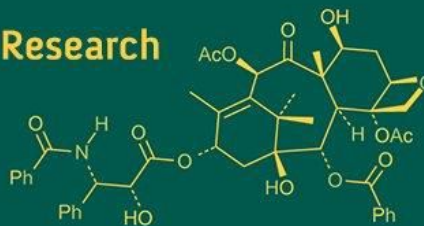


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Harnessing traditional organic formulations: Impact of jeevamrutham and panchagavya on soil nutrition and health

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Abstract

In this research investigates the impact of traditional organic preparations, Jeevamrutham and Panchagavya, on soil's physical and chemical characteristics, microbial communities, and enzyme activities, using tomato (*Solanum lycopersicum* L.) as a test crop. A controlled pot experiment was set up with seven treatments: a control group, a full recommended fertilizer dose (RDF), and various mixtures of Jeevamrutham and Panchagavya. The combined use of Jeevamrutham and Panchagavya (T₇) led to significant improvements in soil organic carbon (0.53%), microbial populations (e.g., total bacteria: 154.5×10^6 CFU g⁻¹), and enzyme activities (e.g., dehydrogenase: $5.06 \mu\text{g TPF g}^{-1} \text{ day}^{-1}$) compared to individual applications and synthetic fertilizers. Notable increases in phosphorus- and potassium-solubilizing bacteria and nitrogen-fixing microorganisms were observed, indicating that these formulations created favourable conditions for microbial growth. Soil pH showed a moderate decrease, whereas electrical conductivity remained within the optimal range, reducing salinity risks. These results highlight the potential of integrated organic formulations in sustainable agriculture by enhancing soil structure, nutrient cycling, and microbial diversity. Jeevamrutham and Panchagavya offer viable substitutes for chemical fertilizers, promoting improved crop productivity and ecosystem conservation. Further research should examine their effectiveness across various agroecological settings to confirm their roles in fostering long-term agricultural sustainability.

Keywords: Jeevamrutham, panchagavya, sustainable agriculture, yeast, and tomato

Introduction

The productivity of tomatoes (*Solanum lycopersicum* L.) across the globe is estimated at 36.97 t/ha in 2022, with a total production of 186.82 million tonnes on approximately 5 million hectares of land, as reported by FAOSTAT (2022). Tomatoes are a valuable source of essential nutrients, including vitamins A and C, minerals, proteins, amino acids, and healthy fats, such as linoleic and linolenic acids (Chabi *et al.* 2024) ^[1]. They also contain compounds, such as flavonoids and hydroxycinnamic acid derivatives, chlorogenic, homovanillic acid, and ferulic acid, making them one of the most widely consumed fresh vegetables and a crucial ingredient in processed foods (Toor *et al.* 2005; Borguini *et al.* 2009; Kumar *et al.* 2012) ^[2, 3, 4]. In the face of the 21st century's unparalleled challenges to agriculture, such as population growth projected to reach 9.7 billion by 2050 (Devaux *et al.* 2020) ^[5], the need for sustainable agricultural practices that can ensure food security while preserving ecosystem health is becoming increasingly pressing. The agricultural sector must confront ramifications of climate change, soil degradation, and environmental consequences of intensive farming methods. This calls for a paradigm shift towards more sustainable agricultural practices that can help address these pressing issues. Modern agriculture, which relies heavily on synthetic fertilizers and pesticides (Prashar, and Shah, 2016) ^[6], has played a crucial role in increasing crop yields over the past century. However, long-term use of these chemical inputs has led to significant environmental concerns, including soil degradation, water pollution, and biodiversity loss, reduced input efficiency, decreased food quality, reduce microbial diversity, resistance development in different weeds, diseases, insects (Kumar *et al.* 2019) ^[7]. Moreover, energy-intensive production of synthetic fertilizers contributes substantially to greenhouse gas emissions and exacerbates climate change (Erisman *et al.* 2013; Mrówczyńska-Kamińska *et al.* 2021; Omotoso and Omotayo, 2024) ^[8, 9, 10]. These issues have spurred renewed interest in alternative, sustainable farming

practices that can maintain or improve crop yields while minimizing environmental impacts (Omotoso and Omotayo, 2024) ^[10].

In recent years, traditional organic preparations have gained attention as potential alternatives to synthetic fertilizers (Carvalho, 2006; Panday *et al.* 2024) ^[11, 12]. Among these, Jeevamrutham and Panchagavya, two formulations rooted in ancient Indian agricultural practices, have shown promise in enhancing soil fertility and crop productivity (Saharan *et al.* 2023; Behera *et al.* 2024) ^[13, 14]. Jeevamrutham, a fermented mixture of cow dung, urine, jaggery, pulse flour, and soil, is rich in beneficial microorganisms and plant growth-promoting substances (Kumar *et al.* 2021; Saharan *et al.* 2023) ^[18, 13]. Panchagavya, which is composed of five cow-derived products, along with other organic materials, is believed to enhance plant growth and provide resistance against pests and diseases (Kumar and Singh, 2020; Sharma *et al.* 2022; Rai *et al.* 2022) ^[19, 41, 20].

This study aimed to address these knowledge gaps by conducting a comprehensive, controlled experiment comparing the effects of Jeevamrutham and Panchagavya fertilizers with conventional chemical fertilizers on soil properties. Using tomato (*Solanum lycopersicum* L.) as a model crop, we investigated the effects of these treatments on the soil physicochemical properties, microbial populations, and enzyme activities.

In the following sections, we present our methodology, results, and discussion of our findings, providing insights into the potential of Jeevamrutham and Panchagavya as sustainable alternatives to modern agriculture. This study not only advances our understanding of organic soil preparation but also paves the way for future research into innovative, eco-friendly agricultural practices that can help meet the growing global demand for food while preserving our planet's ecosystems.

2. Material and Methods

2.1 Experimental Design.

A pot culture experiment was conducted at the Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana Agricultural University (PJTUA), Hyderabad, India (17.3924° N, 78.3237° E), during the Rabi season. The study, which included seven treatments and three replicates were used to investigate the effects of organic preparation application on soil characteristics. Each experimental unit consisted of a pot (30 cm diameter and 30 cm height) containing 15 kg of sterilized red soil. Red soil was collected from Student Farm, College of Agriculture, Rajendranagar, PJTUA, and Hyderabad. The soil was air-dried, sieved through a 2 mm mesh, and sterilized by autoclaving at 121 °C for 1 hour on three consecutive days. This sterilization process ensures the elimination of native microorganisms, allowing for controlled assessment of the introduced treatments.

2.2 Treatments

Seven treatments were applied to evaluate the effects of organic preparations compared with conventional fertilizers.

1. **T₁**: Absolute Control (no organic preparations and chemical fertilizers)
2. **T₂**: 100% Recommended Dose of Fertilizer (RDF)
3. **T₃**: 50% Jeevamrutham
4. **T₄**: 100% Jeevamrutham

5. **T₅**: 50% Panchagavya

6. **T₆**: 100% Panchagavya

7. **T₇**: 100% Jeevamrutham + 100% Panchagavya

2.3. Preparation of traditional organic formulations and Application

2.3.1. Jeevamrutham Preparation

Jeevamrutham was prepared following the method (Palekar, 2006) ^[21]. First, 10 kg of fresh cow dung, 10 liters of cow urine, 2 kg of jaggery, 2 kg of pulse flour, and a small amount of soil collected from the field bund were mixed thoroughly. This mixture was then fermented in 200 liters of water for 12 days to ensure that it was kept in the shade to facilitate proper fermentation. The mixture was agitated twice daily to promote uniform fermentation and ensure the development of beneficial microbial activity.

2.3.2. Panchagavya Preparation

Panchagavya were prepared according to the method (Natarajan, 2002) ^[22]. The preparation began by mixing 7 kg of fresh cow dung, 10 liters of cow urine, 3 liters of cow milk, 2 liters of curd, 3 litres of tender coconut water, 3 kg jaggery, and 12 bananas. This nutrient-rich blend was then allowed to ferment for 24 d, and the mixture was stirred twice daily to encourage the proliferation of beneficial microorganisms and breakdown of organic components into a form that is readily usable by plants. For the pot experiments, the recommended dose of fertilizers (RDF) was applied at a rate of 150:100:60 kg ha⁻¹ N: P₂O₅: K₂O, adjusted based on soil volume, resulting in the application of 1.15 g of Nitrogen (N), 0.77 g of Phosphorus (P), and 0.46 g of Potassium (K) per pot. Nitrogen was applied in two splits: 50% as a basal dose and 50% as a top dressing four weeks after transplantation (WAT), whereas phosphorus and potassium were applied entirely as basal doses. The application of Jeevamrutham followed the rates of 500 L ha⁻¹ (equivalent to 3.85 mL per pot) for the 100% treatment and 250 L ha⁻¹ (equivalent to 1.93 mL per pot) for the 50% treatment. Jeevamrutham and Panchagavya were applied at 15-day intervals until the crop was harvested.

2.4. Crop Management and sampling collection

Three-week-old tomato (*Solanum lycopersicum* L.) hybrid SWEAKAR-448 seedlings were transplanted into individual pots, with three seedlings placed in each pot. After the seedlings were established, they were thinned to retain only one plant per pot. Soil samples were collected at a depth of 0-15 cm at three stages: 30 DAT (seedling stage), 60 DAT (flowering stage), and harvest. Composite samples were created for each pot by combining the soil from five distinct locations. These composite samples were subsequently analyzed to assess their microbial populations, enzyme activities, and physicochemical properties.

2.5. Soil Analysis

Soil physicochemical properties were analyzed following standard methods: pH and electrical conductivity (EC) in a 1:2.5 soil: water suspension (Jackson, 1973) ^[23], organic carbon by wet oxidation method (Walkley and Black, 1934) ^[27] ^[24], available nitrogen by alkaline permanganate method (Subbiah and Asija, 1956), available phosphorus by Olsen's method (Olsen, 1954) ^[26], available potassium by ammonium acetate extraction (Jackson, 1973) ^[23], and soil moisture content using the gravimetric method (Black,

1965)^[27] during the initial and harvesting stages. Microbial populations were enumerated using the serial dilution spread plate technique on the following selective media: Nutrient Agar for total bacteria, Pikovskaya's medium for phosphorus-solubilizing bacteria, Aleksandrow agar for potassium-solubilizing bacteria, Rennie semi-solid medium for nitrogen-fixing bacteria, Ken Knight's medium for actinobacteria, and Sabouraud dextrose agar for yeast. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 24-72 hours, depending on the microbial group. Colony-forming units (CFU) were counted and expressed as CFU g^{-1} air dry soil during the three stages. Soil enzyme activity was measured using the following methods: dehydrogenase activity by the triphenyl tetrazolium chloride (TTC) reduction method (Casida *et al.* 1964)^[28], urease activity by colorimetric determination of NH_4^+ released (Kandeler and Gerber, 1988)^[29], and acid and alkaline phosphatase activities using the p-nitrophenol release method (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977)^[30, 31] during three stages.

2.6. Statistical analysis

All data were subjected to two-way analysis of variance (ANOVA) using Origin Lab and R version 4.1.0 (R Core Team, 2021). The significance of treatment effects was determined at $p \leq 0.05$. When ANOVA results were significant, treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$. Pearson's correlation coefficients were calculated to determine the relationships between soil properties, microbial populations, and enzyme activities. Principal Component Analysis (PCA) was conducted to reduce the dimensionality of the dataset and identify patterns in soil parameters across treatments (Jolliffe and Cadima, 2016)^[32].

3. Results

3.1 Impact of Jeevamrutham and Panchagavya on Soil pH, EC, Organic Matter, and Macro-Nutrient Availability

3.1.1 Soil pH

The treatments resulted in soil pH levels ranging from 7.46 ± 0.53 in T_7 to 7.93 ± 0.95 in the control group (T_1) of table 1. Compared to the initial pH of 8.08 ± 0.18 , most treatments caused a minor reduction in soil pH. Table 1 indicating that treatment T_7 , which combined Jeevamrutham and Panchagavya, was notably effective in lowering the soil pH, indicating its capacity to mildly acidify the soil. This can enhance nutrient availability and promote microbial activity.

3.1.2 Soil Electrical Conductivity (EC)

Significant variations in soil EC were observed among the treatments (Table 1). T_2 (RDF) exhibited the highest EC at $0.46 \pm 0.11 \text{ dSm}^{-1}$, while T_7 showed the lowest at $0.31 \pm 0.10 \text{ dSm}^{-1}$. The initial EC was $0.38 \pm 0.02 \text{ dSm}^{-1}$. The elevated EC in the RDF treatment likely stems from the salts in the chemical fertilizers, potentially increasing soil salinity. Conversely, organic treatments, particularly T_7 , maintained lower EC levels, which may benefit crop growth by minimizing the risk of salt stress.

3.1.3 Soil Organic Carbon (SOC)

Treatments significantly affected soil OC content, with values spanning from $0.29 \pm 0.03\%$ in T_1 to $0.54 \pm 0.04\%$ in

T_7 (Table 1). The initial OC was $0.23 \pm 0.01\%$, indicating an overall improvement in soil organic matter across all treatments. T_7 demonstrated the highest OC, suggesting that the combined use of Jeevamrutham and Panchagavya boosted soil carbon sequestration. This increase could lead to improvements in the soil structure, water retention capacity, and microbial habitat.

3.1.4 Available Soil Moisture

Soil moisture content exhibited less significant variations among the different treatments, with organic amendments outperforming both the control and chemical fertilizer applications (Fig 2). The treatment combining full doses of Jeevamrutham and Panchagavya (T_7) consistently yielded the highest moisture retention throughout all growth stages, peaking at 16% at 30 DAT. This enhanced water-holding capacity was attributed to improvements in soil structure and organic matter levels. Other organic treatments, such as full doses of Panchagavya (T_6) and Jeevamrutham (T_4), also maintained elevated soil moisture levels, while partial applications (T_3 and T_5) resulted in moderate retention. In contrast, the control (T_1) exhibited the lowest moisture content, declining significantly by harvest time. Chemical fertilizers (T_2) offered slight improvements over the control but remained considerably less effective than organic treatments.

Progressive analysis revealed a general trend of decreasing soil moisture from 30 DAT to harvest across all treatments. However, organic amendments were more effective in mitigating this reduction compared to non-organic alternatives. Treatments T_7 , T_6 , and T_4 demonstrated superior moisture retention, highlighting their capacity to support crop growth during crucial developmental stages and minimize water stress.

3.1.5 Soil Available Nitrogen (N)

The available nitrogen content varied widely, with T_2 (RDF) exhibiting the highest value ($202.02 \pm 3.60 \text{ Kg ha}^{-1}$), significantly higher than the initial $157.50 \pm 3.41 \text{ Kg ha}^{-1}$. Organic treatments, particularly T_7 , also increased nitrogen availability, although not as dramatically as RDF (Table 1). These results indicate that, while RDF provides a quick release of nitrogen, organic preparations, such as Jeevamrutham and Panchagavya, contribute to a more gradual increase, likely supporting sustained nitrogen release over time.

3.1.6 Soil Available Phosphorus (P)

Available phosphorus was highest in T_2 ($95.73 \pm 5.10 \text{ Kg ha}^{-1}$), followed by T_7 ($71.66 \pm 3.28 \text{ Kg ha}^{-1}$), compared to the initial $55.25 \pm 1.20 \text{ Kg ha}^{-1}$ (Table 1). This suggests that both chemical and organic treatments can significantly enhance phosphorus availability. Organic treatments, particularly when combined, appear to facilitate phosphorus solubilization, likely through increased microbial activity that enhances P cycling in the soil.

3.1.7 Soil Available Potassium (K)

Significant variation in the available potassium content were observed among treatments with highest in T_7 ($123.42 \pm 4.46 \text{ Kg ha}^{-1}$), compared to the initial $135.00 \pm 2.21 \text{ Kg ha}^{-1}$, indicating that the combination of organic preparations not only maintained but also slightly enhanced potassium availability (Table 1). This contrasts with the general trend

of K depletion in the other treatments, suggesting that Jeevamrutham and Panchagavya may improve K retention in the soil, possibly by reducing leaching or enhancing the exchangeable K pool.

3.1.8 Available Soil Moisture

Soil moisture content exhibited less significant variations among the different treatments, with organic amendments outperforming both the control and chemical fertilizer applications (Figure 1). The treatment combining full doses of Jeevamrutham and Panchagavya (T_7) consistently yielded the highest moisture retention throughout all growth stages, peaking at 16% at 30 DAT. This enhanced water-holding capacity was attributed to improvements in soil structure and organic matter levels. Other organic treatments, such as full doses of Panchagavya (T_6) and Jeevamrutham (T_4), also maintained elevated soil moisture levels, while partial applications (T_3 and T_5) resulted in moderate retention. In contrast, the control (T_1) exhibited the lowest moisture content, declining significantly by harvest time. Chemical fertilizers (T_2) offered slight improvements over the control but remained considerably less effective than organic treatments.

Impact of Jeevamrutham and Panchagavya on Soil Microbial Dynamics

3.1.9. Total Bacteria (TB)

The total bacterial population was significantly improved with time across all treatments; T_7 (53×10^6 CFU g^{-1}) had the greatest population of total bacterial counts, followed by T_6 (49×10^6 CFU g^{-1}) (100% Panchagavya) at 30 Days After Transplanting (DAT) (Figure 2). The total bacterial population elevated in all treatments when the crops reached 60 DAT, with T_7 sustaining the greatest levels (122.33×10^6 CFU g^{-1}), suggesting a cumulative impact of the organic preparations. The fact that T_7 still had the largest bacterial population at harvest (154.5×10^6 CFU g^{-1}) suggests that combining Jeevamrutham with Panchagavya continued to be beneficial. However, Harvest stage had more total bacterial population among all the treatments compared with other growth stages. Control treatment (T_1) had fewer populations of total bacteria compared to other treatments across the growth stages of the crop due to without application of organic preparations.

3.1.10 Phosphorus Solubilizing Bacteria (PSB)

PSB population was showed significant variations among all the treatments throughout the crop cycle (Figure 2). However, it was observed that T_7 demonstrated the highest PSB population (56×10^4 CFU g^{-1}) followed by T_6 (51.33×10^4 CFU g^{-1}) and T_4 (48.33×10^4 CFU g^{-1}) at 30 DAT. At 60 DAT, T_7 (100.33×10^4 CFU g^{-1}) and T_6 (96.3×10^4 CFU g^{-1}) consistently showed the highest count of PSB indicated that the benefits of these treatments were sustained and increased with the duration of time. More importantly, at the time of harvesting, T_7 (233.5×10^4 CFU g^{-1}) maintained the highest PSB population, indicated that combinations of these organic preparations supported phosphorus solubilization process during crop growth stages. The treatments with organic preparations ($T_7 > T_6 > T_4 > T_5 > T_3$) showed significantly highest PSB population count at 30 DAT, and 60 DAT but Harvest stage, Panchagavya applied treatments had more PSB population compared with Jeevamrutham applied treatments. While, the chemical fertilizer (T_2) and control (T_1) were the least effective in

promoting PSB at all crop stages. These findings suggest that P-solubilizing bacteria may have found a more favourable environment to develop and multiply in the organic preparations, which might by constant release of nutrients and healthy substances.

3.1.11 Potassium Solubilizing Bacteria (KSB)

The analysis of Potassium Releasing Bacteria (KRB) among different treatments showed notable trends in their population changes (Figure 2). At 60 DAT, the combination of Jeevamrutham and Panchagavya (T_7) resulted in the highest KRB population (73×10^4 CFU g^{-1}) followed by 100% Panchagavya treatment (T_6) that sustained 70.83×10^4 CFU g^{-1} , alongside 100% Jeevamrutham (T_4) which showed 69×10^4 CFU g^{-1} of soil. In comparison, the chemical fertilizer treatment (RDF) revealed significantly reduced KRB populations at 14.5×10^4 CFU g^{-1} .

During the various growth stages (30 DAT, 60 DAT, and harvest), T_7 consistently exhibited the highest KRB population, showcasing the enduring positive influence of combining these organic amendments on potassium solubilization processes. The treatments using organic preparations (T_7 , T_6 , and T_4) exhibited notably greater KRB populations in comparison to the chemical fertilizer (T_2) and control (T_1) treatments, indicating that organic inputs foster more beneficial conditions for the growth and multiplication of potassium-releasing bacteria. Although Panchagavya and Jeevamrutham were effective on their own, their mixture in T_7 demonstrated the greatest benefit for improving KRB populations in the soil.

3.1.12 Nitrogen-fixing bacteria (NFB)

The communities of nitrogen-fixing bacteria displayed varied temporal trends between treatments, with the combined organic preparations displaying the largest microbial populations of NFB (Figure 2). The treatment group T_7 had the greatest NFB (16.66×10^4 CFU g^{-1} , 33.0×10^4 CFU g^{-1} , and 66.0×10^4 CFU g^{-1}) at 30 DAT, 60 DAT, and harvest stages, which was significantly better than treatments with single organic preparation applied, RDF treatment and those without any organic preparation. These synergistic effects were visible even in the early development stages, with levels reaching up to 357% and 455% higher than the control (T_1) and chemical fertilizer treatment (T_2) at 30 DAT. This enhancement was visible at harvest, demonstrating that overall organic inputs during the growing season greatly increased NFB proliferation.

Both 100% Jeevamrutham (T_4) and 100% Panchagavya (T_6) showed similar effectiveness when compared, having recorded values of 26.66×10^4 CFU g^{-1} and 26×10^4 CFU g^{-1} respectively at 60 DAT (Figure 2). Even at harvest, these single-input treatments-maintained activity, recording populations that exceeded the control by 261% (T_4) and 241% (T_6). Conversely, the recommended dose of fertilizer (RDF) induced lower growth (3.66×10^4 CFU g^{-1} , 11.3×10^4 CFU g^{-1} and 19.0×10^4 CFU g^{-1} at 30, 60 DAT and Harvest stage respectively) of NFB. The significantly better effect of organic as compared to chemical treatments, though, stands out over the years, highlighting the incredible capacity of organic formulations to create perfect environment conducive to nitrogen fixing bacteria, facilitating beneficial communication between them, especially when they are combined. The sustained prevalence of the T_7 through each growth stage suggests that

the combination of Jeevamrutham and Panchagavya creates favourable conditions in the rhizosphere for prolonged NFB populations.

3.1.13 Actinobacteria

The population dynamics of Actinobacteria exhibited clear differences across treatments, with organic formulations clearly outperforming conventional methods (Figure 2). T₇ exhibited the highest Actinobacterial count with 48×10^3 CFU g⁻¹, 120.83×10^3 CFU g⁻¹, and 201.16×10^3 CFU g⁻¹ of soil at 30 DAT, 60 DAT, and at harvesting stages, respectively. A notable difference was observed among the single organic preparations for treatments T₆ (185.83×10^3 CFU g⁻¹), T₄ (177.16×10^3 CFU g⁻¹), T₅ (155.3×10^3 CFU g⁻¹), and T₃ (137.83×10^3 CFU g⁻¹) during the crop's harvest stage. Conversely, during the initial phases of the crop, no notable differences were observed in actinobacteria populations among treatments with single organic preparations. Nevertheless, the control (T₁) and RDF applied Treatment (T₂) demonstrated the lowest populations of actinobacteria during the crop stages.

3.1.14 Yeast

The dynamics of yeast populations changed considerably between treatments, with organic formulations showing enhanced effectiveness in encouraging yeast growth (Figure 2). The joint use of Jeevamrutham and Panchagavya (T₇) noted the highest yeast count, achieving 16.6×10^3 CFU g⁻¹, 34.6×10^3 CFU g⁻¹, and 56.3×10^3 CFU g⁻¹ of soil at 30 DAT, 60 DAT, and harvesting periods, respectively. While, Control (T₁) was limited growth of yeast population with 4.16×10^3 CFU g⁻¹, 11.6×10^3 CFU g⁻¹, and 15.6×10^3 CFU g⁻¹ of soil at 30 DAT, 60 DAT, and harvesting stages, respectively. A notable significant difference existed between treatments with organic preparations and those without, specifically the Control (T₁) and the Chemical fertilizer applied treatment (T₂) throughout the crop stages. Conversely, no significant difference was observed among the treatments of organic preparations during the crop's growth stages.

3.2 Comprehensive Analysis of Soil Enzymatic Activities Under Different Treatments

Significant variations in soil enzyme activities were observed across different treatments and crop growth phases, with organic formulations showing notable effects. Among all treatments, T₇ demonstrated the best results for enzymatic activity of dehydrogenase, urease, and alkaline phosphatases were studied in the figure 3. At 60 DAT, T₇ achieved peak activities in dehydrogenase ($5.06 \mu\text{g TPF/g/day}$), urease ($77.16 \mu\text{g NH}_4^+ \text{ h}^{-1} \text{ g}^{-1}$), and alkaline phosphatase ($45.32 \mu\text{mol h}^{-1} \text{ g}^{-1}$). The consistent top performance of T₇ throughout all growth stages and for all enzymes indicates that combining both organic preparations may have a synergistic impact on overall soil enzymatic activity.

Among the individual organic treatments, T₆ (100% Panchagavya) showed the second-best performance with high enzyme activities at 60 DAT: dehydrogenase ($3.92 \mu\text{g TPF/g/day}$), urease ($70.51 \mu\text{g NH}_4^+ \text{ h}^{-1} \text{ g}^{-1}$), and alkaline phosphatase ($39.64 \mu\text{mol h}^{-1} \text{ g}^{-1}$), followed by T₅ (50% Panchagavya) and T₄ (100% Jeevamrutham), which showed moderate levels of enzyme activity. The effectiveness of treatments followed the order T₇ > T₆ > T₅ > T₄ > T₃ > T₂ >

T₁, clearly demonstrating the superiority of organic inputs over chemical fertilizers and controls. Notably, T₂ (RDF chemical fertilizers) showed only a marginal improvement over the control (T₁), indicating the limited impact of chemical fertilizers on soil biological activity.

Temporal analysis revealed a general trend of increasing enzymatic activities from 30 to 60 DAT across all treatments, followed by a slight decline at harvest. This pattern was particularly evident in T₇, T₆, T₄, and T₅ indicating that the integrated organic treatments enhanced and maintained higher levels of soil enzyme activities throughout the plant growth cycle. The control treatment (T₁) consistently showed the lowest enzymatic activities (dehydrogenase: $1.1\text{--}1.6 \mu\text{g TPF/g/day}$; urease: $24\text{--}38 \mu\text{g NH}_4^+ \text{ h}^{-1} \text{ g}^{-1}$; alkaline phosphatase: $10\text{--}22 \mu\text{mol h}^{-1} \text{ g}^{-1}$), highlighting the crucial role of organic amendments in stimulating soil biological activity.

Statistical analysis using Tukey's test at a 0.05 significance level revealed significant differences between treatments, particularly between organic and non-organic interventions for enzymatic activity of dehydrogenase, urease, and alkaline phosphatases. Error bars indicate the consistency and reliability of the treatment effects, with T₇ showing statistically significant superiority over all other treatments at 60 DAT across the enzymatic activity of dehydrogenase, urease, and alkaline phosphatases. These findings strongly support the beneficial impact of integrated organic formulations on comprehensive soil enzymatic activities, suggesting their potential role in enhancing soil health and biological functions in agricultural systems. The enhanced activities of dehydrogenase, urease, and alkaline phosphatase under organic treatments, particularly T₇, indicate improved microbial activity, nitrogen cycling, and phosphorus mineralization processes in the soil.

3.3 Multivariate analysis

3.3.1 Principal component analysis

The principal components PC1 (52.6%) and PC2 (19.5%) of the PCA biplot showed a significant variance of 72.1%. The biplot showed clear differentiation of different treatments consistent with their effects on various soil properties and microbial communities at harvesting stage (Figure 4). Treatment groups T₁ and T₂ were distinct from the other treatments (T₃–T₇) along PC1, highlighting significant differences in the soil properties. Treatment 7 was found to have a positive correlation in the PC1 domain, that indicated it had a significant positive effect on soil microbial populations and enzymatic activities like dehydrogenase, urease, and alkaline phosphatase. A mixture of organic preparations (Jeevamrutham and Panchagavya) improved soil fertility, and the soil microbial population and enzymatic activities were beneficial for the nutrient pathways. In the negative PC2 zone, the biplot that represented T₂ had a significant impact on soil nutrient availability. Within each of these categories, there were strong positive correlations found between the total populations of soil microbes (including actinobacteria, total bacteria, and nitrogen-fixing bacteria) and plant growth indicators. These connections implied that the treatments that were used had a combined impact. Treatment 1 (Absolute Control) is still separated, indicating its poor performance and emphasizing the need for external inputs to attain perfect soil and plant health. Treatments that involved the exclusive application of Jeevamrutham or Panchagavya (Treatments 3, 4, 5 and 6) occupied intermediate positions,

indicating moderate improvements when contrasted with the combined application. The inverse correlation identified between soil pH and electrical conductivity further elucidates the intricate dynamics that govern soil chemistry under varying treatment conditions. Collectively, the PCA reveals that integrated organic preparations enhance soil biological activity, advocating for their incorporation into sustainable agricultural methodologies aimed at effectively improving soil quality and crop yields.

3.3.2 Multivariate Correlation Analysis of Soil Properties, Enzymatic Activity, and Microbial Dynamics

Multivariate correlations revealed significant relationships among soil microorganisms, enzymatic activities, and chemical properties. Figure 5 indicated that the correlation matrix showed strong positive correlations between different microbial communities (TB, PSB, KRB, NFB, and Actinobacteria), with correlation coefficients ranging from $r = 0.92$ to 0.98 , indicating a synergistic relationship between these microbial populations in the soil ecosystem. Yeasts show slightly lower but still positive correlations (0.92 - 0.96) with other microbial groups, indicating that they are also well integrated into the microbial community structure. Soil enzyme activities (Dehydrogenase, Urease, and Alkaline phosphatase) showed significant positive correlations ($r = 0.84$ - 0.88), indicating coordinated enzymatic activity in the soil. Dehydrogenase exhibits substantial relationships ($r = 0.67$ - 0.71) with microbial populations, as expected, given its significance as a measure of overall microbial activity. Urease and Alkaline Phosphatase exhibited positive correlations with microbial communities, albeit only modestly ($r = 0.80$ - 0.87). Soil pH displayed mild negative correlations with most measures, with values ranging from $r = -0.03$ to -0.14 , showing that the soil parameters studied were pH-independent within the observed range. Soil electrical conductivity (EC) showed a moderately negative correlation with microbial populations ($r = -0.098$ to -0.27), implying that higher salt concentrations may inhibit microbial growth. Soil Organic Carbon (OC) displayed moderate positive connections with microbial populations ($r = 0.33$ - 0.48) and enzyme activities ($r = 0.41$ - 0.49), indicating the significance of organic matter in microbial growth and activity. Soil moisture content had slight to moderate positive connections ($r = 0.09$ - 0.22) with many biological factors, indicating suitable moisture levels for microbial function. Available soil nitrogen was significantly positively correlated with enzyme activities (0.21 - 0.45), especially dehydrogenase activity ($r = 0.45$), suggesting a significant role of microbes in nitrogen cycling. An organic carbon correlation of 0.42 implies that the decomposition of organic matter is significant for soil nitrogen availability. Soil available phosphorus had strong positive relationships with soil EC (0.55) and organic carbon (0.52), indicating that soil salinity and organic matter content had a significant impact on phosphorus availability. Potassium availability in the soil was positively correlated with available nitrogen (0.59), indicating aligned nutrient cycling processes. Available potassium was positively correlated with organic carbon (0.30), indicating that SOC influences available potassium in the soil. These relationships provide valuable insights for soil management and ecosystem health assessment, highlighting the interconnected nature of the biological, chemical, and physical properties of soil. These data suggest that maintaining optimal conditions for one parameter is likely to have cascading positive effects on other soil properties and processes.

4. Discussion

4.1 Soil physicochemical activities

This research examined how organic preparations (Jeevamrutham and Panchagavya) and chemical fertilizers affected soil's physicochemical characteristics and biological functions. The joint use of Jeevamrutham and Panchagavya (T_7) outperformed individual applications and chemical fertilizers in improving soil health indicators. All treatments resulted in a moderate decrease in soil pH from the initial 8.08 , with T_7 showing the lowest pH (7.46). Individual organic preparations applied treatments T_6 (pH 7.86), T_4 (pH 7.50) were showed reduced pH compared with initial value. The growth in microbial populations contributes to the creation of an acidic environment through organic matter breakdown, which may result in a decrease in soil pH (Kulkarni *et al.* 2019) [33]. The pH of the panchgavya solution was lowered by the production of certain organic acids during fermentation. The pH of the formulation and soil rhizosphere may have been lowered by the organic acids produced during the fermentation of Panchagavya by *Lactobacillus*, *Saccharomyces*, *Streptomyces*, *Rhodopseudomonas*, and *Aspergillus*, according to similar findings published (Kumawat *et al.* 2013) [34]. The rise in soil organic carbon (SOC) under organic treatments, especially T_7 (0.53%), was consistent with significant SOC improvements from organic preparation applications (Kumar *et al.* 2024) [35]. The higher SOC content can be attributed to continuous organic matter supply and increased microbial biomass, contributing to carbon sequestration (Gurjar *et al.* 2024) [36]. The lower SOC in chemical fertilizer treatment (T_2) compared to organic treatments underscores the limitations of synthetic fertilizers in building soil organic matter.

4.2 Soil microbial communities

The research demonstrated a notable increase in microbial communities under organic treatment conditions. T_7 's exceptional ability to boost the overall bacterial count (154.5×10^6 CFU g^{-1}) was consistent that combined organic preparations create synergistic effects on microbial proliferation. The growth in bacterial populations can be linked to the varied nutrient compositions and growth-stimulating substances found in both Jeevamrutham and Panchagavya (Palekar, 2006) [21]. The fermented organic liquid (jeevamrut) comprises microbial population and plant-promoting chemicals, which were useful in buffering the rhizosphere for continued co-multiplication of these bacteria (Nitin *et al.* 2021) [37]. Phosphorus and potassium solubilizing bacteria exhibited remarkable growth under organic treatments, particularly T_7 . This improvement may be attributed to beneficial compounds in organic preparations that foster microbial growth and activity (Devakumar *et al.* 2014) [38]. The higher concentrations of nitrogen-fixing bacteria in T_7 (66.0×10^4 CFU g^{-1}) compared to chemical fertilizers (19.0×10^4 CFU g^{-1}) indicate that organic preparations establish more conducive environments for beneficial microorganisms.

4.3 Soil Biochemical activities

Soil enzyme activities showed substantial enhancements under organic treatments, with T_7 displaying the highest levels for dehydrogenase ($5.06 \mu g$ TPF/ g/day), urease ($77.16 \mu g$ NH_4^+ h^{-1} g^{-1}), and alkaline phosphatase ($45.32 \mu mol$ h^{-1} g^{-1}). Several studies have demonstrated that soils enhanced with farmyard manure (FYM) and organic manure displayed greater dehydrogenase activity levels than those treated with mineral fertilizers (Mangalassery *et al.* 2019) [39]. Kaur *et al.*

(2005) ^[40] noted that microbial activity were increased due to the enzymes produced by microbes that consume nutrients provided by organic matter. Singh *et al.* (2012) ^[41] and Doan *et al.* (2013) ^[42] found that using organic manures boosts enzymatic activity in tomato crop. The elevated enzymatic activity suggests enhanced soil biological functioning and nutrient cycling.

4.4 Multivariate analysis

Principal Component Analysis indicated that organic treatments, particularly T₇, exhibited strong positive associations with biological soil parameters and nutrient accessibility. This multidimensional relationship implies that combined organic preparations yield a more comprehensive and advantageous impact on soil health indicators compared to individual applications or synthetic fertilizers. The robust positive correlations between microbial communities and enzymatic activities ($r > 0.85$) pointed to a mutually beneficial relationship among soil biological components. These outcomes align with the

findings of Yadav *et al.* (2023) ^[43], who observed comparable positive correlations in organically managed soils. The moderate positive associations between soil organic carbon and biological parameters ($r = 0.33-0.48$) indicate that organic matter acts as a key catalyst for soil biological activity. This research demonstrated that the combined application of Jeevamrutham and Panchagavya (T₇) presents a promising method for improving soil health by enhancing biological activities and nutrient availability. This eco-friendly management approach could serve as an alternative to chemical fertilizers, while fostering soil biological health and fertility.

The findings of this study have important implications for sustainable agriculture. Jeevamrutham and Panchagavya can be used to improve soil health and nutrient availability, thereby enhancing crop productivity and reducing the need for chemical fertilizers. Further research is needed to evaluate the effectiveness of these biofertilizers in different soil types and under varying environmental conditions.

Table 1: Physio-chemical and chemical properties of soil

Treatments	Soil pH	Soil EC (dSm ⁻¹)	Soil OC (%)	Soil Available Nutrients (Kg ha ⁻¹)		
				N	P	K
T ₁ : Absolute Control	7.93 ± 0.95	0.26 ± 0.09	0.29 ± 0.03 ^d	129.99 ± 2.56 ^d	49.60 ± 0.31 ^c	115.53 ± 0.77 ^b
T ₂ : RDF	7.58 ± 1.41	0.46 ± 0.11	0.48 ± 0.03 ^{ab}	202.02 ± 3.60 ^a	95.73 ± 5.10 ^a	115.01 ± 3.27 ^b
T ₃ : 50% Jeevamrutham	7.75 ± 0.66	0.35 ± 0.04	0.44 ± 0.04 ^{bc}	126.67 ± 1.93 ^d	53.12 ± 0.35 ^{de}	109.31 ± 0.67 ^b
T ₄ : 100% Jeevamrutham	7.50 ± 0.51	0.33 ± 0.06	0.50 ± 0.03 ^{ab}	128.17 ± 7.25 ^d	56.68 ± 0.16 ^d	108.36 ± 2.46 ^b
T ₅ : 50% Panchagavya	7.90 ± 0.66	0.37 ± 0.07	0.36 ± 0.04 ^{cd}	145.03 ± 4.50 ^c	55.56 ± 0.72 ^{de}	108.08 ± 3.71 ^b
T ₆ : 100% Panchagavya	7.87 ± 0.01	0.36 ± 0.06	0.39 ± 0.05 ^c	176.95 ± 3.59 ^b	63.14 ± 0.40 ^c	111.75 ± 2.00 ^b
T ₇ : Jeevamrutham + Panchagavya	7.46 ± 0.53	0.31 ± 0.10	0.54 ± 0.04 ^a	188.15 ± 8.69 ^{ab}	71.66 ± 3.28 ^b	123.42 ± 4.46 ^a
Initial Values	8.08 ± 0.18	0.38 ± 0.02	0.23 ± 0.01	157.50 ± 3.41	55.25 ± 1.20	135.00 ± 2.21

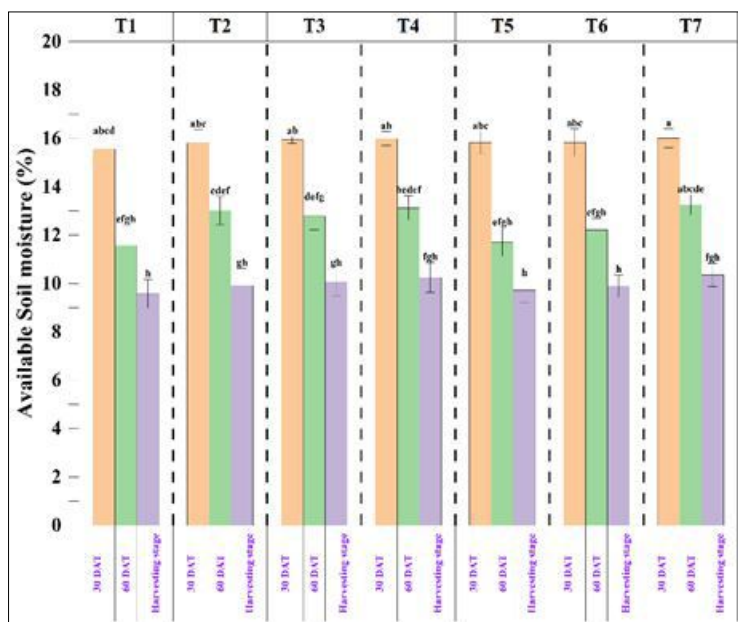


Fig 1: The available soil moisture content (%) under seven fertilizer treatments at various stages

The treatments include the absolute control (T₁), which involved no organic or chemical fertilizers, and the 100% recommended dose of fertilizer (RDF) as T₂. Organic treatments such as 50% Jeevamrutham (T₃), 100% Jeevamrutham (T₄), 50% Panchagavya (T₅), and 100% Panchagavya (T₆) were also evaluated. The combined

organic treatment of 100% Jeevamrutham + 100% Panchagavya is represented as T₇. Soil moisture was measured at different stages: 30 Days After Treatment (DAT), 60 DAT, and harvesting stage, with Tukey's test (significance level 0.05) used to assess statistical differences across treatments.

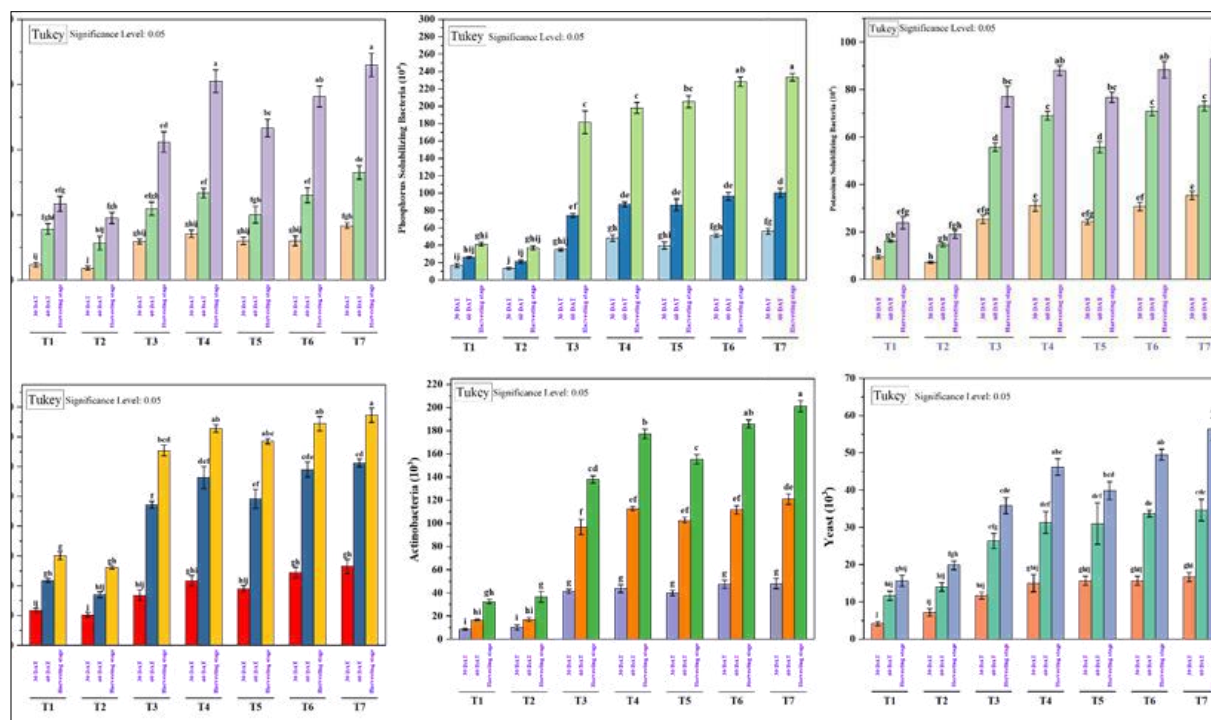


Fig 2: Impact of different fertilizer treatments on microbial populations: Nitrogen-Fixing Bacteria, Phosphate Solubilizing Bacteria (PSB), Potassium Solubilizing Bacteria (KRB), Total Bacteria, Actinobacteria, and Yeast at various stages.

The treatments include the absolute control (T₁), which involved no organic or chemical fertilizers, and the 100% recommended dose of fertilizer (RDF) as T₂. Additionally, organic treatments such as 50% Jeevamrutham (T₃), 100% Jeevamrutham (T₄), 50% Panchagavya (T₅), and 100% Panchagavya (T₆) were tested. The combined organic

treatment of 100% Jeevamrutham + 100% Panchagavya is represented as T₇. Microbial populations were assessed at different days after transplanting (DAT). The differences in microbial populations among treatments were analyzed using Tukey's test (significance level 0.05).

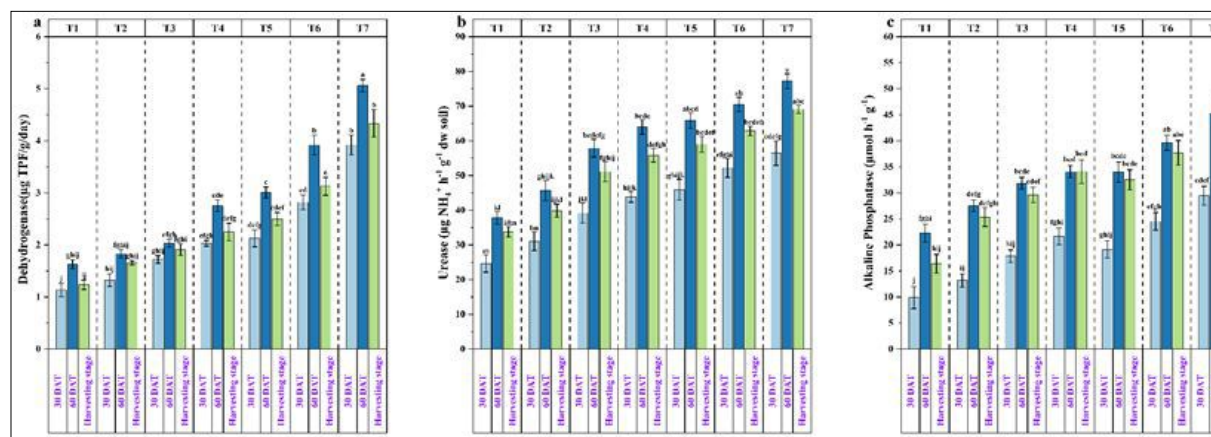


Fig 3: The activity of key soil enzymes, including dehydrogenase (a), urease (b), and alkaline phosphatase (c), under seven fertilizer treatments.

The enzyme activities were measured at different stages: 30 Days After Treatment (DAT), harvesting stage, and 30

DAT, with Tukey's test (significance level 0.05) used to assess statistical differences across treatments.

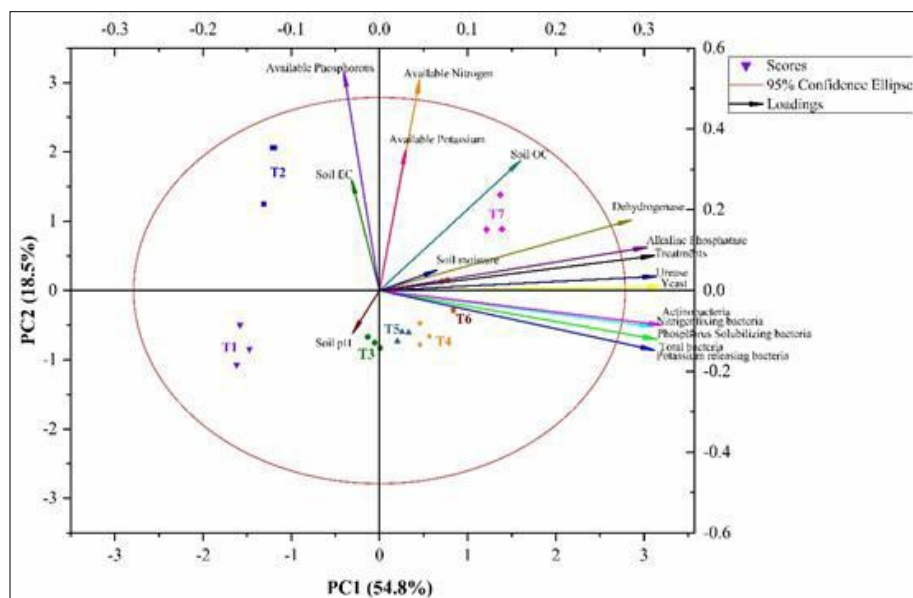


Fig 4: Principal Component Analysis (PCA) biplot shows the distribution of seven fertilizer treatments (T₁ to T₇) based on soil and microbial properties.

Treatment points are plotted with 95% confidence ellipses, illustrating the clustering and relationships between treatments.

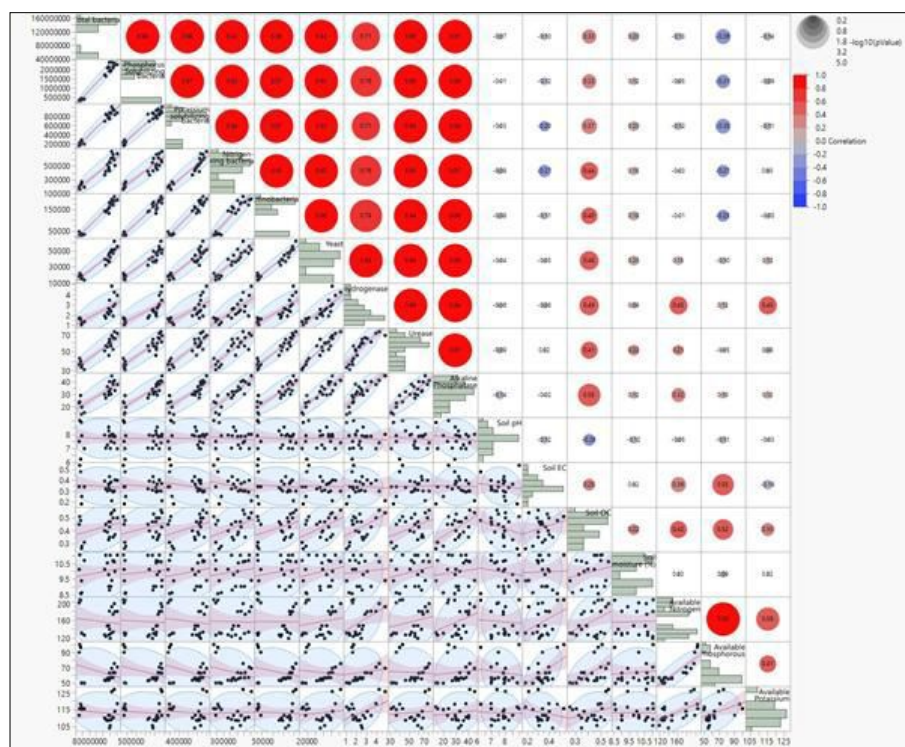


Fig 5: Pearson's Correlation Matrix of Soil and Microbial Properties under Different Fertilizer Treatments.

Correlation coefficients are represented within the circles, with red indicating strong positive correlations and blue representing negative correlations. Scatter plots below the correlation matrix provide a visual representation of the relationships between paired variables.

4. Conclusion

This study revealed that combining organic formulations, specifically Jeevamrutham and Panchagavya, markedly improved the physicochemical properties of soil, increased microbial populations, and enhanced biochemical functions

compared to using them individually or relying on chemical fertilizers. The combined treatment (T₇) notably decreased soil pH, boosted organic carbon levels, and encouraged the growth of microbial communities, including total bacteria, phosphorus and potassium solubilizers, and nitrogen-fixing bacteria. The increase in microbial biomass and activity was linked to higher levels of essential enzymes such as dehydrogenase, urease, and alkaline phosphatase, highlighting the importance of organic inputs in fostering nutrient cycling driven by microbes and overall soil health. Multivariate analyses further confirmed the positive and

synergistic relationships among microbial communities, enzymatic activities, and nutrient availability in organically treated soils. The strong correlations ($r > 0.85$) among these biological indicators underscored the interconnectedness of soil health parameters under organic management. Notably, T₇ treatment proved to be a comprehensive and effective strategy for enhancing soil fertility and function, indicating its potential as a sustainable alternative to synthetic fertilizers. These results advocate for the broader use of organic amendments to ensure long-term soil productivity and emphasize the need to incorporate ecological principles into soil fertility management. Further site-specific studies are necessary to confirm these findings in other agroecosystems.

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Credit Author Statement

Sudheer, K: Writing draft, review & editing, Visualization, Validation, Software, Conceptualization. S. Triveni: Resources, Conceptualization, Methodology, Investigation, Data Curation, Review, editing. P. C. Latha: Review & Editing, Conceptualization, Methodology, Investigation, Data Curation.

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Compliance with Ethical Standards

This article does not involve any studies with human participants or animals performed by any of the authors.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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