



## Microbial analysis of jeevamrutha prepared from different cow breeds of desi and cross cow breeds cow dung and cow urine

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### ABSTRACT

A laboratory experiment was conducted on shelf-life of jeevamrutha at Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udipi. In this study three different desi breeds viz., Malnad gidda, Gir, Sahiwal and three different cross breeds viz., Holstein Friesian (HF), Jersey and crossbred Jersey, cow dung and cow urine were collected aseptically and separately to prepare Jeevamrutha, after preparation of Jeevamrutha from 1<sup>st</sup> day to 15<sup>th</sup> days samples were collected (daily) and enumerated the general microorganisms viz., bacteria, fungi and actinomycetes with their respective media. Among the desi and cross cow breeds jeevamrutha, desi cow breeds jeevamrutha contains higher microbial population compared to cross cow breeds jeevamrutha. In desi cow breeds jeevamrutha, Malnad gidda cow breed jeevamrutha contains the maximum microbial population. In general, the highest microbial population was noticed between 7<sup>th</sup> to 9<sup>th</sup> days after preparation (DAP) of jeevamrutha in all the cow breeds. Hence, it's considered as a best time for the application of jeevamrutha to soil to improve the soil organic carbon.

**KEY WORDS:** *Jeevamrutha; Organic carbon; Desi breeds; Cross breeds*

## 1. Introduction

The cost of inorganic fertilizers is increasing enormously to an extent that they are out of reach small and marginal farmers. Use of inorganic fertilizers and insecticides, the population of beneficial organism's decrease and natural regeneration of nutrition in the soil cease (Rama and Naik, 2017; Dakshayini *et al.*, 2016; Reddy *et al.*, 2015). Soil becomes barren and soil fertility decreases. The use of fermented liquid manures in such situation is, therefore practically a paying proposal. Application of these organic liquid formulations will enhance the soil microbial activity and population to a larger extent. This in-turn has a positive effect on growth and yield of

crops. Similarly, Subhash Palekar is one of the progressive farmers of Maharashtra, India; in his workshop on Philosophy and Technology of Zero Budget Natural Farming (ZBNF) he used a new biodynamic formulation termed jeevamrutha prepared from desi cow dung and cow urine. The desi cow or indigenous breed of cows is the backbone of ZBNF. For centuries, dung and urine from desi cows have been used in farming. Although the milk productivity of Indian cow breeds is low, they are very useful in production of cow dung and urine which will have a very high beneficial property. According to Subhash Palekar, one gram of desi cow dung contains 300

to 500 crore beneficial effective microbes as against 50 to 70 lakh microbes in cross bred cow dung. Hence, Cross bred jersey and Holstein Friesian (HF) cows should not be used in ZBNF (Palekar, 2006). Vanaja *et al.* (2009) stated that jeevamrutha is a plant growth-promoting substance containing beneficial microorganisms that provides the necessary nutritional requirement for growth and yield of a crop.

Cow dung was used as major ingredient for the preparation of jeevamrutha. It contains numerous microorganisms; they are *Azotobacter*, *Acetobacter*, *Azospirillum* (nitrogen supplier), *Pseudomonas* (phosphorus-solubilizer) and *Bacillus silicus* (potash-solubilizer) and others. Once jeevamrutha was incorporated to soil, these organisms are well activated and maintain the soil productivity. Manjunatha *et al.* (2009) reported that the use of jeevamrutha (indigenous species cow dung and cow urine, pulse flour, jaggery, rhizosphere soil solution) treated organics, improves the physico-chemical and biological properties of soil (Arpitha and Dakshayini, 2024), besides improving the efficiency of applied farmyard manure. They also confirmed that the potential of jeevamrutha is to supply materials and to act as food support for beneficial microbes.

## 2. Material and Methods

A laboratory experiment was conducted at Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udipi.

Three desi cow breeds (Malnad Gidda, Gir and Sahiwal) and three cross cow breeds (Holstein Friesian (HF), Jersey and cross Jersey) were selected for the experiment.

### 2.1 Preparation of Jeevamrutha

All the cow breeds cow dung and cow urine was collected aseptically and separately to prepare Jeevamrutha. A standard procedure was used to prepare Jeevamrutha (Palekar, 2006); 1.25 kg of cow dung, 1.25 lit. of cow urine, 250 g of pulse flour, 250 g of jaggery, one handful of soil and 25 lit. of tap water were used to prepare 25 lit. of jeevamrutha. All the ingredients were mixed in a plastic bucket; the mouth of the bucket was covered with gunny cloth and the bucket was kept in the room temperature for 15 days. Each day the content was mixed thoroughly with a wooden stick and the sample was collected in a sterile polythene bottle to analyse the microbial population.

### 2.2 Microbial analysis

The biological properties such as total microbial population of bacteria, fungi and actinomycetes were analysed (Rama *et al.*, 2015). The method advocated for the enumeration was serial dilution and plate count technique with appropriate medium. Enumeration of microbial population was carried out using Nutrient agar for bacteria, Martin's Rose Bengal Agar (MRBA) for fungi, Actinomycetes selective media for actinomycetes at  $10^6$ ,  $10^4$  and  $10^3$  dilutions respectively and the plates were incubated at  $28 \pm 2$  °C.

### 2.3 Statistical analysis

The data obtained from experimentation were statistically analysed using completely randomized design (CRD). The statistical analysis was done by using WASP: 2.0 (Web Agri. Stat Package 2) statistical tool ([www.icargoa.res.in/wasp2/index.php](http://www.icargoa.res.in/wasp2/index.php)) and mean

were separated by Duncan Multiple Range Test (DMRT).

### 3. Results and Discussion

The total microbial population *viz.*, bacteria, fungi and actinomycetes, were significantly influenced by different storage days (1<sup>st</sup> day after preparation to 15<sup>th</sup> days after preparation). The pronounced increase in microbial population during ageing is clearly evident from Table 1, 2 and 3.

The higher microbial population were noticed in desi cow breeds jeevamrutha compared to cross cow breeds jeevamrutha. In desi cow breeds, maximum bacterial population were noticed in Malnad Gidda breed jeevamrutha ( $90.33 \times 10^6$ / ml of jeevamrutha) and the next best was Gir breed jeevamrutha ( $79.33 \times 10^6$ / ml of jeevamrutha). Among cross cow breeds, higher bacterial

population were recorded in HF cow breed jeevamrutha ( $20.66 \times 10^6$ / ml of jeevamrutha), at 7<sup>th</sup> DAP of jeevamrutha. The population was gradually increased in the middle of storage (1<sup>st</sup> DAP to 7<sup>th</sup> DAP) and further decreased gradually (8<sup>th</sup> DAP to 15<sup>th</sup> DAP) in jeevamrutha, similar trend was observed in fungal population (Table 1 and 2).

Actinomycetes population was maximum on 9<sup>th</sup> DAP of jeevamrutha in all the desi breeds, however jeevamrutha prepared with Malnad Gidda ( $20.33 \times 10^3$ / ml of jeevamrutha) recorded the highest population of Actinomycetes compared to all other desi breeds of jeevamrutha at 9<sup>th</sup> DAP (Table 3). Radha and Rao, (2014) also reported the slow growth of actinomycetes in freshly prepared fermented liquid organic formulation compared to bacteria and fungi population. Devakumar *et al.* (2014) observed

Table 1: Bacterial population of Jeevamrutha prepared from dung and urine of different cow breeds

| Days After Preparation | Bacterial population in Jeevamrutha (CFU $\times 10^6$ per ml of Jeevamrutha) |                     |                     |                    |                     |                    |
|------------------------|---|---------------------|---------------------|--------------------|---------------------|--------------------|
|                        | Malnad Gidda  | Gir                 | Sahiwal             | HF                 | Jersey              | Cross jersey       |
| 1                      | 40.33 <sup>h</sup>  | 35.66 <sup>i</sup>  | 32.00 <sup>jk</sup> | 10.33 <sup>h</sup> | 9.66 <sup>i</sup>   | 7.66 <sup>h</sup>  |
| 2                      | 48.33 <sup>g</sup>  | 38.00 <sup>i</sup>  | 33.00 <sup>ij</sup> | 12.66 <sup>g</sup> | 11.33 <sup>h</sup>  | 8.66 <sup>g</sup>  |
| 3                      | 57.33 <sup>f</sup>  | 48.66 <sup>h</sup>  | 35.66 <sup>hi</sup> | 13.66 <sup>f</sup> | 12.66 <sup>ef</sup> | 9.66 <sup>e</sup>  |
| 4                      | 61.66 <sup>e</sup>  | 56.33 <sup>g</sup>  | 38.33 <sup>h</sup>  | 14.66 <sup>e</sup> | 13.66 <sup>d</sup>  | 10.33 <sup>d</sup> |
| 5                      | 77.33 <sup>bc</sup>   | 62.33 <sup>ef</sup> | 55.66 <sup>d</sup>  | 16.33 <sup>d</sup> | 15.33 <sup>c</sup>  | 12.33 <sup>c</sup> |
| 6                      | 86.66 <sup>a</sup>  | 72.33 <sup>b</sup>  | 68.00 <sup>b</sup>  | 18.66 <sup>b</sup> | 16.33 <sup>b</sup>  | 14.33 <sup>b</sup> |
| 7                      | 90.33 <sup>a</sup>  | 79.33 <sup>a</sup>  | 77.33 <sup>a</sup>  | 20.66 <sup>a</sup> | 18.33 <sup>a</sup>  | 16.33 <sup>a</sup> |
| 8                      | 81.33 <sup>b</sup>  | 69.33 <sup>bc</sup> | 62.33 <sup>c</sup>  | 18.66 <sup>b</sup> | 15.33 <sup>c</sup>  | 12.33 <sup>c</sup> |
| 9                      | 73.33 <sup>cd</sup>   | 67.33 <sup>cd</sup> | 51.00 <sup>e</sup>  | 17.66 <sup>c</sup> | 13.33 <sup>de</sup> | 10.33 <sup>d</sup> |
| 10                     | 70.00 <sup>d</sup>  | 64.33 <sup>de</sup> | 49.33 <sup>ef</sup> | 15.33 <sup>e</sup> | 12.33 <sup>fg</sup> | 9.33 <sup>ef</sup> |
| 11                     | 65.33 <sup>e</sup>  | 59.66 <sup>fg</sup> | 47.00 <sup>fg</sup> | 13.66 <sup>f</sup> | 11.66 <sup>gh</sup> | 9.00 <sup>fg</sup> |
| 12                     | 51.00 <sup>g</sup>  | 47.00 <sup>h</sup>  | 46.00 <sup>g</sup>  | 12.00 <sup>g</sup> | 11.00 <sup>h</sup>  | 7.66 <sup>h</sup>  |
| 13                     | 49.33 <sup>g</sup>  | 35.00 <sup>i</sup>  | 35.33 <sup>hi</sup> | 10.66 <sup>h</sup> | 9.66 <sup>i</sup>   | 6.33 <sup>i</sup>  |
| 14                     | 29.33 <sup>i</sup>  | 29.66 <sup>j</sup>  | 29.00 <sup>k</sup>  | 10.33 <sup>h</sup> | 9.33 <sup>i</sup>   | 6.00 <sup>ij</sup> |
| 15                     | 19.66 <sup>j</sup>  | 13.00 <sup>k</sup>  | 13.00 <sup>l</sup>  | 10.00 <sup>h</sup> | 9.00 <sup>i</sup>   | 5.66 <sup>j</sup>  |

Note: Means with same superscript, in a column do not differ significantly at  $P < 0.05$  as per Duncan Multiple Range Test (DMRT).

Table 2: Fungi population of Jeevamrutha prepared from cow dung and cow urine of different cow breeds

| Days After Preparation | Fungi population of Jeevamrutha (CFU $\times 10^4$ per ml of Jeevamrutha) |                    |                     |                     |                     |                    |
|------------------------|---|--------------------|---------------------|---------------------|---------------------|--------------------|
|                        | Malnad Gidda  | Gir                | Sahiwal             | HF                  | Jersey              | Cross jersey       |
| 1                      | 10.00 <sup>j</sup>  | 9.66 <sup>h</sup>  | 7.33 <sup>h</sup>   | 5.66 <sup>j</sup>   | 6.00 <sup>h</sup>   | 5.00 <sup>l</sup>  |
| 2                      | 16.33 <sup>i</sup>  | 14.66 <sup>f</sup> | 12.00 <sup>g</sup>  | 6.33 <sup>ij</sup>  | 7.66 <sup>g</sup>   | 6.33 <sup>k</sup>  |
| 3                      | 20.66 <sup>ef</sup>   | 19.66 <sup>d</sup> | 17.66 <sup>e</sup>  | 7.00 <sup>hi</sup>  | 8.00 <sup>g</sup>   | 7.00 <sup>j</sup>  |
| 4                      | 25.66 <sup>c</sup>  | 23.66 <sup>c</sup> | 22.66 <sup>c</sup>  | 8.00 <sup>g</sup>   | 10.00 <sup>f</sup>  | 7.66 <sup>i</sup>  |
| 5                      | 27.66 <sup>b</sup>  | 26.66 <sup>b</sup> | 25.66 <sup>b</sup>  | 9.66 <sup>f</sup>   | 11.33 <sup>e</sup>  | 8.33 <sup>h</sup>  |
| 6                      | 29.33 <sup>a</sup>  | 28.33 <sup>a</sup> | 27.33 <sup>a</sup>  | 10.00 <sup>f</sup>  | 12.00 <sup>e</sup>  | 10.00 <sup>f</sup> |
| 7                      | 30.66 <sup>a</sup>  | 29.33 <sup>a</sup> | 26.33 <sup>ab</sup> | 15.33 <sup>b</sup>  | 15.66 <sup>c</sup>  | 13.33 <sup>c</sup> |
| 8                      | 25.66 <sup>c</sup>  | 24.66 <sup>c</sup> | 22.33 <sup>c</sup>  | 17.00 <sup>a</sup>  | 18.00 <sup>b</sup>  | 15.00 <sup>b</sup> |
| 9                      | 23.66 <sup>d</sup>  | 20.00 <sup>d</sup> | 19.66 <sup>d</sup>  | 14.66 <sup>b</sup>  | 18.66 <sup>ab</sup> | 15.66 <sup>a</sup> |
| 10                     | 22.00 <sup>e</sup>  | 18.33 <sup>e</sup> | 17.66 <sup>e</sup>  | 13.00 <sup>c</sup>  | 19.00 <sup>a</sup>  | 13.66 <sup>c</sup> |
| 11                     | 19.00 <sup>gh</sup>   | 18.00 <sup>e</sup> | 17.33 <sup>e</sup>  | 12.66 <sup>cd</sup> | 15.66 <sup>c</sup>  | 12.00 <sup>d</sup> |
| 12                     | 19.33 <sup>fg</sup>   | 15.66 <sup>f</sup> | 15.66 <sup>f</sup>  | 12.00 <sup>d</sup>  | 14.66 <sup>d</sup>  | 11.33 <sup>e</sup> |
| 13                     | 17.66 <sup>hi</sup>   | 12.33 <sup>g</sup> | 11.66 <sup>g</sup>  | 11.00 <sup>e</sup>  | 11.66 <sup>e</sup>  | 9.00 <sup>g</sup>  |
| 14                     | 11.00 <sup>j</sup>  | 7.66 <sup>i</sup>  | 7.33 <sup>h</sup>   | 8.00 <sup>g</sup>   | 10.00 <sup>f</sup>  | 7.66 <sup>i</sup>  |
| 15                     | 9.66 <sup>j</sup>   | 5.33 <sup>j</sup>  | 4.66 <sup>i</sup>   | 7.66 <sup>gh</sup>  | 9.66 <sup>f</sup>   | 5.00 <sup>l</sup>  |

Note: Means with same superscript, in a column do not differ significantly at  $P < 0.05$  as per Duncan Multiple Range Test (DMRT)

Table 3: Actinomycetes population of Jeevamrutha prepared from dung and urine of different cow breeds

| Days After Preparation | Actinomycetes population of Jeevamrutha (CFU $\times 10^3$ per ml of Jeevamrutha) |                     |                    |                    |                    |                    |
|------------------------|---|---------------------|--------------------|--------------------|--------------------|--------------------|
|                        | Malnad Gidda  | Gir                 | Sahiwal            | HF                 | Jersey             | Cross jersey       |
| 1                      | 6.66 <sup>i</sup>   | 4.33 <sup>k</sup>   | 4.00 <sup>i</sup>  | 4.00 <sup>g</sup>  | 4.00 <sup>hi</sup> | 1.00 <sup>k</sup>  |
| 2                      | 8.66 <sup>h</sup>   | 6.33 <sup>j</sup>   | 4.66 <sup>h</sup>  | 5.00 <sup>f</sup>  | 4.66 <sup>fg</sup> | 1.66 <sup>j</sup>  |
| 3                      | 10.33 <sup>g</sup>  | 8.33 <sup>h</sup>   | 5.33 <sup>g</sup>  | 5.66 <sup>e</sup>  | 5.00 <sup>f</sup>  | 5.66 <sup>g</sup>  |
| 4                      | 12.33 <sup>f</sup>  | 11.33 <sup>f</sup>  | 6.66 <sup>de</sup> | 7.33 <sup>d</sup>  | 6.00 <sup>e</sup>  | 6.66 <sup>f</sup>  |
| 5                      | 13.33 <sup>e</sup>  | 12.66 <sup>e</sup>  | 7.00 <sup>d</sup>  | 8.00 <sup>c</sup>  | 7.66 <sup>c</sup>  | 9.00 <sup>e</sup>  |
| 6                      | 14.00 <sup>e</sup>  | 13.66 <sup>d</sup>  | 9.00 <sup>b</sup>  | 9.66 <sup>a</sup>  | 9.33 <sup>ab</sup> | 11.66 <sup>c</sup> |
| 7                      | 16.66 <sup>c</sup>  | 15.33 <sup>c</sup>  | 9.33 <sup>ab</sup> | 10.00 <sup>a</sup> | 9.66 <sup>a</sup>  | 11.33 <sup>c</sup> |
| 8                      | 19.33 <sup>b</sup>  | 18.33 <sup>b</sup>  | 9.66 <sup>a</sup>  | 8.66 <sup>b</sup>  | 9.00 <sup>b</sup>  | 13.66 <sup>b</sup> |
| 9                      | 20.33 <sup>a</sup>  | 19.33 <sup>a</sup>  | 7.66 <sup>c</sup>  | 7.00 <sup>d</sup>  | 7.00 <sup>d</sup>  | 15.00 <sup>a</sup> |
| 10                     | 15.66 <sup>d</sup>  | 13.66 <sup>d</sup>  | 6.33 <sup>ef</sup> | 6.00 <sup>e</sup>  | 6.66 <sup>d</sup>  | 10.00 <sup>d</sup> |
| 11                     | 13.66 <sup>e</sup>  | 13.33 <sup>de</sup> | 6.00 <sup>f</sup>  | 5.66 <sup>e</sup>  | 6.00 <sup>e</sup>  | 6.33 <sup>f</sup>  |
| 12                     | 10.33 <sup>g</sup>  | 10.00 <sup>g</sup>  | 5.00 <sup>gh</sup> | 4.00 <sup>g</sup>  | 4.33 <sup>gh</sup> | 5.33 <sup>g</sup>  |
| 13                     | 8.66 <sup>h</sup>   | 7.33 <sup>i</sup>   | 4.66 <sup>h</sup>  | 2.66 <sup>h</sup>  | 3.66 <sup>ij</sup> | 4.33 <sup>h</sup>  |
| 14                     | 8.33 <sup>h</sup>   | 6.66 <sup>ij</sup>  | 3.00 <sup>j</sup>  | 2.00 <sup>i</sup>  | 3.33 <sup>j</sup>  | 4.66 <sup>h</sup>  |
| 15                     | 5.33 <sup>j</sup>   | 4.33 <sup>k</sup>   | 1.66 <sup>k</sup>  | 1.66 <sup>i</sup>  | 2.00 <sup>k</sup>  | 3.66 <sup>i</sup>  |

Note: Means with same superscript, in a column do not differ significantly at  $P < 0.05$  as per Duncan Multiple Range Test (DMRT)

higher colony forming units of bacteria, actinomycetes, fungi and nitrogen fixers in Jeevamrutha at 7<sup>th</sup> DAP. Babu (2011) reported that uncountable rate of *Bacillus*. The higher microbial population of these liquid organic formulations made them as a potent source to maintain soil fertility and to enhance the nutrient availability by helping in faster decomposition of bulky organic manures (Kumar *et al.*, 2023; Shilpa *et al.*, 2015).

The over-all results reviewed that; the highest microbial population were observed between 7<sup>th</sup> to 9<sup>th</sup> days after preparation of jeevamrutha. Hence, it's considered as a best time for the application of jeevamrutha, out of six cow breeds, jeevamrutha prepared with Malnad Gidda showed maximum microbial population compared to other cow breeds jeevamrutha. These microbes help to improve the plant growth by different mechanisms such as fixing of atmospheric nitrogen, solubilization of unavailable form phosphorus, potassium, zinc, organic matter decomposition *etc.*, and also improve the soil fertility by increase with soil organic carbon.

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