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Physico-Chemical Parameters and Microbial Population Dynamics in *Jeevamrutha* Bioformulations of Different Cow Breeds

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ABSTRACT

Jeevamrutha is a complex biodynamic microbial liquid formulation prepared from indigenous ingredients such as cow dung, cow urine, pulse flour, jaggery, soil and water. *Jeevamrutha* has been used by Indian farming community for centuries and deeply rooted in traditional organic farming practices. To understand the physico-chemical dynamics of *jeevamrutha* during the incubation period, pH and EC were analysed up to 7th day of incubation. The C/N ratio and microbial population were estimated up to 8th day to determine if microbial load reduces beyond 7th day. The final pH 5.05, 5.05, 5.20 and 5.20, electrical conductivity 1.38, 1.44, 1.44 and 1.43 dSm^{-1} and the decrease in C/N ratio from 20.77 to 13.55, 19.19 to 11.77, 21.42 to 14.63 and 20.88 to 13.48 in Hallikar, Deoni, Ongole and HF *jeevamrutha,* respectively. The decrease in C/N ratio of *jeevamrutha* during the incubation period corresponds to increase in the microbial population. The relatively high microbial population on 7th day justifies the field application of jeevamrutha by farmers after 7-8 days of incubation.

Keywords : Electrical conductivity, Jeevamrutha, Microbial load, pH

ORGANIC farming has become a key focus in global development due to increasing demand for safe and healthy food, long-term sustainability and concerns about environmental pollution from the negligent use of agrochemicals (Mahdi *et al*., 2012). Practicing sustainable agriculture can prevent pesticides and toxic substances from entering the food chain while also help to avoid soil and water pollution (Boraiah *et al*., 2017). Organic farming mainly focuses on use of on-farm organic resources to sustain soil health (Nath *et al*., 2023). The extensively applied organic manures like farm yard manure, green manures, vermicompost and non-edible oil cakes are needed to accomplish the nutritional requirements of crops. The narrow availability of these bulky organic manures requires their integration with supplementary available options. Liquid organic fertilizer formulations like *Panchagvya*, *Beejamrutha* and *Jeevamrutha* are often used as plant growth enhancing substances prepared with material available with farmers. They are rich sources of beneficial microflora that support and promote plant growth, leading to improved vegetative growth and higher-quality yields (Devakumar *et al*., 2011).

Natural Farming is a chemical-free agricultural system rooted in Indian tradition, enriched by modern ecological knowledge, resource recycling and on-farm resource optimization. It is viewed as an agroecology-based diversified farming system that integrates crops, trees and livestock with functional biodiversity. Nearly 10 lakh hectares of land is covered under natural farming in India

(National Mission on Natural Farming Management and Knowledge Portal, 2022). The use liquid formulations like *jeevamrutha*, *beejamrutha* and *panchagavya* are integral part of natural farming.

Jeevamrutha is a complex biodynamic microbial liquid formulation prepared from indigenous ingredients such as cow dung, cow urine, jaggery, pulse flour, soil and water. *Jeevamrutha* has been used by Indian farming community for centuries and deeply rooted in Indian natural farming practices (Palekar, 2006). Microflora in *jeevamrutha* play a vital role in improving soil quality. The beneficial microbes in *jeevamrutha* directly or indirectly improves plant growth by the secretion of growth hormones, biofertilization, stimulation of root growth, rhizoremediation and plant stress control (Prasad *et al*., 2017). With this view, a study was carried out to study the changes in pH and electrical conductivity and realize the influence of nutrient content mainly the C/N ratio on microbial population during its preparation.

Material and Methods

Sample Collection

The cow dung and urine samples of milch breeds of Hallikar, Deoni, Ongole and Holstein- Friesian (HF) were sourced from Rashtrotthana Goshala, Doddaballapur, Karnataka (13.40°N, 77.52°E). The average age of these cows were 4-5 years. The cow's feeding habit did not include any feed concentrates and were not administered any hormonal injections. The samples were placed in ice box, transported to laboratory and stored at 4ºC for further studies.

Plate 1 : List of ingredients used for the preparation of *jeevamrutha*

Preparation of *Jeevamrutha*

Materials required (1L): Cow dung-50g; Cow urine- 50mL; Jaggery-10g; Chickpea (gram) flour-10g; Soil-2.5g; Water- to make up the volume. Gram (chickpea) flour and jaggery samples were sourced from organically certified seller. Gram flour is rich source of proteins, carbohydrates and fatty acids, where jaggery is natural source of sucrose, fructose, glucose, minerals and vitamins (Hend *et al*., 2020 and Sharifi-Rad *et al*., 2023). The top soil was collected from Mahatma Gandhi Botanical Garden, University of Agricultural Sciences, Bangalore. These materials in exact proportion were mixed together into a non-metal container and water was added to make the volume to 1L. This preparation was mixed twice every day and allowed to ferment for 7 days (Palekar, 2006).

Determination of Physicochemical Properties and Microbial Load of *Jeevamrutha*

Measurement of pH, Electrical Conductivity (EC) and C/N Ratio of *Jeevamrutha*

Jeevamrutha samples were harvested at 24 h intervals starting from 0-day to 7-day of incubation. Changes in pH and electrical conductivity (EC) of *jeevamrutha* were measured using pH and EC meter (Rayment and Higginson, 1992). The organic carbon content in *jeevamrutha* was determined using wet oxidation method by Walkley and Black (1934) while, nitrogen content was determined using Micro-Kjeldahl method described by Allison (1965) up to 8 days of incubation at every 24 hours intervals.

Microbial Population in *Jeevamrutha*

Microbial population was estimated by standard plate count technique at 24 hours intervals up to 8 days of incubation. Different dilutions of *jeevamrutha* bioformulation were plated on nutrient agar, Martin's rose bengal agar and Kusters agar to enumerate general bacteria, fungi, actinomycetes population respectively. The plates were incubated at 28 ± 2 ºC and the observations were recorded after 24 hours of incubation for bacteria, 4872 hours of incubation for fungi and actinomycetes. Microbial count were

recorded as colony forming units per mL (cfu/mL) of *jeevamrutha* sample tested.

Statistical Analysis

All the experimental data were subjected to statistical analysis using Web Agri Statistical Package (WASP 2.0) software and means were compared using Duncan's multiple range test (DMRT).

Results and Discussion

Jeevamrutha undergoes a series of physicochemical and biological changes during incubation.

pH and Electrical Conductivity (EC) Parameters of *Jeevamrutha*

An initial pH of 7.8 was recorded on 0th day in Hallikar *jeevamrutha*. A sharp drop in the pH to 4.0 was observed after 24 h of incubation. This was followed by a gradual rise in pH until which stabilized at 5.05. Simultaneously, rise in EC value from 0.90 to 1.38 dSm^{-1} was observed (Fig. 1), during incubation period. Similarly, in Deoni *jeevamrutha*, pH dropped from 8.0 (0th day) to 4.0 (1st day) followed by stable increase in pH to 5.05 on 7th day while, EC increased from 0.96 to 1.44 dSm^{-1} (Fig. 2).

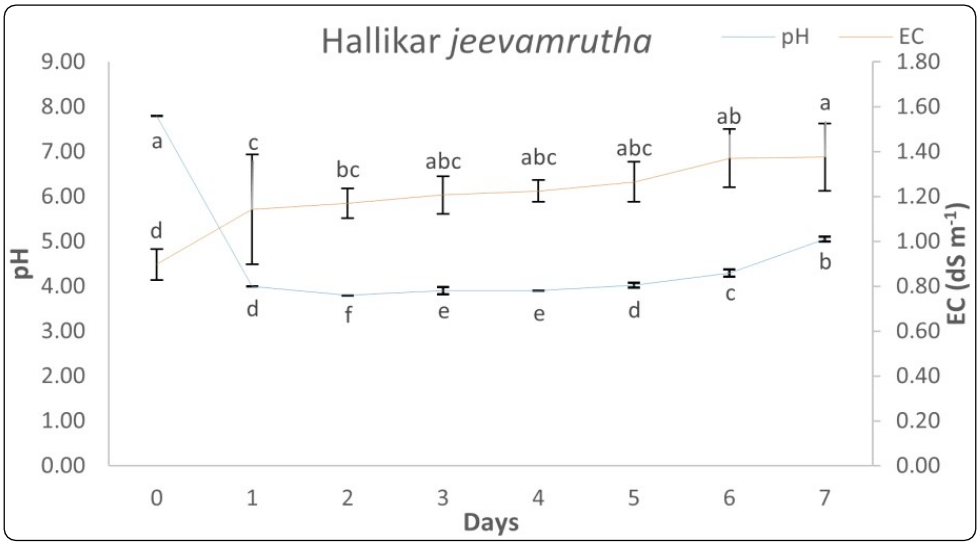

Fig. 1 : A graphical representaion of pH and EC in Hallikar *jeevamrutha* during incubation period

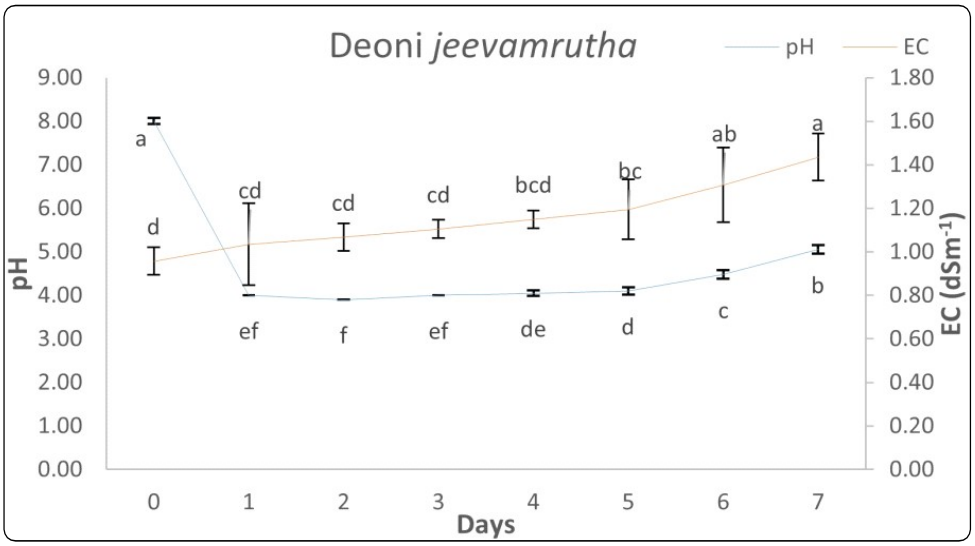

Fig. 2 : A graphical representaion of pH and EC in Deoni *jeevamrutha* during incubation period

The pH decreased from 7.90 to 5.20 and EC increased from 1.01 to 1.44 dSm^{-1} from 0^{th} day to 7^{th} day in Ongole *jeevamrutha* (Fig. 3) while, pH lowered to 5.20 and EC increased to 1.43 dSm^{-1} on 7^{th} day in HF *jeevamrutha* (Fig. 4).

The demand for carbon in the presence of complex nutrients is primarily satisfied by free sugars during fermentation. Sugars are converted to volatile fatty acids (VFAs) and organic acids (OAs) during this process thus lowering the system's pH (Ferreira and Mendes-Faia, 2020). Further, on the depletion of sugars, microbes utilize protein, releasing ammonia from amino acids, thereby increasing pH (Ferreira & Mendes-Faia, 2020 and Mira-de-Orduna *et al*., 2001). The increase in number of free ions due to the breakdown of complex substrates into simpler ones explains the increase in electrical conductivity during incubation period. The cow breeds do not have influence on changes in pH and EC, however, the nutrient content and microbial load of jeevamrutha depends on feeding habit of the animal.

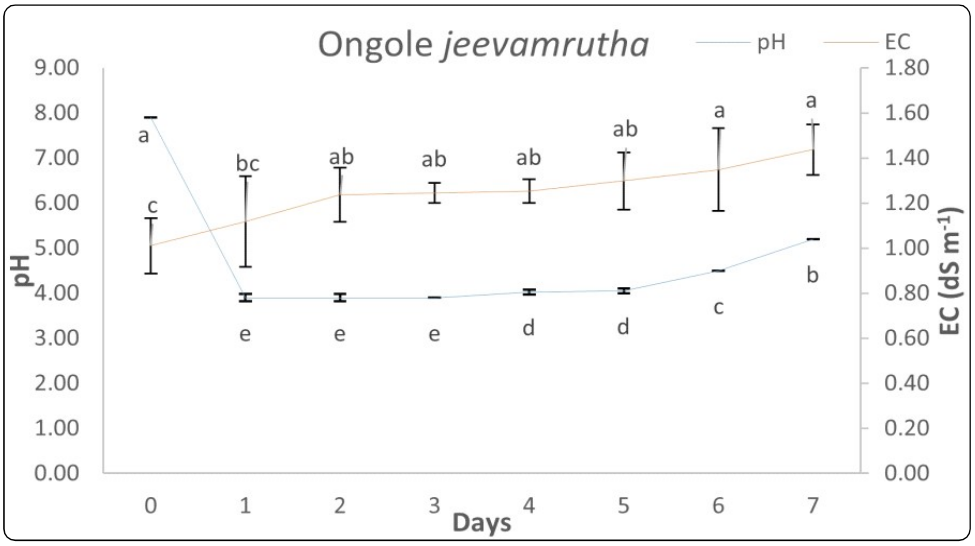

Fig. 3 : A graphical representaion of pH and EC in Ongole *jeevamrutha* during incubation period

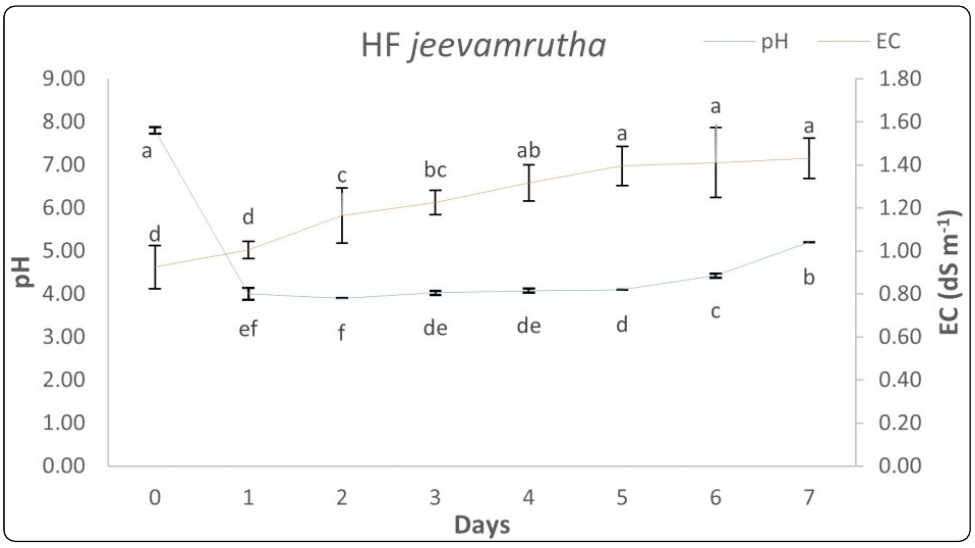

Fig. 4 : A graphical representaion of pH and EC in HF *jeevamrutha* during incubation period

Microbial Load and C/N Ratio of *Jeevamrutha*

The C/N ratio declined from 20.77 (0th day) to 13.55 (8th day) in Hallikar *jeevamrutha* while, Deoni *jeevamrutha* showed a drop in C/N ratio from 19.19 to 11.77 during incubation period. Similarly, a decline in C/N ratio from 21.42 to 14.63 and 20.88 to 13.48 was recorded in Ongole and HF *jeevamrutha* respectively. This indicates the continuous utilization of C and N rich substrates by microbes for their multiplication. But an increase in C/N ratio in all *jeevamrutha* samples on 5th day suggested the release of sugars from complex polysaccharides like cellulose and hemicellulose in the fermenting medium (Table 1). Subsequent drop in C/N ratio beyond 7th day may be due to release of nitrogen from protein in the form of ammonia (Duraivadivel *et al*., 2022).

There was an increase in bacterial population from $23.75X10^6$ to $38.00X10^6$ cfu/mL, $18.50X10^6$ to $32.75X10^6$ cfu/mL, $35.00X10^6$ to $49.25X10^6$ cfu/mL and $22.50X10^6$ to $36.00X10^6$ in Hallikar, Deoni (Table 1), Ongole and HF *jeevamrutha* from 0th day to 1st day respectively (Table 2). The readily available

Table 1

Microbial load and C/N ratio of Hallikar and Deoni *jeevamrutha* during 8 days of incubation

Parameters	Day-0	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	Day-8
Hallikar									
C/N ratio	20.77 ^{a}	19.39 ^{b}	18.66 ^{c}	17.49 ^{d}	15.47 ^{e}	13.30 ^{h}	14.15 ^{g}	14.61 ^{f}	13.55 ^{h}
Bacteria(10^6 cfu/mL)	23.75 ^{g}	38.00 ^{e}	30.25 ^{f}	31.00 ^{f}	39.50 ^{e}	50.25 ^{d}	68.50 ^{c}	79.00 ^{a}	72.75 ^{b}
Fungi(10^4 cfu/mL)	10.25 ^{ef}	8.50 ^{f}	12.75 ^{bcd}	13.00 ^{bcd}	11.25 ^{de}	12.00 ^{cde}	13.25 ^{bc}	14.50 ^{b}	16.75 ^{a}
Actinobacteria (10^3 cfu/mL)	1.25 ^{c}	1.75 ^{bc}	2.25 ^{abc}	2.25 ^{abc}	2.25 ^{abc}	1.00 ^{c}	2.50 ^{abc}	3.25 ^{ab}	3.75 ^{a}
Deoni									
C/N ratio	19.19 ^{a}	17.58 ^{b}	16.86 ^{c}	15.72 ^{d}	13.63 ^{e}	11.52 ^{h}	12.48 ^{g}	12.88 ^{f}	11.77 ^{h}
Bacteria(10^6 cfu/mL)	18.50 ^{f}	32.75 ^{de}	28.00 ^{e}	30.25 ^{e}	37.00 ^{d}	49.50 ^{c}	65.25 ^{b}	75.50 ^{a}	75.50 ^{a}
Fungi(10^4 cfu/mL)	8.75 ^{de}	9.75 ^{e}	11.75 ^{abc}	12.75 ^{ab}	8.75 ^{bcd}	10.25 ^{abcd}	11.00 ^{cd}	13.75 ^{a}	13.75 ^{a}
Actinobacteria (10^3 cfu/mL)	1.50 ^{c}	2.00 ^{bc}	2.25 ^{bc}	2.25 ^{bc}	2.25 ^{bc}	1.25 ^{c}	2.50 ^{bc}	3.00 ^{ab}	4.00 ^{a}

Note : Means with different superscripts within the rows are significantly different (n=3)

Table 2

Microbial load and C/N ratio of Ongole and HF *jeevamrutha* during 8 days of incubation

Parameters	Day-0	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	Day-8
Ongole									
C/N ratio	21.42 ^{a}	20.45 ^{b}	19.57 ^{c}	18.39 ^{d}	16.48 ^{e}	14.38 ^{g}	15.06 ^{g}	15.65 ^{g}	14.63 ^{f}
Bacteria(10^6 cfu/mL)	35.00 ^{f}	49.25 ^{d}	41.00 ^{e}	43.25 ^{e}	52.00 ^{d}	65.75 ^{c}	81.25 ^{b}	91.50 ^{a}	90.25 ^{a}
Fungi(10^4 cfu/mL)	11.00 ^{d}	12.75 ^{cd}	12.50 ^{cd}	14.25 ^{bc}	12.00 ^{cd}	13.00 ^{cd}	14.50 ^{bc}	16.50 ^{ab}	17.50 ^{a}
Actinobacteria (10^3 cfu/mL)	1.00 ^{c}	2.50 ^{abc}	2.00 ^{bc}	2.50 ^{abc}	2.00 ^{bc}	0.75 ^{c}	2.00 ^{bc}	3.25 ^{ab}	4.00 ^{a}
HF									
C/N ratio	20.88 ^{a}	19.30 ^{b}	18.23 ^{c}	17.32 ^{d}	15.31 ^{e}	13.23 ^{g}	13.90 ^{g}	14.43 ^{g}	13.48 ^{f}
Bacteria(10^6 cfu/mL)	22.25 ^{f}	36.00 ^{d}	28.25 ^{e}	30.50 ^{e}	38.75 ^{d}	51.75 ^{c}	68.00 ^{b}	80.00 ^{a}	78.50 ^{a}
Fungi(10^4 cfu/mL)	12.25 ^{cd}	11.25 ^{d}	13.75 ^{bc}	15.00 ^{ab}	12.50 ^{cd}	13.75 ^{bc}	14.75 ^{ab}	15.00 ^{ab}	16.50 ^{a}
Actinobacteria (10^3 cfu/mL)	2.00 ^{b}	3.50 ^{a}	2.50 ^{ab}	1.75 ^{b}	2.50 ^{ab}	1.50 ^{b}	2.50 ^{ab}	2.50 ^{ab}	3.25 ^{a}

Note : Means with different superscripts within the rows are significantly different (n=3)

sugars like glucose, fructose and sucrose are used rapidly. However, the narrow access to these sugars from jaggery causes subsequent decline in population on 2nd day. An upward trend in bacterial population was observed beyond 3rd day upto 7th day in all *jeevamrutha* samples because of availability of nutrients from other complex polysaccharides like cellulose and hemicellulose which are sourced through cow dung. A drop in bacterial population after 7th day may be due to exhaustion of nutrients.

An increase in fungal population from $10.25x10^4$ to $16.75x10^4$ cfu/mL, $8.75x10^4$ to $13.75x10^4$ cfu/mL, $11.00x10^4$ to $17.50x10^4$ cfu/mL and $12.25x10^4$ to $16.50x10^4$ cfu/mL from 0th day to 8th day was recorded in Hallikar, Deoni, Ongole and HF *jeevamrutha,* respectively. Fungal population continues to increase beyond 8 days (Devakumar *et al*., 2014) as they prefer acidic to near neutral pH and because of their natural ability to degrade the undigestible and partially degraded polysaccharides better than other microbial groups. Similarly, an upward trend was observed in actinobacterial population from 0th day to 8th day of incubation (Table 1 & Table 2) in all *jeevamrutha* samples. Acidic pH may be the reason for low count of actinobacteria during incubation period as they favour neutral to alkaline pH (Hamid *et al*., 2015).

The acidic pH of *jeevamrutha* at the end of incubation lead to the conclusion that it is a fermentative type of product. There was an increase in electrical conductivity due to the build-up of free ions at the end of fermentation period. The cow breeds as such do not have influence on pH and EC changes, but the grazing habit and feed source can influence the nutrient content and microbial population in *jeevamrutha*. The decrease in C:N ratio of *jeevamrutha* during the incubation period corresponds to increase in the microbial population. Farmer's practise of field application of *jeevamrutha* after 7-8 days of incubation could be justified by the relatively high microbial load observed during these days.

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