Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant) and T_{18}: Control (No bio-stimulant and bio-fertilizer)

plant biomass (89.07 g) was observed with T_{18}. The minimum success rate (80.00%) and proportion of saleable plants (66.67%) was also recorded in T_{18}. The data depicted in Fig. 1 outlines that T_{17} - Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant) exhibited marked increase in linear growth (34.46%), stock diameter (81.96%), scion diameter (81.47%), survival of plants (25.0%), proportion of saleable plants (49.99%), total root length (109.30%) and total plant biomass (100.16%) over control treatment.

The significantly higher growth of apple nursery plants with the increased dose of bio-stimulant could be attributed to the fact that these substances have similar action as auxins, gibberellins and cytokinins (Yaronskaya *et al.,* 2006). PGPR and bio-stimulants are capable of synthesizing auxins, cytokinins and gibberellins which promote vegetative growth in plants (Aslantas *et al.,* 2007) via cell division and expansion (Taiz and Zeiger, 2002) or by improving nutrient availability (Glick, 1995). According to Jensen (2004), seaweeds play an important role in enhancing plant growth by improving soil texture, water holding capacity of soil and act as a transmissive media for nutrient uptake and stimulation of soil micro-organisms activity, which ultimately helps in increasing the vegetative growth of plants. Ascorbic acid, an essential constituent of bio-stimulants has action analogous to auxin (Wassel *et al.*, 2007) with respect to improving many metabolic and physiological processes besides enhancing the synthesis of carbohydrates and hence results in increments of shoot and root growth. Biological microorganisms also release plant growth stimulating hormones (Pathak *et al.,* 2017) which enhanced the growth of plants. Application of biostimulants enhanced plant defenses and participated in the biosynthesis of organic compounds viz., amines, purines, pyrimidines, vitamins for better nutrient acquisition (Kocira *et al.,* 2018). The findings of present study are in conformity with those of Haggag *et al.* (2016), who recorded significantly higher vegetative growth in the seedlings of olive on the application of biostimulant (humic acid).

The improvement in plant biomass with the administration of PGPR might be attributed to their several roles

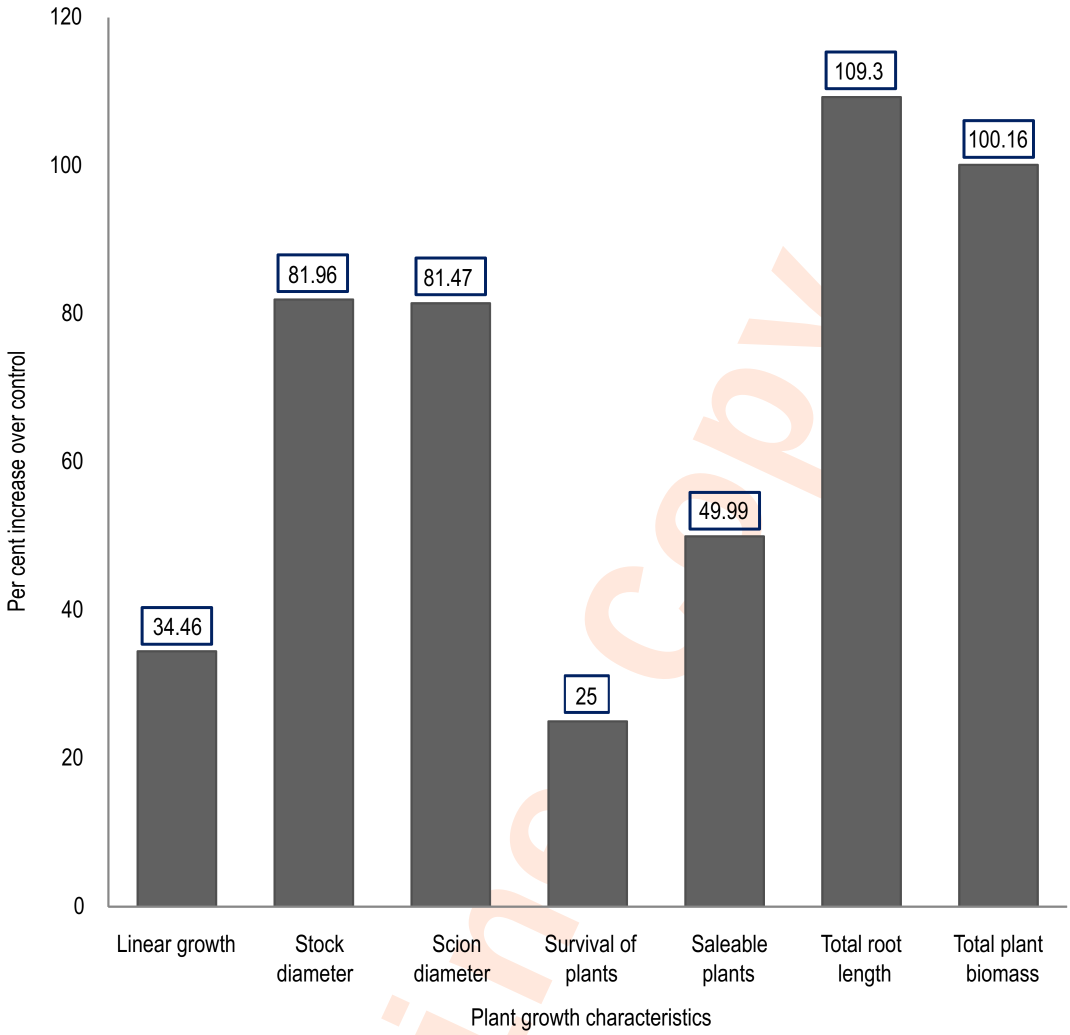

Fig. 1: Per cent increment in growth characteristics of apple nursery plants with treatment 17 over control. *treatment 17 - Biostimulant (200 g m^{-2} nursery bed) + AM fungi (20 g per plant) + PGPR (50 ml per plant).

in the soil viz., nutrient recycling, phyto-hormone production and solubilization of nutrients (Gange and Gadhave, 2018), which further activates other physiological processes for rapid absorption and utilization of nutrients for primary metabolic processes and as a result dry matter accumulates in plant tissues. Increase in shoot, root and plant biomass with AM fungi inoculation was reported by clonal rootstocks of apple (Hosseini and Gharaghani, 2015) and persimmon seedlings (Machineski *et al.*, 2018) .

Almadi *et al.* (2020) recorded higher plant biomass of young olive trees with the fertigation of biostimulant (comprising amino acids – glycine, proline and hydroxyproline). Di Vaio *et al.* (2021) recorded an increase in vegetative growth (total leaf area, fresh and dry weight of the shoots, roots and total dry matter) with the application of Osmocote (Trichoderma harzianum – based) treatment for the nursery production of young olive trees.

The data presented in Table 2 indicate that biostimulant and biofertilizers significantly improved the status of available soil nutrients (N, P and K) and rhizosphere microflora. The status of available nutrients increased with increasing doses of biostimulant and biofertilizers. The highest available soil nitrogen (405.04 kg per ha), phosphorus (75.07 kg per ha) and potassium (385.05 kg per ha) were recorded in the nursery beds treated with T_{17} treatment. Increase in available soil N, P and K with increasing doses may be attributed to the stimulatory action of bio-stimulants and biofertilizers on soil microbial activity, transformation of insoluble nutrients into soluble form and effective mobilization of nutrients to make them available to plants. VAM inoculation results in better mobilization of fixed phosphorus (Manjunath *et al.,* 1983). According to Vaughan and

Malcom (1985), biostimulants improve soil structure, water holding capacity of soil, provide energy for the beneficial organisms within the soil and release plant nutrients from soft minerals. Humic substances are natural, polymeric and organic materials produced by the good rotten organic matter (Sharif *et al.,* 2002) and the most active component of soil and organic matter (Trevisan *et al.,* 2010), which increases nutrient availability and other important physico-chemical and biological properties of soil. Biostimulant (humic acid) increases the permeability of cell membranes through which mineral elements move back and forth thereby increasing the availability and transport of various mineral nutrients. There seems to be a synergistic interaction between VAM and PSB (Phosphate solubilizing bacteria). VAM hyphal network in the soil-plant continuum have great bearing on exploration of soil volume by the crop, leading to higher nutrient efficiency especially P. This may be ascribed to mobilization of soil NPK from organic and inorganic complexes by the action of VAM fungi due to its mycelial growth or release of organic acids in rhizosphere (Suri *et al.*, 2011).

Application of bio-stimulant and biofertilizers had a significant effect on viable microbial count with maximum population of fungi (12.00 x 10^4 cfu g^{-1}), bacteria (35.00 x 10^6 cfu g^{-1}) and actinomycetes (16.00 x 10^4 cfu g^{-1} in the nursery beds subjected to T_{17}. This treatment was statistically at par with T_{14}, T_{15} and T_{16} treatments with respect to fungi and actinomycetes count but significantly superior to all other treatments with respect to bacterial count. The increase in viable microbial count with biostimulant and biofertilizers may be ascribed to non-chemical supplements that provide food and conducive micro-environment for multiplication and growth of microbes (Khattak *et al.,* 2013). Biostimulants like humic substances and seaweed extract improves physical and biological properties of soil (Cardozo *et al.,* 2007) and enhances the growth of beneficial soil microbes as well as secretion of soil conditioning substances by these microbes. The results of present investigation corroborates with the findings of Sahain *et al.* (2007) who reported an increase in rhizosphere microflora of apple orchard soil with the application of biostimulant. Similar increase in rhizobacterial count of guava orchard soils was reported by Ram and Pathak (2007). Wang *et al.* (2017) had observed considerable increase in the number of beneficial microbes and fungi/bacteria ratio of *Malus hupehensis* rhizosphere on applying sea weed extract based biostimulant.

On the basis of significantly higher plant growth, available soil nutrients and soil microbial count, application of biostimulant (200g m^{-2} nursery bed) + AM fungi (20g per plant) + PGPR (50ml per plant) proved to be the most effective treatment for production of good quality nursery plants of apple with well developed root and shoot system.

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Add-on Information

Authors' contribution: J. Thomas: Data analysis, Methodology, Writing original draft; **N.C. Sharma:** Resources, Methodology, Review and editing; **P. Kumar:** Review and editing; **A. Chauhan:** Resources, validation, writing original draft and **P. Chauhan:** Data analysis, writing final draft.

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