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Effects of Cow Dung Quantity on the Physicochemical, Microbial, and Enzymatic Characteristics of *Jeevamrit*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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Abstract

Jeevamrit is a fermented input used in natural farming, but the effects of cow dung quantity on its physicochemical, microbial, and enzymatic characteristics remain insufficiently characterised. This study compared different cow dung proportions and determined their effects on the physicochemical, microbial, and enzymatic characteristics of *Jeevamrit*. The experiment was conducted at the Department of Microbiology, Gujarat Vidyapith, Ahmedabad, from January to April 2026 using a completely randomised design comprising five treatments, each represented by five independently prepared fermentation units. Each formulation contained 3 L of water, 150 mL of cow urine, 30 g each of jaggery and gram flour, 2 g of rhizosphere soil, and 75, 150, 225, 300, or 375 g of cow dung, designated T_1–T_5, respectively. The formulations were fermented for 10 days and mixed twice daily. On the 10th day, 15 physicochemical, microbial, and enzymatic parameters were assessed. Treatment means were compared using analysis of variance, with statistical significance evaluated at the 5% level. Treatment effects were non-significant for calcium, amylase, total bacterial count, and total fungal count; therefore, numerical differences in these parameters were considered descriptive. Among the parameters showing significant differences, T_5 recorded

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the highest pH, electrical conductivity, phosphorus, organic carbon, nitrogen, alkaline phosphatase, and protease values, whereas T_2 recorded the highest potassium, acid phosphatase, and urease values. No single formulation was consistently superior across all measured parameters. The findings represent laboratory-level formulation responses, and controlled pot experiments, field validation, and biosafety assessment are required before recommending a standard formulation.

Keywords: Jeevamrit; cow dung; natural farming; fermented bio-input; soil enzymes; microbial population; nutrient composition.

1. Introduction

India witnessed a 35% increase in urea production and a 44% increase in phosphatic fertiliser production in 2024–2025 compared with 2013–2014 (Press Information Bureau, 2025). Although these production trends reflect the continuing importance of mineral fertilisers in Indian agriculture, they also reinforce the need to scientifically evaluate complementary biological inputs that may support nutrient cycling and soil microbial functioning. Increased application of chemical fertilisers may increase or maintain soil productivity, but their long-term injudicious use may disrupt soil microbial ecology (Singh et al., 2001). Soil microbes play an important role in nutrient cycling. Disturbance of soil microbial communities may impair nutrient transformation and reduce the efficiency with which plants acquire mineral nutrients, potentially increasing dependence on externally supplied fertilisers (Geisseler & Scow, 2014; Jacoby et al., 2017). One complementary approach to maintaining soil biological activity is the use of fermented natural-farming inputs such as *Jeevamrit* and Bijamrit. Rather than functioning solely as concentrated fertilisers, these formulations may act as microbial inoculants or biostimulant-type inputs by introducing metabolically active microorganisms and compounds involved in nutrient transformation. Recent studies have identified plant growth-promoting microorganisms in *Jeevamrit* and related organic inputs (Dhiman et al., 2023; Srivastava et al., 2023). Recent culture-independent investigations have further demonstrated that *Jeevamrit* contains taxonomically diverse microbial communities and metabolically active bacterial genera associated with nutrient mobilisation and plant growth promotion (Mukherjee et al., 2025; Gajjar et al., 2026). These findings support its characterisation as a biologically active fermented formulation, although the abundance and functional expression of its microorganisms may depend on the ingredients and preparation conditions used. More recent characterisation studies have further shown that ingredient quality, preparation methods, and mixing conditions can influence microbial composition, nutrient transformation, and formulation consistency (Gajjar et al., 2026; Jain et al., 2026; Mukherjee et al., 2025). In particular, differences in mixing and oxygenation can alter microbial-community composition, nutrient regimes, and the biological properties of the resulting formulation (Jain et al., 2026). Variation in the amount of cow dung may likewise affect the supply of organic carbon, nitrogenous substrates, minerals, and indigenous microorganisms available during fermentation, but this specific formulation factor has received comparatively limited experimental attention.

Cow dung is a major biological and organic component of *Jeevamrit* because it contributes microorganisms, organic matter, nitrogenous compounds, and mineral constituents to the fermentation mixture. Increasing its quantity could therefore enhance substrate and microbial availability; however, an excessive concentration could also alter aeration, ionic strength, substrate balance, and microbial interactions. The graded quantities of 75–375 g used in the present experiment were selected to represent progressively increasing cow-dung inputs while keeping the quantities of cow urine, jaggery, gram flour, rhizosphere soil, and water constant. This design enabled the effect of cow-dung quantity to be examined independently under otherwise comparable preparation conditions.

1.1 Research Gap

The inputs and general preparation method for *Jeevamrit* described by NITI Aayog (n.d.) are followed in natural-farming practice; however, published information directly comparing graded quantities of cow dung in otherwise comparable *Jeevamrit* formulations remains limited.

1.2 Objective

The present study aimed to compare graded quantities of cow dung and determine their effects on the physicochemical, microbial, and enzymatic characteristics of *Jeevamrit* after 10 days of fermentation. It was

hypothesised that increasing the quantity of cow dung would produce measurable changes in nutrient composition and enzyme activity, but that the responses would not necessarily be linear because fermentation depends on the balance among substrate availability, microbial growth, aeration, and nutrient transformation.

2. Material and Methods

2.1 Experimental Design and Preparation of *Jeevamrit*

The experiment was conducted at the Department of Microbiology, Gujarat Vidyapith, Ahmedabad, from January to April 2026. A completely randomised design was used with five treatments and five independently prepared fermentation units per treatment, giving 25 experimental units. Each unit was prepared in a 5 L plastic bucket using 3 L of tap water, 150 mL of cow urine, 30 g of jaggery, 30 g of gram flour, and 2 g of rhizosphere soil. Cow dung was added at 75 g (T_1), 150 g (T_2), 225 g (T_3), 300 g (T_4), or 375 g (T_5). Rhizosphere soil was collected from the root zone of a *Citrus limon* plant and used as an inoculum in all treatments. Treatments were allocated using a random-number table (Gomez & Gomez, 1984). The buckets were covered with jute cloth to reduce contamination and evaporative loss, and each formulation was mixed twice daily with a wooden stick. Fermentation was continued for 10 days.

2.2 Analytical Measurements

Before use, cow dung, cow urine, and soil, as well as fermentation samples collected on the 10th day, were analysed for their physicochemical, microbial, and enzymatic characteristics using standard methods: pH (Jackson, 1958), EC (Jackson, 1958), nitrogen (Kjeldahl, 1883), organic carbon (Walkley & Black, 1934), potassium (Sparks, 1996), sodium (Sparks, 1996), phosphorus (Olsen et al., 1954), calcium (Schwarzenbach, 1957), total bacterial count (Weaver et al., 1994), total fungal count (Weaver et al., 1994), acid phosphatase (Tabatabai & Bremner, 1969), alkaline phosphatase (Tabatabai & Bremner, 1969), urease (Alef & Nannipieri, 1995), protease (Alef & Nannipieri, 1995), and amylase (Miller, 1959). The results of the preliminary analysis of the inputs used for *Jeevamrit* preparation are presented in Table 1.

Table 1. Preliminary physicochemical, microbial, and enzymatic characteristics of the inputs used to prepare *Jeevamrit*

Parameter	Cow dung	Cow urine	Rhizosphere soil
Total bacterial count	47.00 x 10^7 CFU/g	31.00 x 10^7 CFU/mL	54.00 x 10^7 CFU/g
Total fungal count	27.00 x 10^6 CFU/g	23.00 x 10^6 CFU/mL	34.00 x 10^6 CFU/g
pH	8.340	7.890	7.320
Electrical conductivity (mS cm^{-1})	0.341	1.232	1.510
Acid phosphatase	48.00 (µg/mL/g)	32.00 (µg/mL)	57.00 (µg/mL/g)
Alkaline phosphatase	121.0 (µg/mL/g)	78.00 (µg/mL)	172.0 (µg/mL/g)
Urease	37.00 (µg/mL/g)	54.00 (µg/mL)	45.00 (µg/mL/g)
Protease	19.00 (µg/mL/g)	15.00 (µg/mL)	8.000 (µg/mL/g)
Amylase	28.00 (µg/mL/g)	22.00 (µg/mL)	21.00 (µg/mL/g)
Total nitrogen (%)	0.657	1.078	0.053
Total phosphorus (%)	0.055	0.032	0.045
Total potassium (%)	0.141	0.423	0.788
Organic carbon (%)	11.00	2.545	1.569
Calcium (%)	0.031	0.065	0.067
Sodium (%)	0.534	0.672	0.0370

Note. Enzyme values are reported on the analytical basis used in the original laboratory assays; assay-specific product, time, and sample-normalisation units should be verified from the laboratory records.

2.3 Statistical Analysis

Treatment means were compared using one-way analysis of variance appropriate for a completely randomised design. Standard errors and critical differences were calculated at the 5% and 1% significance levels. Means sharing a common superscript letter were considered statistically comparable. Microbial-count and amylase results were interpreted descriptively when the omnibus treatment effect was non-significant. Pearson

correlation coefficients were calculated from the five treatment means to explore covariation among the physicochemical, microbial, and enzymatic characteristics. Because each coefficient was based on only five treatment-level observations, the correlations were interpreted descriptively and were not used to infer causation or direct biological regulation.

3. Results and Discussion

Although the numerical means for total bacterial count differed among treatments, the overall treatment effect was not statistically significant. Consequently, the observed ranking should be regarded as descriptive and does not demonstrate that any cow-dung quantity consistently stimulated or inhibited bacterial proliferation. For total fungal count, T_2 showed the highest numerical value and T_1 the lowest; however, the absence of a statistically significant treatment effect indicates that these differences could not be distinguished reliably from within-treatment variability. The results therefore do not establish that the formulations created consistently different conditions for fungal proliferation.

The microbial populations detected in *Jeevamrit* are derived from its constituent materials, including cow dung, cow urine, gram flour, rhizosphere soil, and jaggery (Naik et al., 2022). Recent culture-dependent and culture-independent studies have shown that *Jeevamrit* may contain diverse bacterial populations with nutrient-solubilising and other plant growth-promoting traits; however, microbial abundance and community composition vary with cattle source, ingredient quality, oxygen availability, and preparation conditions (Jain et al., 2026; Mukherjee et al., 2025; Sagar et al., 2026). Accordingly, total plate counts alone provide only a broad measure of cultivable microbial abundance and do not establish the taxonomic composition, functional activity, or agronomic value of the microorganisms present. The cow dung quantities used were 75 g in T_1, 150 g in T_2, 225 g in T_3, 300 g in T_4, and 375 g in T_5. Increasing the quantity of cow dung would be expected to modify the supply of organic matter, nutrients, and indigenous microorganisms; nevertheless, the microbial response may not increase proportionally because fermentation is also influenced by oxygenation, substrate balance, pH, and interactions among microbial groups (Jain et al., 2026; Gajjar et al., 2026). The maximum microbial population in *Jeevamrit* has been observed on the 10th day after preparation (Reddy & Menon, 2021).

Overall, the treatments showed numerical variation in total bacterial and fungal counts, but the non-significant treatment effects do not provide evidence that cow-dung quantity alone altered cultivable microbial abundance after 10 days of fermentation. Species-level identification, community profiling, and functional assays would be required to determine whether the treatments nevertheless differed in microbial composition or metabolic potential.

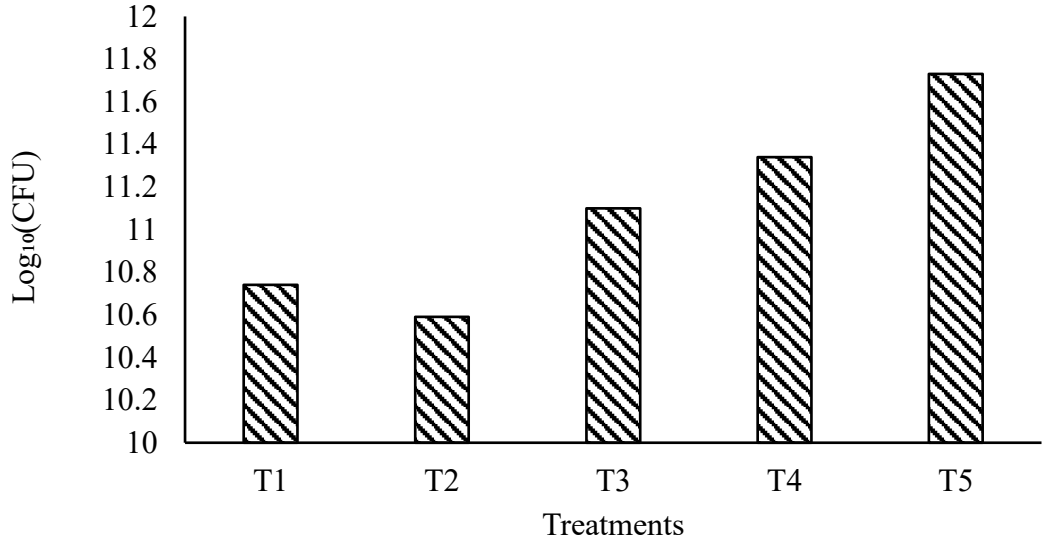

Fig. 1. Effect of treatments on Total Bacterial Count (Log_{10} CFU mL^{-1} *Jeevamrit*)

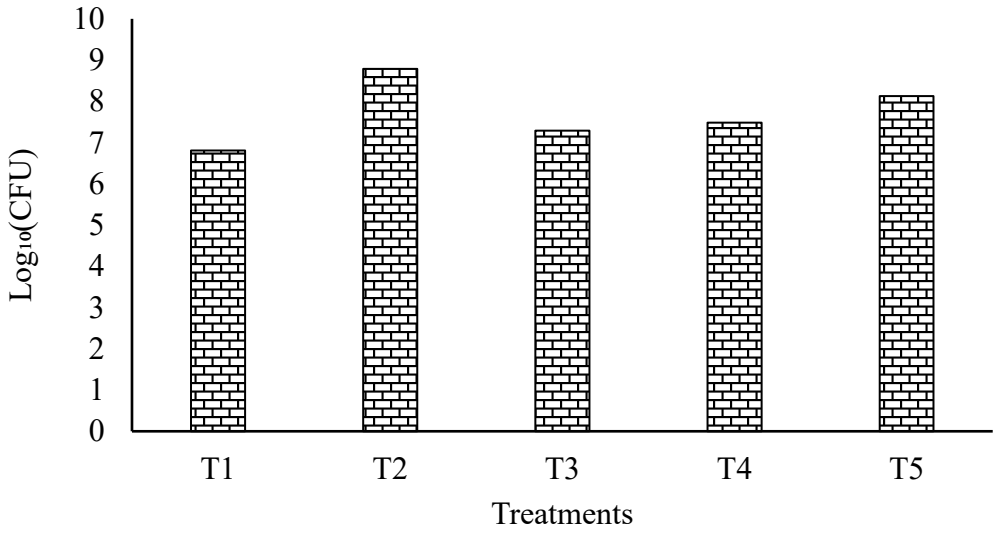

Fig. 2. Effect of treatments on Total Fungal Count (Log_{10} CFU mL^{-1} *Jeevamri*)

Data presented in Table 2 illustrate the activities of various enzymes in the different *Jeevamrit* treatments on *the* 10th day after preparation. The activities of acid phosphatase, alkaline phosphatase, urease, and protease differed among treatments, whereas the treatment effect on amylase was non-significant. Because enzyme activities can be influenced by substrate availability, extracellular-enzyme stability, microbial community composition, and physicochemical conditions, the present endpoint measurements indicate treatment-associated differences but do not independently identify the underlying regulatory mechanisms.

Regarding acid phosphatase activity, T_2 (78.23 µg/mL) showed the highest measured activity under the assay conditions. In contrast, T_5 (30.61 µg/mL) showed the lowest enzymatic activity. Treatments T_1, T_3, T_4, and T_5 were statistically non-significant relative to one another, whereas T_2 showed a significantly higher value than the other treatments. The higher acid phosphatase activity in T_2 indicates a treatment-associated difference under the assay conditions; however, the present study does not establish whether this response resulted from substrate availability, microbial composition, enzyme stabilisation, or phosphorus status. This parameter was statistically significant at both the 1% and 5% levels. Microorganisms generally produce phosphatase under phosphorus-limiting conditions. The lower activity recorded in T_5 may reflect differences in phosphorus status, substrate balance, enzyme stability, or microbial-community composition; however, feedback inhibition cannot be confirmed without measurements of soluble phosphate, enzyme kinetics, and microbial functional activity.

Regarding alkaline phosphatase activity, T_4 and T_5 (6.05 µg/mL) showed the highest measured values. These results indicate greater assayable alkaline phosphatase activity under the experimental conditions, but they do not directly demonstrate increased phosphorus mineralisation or plant availability. In contrast, T_1 (2.70 µg/mL) showed the lowest activity. Treatments T_1 and T_2 did not differ significantly from one another, whereas T_3, T_4, and T_5 were statistically comparable. This parameter was statistically significant at both the 1% and 5% levels. Alkaline phosphatase activity was lower than acid phosphatase activity on the 10th day, when the measured pH of all formulations ranged from 4.28 to 4.99. This pattern is consistent with the general sensitivity of phosphatase activity to pH, although the present study did not measure enzyme-specific pH optima or changes in enzyme activity over the course of fermentation. The difference between the two phosphatases should therefore be interpreted as an association with the acidic endpoint conditions rather than proof that pH alone controlled their activities.

Urease activity was highest in T_2 (46.81 µg/mL) and lowest in T_5 (19.33 µg/mL). Higher urease activity indicates greater potential for urea hydrolysis under the assay conditions; however, actual nitrogen mineralisation cannot be confirmed without measuring urea depletion and ammonium accumulation. T_2 recorded the highest urease activity and was statistically comparable with T_3 and T_1, whereas T_4 and T_5 formed the lower activity group. The observed treatment difference may reflect variation in substrate availability, pH,

microbial composition, or enzyme stability, although these mechanisms were not evaluated directly. Cow urine contributes urea and uric acid to the formulation, providing nitrogenous substrates that may influence urease activity (Singh, 2019). Nevertheless, the lower activity in T_5 cannot be attributed to substrate inhibition or feedback regulation because the concentrations of urea, ammonium, and other nitrogen fractions were not measured.

Protease activity was highest in T_5 (56.56 µg/mL), whereas T_1 (20.86 µg/mL) showed the lowest activity. The result demonstrates higher assayable protease activity in T_5 rather than direct evidence of greater protein mineralisation within the formulation. Protease activity differed significantly among all five treatments at the 5% level. The higher protease activity in T_5 may be associated with the greater cow-dung input, but the present data do not determine whether this response resulted from protein availability, microbial abundance, or other fermentation conditions. Proteases catalyse the hydrolysis of proteinaceous substrates, but the extent of protein degradation within the formulations was not measured directly.

Amylase activity was numerically highest in T_2 (60.18 µg/mL) and lowest in T_5 (28.71 µg/mL); however, the treatment effect was not statistically significant. The numerical ranking should therefore not be interpreted as evidence that T_2 provided greater carbohydrate-degrading activity than the other formulations. Because jaggery and gram flour were supplied at the same quantities in all treatments, the result suggests that any treatment-related variation in assayable amylase activity was small relative to the variability among replicates (Dick, 2011; Sagar et al., 2024).

Table 2. Effect of treatments on content of various enzymes

Treatments	Enzymes (µg/mL)				
	Acid phosphatase	Alkaline phosphatase	Urease	Protease	Amylase
T_1	33.30[a]	2.70[a]	33.54[ab]	20.86[a]	47.94
T_2	78.23[b]	3.10[a]	46.81[b]	29.66[b]	60.18
T_3	47.60[a]	4.80[b]	41.35[b]	48.00[d]	33.86
T_4	45.36[a]	6.05[b]	22.51[a]	37.12[c]	42.59
T_5	30.61[a]	6.05[b]	19.33[a]	56.56[c]	28.71
SE (±)	8.894	0.654	6.921	3.376	17.10
CD(5%)	18.55	1.364	14.43	7.022	NS
CD(1%)	25.30	1.861	19.69	9.578	NS

Note. Means within a column sharing at least one superscript letter are not significantly different according to the critical-difference procedure at the 5% level. NS = non-significant.

Data presented in Table 3 illustrate the physicochemical properties of the different *Jeevamrit* treatments containing varying proportions of cow dung on the 10th day after preparation. As these data represent measurements made at a single endpoint, they permit comparison among treatments on day 10 but do not independently demonstrate progressive increases or decreases during fermentation. Any temporal interpretation should therefore be restricted to variables for which repeated measurements were actually collected and reported.

The pH ranged from 4.28 in T_1 to 4.99 in T_5. The treatments differed significantly at both the 1% and 5% levels. T_1 and T_4 did not differ significantly, while T_2, T_3, and T_4 were statistically at par. T_5 showed the highest significant value. On the 10th day, all treatments were acidic, consistent with the findings of Sudheer et al. (2025). This may be attributable to microbial metabolism, which uses sugars, proteins, and other compounds as substrates and produces organic acids as by-products. These acids lower the pH and make the mixture more acidic over time (Aulakh et al., 2013; Gurjar et al., 2024).

EC ranged from 1.93 mS/cm in T_1 to 4.24 mS/cm in T_5. The treatments differed significantly at both the 1% and 5% levels. T_1 and T_2 did not differ significantly, whereas T_3, T_4, and T_5 differed significantly from one another. T_5 showed the highest significant value. On day 10, EC increased across the treatment sequence from 1.93 mS/cm in T_1 to 4.24 mS/cm in T_5, although this endpoint pattern does not establish the direction of change within individual treatments during fermentation. The higher EC values at greater cow-dung quantities are

consistent with a higher concentration of soluble ionic constituents, but direct evidence of mineralisation would require time-resolved measurements of specific dissolved ions.

The potassium concentration ranged from 0.0565% in T_5 to 0.0775% in T_2. The treatments differed significantly at both the 1% and 5% levels. T_1 and T_3 did not differ significantly, T_1 and T_4 were statistically at par, and T_4 and T_2 also did not differ significantly. The higher potassium concentration in T_2 and lower concentration in T_5 indicate a non-linear treatment response. Because potassium solubilisation, adsorption, precipitation, and microbial immobilisation were not measured separately, the underlying mechanisms cannot be determined from the present data. Because potassium was reported only for the 10th-day samples, the present results demonstrate differences among treatments at that endpoint but do not establish that potassium decreased during fermentation (Naik et al., 2022; Sagar et al., 2024).

Sodium concentration was highest in T_4 and lowest in T_5. The treatments differed significantly at both the 1% and 5% levels. T_2, T_3, and T_4 did not differ significantly from one another, whereas T_1 and T_5 differed significantly from all other treatments. The observed pattern demonstrated a non-linear response to increasing cow-dung quantity. Because sodium speciation and ionic transformations were not measured, the mechanisms underlying these treatment differences remain uncertain. Comparable treatment-dependent variation in the mineral characteristics of cow-dung-based formulations has been reported previously (Sagar et al., 2024).

The phosphorus concentration ranged from 0.0008% in T_1 to 0.0132% in T_5. The treatments differed significantly at both the 1% and 5% levels. T_1 and T_2 did not differ significantly, while T_5 and T_4 were statistically at par. T_3 differed significantly from all other treatments. The highest value in T_5 may be attributable to the high concentration of organic phosphorus supplied by the greater quantity of cow dung. A greater cow-dung input could increase the total phosphorus introduced into the mixture, but the present analysis does not distinguish organic phosphorus from soluble inorganic phosphate. Recent *Jeevamrit* studies have shown that phosphorus-related outcomes depend on both formulation composition and microbial solubilisation traits; consequently, the higher total phosphorus in T_5 should not be interpreted automatically as greater plant-available phosphorus (Jain et al., 2026; Mukherjee et al., 2025). Cow dung can contribute phosphorus-containing compounds to the formulation, but conversion to plant-available phosphate and subsequent crop uptake require confirmation through phosphorus-fractionation, soil-incubation, or plant-response studies. The data are similar to those of a previous experiment (Bhattacharjee & Uppaluri, 2025).

The calcium concentration ranged from 0.0568% in T_4 to 0.0776% in T*5*. The treatments did not differ significantly at either the 1% or 5% level. Calcium in *Jeevamrit* is mainly derived from soluble salts and mineral ash present in cow dung and urine. Although cow-dung quantity differed among treatments, calcium concentrations remained statistically comparable, suggesting that any treatment-related variation was small relative to within-treatment variability.

The organic carbon concentration ranged from 0.0048% in T_4 to 0.0185% in T_5. The organic carbon values should be interpreted in relation to the analytical basis used for the fermented liquid, because dilution and sample processing can substantially influence the measured concentration. The treatments differed significantly at both the 1% and 5% levels. T_3, T_2, and T_4 did not differ significantly from one another, while T_1, T_2, and T_3 were statistically at par. T_1 and T_2 also did not differ significantly. T_5 showed the highest significant value among all treatments. The high value in T_5 may be attributable to its greater cow dung concentration. Cow dung is the initial source of organic carbon. Fermentation can redistribute carbon among particulate, dissolved, microbial-biomass, and gaseous pools; therefore, differences in measured organic carbon should not be attributed solely to microbial proliferation without identifying the carbon fraction quantified by the analytical method. The lower value in T_4 may reflect treatment-specific carbon transformation, although microbial utilisation and respiratory losses were not measured directly.

The nitrogen concentration ranged from 0.352% in T_1 to 1.933% in T_5. The treatments differed significantly at both the 1% and 5% levels. T_1 and T_2 did not differ significantly, while T_4 and T_3 were statistically at par. T_5 showed the highest significant value among all treatments. The significantly higher nitrogen concentration in T_5 may partly reflect the greater nitrogen input associated with the larger quantity of cow dung. Although *Jeevamrit* can contain bacteria with nitrogen-fixation potential, the present study did not measure diazotrophic abundance, nitrogenase activity, isotopic nitrogen incorporation, or changes in individual nitrogen fractions; nitrogen fixation should therefore not be invoked as an explanation for the observed treatment difference

(Mukherjee et al., 2025). The result should be interpreted primarily as a difference in total nitrogen measured at the 10-day endpoint.

The nutrient results collectively indicate that increasing cow-dung quantity modified several measured characteristics, but the responses were parameter-specific and sometimes non-linear. This pattern agrees with recent evidence that *Jeevamrit* properties depend on interacting formulation and preparation factors rather than on a single ingredient in isolation (Gajjar et al., 2026; Jain et al., 2026). Moreover, studies of modified *Jeevamrit* formulations have shown that nutrient concentrations can change substantially when additional organic precursors are incorporated, underscoring the need to interpret the present values as specific to the formulation and analytical conditions used here (Bhattacharjee & Uppaluri, 2025).

Table 3. Physicochemical characteristics of *Jeevamrit* on day 10

Treatments	Physico-chemical properties							
	pH	EC (mS/cm)	Potassium (%)	Sodium (%)	Phosphorus (%)	Calcium (%)	OC (%)	Nitrogen (%)
T_1	4.28^{a}	1.93^{a}	$0.0716^{a,b}$	0.0714^{a}	0.0008^{a}	0.0608	$0.0155^{b,c}$	0.352^{a}
T_2	4.65^{b}	1.97^{a}	0.0775^{c}	0.0809^{b}	0.0018^{a}	0.0757	$0.0117^{a,b,c}$	0.447^{a}
T_3	4.63^{b}	3.07^{b}	0.0703^{a}	0.0790b	0.0090^{b}	0.0612	$0.0084^{a,b}$	0.655^{b}
T_4	$4.39^{a,b}$	3.54^{c}	$0.0753^{b,c}$	0.0815b	0.0125^{c}	0.0568	0.0048^{a}	0.616^{b}
T_5	4.99^{c}	4.24^{d}	0.0565^{d}	0.0650^{c}	0.0132^{c}	0.0776	0.0185^{c}	1.933^{c}
SE (±)	0.09	0.10	0.0010	0.0011	0.0007	0.0122	0.0032	0.039
CD(5%)	0.19	0.21	0.0022	0.0023	0.0015	NS	0.0068	0.082
CD(1%)	0.27	0.29	0.0031	0.0032	0.0021	NS	0.0093	0.111

Note. Means within a column sharing at least one letter are not significantly different according to the critical-difference procedure. Similar alphabet represents that treatments are at par between themselves. NS = non-significant.

Exploratory Pearson correlations were calculated from the five treatment means to describe covariation among the measured characteristics. Because of the small number of treatment-level observations and the common influence of the cow-dung gradient, the coefficients were considered descriptive and were not interpreted as evidence of causation.

Table 4 presents the correlation coefficient analysis conducted to evaluate the relationships between the different physicochemical parameters of the treatments and the total microbial count in *Jeevamrit*. The treatment-mean correlations showed both positive and negative patterns between microbial counts and physicochemical characteristics. Total bacterial count was positively associated with pH, electrical conductivity, phosphorus, organic carbon, and nitrogen and negatively associated with potassium and sodium. Total fungal count showed positive associations with electrical conductivity, sodium, and phosphorus and negative associations with pH, calcium, and organic carbon. These patterns may reflect common responses of the measured variables to differences in cow-dung quantity and fermentation conditions. Because the coefficients were based on only five treatment means, they were considered hypothesis-generating and were not used to establish direct effects of physicochemical conditions on microbial abundance.

Table 4. Exploratory Pearson correlations between microbial counts and physicochemical characteristics based on treatment means

Physicochemical properties	Microbial properties	
	Total Bacterial Count	Total Fungal Count
pH	0.73	-0.13
EC	0.90	0.63
Potassium	-0.88	0.06
Sodium	-0.68	0.38
Phosphorus	0.79	0.78
Calcium	0.42	-0.57
OC	0.42	-0.74
Nitrogen	0.97	0.07

Note. Coefficients were calculated from five treatment means (n = 5). They are presented descriptively and should not be interpreted as evidence of direct or causal biological relationships

Table 5 presents the correlation coefficient analysis conducted to evaluate the relationships between the different enzymatic parameters of the treatments and the total microbial count in *Jeevamrit*. Acid phosphatase, urease, and amylase showed negative treatment-mean correlations with both bacterial and fungal counts, whereas alkaline phosphatase and protease showed positive correlations. These associations may reflect simultaneous responses of microbial counts and enzyme activities to formulation composition rather than direct regulation of enzyme production by microbial abundance. The coefficients should therefore be treated as exploratory patterns requiring confirmation using replicate-level observations and direct measurements of microbial community composition and enzyme-producing populations.

Table 5. Exploratory Pearson correlations between microbial counts and enzyme activities on day 10 based on treatment means

Enzymatic properties	Microbial Properties	
	Total Bacterial Count	**Total Fungal Count**
Acid phosphatase	-0.56	-0.21
Alkaline phosphatase	0.78	0.79
Urease	-0.80	-0.48
Protease	0.81	0.40
Amylase	-0.76	-0.45

Note. Coefficients were calculated from five treatment means (n = 5). The correlations are descriptive and do not establish that microbial abundance directly controlled enzyme activity

Table 6 presents the correlation coefficient analysis conducted to evaluate the relationships between the enzymatic and physicochemical parameters of the treatments in *Jeevamrit*. Several relatively strong treatment-mean correlations were observed between physicochemical and enzymatic characteristics. Electrical conductivity was positively correlated with alkaline phosphatase and protease and negatively correlated with urease and amylase. Phosphorus showed a strong positive correlation with alkaline phosphatase and protease and negative correlations with urease and amylase. Nitrogen was positively correlated with alkaline phosphatase and protease and negatively correlated with acid phosphatase, urease, and amylase. These patterns may reflect shared responses to formulation composition, substrate concentration, or fermentation conditions. Because only five treatment means contributed to each coefficient, the correlations should be interpreted as preliminary patterns rather than evidence of direct biochemical regulation.

Table 6. Exploratory Pearson correlations between physicochemical characteristics and enzyme activities on day 10 based on treatment means

Physicochemical characteristic	Acid phosphatase	Alkaline phosphatase	Urease	Protease	Amylase
pH	−0.004	0.457	−0.184	0.822	−0.481
Electrical conductivity	−0.509	0.966	−0.779	0.882	−0.842
Potassium	0.684	−0.500	0.610	−0.735	0.805
Sodium	0.719	−0.135	0.540	−0.345	0.534
Phosphorus	−0.421	0.997	−0.724	0.838	−0.791
Calcium	0.272	−0.033	0.066	0.338	0.050
Organic carbon	−0.373	−0.231	−0.178	0.110	−0.168
Nitrogen	−0.476	0.666	−0.653	0.828	−0.726

*Note. Coefficients were calculated from five treatment means (*n = *5) and are presented as exploratory descriptive statistics.*

Overall, the correlation analysis identified potential relationships for future investigation but did not establish causation. Replicate-level analysis using the 25 independent fermentation units, together with time-resolved measurements, nutrient-fraction analysis, and microbial-community profiling, would be required to assess whether these associations are reproducible and biologically meaningful.

4. Conclusions

The proportion of cow dung used in *Jeevamrit* influenced several physicochemical and enzymatic characteristics after 10 days of fermentation. Among the evaluated formulations, T_5, containing 375 g cow dung, recorded the

highest pH, electrical conductivity, phosphorus, organic carbon, nitrogen, alkaline phosphatase, and protease values. T_2, containing 150 g cow dung, recorded the highest potassium, acid phosphatase, and urease values among the parameters that differed significantly. However, calcium, amylase, total bacterial count, and total fungal count did not differ significantly among treatments. These findings show that formulation performance varied according to the parameter considered and that no treatment was consistently superior across all measurements. T_5 produced higher laboratory values for several nutrient-related and enzymatic measurements, whereas T_2 produced higher values for potassium and selected enzyme activities. These differences should not be interpreted as evidence of superior agronomic performance until crop response, soil effects, formulation stability, and biosafety are evaluated. The findings therefore support parameter-specific laboratory characterisation rather than selection of a universally superior formulation. Confirmation through controlled pot experiments and field trials is necessary before practical agricultural recommendations are made.

5. Limitations and Future Research

The study was conducted under laboratory conditions using one fermentation period and did not include time-series sampling, crop-response trials, soil application, shelf-life testing, phytotoxicity assessment, pathogen screening, or field validation. Total plate counts did not resolve microbial identity or functional capacity, and the study did not quantify individual nitrogen or phosphorus fractions. The analytical basis, assay-specific units, and quality-control procedures require more detailed reporting to support replication and cross-study comparison. The use of a single ingredient source also limits generalisation across cattle breeds, diets, seasons, and farms. Multi-batch experiments should therefore evaluate formulation reproducibility under controlled temperature and aeration, incorporate microbial-community profiling and biosafety screening, and test agronomic performance across soils, crops, application rates, and agroecological conditions.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all authors, including contributions to the study design, data generation, content development, results, interpretation, and related scholarly work. The authors acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, and reference formatting. These AI-assisted tools were not used as authors and did not replace the authors' intellectual contributions or scholarly judgement. All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the authors. The authors accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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