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Impact of *Jeevamrut* formulations and biofertilizers on soil microbial and chemical attributes during potato cultivation

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ABSTRACT

Application of *jeevamrut*, fortified with neem cake or vermitea, in combination with consortia of plant growth-promoting microorganisms such as *Azotobacter*, Vesicular Arbuscular Mycorrhiza (VAM) or Phosphate Solubilizing Bacteria (PSB) would attenuate the negative effects of synthetic fertilizers over microbial and physicochemical properties of soil. The present study was carried out during 2021–2022 and 2022-23 with two factors, namely Factor-J (*Jeevamrut*) of 4 levels and Factor-B (Biofertilizers) of 5 levels. Four levels of factor-J include three *jeevamrut* formulations with one control while five levels of factor-B include four biofertilizer combinations with one control. Observations of various soil-based parameters confirm a significant reduction in soil pH and electrical conductivity of soil after harvesting potato crops (variety Kufri Bahar) under different treatments in comparison to controls and the initial value. Further, the organic carbon, available nitrogen, phosphorus, and potassium of soil were reported to be enhanced after the application of *jeevamrut* fortified with vermitea or neem cake. The efficacy of fortified *jeevamrut* can be further improved by adding a consortia of biofertilizers consisting of PSB and *Azotobactor* or VAM fungi. These treatments have also a significant effect on enhancing soil microbial activities.

1. INTRODUCTION

The intensive use of synthetic or inorganic fertilizers is one of the common practices used by potato crop growers for higher marketable yields. This has raised serious concerns over soil health and soil fertility in the long run. Due to the overuse of chemical fertilizers, society is facing the biggest challenge of productivity declining, natural resource depletion, soil fertility reduction, the threat to life on land and in water, adverse effects on climate, and unsecured healthy lives and well-being for human [1-4]. Further, the sole application of fertilizers has caused the depletion of microbial biomass carbon, soil respiration, dehydrogenase, acid phosphatase, and β-glucosidase activities, microbial population, and soil aeration in the rhizosphere [5].

The rhizosphere is attributed with non-metabolic secretions by roots (called root exudates) which are involved in rhizospheric communication between soil, roots, and microbial populations [6-8]. In addition, roots also release some metabolically produced compounds such as mucilage or lysates which act as chemo-attractants for rhizobacteria. The healthy rhizosphere facilitates plant growth promoting rhizobacteria (PGPR) to establish the endophytic association [9,10] or non-symbiotic associations with host plants. However, indiscriminate use of chemicals has resulted a detrimental impact on the health of the rhizosphere ecosystem. The practice of a natural farming system offers some hope; however, the complete package of practices based on natural farming needs to be worked out for proper recommendations to the farmers. The natural farming system, also known as organic or sustainable farming, has received popularity for its emphasis on environmental stewardship, soil health, and reduced dependence on synthetic inputs. Thus, in the current investigation, we have explored the integration of *jeevamrut*, vermitea, or neem cake with biofertilizers, including phosphate solubilizing bacteria (PSB), *azotobacter,* and vesicular arbuscular mycorrhiza (VAM).

Jeevamrut is considered one of the important components of Zero Budget Natural Farming [11]. It contains beneficial microorganisms which ensure the mineralization of organic matter or humus to mobilize fixed nutrients [12] and acts as a nutrient reservoir that can improve soil fertility and crop productivity [13]. Vermitea is an aqueous extract from vermicompost that is rich in major nutrients and PGPR [14]. It also contains plant growth promoter's analog to auxins and cytokinin which could be accountable for improved nutrient and microbial levels of *jeevamrut*. Adding neem cake will improve the nutritional value as well as the insecticidal potential of *jeevamrut*. The fortification of *jeevamrut* with vermitea or neem cake could be a novel

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approach for the improvement of soil nutrient status for commercial potato cultivation.

Biofertilizers, consortia of living microorganisms, have the ability to dissolve the fixed and unavailable nutrients for making them available to the plants [15,16]. *Azotobacter* is a free-living bacterium that promotes nitrogen sequestration from atmosphere, synthesizes plant growth promoters such as auxin, cytokinin, and gibberellin, and stimulates microbial activities in rhizosphere to enhance the uptake of nutrients by plants [17,18]. The PSB increase phosphatase activities and release organic acids to lower the pH of the rhizosphere [19,20]. The acid hydrolysis of a bound form of phosphate results in its solubilization and increases the level of available phosphorus in soil [21]. The VAM fungi are involved in the mineralization of organic matter, the production of growth-promoting substances, and the development of tolerance to soil-based stresses in plants. Co-inoculation of VAM with PSB has many fold advantages by decomposition and mineralization of organic matter to release major nutrients in the soil [22].

Although a number of studies have been carried out to explore the potential of various biofertilizers to improve the productivity and fertility of the soil, it is essential to work out the physicochemical and microbial parameters of soil in relation to the application of microbial consortia (PSB and *Azotobacter* or VAM) in combination with the enriched *jeevamrut* formulation. Thus, the investigation was carried out with the hypothesis that the combined application of fortified *jeevamrut* and plant growth-promoting microorganisms would attenuate the negative effects of synthetic fertilizers over microbial and physicochemical properties of soil.

2. MATERIALS AND METHODS

2.1. Experimental Area and Materials

The experiment under the above topic was performed at the CRC farm of agriculture at ITM University, Gwalior, India. The investigation site is located under humid and subtropical climatic conditions and located at an elevation of 412 meters above mean sea level in the gird region of north Madhya Pradesh. The experiment was repeated for 2 years from 2021–2023. Kufri Bahar, a hybrid of Kufri Red × Gineke, which was released by Central Potato Research Institute, Shimla, was used for experimentation due to its commercial acceptability in the region. It has a semi-compact canopy and matures within 100–120 days with an average potential yield of 300–350 q ha^{-1}.

2.2. Experimental Details

2.2.1. Treatment details

The treatments consisted of two factors: Factor-J (*Jeevamrut*) of 4 levels and Factor B (Biofertilizers) of 5 levels, resulting in 20 treatment combinations. The gross experimental area was 528 m^2 while the net experimental area was 345.6 m^2 and planting was done at the spacing of 60 cm (row) × 20 cm (plant). The fertilizer application was done as per the recommended dose of N: P:K at the rate of 180:80:120 kg ha^{-1} (recommended by Central Potato Research Institute–Regional Station, Gwalior). The details of doses and applications of fertilizers and treatments are discussed in Table 1.

2.2.2. Climatic conditions and soil attributes of the experimental area

The experimental area received an average annual rainfall of 900 mm where monsoon started in June and remained active till September. Although occasional showers were also reported during an investigation (winter season), it was a cool and dry period. The initial properties of the soil are: the sand: silt: clay as 66:22.50:15.10 (sandy loam), pH of 8.04, electrical conductivity (EC) of 0.25 dSm^{-1}, organic carbon (OC) of 0.65 %, available nitrogen of 198.45 kg ha^{-1}, available phosphorus of 12.55 kg ha^{-1}, exchangeable potassium of 155.65 kg ha^{-1}. The details of chemical and microbial attributes of experimental soil and different formulations applied during an investigation are given in Table 2.

2.2.3. Agronomical operations

After harvesting of previous crop (rice), the primary tillage practices were carried out to give a proper tilth. The farm yard manure of 10 tons per hectare was incorporated 30 days before the sowing of potatoes. Seed potato tubers were taken out from cold storage and kept in the shade for 15 days before planting to accelerate the sprouting. The seeds of uniform size (45–50 mm diameter) were planted manually at a uniform distance of ten centimeters between plants. The basal application of fertilizers and other treatments was carried out as per details given in Table 1 at 3 days before planting in the experimental field. Earthing up was done 30 days after planting along with the manual weeding. The exposed tubers were covered to avoid disease and rotting

Table 1: Details of treatment formulation and application.

Factor J	*Jeevamrut* formulation	Details of formulation and application
J_0	No *Jeevamrut*	Only inorganic fertilizers were applied*
J_1	*Jeevamrut*	100 l of water + 10 kg of cow dung + 10 l of cow urine + 2 kg of *jaggery* + 2 kg of gram flour. The mixture was allowed for fermentation for 10 days
J_2	*Jeevamrut* + Vermitea	10 l of Vermitea were mixed with J_1 at the time of application
J_3	*Jeevamrut* + Neem cake	10 kg of Neem cake powder was mixed with J_1 at the time of application
Factor B	**Biofertilizer (s)**	**Details of formulation and application**
B_0	No biofertilizers	Only inorganic fertilizers were applied*
B_1	*Azotobacter*	100 ml of *Azotobacter* in 10 l of water
B_2	VAM (Vesicular Arbuscular Mycorrhiza)	100 g of VAM in 10 l of water
B_3	PSB (Phosphate Solubilizing Bacteria) + *Azotobacter*	100 ml of *Azotobacter* 100 mL of PSB in 10 l of water
B_4	PSB + VAM (Vesicular Arbuscular Mycorrhiza)	100 g of VAM and 100 mL of PSB in 10 liters of water

*Only N: P: K @ 180:80:120 kg ha^{-1} (recommended by Central Potato Research Institute-Regional Station, Gwalior.

Table 2: Details of chemical and microbial properties of soil along with various formulations of J*eevamrut*.

Microbial count	J_1: *Jeevamrut*	J_2: *Jeevamrut* + Vermitea	J_3: *Jeevamrut* + Neem cake	Experimental Soil (dry)
Bacterial count (cfu mL^{-1})	8.75×10^6	48.42×10^6	35.67×10^6	66×10^6 cfu g^{-1}
Fungal count (cfu mL^{-1})	1.35×10^4	4.06×10^4	3.88×10^4	1.21×10^6 cfu g^{-1}
Actinomycetes (cfu mL^{-1})	3.78×10^4	18.78×10^4	12.46×10^4	0.82×10^6 cfu g^{-1}
Chemical Properties	**J_1: *Jeevamrut***	**J_2: *Jeevamrut* + Vermitea**	**J_3: *Jeevamrut* + Neem cake**	**Soil**
pH	4.65	4.43	5.57	8.04
EC (dSm^{-1})	0.56	0.67	0.66	0.25
Carbon (g l^{-1})	6.78	6.81	6.79	0.65%
Nitrogen (g l^{-1})	0.35	0.56	0.71	198.45 kg ha^{-1}
Phosphorus (g l^{-1})	0.05	0.22	0.19	12.55 kg ha^{-1}
Potassium (g l^{-1})	0.65	0.83	0.81	155.65 kg ha^{-1}

of tubers and to prevent the synthesis of solanin which is responsible for the greening of potato tubers. Five light irrigations (5 cm) were given at 15, 30, 45, 60, and 75 days after the sowing of the potato crop. Harvesting was done in March 2021–2022 and 2022–2023 manually with the help of *kudal*.

2.3. Observations Recorded

2.3.1. Physical properties of soil

Bulk density and particle density value for soil was calculated using the formula [23]:

$$Bultk\ density = \frac{Mass\ (g) oven\ dry\ soil}{Total\ soil\ volume\ (cubic\ cm)}$$

$$Particle\ density = \frac{Mass\ (g) oven\ dry\ soil}{Volume\ of\ solids\ (cubic\ cm)}$$

The porosity of soil was estimated by the Piper method (1966) as per the given formula:

$$\Pr osity(\%) = \left(1 - \frac{Bulk\ Density}{Particle\ Density}\right) \times 100$$

The soil particles were segregated as per the given particle diameter: sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (<0.002 mm). To determine the soil texture, the percentages of sand, silt, and clay were calculated from the laboratory as per the given formula [23], and soil texture was determined using a soil triangle:

$$Sand(\%) = \frac{mass(g) of\ sand \times 100}{mass(g) of\ sand + silt + clay}$$

$$Silt(\%) = \frac{mass(g) of\ silt \times 100}{mass(g) of\ sand + silt + clay}$$

$$Clay(\%) = \frac{mass(g) of\ clay \times 100}{mass(g) of\ sand + silt + clay}$$

2.3.2. Microbial properties of soil

The microbial populations were estimated by the serial dilution and pour plate techniques using agar media [24]. The media were prepared and sterilized in an autoclave at 121°C and 15 psi for 15 min, while pre-sterilized agar plates were used for plating of diluted samples in triplicates. The agar plates for microbes were incubated at 30+1°C in an inverted position for 5–7 days until countable colonies were developed. The respective colonies were counted on the basis of their morphological characteristics and growth pattern and the microbial population was expressed as colony-forming units (cfu) ml^{-1}.

2.3.3. Chemical properties of soil

The soil samples were randomly collected from four points of each plot and thoroughly mixed to make representative samples for further analysis before planting and after harvesting of crops. Standard protocols were used to determine the various soil parameters. OC, available nitrogen, phosphorus, and potassium were estimated in the process as advocated by Jackson [25]. Soil pH was determined using a pH meter through a suspension of 1: 2.5 soil in water [25] as described by Schwyter and Vaughan [23]. EC was determined using the procedure given by Schwyter and Vaughan [23] with the help of an EC meter. The details of the physico-chemical and microbial properties of soil along with various formulations of *jeevamrut* is given in Table 2. This value was taken as the initial control for estimating the post-harvest properties of the experimental field.

2.4. Statistical Analysis

The statistical analysis was carried out on the observations recorded on various microbial, physical, and chemical parameters of soil using a two-way analysis of variance at 0.05 probability level in Excel and the SPSS software. The strength of the relationship of initial microbial count in *jeevamrut* with the various soil attributes was estimated as Pearson's correlation coefficient. The principal component analysis (PCA) was carried out using OPSTAT software to reveal the existing unexpected associations among variables.

3. RESULTS AND DISCUSSION

3.1. Soil Physical Attributes

The physical attributes of soil including bulk density, particle density, and soil texture (sandy, silt, and clay) were estimated before planting (taken as IC-Initial Control) and after harvesting [Figure 1] in both the years of observation. Bulk density has having strong correlation with a stock of carbon and nutrients in soil [26]. Soil porosity is primarily dependent on soil structure; however, the impact of vegetation and soil use patterns cannot be ignored. As per the available proportion of sand, silt, and clay particles (66: 22.50: 15.10), the soil of the experimental area was reported as silt loam. There was no noticeable variation reported before and after the crop. The bulk density was noticed to range from 1.53 to 1.55 g cm^{-3} in year 1 and 1.55 to 1.56 g cm^{-3} in

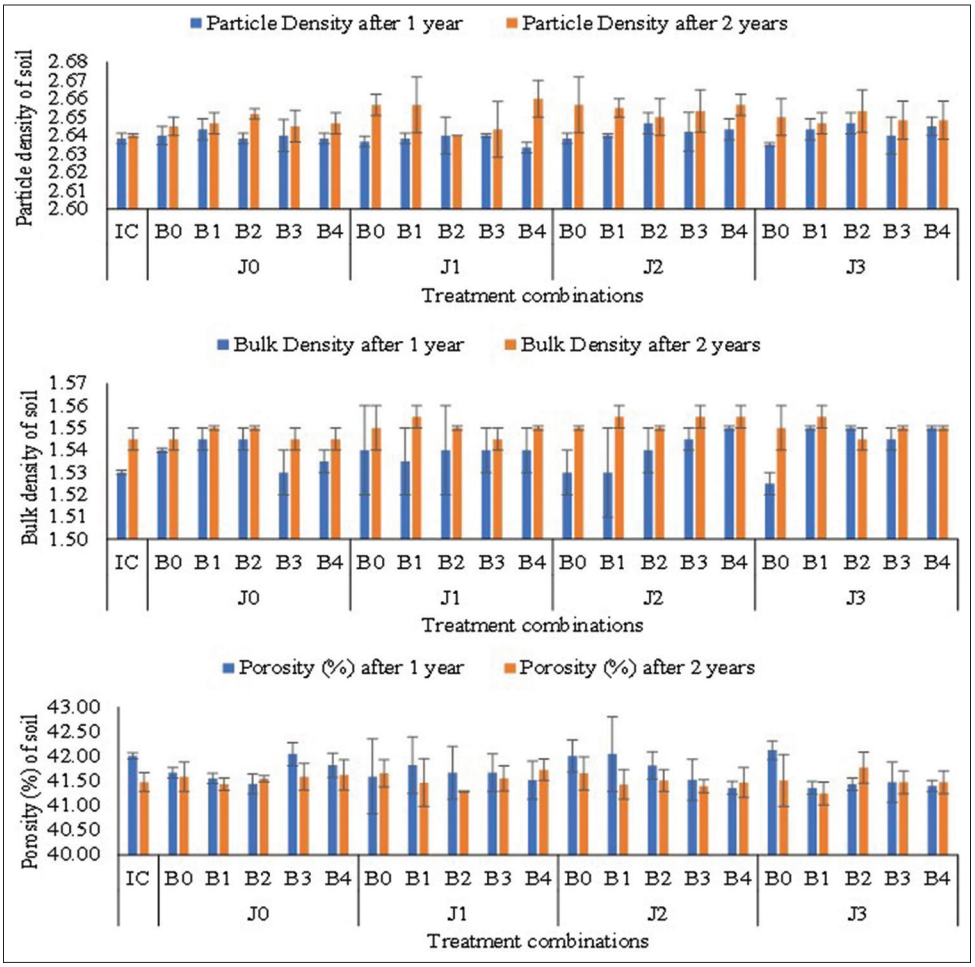

Figure 1: Physical properties of soil after harvesting of crops under different treatment combinations (J_0: No *Jeevamrut*, J_1: *Jeevamrut*, J_2: *Jeevamrut* + Vermitea, J_3: *Jeevamrut* + Neem cake, B_0: No biofertilizers, B_1: Azotobacter, B_2: Vesicular Arbuscular Mycorrhiza [VAM], B_3: Phosphate Solubilizing Bacteria [PSB] + Azotobacter, B_4: PSB + VAM, IC: Initial control).

year 2 with initial control of 1.53 and 1.55 g cm^{-3}, respectively. The particle density was varied from 2.63 to 2.65 g cm^{-3} in year 1 and 2.64 to 2.66 g cm^{-3} in year 2 with initial control of 2.64 g cm^{-3}. The porosity of the experimental soil was reported to range from 41.36 to 42.13 in year 1 and from 41.25 to 41.77 in year 2.

The present study observed a non-significant variation in porosity, particle density, and bulk density in the experimental areas which could be due to the static nature of these physical attributes in a short duration. However, the climatic factor and land use pattern are not static in the long term and are closely related to the hydro-ecological and bio-geochemical cycling within that ecosystem [27]. These favors, the ecological succession to bring alteration in the structure and porosity of the soil. The distribution and magnitude of soil porosity regulate the water holding capacity, soil aeration, and microbial diversity which in turn play a significant role in biogeochemical and hydrological cycling so any change in soil structure due to compaction is one of the serious threats to vegetation and biogeochemical cycling [28]. Hence, it is important to maintain proper aeration and drainage in soil for effective improvement in soil nutrient availability. While most ecological models consider porosity as a constant parameter, its dynamic nature is also being recognized in response to changes in climate and land use patterns in the long run [29,30]. Bulk density of soil is used to determine stocks of carbon which is one of the three sub-indicators used to calculate the extent of land degradation under UN SDG Indicator 15.3.1 [26,31].

3.2. Soil Microbial Properties

The soil microbial population was significantly improved due to the application of different formulations of *jeevamrut* or biofertilizers alone or in combination [Table 3, Supplementary Table 1, Figure 2] in comparison to estimates taken before the experiment [Table 2]. This might be due to the existence of favorable conditions in the rhizosphere region after the addition of *jeevamrut* or biofertilizers under different treatments [32]. The fermented organic liquid (*jeevamrut*) contains microbial population and plant-promoting substances which was helpful in buffering the rhizosphere for further co-multiplication of these microbes [33]. Further, the addition of vermitea (J_2) or neem cake (J_3) during the preparation of *jeevamrut*-based formulation improved microbial counts. These additives acted as bio-enhancers or catalysts and supplied essential nutrients for the growth and multiplication of beneficial microbes [34,35]. The microbial strength present in *jeevamrut* or biofertilizers secrete proteins, organic acids, and antioxidants to transform soil organic matter into energy which are supportive of the growth of useful microbes in the rhizosphere [36,37].

Table 3: Microbial count in the soil after harvesting of crops under different treatments.

Factors	Bacterial count (10^6 cfu g^{-1})	Fungal count (10^4 cfu g^{-1})	Actinomycetes count (10^4 cfu g^{-1})
J_0 (control)	76.91±7.91^c	155.61±24.44^c	96.01±8.28^d
J_1	79.93±7.99^c	156.22±23.70^b	98.76±7.36^c
J_2	95.83±7.83^a	157.40±24.18^a	108.00±7.69^a
J_3	90.70±7.80^b	157.54±23.70^a	103.96±6.44^b
CD (at 0.05)	0.427	0.608	0.855
SE (m) ±	0.149	0.211	0.298
P-value	2.65×10^{-17}**	9.47×10^{-05}**	4.37×10^{-09}**
B_0 (control)	77.29±9.12^d	125.98±1.19^e	93.09±5.81^e
B_1	85.63±8.83^c	136.80±1.20^d	101.28±4.87^c
B_2	79.35±8.85^d	170.94±0.92^b	96.12±5.69^d
B_3	96.33±8.84^a	167.72±0.87^c	111.04±4.57^a
B_4	90.61±8.94^b	182.02±0.90^a	106.89±6.11^b
CD (at 0.05)	0.478	0.679	0.956
SE (m) ±	0.166	0.236	0.333
P-value	1.88×10^{-16}**	6.34×10^{-21}**	1.57×10^{-10}**

All values are mean±SD values of three replications, J0: No *Jeevamrut* (control), J_1: *Jeevamrut*, J_2: *Jeevamrut* + Vermitea, J_3: *Jeevamrut*+Neem cake, B_0: No biofertilizers (control), B_1: Azotobacter, B_2: VAM (Vesicular Arbuscular Mycorrhiza), B_3: PSB (Phosphate Solubilizing Bacteria) + Azotobacter, B_4: PSB + VAM (Vesicular Arbuscular Mycorrhiza),*0.05 level of significance, **0.01 level of significance.

Bacterial and actinomycetes counts were significantly improved due to the application of *Azotobacter* and/or PSB ($B_3 > B_4 > B_1$) in combination with *jeevamrut* while the fungal count was enhanced due to application of VAM ($B_4 > B_2$). VAM develops a synergetic interaction with other beneficial microorganisms of the rhizosphere due to the strong capability of co-inoculation with other biofertilizers [22] and better multiplication of these microbes was observed when applied in combinations [38]. The enrichment of soil microbial population after application of *jeevamrut* and biofertilizers might be due to favorable rhizospheric micro-environment developed due to root exudates, soil aggregation, decomposition of root cells and organic matter, availability of plant nutrients, and other physical-biochemical processes resulting a higher microbial abundance [39-44].

3.3. Soil Chemical Attributes

The chemical attributes of soil including pH, EC, OC, available N, P, and K were estimated before planting (initial value) and after harvesting of potato crops [Tables 4 and 5, Figures 3 and 4] in both the years of observation.

3.3.1. Soil pH

The initial soil pH before planting was reported as 8.04 which were significantly reduced to 7.73 and 7.45 after application of *jeevamrut* + vermitea in year 1 and year 2, respectively [Table 4]. A significant reduction (3.33% and 6.14%) in pH was also reported after the application of PSB with *Azotobacter* (7.77 and 7.54) in consecutive years. The interaction effect of *jeevamrut* formulation and biofertilizers was also significant with the lowest pH in J_2B_3 and J_2B_4 [Figure 3 and Supplementary Table 2]. The finding confirms that the application of *jeevamrut* and biofertilizers such as PSB, *Azotobacter, or* VAM with a reduction of inorganic fertilizers up to 50% provided a constant supply of major nutrients to the plants and made the fixed micronutrients easily available. Further, the increased microbial population is responsible for developing an acidic medium through the decomposition of organic matter which could be responsible for lowering of pH of soil [38].

Jeevamrut formulation is dominated with the fungal members of *Ascomycota* and *Basidiomycota* phyla [11,45,46] while vermitea formulation is enriched with bacterial members of Proteobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, and Chloroflexi and the fungal members of *Ascomycota*, Basidiomycota, Cryptomycota, *Entomophthoromycota*, and *Glomeromycota* phyla which are involved in nutrient recycling in agri-ecosystem through microbial action on organic matter [47]. *Jeevamrut* contains cow urine which is a source of amino acid and can impart resistance against the pathogen [12] and various formulations of *jeevamrut* have been reported effective in inhibiting *Alternaria alternata in vitro* with more than 90% mycelial growth inhibition [48]. Further, vermitea has been reported to enhance the suppression of soil or air borne diseases. Application of the commercial compost tea inhibited the growth of *A. solani* mycelium (up to 74%), *Rhizoctonia solani* (isolate 422) (up to 85%), and *R. solani* (isolate 299) (up to 36%) in potato [47]; and suppressed the growth of Gray Mold (*Botrytis cinerea*) in geranium [49]. The efficacy has been reported to be influenced by the method of preparation, dilution ratio, application equipment, timing, rates, spray adjuncts, and supplementing specific microbial antagonists [50]. However, the details of mechanism for imparting disease resistance are yet to be explored. In the current study, the application of vermitea with *jeevamrut* has the additional advantage of developing disease resistance and enhancing the microbial actions on soil organic matters which in turn improves the soil pH, soil EC, and availability of nutrients; however, there was no substantial change in soil OC [51].

3.3.2. Soil EC

The initial soil EC before planting was reported as 0.25 dSm^{-1} which was significantly reduced to 0.238 dSm^{-1} and 0.227 dSm^{-1} after application of *jeevamrut* + vermitea in consecutive years [Table 4]. A significant reduction in EC was also reported after the application of PSB with *Azotobacter* (0.238 dSm^{-1} and 0.233 dSm^{-1}). There was a significant interaction between *jeevamrut* and biofertilizers with the lowest EC in J_2B_3 and J_2B_4 [Figure 3 and Supplementary Table 3]. The finding confirms that the application of *jeevamrut* and biofertilizers such as PSB, *Azotobacter, or* VAM has resulted in better uptake of metallic ions by the plants resulting in lowering of EC of soil. The microbial populations present in soil are involved in the secretion of extracellular enzymes which are involved in electrolytic balance in the soil for improved nutrient absorption and assimilation by the plants [52]. The VAM fungi have a strong impact on lowering of EC and maintaining the ionic balance in soil which might be associated with the ability of these fungi to mobilize the mineral ions by direct uptake and translocation of ions to plants through mycorrhizal hyphae [53]. VAM adheres to the plants rhizoids which lead to the development of fungal hyphae. These hyphae further penetrate and form arbuscules within the root cortical which leads to the significant increase in rhizosphere resulting in improvement of nutrient uptake by plants. Further, these fungi developed intracellular vesicles as terminal swellings between the fungal hyphae and the host plant that acts as a storehouse for complex carbon compounds and mineral nutrients [54].

3.3.3. Soil OC

The initial soil OC before planting was reported as 0.65% which was significantly increased to 0.693% and 0.692% after the application

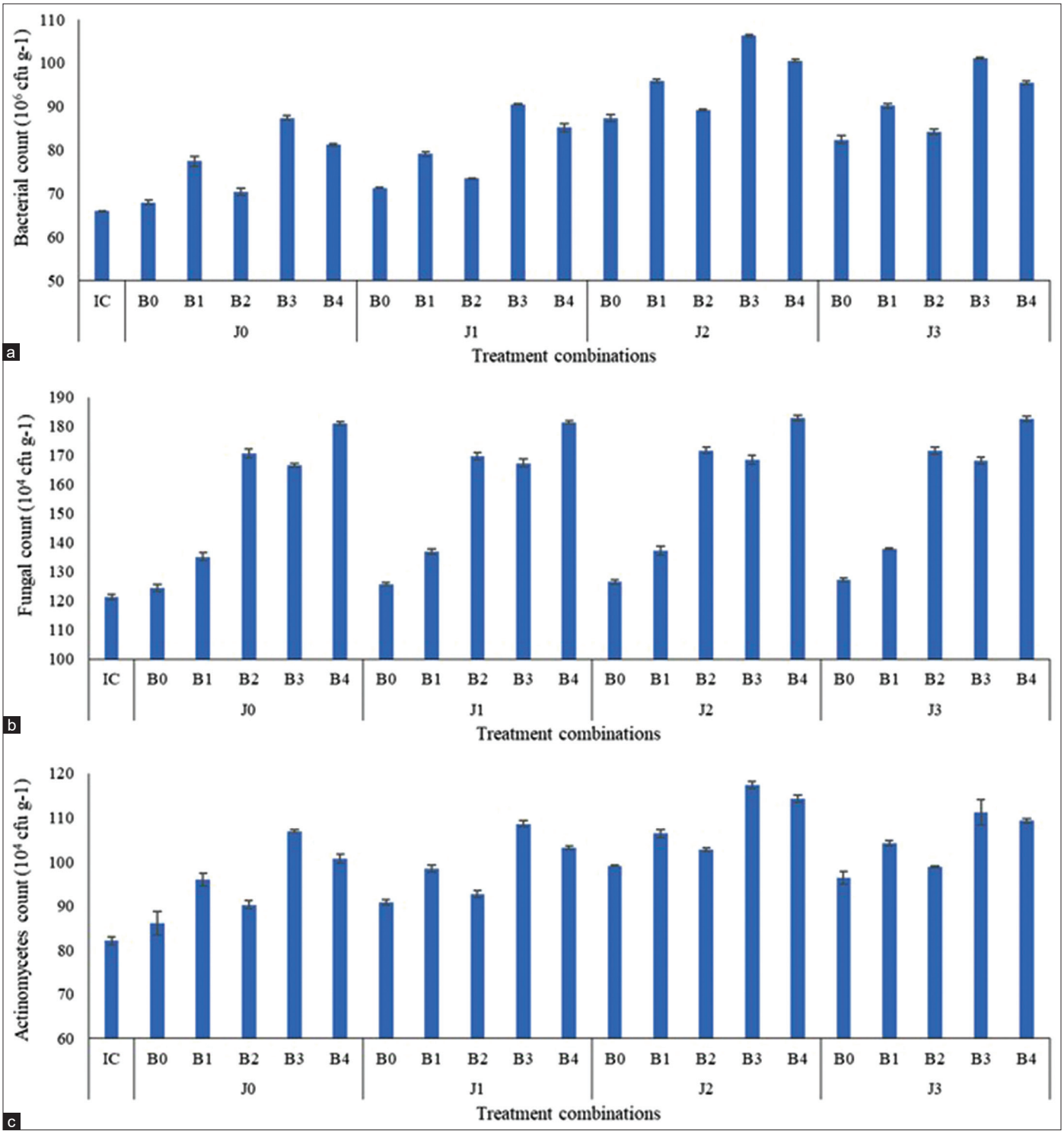

Figure 2: (a-c) Microbial count in the soil after harvesting of crops under different treatment combinations (J_0: No *Jeevamrut*, J_1: *Jeevamrut*, J_2: *Jeevamrut* + Vermitea, J_3: *Jeevamrut* + Neem cake, B_0: No biofertilizers, B_1: Azotobacter, B_2: Vesicular arbuscular mycorrhiza [VAM], B_3: Phosphate solubilizing bacteria [PSB] + Azotobacter, B_4: PSB + VAM, IC: Initial control).

of *jeevamrut* + neem cake and *jeevamrut* + vermitea, respectively, in year 2 [Table 4]. A significant improvement in OC was also reported after the application of PSB + VAM and PSB + *Azotobacter* (0.695% and 0.687%, respectively) in year 2. However, the interaction effect of *jeevamrut* formulation and biofertilizers was also not significant with the highest OC in J_2B_3 and J_2B_4 [Figure 3 and Supplementary Table 4]. Sharma [55] and Adekiya *et al*. [56,57] had also confirmed improvement in the soil physicochemical properties including soil OC after the application of *jeevamrut*. The strength of microbial population in the soil is largely affected by the soil environment which in turn is influenced by the application of fertilizers and the *jeevamrut* and/or biofertilizers in various treatments. The investigation

Table 4: Soil pH, EC (dSm^{-1}), and OC (%) after harvesting of crops in year 1 and year 2 under different treatments.

Factors	pH (after year 1)	pH 2 (after year 2)	EC (after year 1)	EC (after year 2)	OC (after year 1)	OC (after year 2)
J_0 (control)	8.01±0.05^a	7.96±0.08^a	0.249±0.003^a	0.247±0.005^a	0.615±0.017^c	0.660±0.012^b
J_1	7.76±0.08^c	7.53±0.10^c	0.240±0.001^a	0.232±0.003^c	0.638±0.009^b	0.687±0.008^a
J_2	7.73±0.09^d	7.45±0.17^d	0.238±0.004^c	0.227±0.007^d	0.645±0.011^{ab}	0.692±0.011^a
J_3	7.88±0.03^b	7.73±0.06^b	0.240±0.001^b	0.240±0.001^b	0.649±0.011^a	0.693±0.008^a
CD (at 0.05)	0.013	0.036	0.001	0.002	0.011	0.012
SE (m) ±	0.005	0.013	0.001	0.001	0.004	0.004
P-value	1.97×10^{-07}**	2.08×10^{-08}**	3.75×10^{-05}**	4.16×10^{-06}**	6.24×10^{-05}**	4.84×10^{-07}**
B_0 (control)	7.93±0.10^a	7.80±0.19^a	0.243±0.005^a	0.241±0.006^a	0.620±0.022^c	0.670±0.016^b
B_1	7.87±0.10^b	7.72±0.18^b	0.243±0.005^a	0.238±0.009^b	0.638±0.020^b	0.680±0.019^b
B_2	7.86±0.15^b	7.68±0.25^b	0.243±±0.005^a	0.237±0.010^b	0.638±0.015^b	0.683±0.016^{ab}
B_3	7.77±0.14^d	7.54±0.25^d	0.238±0.006^b	0.233±0.010^c	0.642±0.011^{ab}	0.687±0.019^{ab}
B_4	7.81±0.15^c	7.59±0.28^c	0.243±0.005^a	0.233±0.010^c	0.650±0.010^a	0.695±0.010^a
CD (at 0.05)	0.015	0.041	0.001	0.002	0.012	0.013
SE (m) ±	0.005	0.014	0.001	0.001	0.004	0.005
P-value	0.00072**	0.0002**	0.033*	0.021*	0.00092**	0.0092**

All values are mean±SD values of three replications, J_0: No *Jeevamrut* (control), J_1: *Jeevamrut*, J_2: *Jeevamrut*+Vermitea, J_3: *Jeevamrut* + Neem cake, B_0: No biofertilizers (control), B_1: Azotobacter, B_2: VAM (Vesicular Arbuscular Mycorrhiza), B_3: PSB (Phosphate Solubilizing Bacteria) + Azotobacter, B_4: PSB + VAM (Vesicular Arbuscular Mycorrhiza),*0.05 level of significance, **0.01 level of significance.

Table 5: Soil N, P, and K ($kg\ ha^{-1}$) of soil after harvesting of crops under different treatment combinations.

Factors	N1Y	N2Y	P1Y	P2Y	K1Y	K2Y
J_0	210.17±2.36^d	216.47±1.61^c	13.61±0.29^c	14.83±0.53^c	161.07±2.58^d	166.07±2.58^d
J_1	213.40±4.79^c	220.00±3.76^b	13.88±0.23^b	15.01±0.59^b	164.83±2.32^c	169.20±2.47^c
J_2	218.57±5.85^a	226.27±5.71^a	14.45±0.34^a	15.38±0.73^a	171.43±2.24^a	179.67±3.66^a
J_3	216.20±5.68^b	224.07±5.72^a	14.52±0.27^a	15.40±0.62^a	167.17±1.20^b	171.40±0.72^b
CD (at 0.05)	1.686	2.276	0.191	0.120	0.617	0.933
SE (m) ±	0.587	0.792	0.067	0.042	0.215	0.325
P-value	0.0002**	8.23 × 10-05**	5.39 × 10^{-1}1**	2.36 × 10-06**	4.94 × 10-09**	8.51 × 10-09**
B_0	208.08±1.85^d	218.25±2.75^c	13.69±0.39^c	14.18±0.20^c	163.04±5.20^d	167.67±5.36^c
B_1	213.75±2.38^c	220.67±4.30^{bc}	14.03±0.49^b	15.07±0.22^b	165.25±3.49^c	171.33±5.30^b
B_2	213.92±4.01^c	219.75±3.75^{bc}	14.13±0.45^b	15.18±0.30^b	167.17±4.40^b	172.83±6.17^a
B_3	220.46±6.31^a	228.75±8.02^a	14.34±0.42^{ab}	15.61±0.34^a	167.17±4.67^b	172.58±6.59^a
B_4	216.71±4.18^b	221.08±3.21^b	14.38±0.45^a	15.74±0.37^a	168.00±4.14^a	173.50±6.12^a
CD (at 0.05)	1.885	2.545	0.216	0.134	0.690	1.044
SE (m) ±	0.656	0.886	0.074	0.047	0.240	0.363
P-value	3.39×10^{-05}**	0.00024**	1.84×10^{-08}**	5.15×10^{-10}**	4.06×10^{-05}**	0.00026**

All values are mean±SD values of three replications, J0: No *Jeevamrut* (control), J_1: *Jeevamrut*, J_2: *Jeevamrut* + Vermitea, J_3: *Jeevamrut* + Neem cake, B_0: No biofertilizers (control), B_1: Azotobacter, B_2: VAM (Vesicular Arbuscular Mycorrhiza), B_3: PSB (Phosphate Solubilizing Bacteria) + Azotobacter, B_4: PSB + VAM (Vesicular Arbuscular Mycorrhiza),*0.05 level of significance, **0.01 level of significance.

by Vieira and Nahas [58] confirmed that the microbial population was significantly influenced by the nature of soil and plants as the bacterial and fungal counts were higher in agricultural soil (sorghum) followed by eucalyptus and forest soil. They have reported that the counts of spore-forming, gram-negative bacteria, and actinomycetes were higher in forest soil followed by eucalyptus and agricultural soil. The genome metagenomic analysis confirms the presence of proteobacteria including *Rhizobium*, *Pseudomonas,* and *Bacillus*, fungal hyphae, and enzymes regulating the protein and carbohydrate metabolisms in *jeevamrut* which might be contributing factors toward soil OC, soil fertility, and plant growth [11].

3.3.4. Soil available N

The available nitrogen was reported to be 198.45 $kg\ ha^{-1}$ before planting and significantly increased to 218.57 and 226.27 $kg\ ha^{-1}$ after the application of *jeevamrut* + vermitea in year 1 and year 2, respectively [Table 5]. A significant increase in available nitrogen was also reported after the application of PSB with *Azotobacter* (220.46 and 228.75 $kg\ ha^{-1}$) in consecutive years. The interaction effect of *jeevamrut* formulation and biofertilizers was significant in year 1 while non-significant in year 2 with the highest available N in J_2B_3 and J_3B_3 [Figure 4 and Supplementary Table 5]. The finding confirms that the application of *jeevamrut* and biofertilizers like PSB, *Azotobacter, or* VAM has resulted in the fixation of free atmospheric nitrogen in the microbial

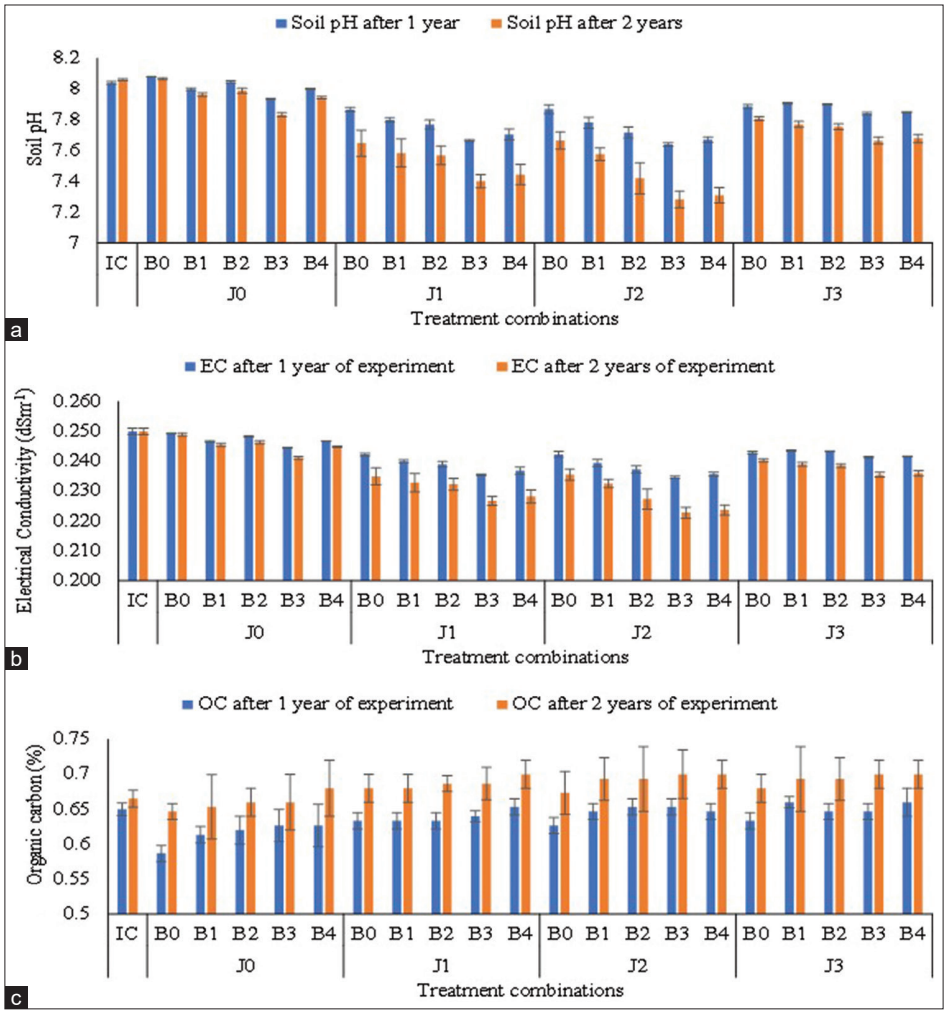

Figure 3: (a-c) Soil pH, EC, and OC after harvesting of crops under different treatment combinations (J_0: No *Jeevamrut*, J_1: *Jeevamrut*, J_2: *Jeevamrut* + Vermitea, J_3: *Jeevamrut* + Neem cake, B_0: No biofertilizers, B_1: Azotobacter, B_2: Vesicular arbuscular mycorrhiza [VAM], B_3: Phosphate solubilizing bacteria [PSB] + Azotobacter, B_4: PSB + VAM, IC: Initial control).

bodies which on decomposition were able to release a significant amount of nitrogen in soil [11,59]. Further, the *jeevamrut* formulation containing vermitea was able to enhance the population of *Azotobacter* to increase the magnitude of nitrogen fixation [14]. Saharan *et al.* [11] had reported approximately 98%, 23%, 62%, 55%, 46%, 439%, and 142% increases in zinc, iron, copper, manganese, OC, phosphorus, and potassium, respectively in soil after application of *jeevamrut* for two consecutive years. *Azotobacter* utilizes atmospheric nitrogen for the synthesis of cellular protein which on mineralization provides nitrogen to the soil. The sequestration of free atmospheric nitrogen by *Azotobacter* is regulated by iron-rich nitrogenases which bring reduction of nitrogen during the process. These bacteria are also known to release siderophores which make the metallic nutrients available to the plants through chelation [60]. Laboratory study by Wang *et al*. [61], aimed to study the dynamic growth of bacteria in incubation soil, reflected the peak of the total bacterial count at the 30th day during inoculation of mixed bacterial addition where the pattern of growth in both, PSB and N_2- fixing bacteria, was different. The growth of PSB declined in the past 30 days while the growth of N_2-fixing bacteria increased. Although the competition of mixed bacteria retarded the peaking time increased the maximum. Thus, the co-inoculation of these bacteria with PSB can be a more effective tool for soil fertility management [62].

3.3.5. Soil available P

The available phosphorus was reported to be 12.55 kg ha^{-1} before planting and significantly increased to 15.40 and 15.38 kg ha^{-1} after application of *jeevamrut* + neem cake and *jeevamrut* + vermitea, respectively, in year 2 [Table 5]. A significant increase in available phosphorus in year 2 was also reported after the application of PSB with VAM (15.74 kg ha^{-1}) and PSB with *Azotobacter* (15.61 kg ha^{-1}). The interaction effect of *jeevamrut* formulation and biofertilizers was significant in year 1 while non-significant in year 2 with the highest available P in J_3B_4, J_2B_4, J_2B_3, and J_3B_3 [Figure 4 and Supplementary Table 6]. The finding confirms that the application of jeevamrut and neem cake in combination with biofertilizers such as PSB, *Azotobacter, or* VAM has resulted in the dissolution of unavailable P to available forms which were able to increase the soil phosphorus. The presence of a greater microbial population in the *jeevamrut* formulation accelerated the decomposition of soil organic matter to improve soil fertility and nutrient availability to plants [33,38]. Although PSB is a non-symbiotic bacteria, it is beneficial for plants in many ways and can effectively be used under saline soil to increase soil phosphorus availability [63]. Further, its co-inoculation with *Azotobacter*, Rhizobium, or VAM fungi has a significant impact on soil nutrient status [64,65]. PSB as a biofertilizer has the potential to nullify the

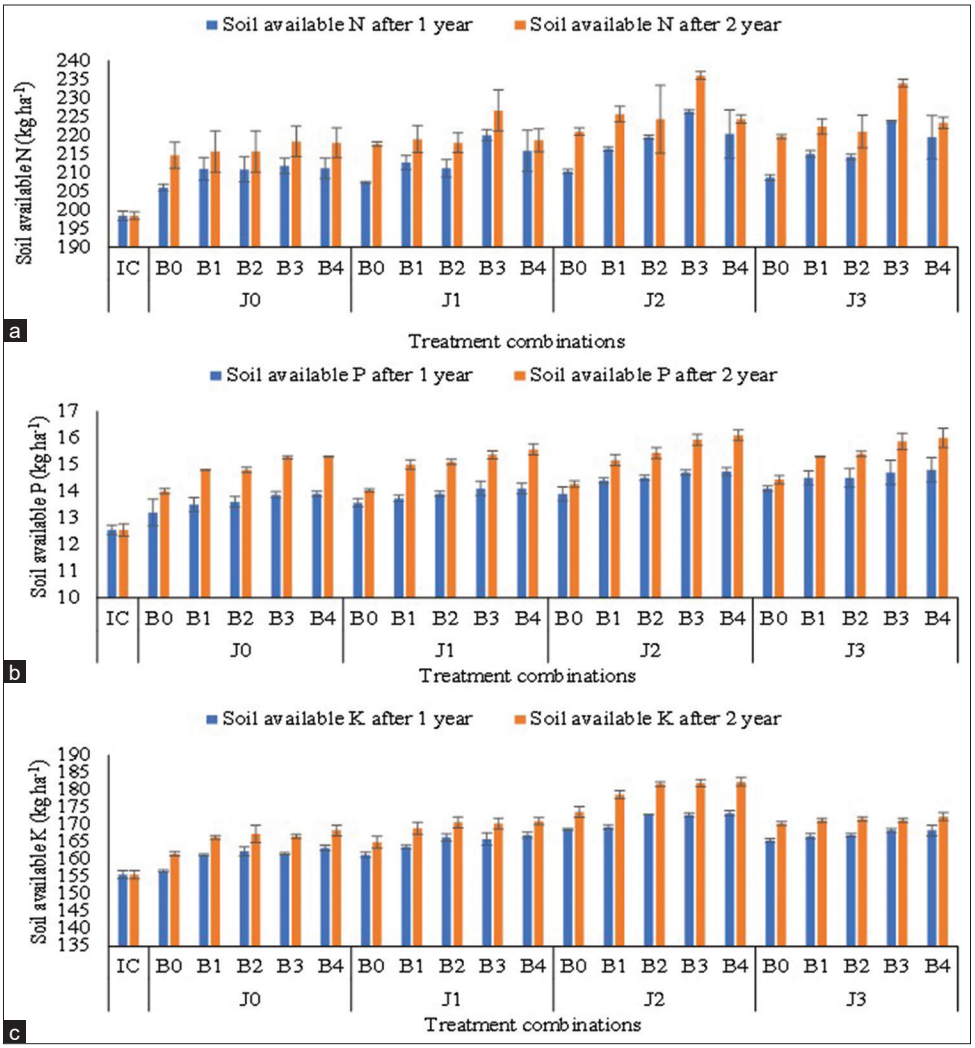

Figure 4: (a-c) Soil Available N, P and K after harvesting of crops under different treatment combinations (J_0: No *Jeevamrut*, J_1: *Jeevamrut*, J_2: *Jeevamrut* + Vermitea, J_3: *Jeevamrut* + Neem cake, B_0: No biofertilizers, B_1: Azotobacter, B_2: Vesicular arbuscular mycorrhiza [VAM], B_3: Phosphate solubilizing bacteria [PSB] + Azotobacter, B_4: PSB + VAM, IC: Initial control).

effect of induced salinity or alkalinity in calcareous soil and improve P availability through soil acidification in this condition [66]. The PSB releases a proton (H^+), phenolics, siderophores, organic acids, and mineral acids which could be involved in the dissolution of precipitated P like $Ca_3(PO_4)_2$ [67-72]. These exudates from PSB are involved in the chelation of the cations bound to phosphate through their hydroxyl and carboxyl groups, thereby converting them into soluble forms Chen *et al*. [73]. It is also able to release plant growth-promoting substances which results in increased availability of micronutrients including iron (Fe^{+2}) and zinc (Zn^{+2}). The PSBs are involved in the conversion of the insoluble form of phosphorus to the available form while *Azotobacter* is both solubilizing and mineralizing P bacteria so in the consortia they work in a complementary manner to improve the availability of P to the plants [72]. Thus, the co-inoculation of these bacteria can be a more effective tool for the improvement of P availability to the plants.

3.3.6. Soil available K

The available potassium was reported to be 155.65 kg ha^{-1} before planting and significantly increased to 179.67 and 171.40 kg ha^{-1} after application of *jeevamrut* + vermitea and *jeevamrut* + neem cake, respectively, in year 2 [Table 5]. A significant increase in available potassium in year 2 was also reported after the application of PSB with VAM (173.50 kg ha^{-1}), VAM alone (172.83 kg ha^{-1}), and PSB with *Azotobacter* (172.58 kg ha^{-1}). The interaction between *jeevamrut* formulation and biofertilizers was significant with the highest available K in J_2B_4, J_2B_3, and J_2B_2 [Figure 4 and Supplementary Table 7]. The finding confirms that the application of *jeevamrut* and vermitea in combination with biofertilizers such as PSB, *Azotobacter, or* VAM has resulted in the dissolution of unavailable K to available form which was able to increase the significant amount of potassium in soil. The microbial species available in *jeevamrut* and biofertilizers were able to improve the quantity of soil microorganisms which are involved in the decomposition (humification and mineralization) of soil organic matters and acted as nutrient reservoirs responsible for soil fertility improvement and stable but dynamic soil ecosystems [74-77]. *Jeevamrut* enriches the soil with nutrients and improves soil fertility by buffering the soil pH in acidic as well as alkaline soil to make the soil nutrients available to the plants [38]. The release of organic acids and enzymes due to co-inoculation of VAM and PSB might be accountable to the enhanced dissolution of complex minerals to available form and increase in K content in the rhizosphere soil

[22]. It has also been observed that the biofertilizer application improved the level of available phosphorus and potassium content in soil when it was supplied in combination with other organic nutrient sources [78].

3.4. Correlation (r) of various soil parameters with microbial count

The correlation study of initial microbial count in *jeevamrut* with the various soil attributes estimated after harvesting of potato crops in year 1 and year 2 was estimated and it was reported that after harvesting of the crop, the bacterial count (BC2), fungal count (FC2) and actinomycetes count (AC2), (OC1 and OC2), available nitrogen (N1Y, N2Y), available phosphorus (P1Y, P2Y), and available K (K1Y and K2Y) in soil were positively affected by bacterial, fungal and actinomycetes population of *jeevamrut* formulation applied [Figure 5a, Supplementary Table 8]. Similarly, the microbial population in the soil after harvest was also having a positive correlation with the chemical parameters of the soil after harvesting [Figure 5b, Supplementary Table 8]. The pH and EC of soil were reported to be negatively affected by the microbial population of *jeevamrut* and soil. The experimental findings confirm that the application of *jeevamrut* and biofertilizers have increase the humification and mineralization of organic matter present in the soil to release the nutrients [12,16]. Further, the acidic medium developed during the decomposition of organic matter might be responsible for reducing soil pH and inducing the dissolution of fixed nutrients in available forms.

3.5. PCA

The inter-relationships between various microbial and chemical attributes were studied using PCA [Table 6 and Supplementary Table 9]. The scree plot of all principal components (PCs) [Figure 6] confirms that the first four PCs are explaining 93 % of the total variable so these components are retained and detailed in the loading of the correlation matrix as shown in Table 6. The PCs-1, explaining 72.90% of the total variances, was significantly contributed to all attributes under study. This could be due to a strong association between the parameters under study and bacterial activity. The negative loading reflected by soil pH and EC in PC-1 suggested a stressed activity of the bacterial community during the utilization of substrate in controls (J_0 and B_0). However, the positive loading in the other parameters indicated significant improvement in bacterial community with the application of *jeevamrut* and biofertilizers. The PC-2, explaining 9.10 % of the total variance, was contributed by FC2, pH1, pH2, EC1, EC2, N1Y, P1Y, and P2Y with positive loading which suggests that application of *jeevamrut* and biofertilizers has enhanced the synergistic influence of the fungal communities over these parameters [5] and plays significant role in improving nutrient use efficiency in the integrated plant nutrient approach [79].

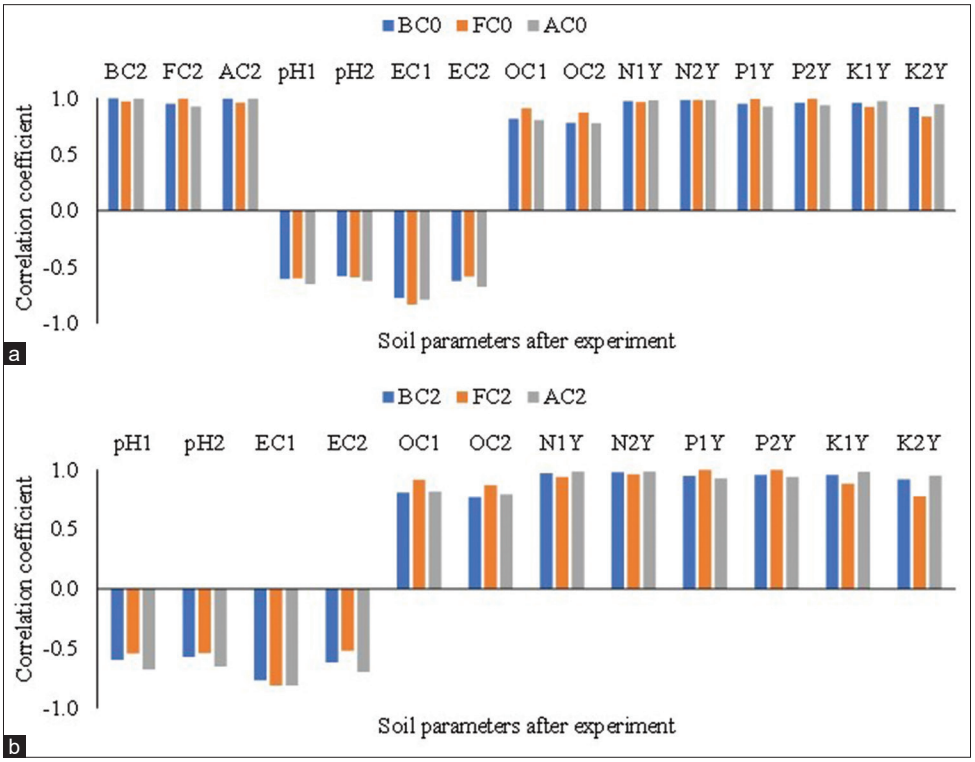

Figure 5: Correlation coefficient (a) initial microbial count with various soil properties after crop harvest and (b) the microbial count with other soil properties after crop harvest (BC0: Initial bacterial counts in *jeevamrut*, FC0: Initial fungal counts in *jeevamrut*, AC0: Initial actinomycetes counts in *jeevamrut*, BC2: Bacterial count in the soil after 2 years of experiment, FC2: Fungal count in the soil after 2 years of experiment, AC2: Actinomycetes count in the soil after 2 years of experiment, pH1: Soil pH after 1 year of the experiment, pH2: Soil pH after 2 years of experiment, EC1: Soil electrical conductivity after 1 year of the experiment, EC2: Soil electrical conductivity after 2 years of experiment, OC1: Soil organic carbon after 1 year of the experiment, OC2: Soil organic carbon after 2 years of experiment, N1Y: Soil available nitrogen after 1 year of the experiment, N2Y: Soil available nitrogen after 2 years of experiment, P1Y: Soil available phosphorus after 1 year of the experiment, P2Y: Soil available phosphorus after 2 years of experiment, K1Y: Soil available potassium after 1 year of experiment, K2Y: Soil available potassium after 2 years of experiment).

Table 6: Principal component loadings after Varimax rotation.

Eigenvalues and parameters	Principal components#			
	PC1	PC2	PC3	PC4
Eigenvalues	10.937	1.363	0.915	0.729
Explained variance (%)	72.90	9.10	6.10	4.90
Loadings (Eigenvectors) of correlation matrix on three retained components				
BC2: Bacterial count in soil after 2 years of experiment	0.275	NS	NS	0.34
FC2: Fungal count in soil after 2 years of experiment	0.185	0.393	0.384	-0.588
AC2: Actinomycetes count in soil after 2 years of experiment	0.276	NS	NS	NS
pH 1: Soil pH after 1 year of experiment	-0.263	0.341	NS	NS
pH 2: Soil pH after 2 years of experiment	-0.268	0.327	NS	NS
EC1: Soil electrical conductivity after 1 year of experiment	-0.248	0.314	NS	NS
EC2: Soil electrical conductivity after 2 years of experiment	-0.243	0.400	NS	0.319
OC1: Soil organic carbon after 1 year of experiment	0.201	NS	0.665	0.383
OC2: Soil organic carbon after 2 years of experiment	0.255	NS	0.431	NS
N1Y: Soil available nitrogen after 1 year of experiment	0.282	0.219	NS	NS
N2Y: Soil available nitrogen after 2 years of experiment	0.263	NS	-0.354	NS
P1Y: Soil available phosphorus after 1 year of experiment	0.279	0.213	NS	NS
P2Y: Soil available phosphorus after 2 years of experiment	0.260	0.369	NS	NS
K1Y: Soil available potassium after 1 year of experiment	0.283	NS	NS	NS
K2Y: Soil available potassium after 2 years of experiment	0.271	NS	NS	NS

The soil parameters are grouped according to the maximum fittings to principal components (correlation coefficients ≥0.25; *n*=150) *NS* Loadings where the correlation coefficient is lower than 0.25, # Only principal components with Eigen values >0.5 and those explaining >5% of the total variance were retained.

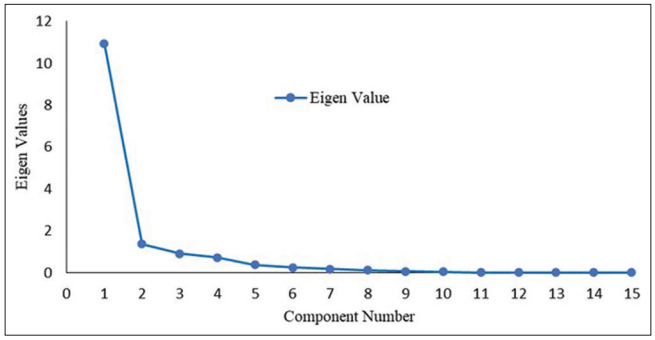

Figure 6: The Scree plot reflecting Eigen value of different principal components (PC-1 to PC-15).

4. CONCLUSIONS

On account of the present experimental findings, it can be concluded that the application of *jeevamrut* fortified with vermitea or neem cake is essential for improving the soil microbial activity and the available nutrient content. The highest bacterial count (95.83 ± 7.83 × 10^6 cfu g^{-1}), actinomycetes count (108.00 ± 7.69 × 10^4 cfu g^{-1}), soil nitrogen (218.57 ± 5.85 and 226.27 ± 5.71 kg ha^{-1}), and soil potassium (171.43 ± 2.24 and 179.67 ± 3.66 kg ha^{-1}) were reported due to application of *jeevamrut* fortified with vermitea. The highest fungal count (157.54 ± 23.70 × 10^4 cfu g^{-1}) and soil phosphorus (14.52 ± 0.27 and 15.40 ± 0.62 kg ha^{-1}) was recorded after application of *jeevamrut* fortified with neem cake which was at par to *jeevamrut* fortified with vermitea.

The application of microbial consortium (co-inoculation of different types of microbes) consisting of PSB and *Azotobacter* or PSB and VAM has enhanced the mobilization of primary nutrients and soil microbial population. The highest bacterial count (96.33 ± 8.84 × 10^6 cfu g^{-1}), actinomycetes count (111.04 ± 4.57 × 10^4 cfu g^{-1}), and soil nitrogen (220.46 ± 6.31 and 228.75 ± 8.02 kg ha^{-1}) were reported due to application of consortia of PSB and *Azotobacter*. The highest fungal count (182.02 ± 0.90 × 10^4 cfu g^{-1}), soil phosphorus (14.38 ± 0.45 and 15.74 ± 0.37 kg ha^{-1}), and soil potassium (168.00 ± 4.14 and 173.50 ± 6.12 kg ha^{-1}) was recorded after application of consortia of PSB and VAM which was at par to PSB and *Azotobacter*.

Thus, application of *jeevamrut* fortified with vermitea or neem cake in combination with consortia of PSB and *Azotobacter* or PSB and VAM in crop nutrient management is necessary to increase soil bacterial, actinomycetes and fungal count; improve soil available nitrogen, phosphorus, and potassium; and balance soil structure, pH, EC and OC.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The data are available with the first and corresponding author as it is from the dissertation work of the first author. It will be made available on request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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