



Enhancement of apple (*Malus domestica*) productivity and soil health through organic fertilization and bio-inoculants under north-western Himalayan region of India

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ABSTRACT

A field experiment was performed to assess the medium-term effect of mineral fertilizer and organic fertilizer (vermicompost) supplemented with microbial inoculants (*Azotobacter* and *Microphos*) on growth parameters of apple (*Malus domestica* Borkh. cv. Oregon Spur) and soil health parameters. Eight different combinations of mineral fertilizer, vermicompost (VC) and microbial inoculants treatments were applied during 2012 to 2014. The percentage increase in trunk cross section area (TCSA) during the three years of experiment was maximum 17.0% and 15.8% with application of 75% of recommended dose of fertilizer (RDF) + 25% N through VC + *Microphos* and 50% of RDF + 50% N through VC, respectively. The pooled data of three years revealed that, maximum fruit yield (20.8 tonnes/ha) and production efficiency (112 g/cm² TCSA) of crop were recorded in the treatment received 50% N through VC and this treatment is similar ($P \leq 0.05$) with treatment having application of 75% of RDF + 25% N through VC + *Microphos* while minimum was recorded in control. Among all treatments, application of 50% N through VC and application of 25% N through VC + *Microphos* performed better for taming chemical fertility of soil. Study also noticed that, continuous three years application of 50% N through VC, the soil organic carbon, soil microbial biomass carbon and dehydrogenase activity of soil increased by 17, 33 and 39%, respectively over control. Overall, study revealed that, plants received 50% of RDF + 50% VC or 25% VC + *Microphos* had highest ability of promoting the sustainability of apple yield, soil biological and chemical fertility of an agro-ecosystem in north-western Himalayan region.

Key words: Apple production, Biofertilizers, Enzyme activity, Integrated nutrient management, Soil microbial biomass carbon

Apple (*Malus domestica* Borkh.) is one of the principal fruit having huge amount of polyphenols (antioxidants) and grown especially in the temperate climate of the world. In India, apple mainly grown in north western Himalayan region (NWHR) having total acreage of 0.28 million ha and production of 2242 million tonnes with productivity of 8.1 tonnes/ha; out of which Jammu and Kashmir accounts for 49% of total area and 74% of total production (NHB 2017). Due to high nutritional facts of apple and ever increasing human population, the demand of apple is immensely increased in the last two decades. At the same time the productivity of crop has also increased from 5.9 tonnes/ha (1991-92) to 8.1 tonnes/ha (2016-17), for which by and large

inorganic fertilization and climatic conditions are the most vital. But the application of mainly nitrogenous fertilizers had adverse effects on soil microbial communities, soil organic matter content, nutrient use efficiency, deficiency of nutrients like B, Zn etc. and ultimately soil health deterioration that led to unsustainable yield.

Even the use of heavy doses of nitrogen based fertilizers, the apple productivity (8.1 tonnes/ha) of country is far behind than the major apple producing countries like China (18.0 tonnes/ha), USA (40.2 tonnes/ha), Italy (45.2 tonnes/ha), Chile (47.2 tonnes/ha) (<http://faostat3.fao.org/home/E>; NHB 2017). Major apprehension of low productivity of apple in Kashmir valley; is that, most of the apple orchards of this region are under old cultivars with traditional or low density plantation and unsynchronized and/or over application of inorganic fertilization that led to soil health deterioration. In general, most of the farmers apply only nitrogenous fertilizers in single dose during dormant period and no fertilizers are applied during vegetative, flowering and fruit growth stages of apple. Therefore, adoption of high density apple orcharding along with an integrated use of inorganic, organic and bio-inoculants at appropriate proportions and their application at particular plant growth

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stage depending upon the age of the tree can alter the soil microbiome and influence plant health and productivity. Sharma and Bhatnagar (2014) noticed application of 50% recommended dose of fertilizer along with 50% N through VC supplemented with bio-fertilizers is most suitable for custard apple plant growth and development. VC/FYM along with specific strain of biofertilizers in sufficient quantities not only supplement NPK requirement from inorganic fertilizer sources, but also reduce the total quantity of inorganic fertilizers required as per fertilizer schedule. Application of VC and FYM not only reduce the inorganic fertilizers requirement but also help in enhancing the resistance and resilience capacity of soil under abiotic stress (Kumar *et al.* 2013 and 2014) by improving structural and functional diversity of soil. Recently, Kumar *et al.* (2018) and Das *et al.* (2016) noticed, through incorporation of FYM in sufficient quantities, a considerable proportion of NPK increased in soil and enhance productivity of apple. However, the methodical information on pooled application of VC, inorganic fertilizers supplemented with microbial inoculants, viz. nitrogen fixing bacteria (*Azotobacter*) and phosphorus solubilizer (*Microphos*) for long term soil health and sustainability are not well understood or characterized under NWHR. Considering all these, the present study was carried out with the objectives to examine the effect of cohesive application of inorganic fertilizers, VC and microbial inoculants on apple productivity and soil health under high density plantation system of apple (cv. Oregon Spur).

MATERIALS AND METHODS

Field experiment was conducted at experimental farm of ICAR-Central Institute of Temperate Horticulture, Srinagar, Jammu and Kashmir, India, during 2012 to 2014. The experimental area is situated at latitude of 34°05'N and longitude of 74°50'E with an altitude of 1640 m above mean seal level. The experimental farm falls under temperate region having cold conditions from November to February and five years mean maximum and minimum temperature of Srinagar climate indicated maximum (30°C) in August and minimum (-2.1°C) in December. The average annual precipitation was 650 mm distributed erratically throughout the year during the course of investigation.

Field experiment was conducted on eight-year-old uniform apple orchard of Oregon Spur variety grafted on seedling root stock, planted with 2.5m × 2.5m intra row spacing or 1600 plants/ha with four replications under randomized block design. The soil of experimental field falls under Inceptisol and sandy loam in texture with 65% sand, 22% silt and 13% clay. The initial chemical characteristics of soil, viz. pH 6.7 (1:2.5 soil-water suspension), electrical conductivity 0.23 dS/m (1:2.5 soil:water suspension), soil organic carbon (0.87%), available-N (263 kg/ha), available-P (16.8 kg/ha), available-K (339 kg/ha) and DTPA extractable Zn, Cu, Fe and Mn are 0.54, 1.03, 10.4, and 7.2 mg/kg, respectively.

The experiment comprised eight treatments, viz.

T₁: Control (without fertilizer); T₂: recommended dose of NPK (RDF); T₃: 75% of RDF; T₄: 75% of RDF + 25% N through vermicompost (VC); T₅: 50% of RDF + 50% N through VC; T₆: 75% of RDF + 25% N through VC + *Azotobacter*; T₇: 75% of RDF + 25% N through VC + *Microphos*; T₈: RDF + 25% additional N from urea. Recommended dose of fertilizers means, 70:35:70 g of NPK/plant for one-year-old plant. The required quantity of VC was applied just after snow cover over from field, while chemical fertilizers were applied before bloom and after fruit set of crops. Nitrogen was applied in 3 split doses, first dose (half the quantity) applied along with full dose of P and K, a fortnight before the bloom, second dose of nitrogen (1/4th of the total quantity) applied three weeks after fruit set and third dose (1/4th of total quantity) applied during fruit development stage, i.e. during last fortnight of May. The biofertilizers were applied with the first dose of chemical fertilizers as per recommended procedure. The experimental field was irrigated by drip irrigation and other cultural practices followed on uniform basis.

The tree trunk girth was recorded before the execution and at the end of each year of experiment (2012-2014). Before the execution of experiment, a ring was made with red paint at a height of 15cm above the ground level in each selected tree (treatment) to record the trunk girth from the same point each year. The trunk cross-sectional area (TCSA) of plant was calculated by using formula $TCSA = Girth^2/4\pi$. Plant yield attributes like fruit number, and fruit weight were recorded at proper growth stage and yield was calculated in t/ha. The production efficiency was calculated by the formula: Production efficiency (g/cm² TCSA) = Fruit yield (g)/TCSA (cm²).

Physico-chemical properties of composite soil samples (i.e. 4 samples from each plant was thoroughly mixed to make one) were collected at 0-20 cm depth (plant canopy area) after 30 days of third dose of fertilizer application for microbiological assay and after harvest of crop for physico-chemical properties of soil. The moisture content of soil was immediately determined by gravimetric method. In a 1:2.5 (soil:water) suspension, soil pH and electrical conductivity were measured. Soil organic carbon was determined using potassium dichromate oxidation method. Available N determined by alkaline permanganate method (Subbaiah and Asija 1956), available P by ascorbic acid method (Watanabe and Olsen 1965), available K by ammonium acetate method (Hanway and Heidal 1952) and DTPA extractable micronutrients by Lindsay and Norvell (1978). Dehydrogenase activity (DHA) was determined using the reduction of 2, 3, 5-triphenyltetrazolium chloride (3%) method (Klein *et al.* 1971), soil microbial biomass carbon (SMBC) was estimated through chloroform fumigation method (Vance *et al.* 1987). Measured variables were submitted for analysis of variance (ANOVA) and comparisons among means were made using the Duncan Multiple Range significant difference test (DMRT) using SAS 9.3 version packages (Chicago, IL, USA). Unless otherwise stated, the level of significance referred to in the

results is $P < 0.05$.

RESULTS AND DISCUSSION

Trunk cross sectional area (TCSA)

Data on trunk cross section area of apple plant as influenced by integrated use of VC, biofertilizers with inorganic fertilizer and over the year (2012-2014) presented in Table 1. Data revealed that, during the initial year of experiments (2012 and 2013) different treatments applied had no significant impact on apple tree TCSA. During the year 2014, plants received 75% RDF of NPK + 25% N through VC + *Microphos* (T_7) recorded significantly higher TCSA ($125/cm^2$) to other treatments where NPK was used through inorganic fertilizer alone (T_2 and T_3) except treatment T_8 . But, treatment (T_7) was statistically at par with treatments (T_4, T_5, T_6 and T_8) which received inorganic fertilizer in combination with VC and biofertilizers in

respect of TCSA. Minimum TCSA ($108/cm^2$) was recorded with T_1 (control) but statistically at par with treatments T_2, T_3 and T_4 in 2014. The data revealed that, application of fertilizer either through inorganic or organic sources as nutrients, had significantly higher percent change in TCSA from 2012 to 2014 in comparison with the controls, but the percent change in TCSA depends upon nature of fertilizer used during experiments. This suggest that, the reduced tree growth in the control treatment was caused by reduced nutrient supply to the plants. The maximum change (17.0%) were recorded under treatment 75% RDF of NPK + 25% N through VC + *Microphos* which was similar ($P \leq 0.05$) to 50% RDF of NPK + 50% N through VC (15.8%). Whereas, both inorganic treatments (T_2 and T_3) were similar to each other and were significantly higher over control in respect of per cent change in TCSA of apple plant. The maximum enhancement in TCSA in VC + biofertilizers treatments over the year as compared to control (T_1) might be due to use of VC along with biofertilizers had a beneficial impact on favorable conditions to soil microorganism which help in transformation of nutrient from unavailable pool to available pool. Bonilla *et al.* (2015) also reported compost made from almond shells, pruning waste, yard waste had significantly higher TCSA and other Avocado plant growth parameters.

Table 1 Effect of INM on trunk cross section area (TCSA) of apple var. Oregon Spur

Treatment	Trunk cross section area (cm^2)			
	2012	2013	2014	% change
T_1	100	104	108 ^C	8.03 ^E
T_2	102	107	113 ^{BC}	11.2 ^{CD}
T_3	103	108	114 ^{BC}	10.1 ^D
T_4	103	111	116 ^{ABC}	12.3 ^{BC}
T_5	107	114	124 ^{AB}	15.8 ^A
T_6	107	114	121 ^{AB}	13.5 ^B
T_7	106	116	125 ^A	17.0 ^A
T_8	111	118	124 ^{AB}	12.5 ^{BC}
Mean	105	111	118	12.6
SE(d)	3.57	3.95	4.65	0.86
LSD(P=0.05)	NS	NS	11.0	2.04

Fruit weight, yield and production efficiency

Data on fruit weight of apple as affected by different INM treatments with VC and biofertilizers is presented in Table 2. Average of three years data revealed that 75% RDF of NPK + 25% N through VC + *Azotobacter* (T_6) and 75% RDF of NPK + 25% N through VC + *Microphos* (T_7) treatments had significantly higher apple fruit weight (113 g) as compared to treatments 75% RDF of NPK + 25% N through VC (105 g) and control (96 g) and it was at par with rest of other treatments. VC and biofertilizers (*Azotobacter* and *Microphos*) has an outright role in nitrogen fixation, phosphorus solubilization, production of vitamins and growth promoting bio-chemical's that help in increased

Table 2 Effect of INM on fruit weight, yield and production efficiency of apple var. Oregon Spur

Treatment	Fruit weight (g)				Yield (t/ha)				Production efficiency (g/cm^2 TCSA)			
	2012	2013	2014	Mean	2012	2013	2014	Mean	2012	2013	2014	Mean
T_1	91	100 ^B	99 ^B	96 ^C	11.2 ^C	12.9 ^C	12.8 ^D	12.3 ^D	70.2 ^B	78.3	74.7 ^D	74.4 ^D
T_2	109	111 ^{AB}	110 ^{AB}	110 ^{AB}	16.8 ^{AB}	17.0 ^B	18.6 ^{BC}	17.5 ^{BC}	103 ^A	99.4	102 ^{BC}	101 ^B
T_3	109	114 ^A	107 ^{AB}	110 ^{AB}	15.2 ^B	16.8 ^{BC}	17.2 ^C	16.4 ^C	92.0 ^A	97.5	94.6 ^{CD}	94.7 ^{BC}
T_4	100	105 ^{AB}	108 ^{AB}	105 ^B	16.8 ^{AB}	17.2 ^{AB}	19.6 ^{BC}	17.9 ^{BC}	101 ^A	97.4	105 ^{ABC}	101 ^B
T_5	103	111 ^{AB}	115 ^A	110 ^{AB}	17.2 ^{AB}	20.5 ^{AB}	24.6 ^A	20.8 ^A	100 ^A	112	123 ^A	112 ^A
T_6	103	115 ^A	120 ^A	113 ^A	17.8 ^A	19.2 ^{AB}	20.2 ^B	19.1 ^{AB}	103 ^A	105	104 ^{ABC}	104 ^{AB}
T_7	105	113 ^{AB}	121 ^A	113 ^A	17.6 ^{AB}	21.3 ^A	23.3 ^A	20.7 ^A	103 ^A	114	117 ^{AB}	111 ^A
T_8	105	107 ^{AB}	114 ^{AB}	109 ^{AB}	15.5 ^{AB}	17.9 ^{AB}	19.8 ^{BC}	17.7 ^{BC}	86.6 ^{AB}	94.7	98.5 ^{BC}	93.2 ^{BC}
Mean	103	109	111	108	16.0	17.9	19.5	17.8	95.1	99.9	102	99.2
SE(d)±	4.26	3.29	3.77	1.69	1.06	1.71	1.24	1.09	7.47	11.2	8.90	3.98
LSD(P=0.05)	NS	7.92	9.07	4.07	2.50	4.06	2.95	2.59	17.6	NS	21.0	9.46

uptake of nutrients accordingly, the fruit size increased, the similar results were also reported by Kumar *et al.* (2018). During the experimentation period, maximum fruit yield (24.6 q/ha) in 2014 and minimum (11.2 q/ha) in 2012 were recorded with 50% RDF + 50% N through VC (T_5) and control (T_1) treatments respectively. The maximum average fruit yield of apple during experiment period were recorded in treatments T_5 (20.8 q/ha) and T_7 (20.7 q/ha), which were statistically at par with 75% RDF of NPK + 25% N through VC + *Azotobacter* (T_6). But these two treatments (T_5 and T_7) were significantly higher as compared to rest of the treatments. The combined use of VC and biofertilizers along with inorganic fertilizer resulted higher yield due to its increased fruit number and weight. This might be due to synchronized demand with supply of nutrient by their slow transformation and biodegradation of organic matter in the soil by biofertilizers. Kirad *et al.* (2009) also delineated similar results in strawberry with 75% of recommended fertilizers rate + 25% VC + rhizosphere bacteria culture treatment, which increase fruits/plant as well as yield of crops. On the basis of mean of three years of production efficiency (PE) data, highest average PE (112 g/cm² TCSA) was recorded in plants received 50% N through inorganic fertilizer and 50% through VC (T_5) followed by treatment T_7 (111 g/cm² TCSA). The treatments T_5 , T_6 and T_7 recorded significantly higher PE as compared to other treatments. The minimum mean production efficiency (74.4) was observed in control (T_1) (Table 2). In treatments T_5 , T_6 and T_7 had highest yield and PE might be due to secretion of ammonia and solubilisation of nutrients into the rhizosphere by microbial inoculants, which helps to better nutrient uptake by plants, thus increase, fruit size and conclusively, the yield and PE of apple. Wang *et al.* (2017) also revealed, continuous application of bioorganic fertilizers led to an accumulation of SOC and total as well as available nutrients,

which ensured more stable and higher apple yields.

Soil properties

Soil physico-chemical properties like soil reaction, soil organic carbon (SOC) content, available N, P and K contents in apple orchard after three years were influenced by integrated treatments listed in Table 3. In general, integrated treatments comprising 75% RDF + 25% N through VC + *Microphos/Azotobacter* improved soil health of apple orchard by improving soil organic matter, available N, P, Zn, dehydrogenase activity as well as soil microbial biomass carbon. Soil pH and electrical conductivity (EC) were recorded in the range of 6.40 - 6.84 and 0.18 - 0.26 dS/m, respectively, under different treatments in the 0-20 cm soil depth after three years of the experiment. Data also revealed that there was a positive impact in soil pH towards neutral by use of VC and biofertilizers but there were no significant effects of different treatments on pH and EC of soil was noticed. The treatment comprised 50% RDF + 50% N through VC (T_5) recorded significantly higher SOC (1.10%) as compared to rest of the applied treatments, except 75% RDF + 25% N through VC + *Azotobacter/Microphos* (T_6 and T_7) while lowest SOC (0.85%) was noticed in control treatment. 50% N replacement by VC and 25% N replacement by VC + biofertilizers resulted similar build up in SOC, this might be because of biofertilizers help in photosynthetic carbon assimilation and root exudation, thereby, accelerated microbial activity or microbial biomass content of soils, eventually resulted in similar organic C accumulation in soil. In general, integrated use of 75% RDF with VC along with biofertilizers (*Azotobacter* and *Microphos*) significantly enhanced the soil available N and P. Addition of 25% extra dose of N through urea resulted significantly highest available N (333 kg/ha) but statistically at par with T_5 and T_6 treatments. Addition of 50% nitrogen

Table 3 Fertility status of post-harvest soil of apple (var. Oregon Spur) orchard as influenced by INM treatments

Treatment	pH	EC (dS/m)	SOC (%)	Available major nutrients (kg/ha)			Available micro nutrients (mg/kg)			
				N	P	K	Zn	Cu	Fe	Mn
T1	6.64	0.25	0.85 ^D	246 ^D	15.4 ^C	321	0.31 ^F	0.80	8.41 ^C	6.24
T2	6.72	0.18	0.93 ^C	298 ^{BC}	21.0 ^{AB}	359	0.40 ^{EF}	1.04	8.90 ^C	6.62
T3	6.40	0.20	0.85 ^D	280 ^C	17.5 ^{BC}	345	0.53 ^{CD}	1.11	11.4 ^{BC}	8.06
T4	6.74	0.20	0.95 ^{BC}	300 ^{BC}	21.3 ^{AB}	357	0.59 ^{BC}	1.21	13.0 ^{AB}	7.97
T5	6.70	0.21	1.10 ^A	309 ^{AB}	21.6 ^A	361	0.58 ^{BC}	1.16	13.6 ^{AB}	8.14
T6	6.73	0.22	1.03 ^{AB}	320 ^{AB}	21.7 ^A	384	0.64 ^{AB}	1.22	15.0 ^{AB}	8.33
T7	6.84	0.26	1.02 ^{AB}	300 ^{BC}	24.2 ^A	376	0.72 ^A	1.24	16.4 ^A	7.76
T8	6.69	0.20	0.90 ^{CD}	333 ^A	20.9 ^{AB}	356	0.46 ^{DE}	1.15	11.8 ^{BC}	7.23
Mean	6.69	0.21	0.95	298	20.4	357	0.53	1.12	12.3	7.54
SE(d)	0.26	0.01	0.03	11.0	1.59	15.7	0.04	0.13	1.75	0.81
LSD (P=0.05)	NS	NS	0.08	26.0	3.77	NS	0.10	NS	4.15	NS

Means followed by same letter are not significantly ($P < 0.05$) different according to Duncan's multiple range test. EC- Electrical conductivity; SOC- Soil organic carbon

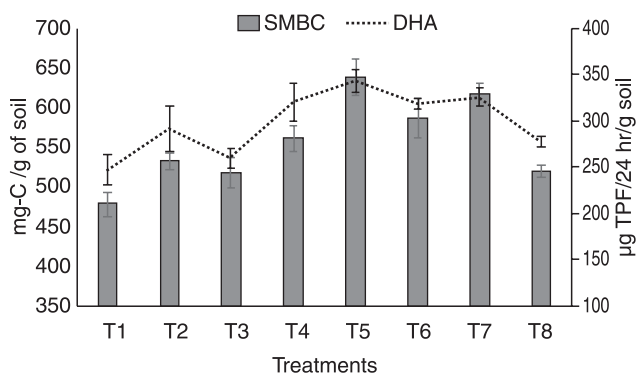


Fig 1 Dehydrogenase activity (DHA) and soil microbial biomass carbon (SMBC) of soil as influenced by INM in apple (var. Oregon Spur) orchard.

through VC led to less N loss, as most of the N associated with organic matter and are in equilibrium with available or mineralizable pool of N, while *Azotobacter* increased N fixation and ultimately both the treatments resulted in higher N availability. The available P was found highest (24.2 kg/ha) in 75% RDF + 25% N through VC + *Microphos* (T_7) treatment, which was statistically superior over control as well as 75% RDF treatments and statistically at par with all other treatments. Highest $\text{NH}_4\text{OAc-K}$ (384 kg/ha) was recorded with T_6 treatment and lowest (321 kg/ha) in control but no significant effect was noticed. During the experiment period, DTPA-Zn content in soil improved significantly from 0.31 in control to 0.72 mg/kg in 75% RDF + 25% N through VC + *Microphos* (T_7) treatment and the treatment T_7 was statistically at par with T_6 treatment and superior over rest of the treatments. DTPA extractable Cu and Mn in post-harvest of soil ranged from 0.80 - 1.24 mg/kg and 6.24 - 8.33 mg/kg respectively, and the contents were not influenced due to application of RDF along with biofertilizer and VC, but increasing trend were noticed by integrated applications of inorganic and organic fertilizers. The significant highest DTPA extractable Fe (16.4 mg/kg) was recorded with 75% RDF + 25% N through VC + *Microphos* (T_7) which was statistically higher over inorganic and control treatments and at par with other integrated treatments. In nut shell, application of VC and biofertilizers enhanced the soil available nutrients as compared to only inorganic fertilizer and unfertilized control treatments indicating long-term improvement in soil physical and chemical properties and buildup of soil fertility.

The activity of dehydrogenase, an oxidoreductase enzyme related to oxidative phosphorylation processes, has been proposed, together with basal respiration, as a measure of the overall microbial activity (Tejada *et al.* 2006). Dehydrogenase activity (DHA) was significantly affected by the different treatments applied in the experiments has been presented in Fig 1. Highest DHA (342 µg TPF/24 hr/g dry soil) was recorded in 50% RDF + 50% N through VC (T_5) treatment, which was statistically similar to integrated treatments, i.e. T_4 , T_6 and T_7 and superior over rest of other inorganic treatments. Lowest

DHA (246 µg TPF/ 24 hr/g soil) was noticed in control. The increase in DHA in VC treated soil might be due to the synergic effect of the stimulation of microbial growth by the organic substrates added, therefore, the intensity of addition of VC correlated with DHA in soil. Higher DHA with increasing rate of organic fertilizer applications might be due to proliferation of microbial populations capable of producing new enzymes has also been reported earlier by several other workers (Kumar *et al.* 2014, Sharma *et al.* 2016). Secondly, continuous application of only inorganic fertilizers or extra nitrogenous fertilizers lead to shift in microbial community structure, which might have led to a lower DHA and decreases susceptibility of soil to abiotic stress. Soil microbial biomass carbon (SMBC), an agent of labile nutrients, is critically important for the soil health establishment and one of the most sensitive indicators of sustainability of management systems. An increase in SMBC in soil following application of compost has been reported earlier by several scholars. However, in the present study, along with VC and biofertilizers, recommended inorganic fertilization also had higher SMBC (Fig 1) than that of unfertilized control indicating that nutrient management practice with sufficient nutrient inputs and supplements are vital key for improvement of SMBC. Present study revealed, application of 50% of recommended dose of N through VC (T_5) was most efficient treatment for improving SMBC and it was statistically at par with treatment T_7 but significantly higher than rest of applied treatments. This could be caused by the higher amount of organic carbon and microbial biomass carbon added by VC.

The present investigation concluded that, apple trees (var. Oregon Spur) under high density plantation are very responsive to the application of 50% RDF + 50% N through VC or 25% N through VC + *Microphos/Azotobacter*, which provides a “win-win” opportunity for sustainable apple production as well as soil health and simultaneously reduce environmental impact.

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