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Shelflife study of jeevamrutha prepared from cow dung and cow urine of different desi breeds

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Abstract

A laboratory experiment was conducted on shelf-life of jeevamrutha at Zonal Agricultural and Horticultural Research Station (ZAHRS), Brahmavar, Udupi. In this study cow dung and cow urine of three different desi cow breeds viz., malnad gidda, gir and sahiwal were collected aseptically and separately to prepare Jeevamrutha. After preparation of jeevamrutha samples were collected daily from 1st day to 15th days and enumerated the general and beneficial microorganisms viz., bacteria, fungi, actinomycetes, phosphate solubilizing microorganisms (PSM), nitrogen fixers, *Pseudomonas*, potassium solubilizing microorganisms (KSM), Zinc solubilizing microorganisms (ZnSM) and *Trichoderma* with their respective media. Among the three desi cow breeds the jeevamrutha prepared from, malnad gidda breed of cow contained the maximum microbial population. In general, the highest microbial population was noticed between 7 to 9 days after preparation (DAP) of jeevamrutha in all the desi breeds. Hence, it's considered as a best time for the application of jeevamrutha to soil to improve the soil microorganisms.

Keywords: jeevamrutha, desi breeds, enumerated, *Pseudomonas* and *Trichoderma*

Introduction

The cost of inorganic fertilizers is increasing enormously to an extent that they are out of reach to small and marginal farmers. Use of inorganic fertilizers and insecticides, the population of beneficial organisms decrease and natural regeneration of nutrition in the soil cease. 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 Zero Budget Natural Farming (ZBNF). For centuries, dung and urine from desi cows have been used in Indian agriculture. 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 HF cows should not be used in ZBNF (Palekar 2006)^[4]. Vanaja *et al.* (2009)^[6] 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 viz., *Azotobacter*, *Acetobacter*, *Azospirillum* (nitrogen supplier), *Pseudomonas* (phosphorus-solubilizer), *Bacillus silicus* (potash-solubilizer) and others. Once jeevamrutha was incorporated to soil, these organisms are well activated and maintains the soil productivity. Manjunatha *et al.* (2009)^[3] 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, 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.

Materials and Methods

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

Three different desi breeds (Malnad Gidda, Gir and Sahiwal) were selected for the experiment.

Preparation of Jeevamrutha

The dung and urine of three desi cow breeds were collected aseptically and separately to prepare Jeevamrutha. A standard procedure was used to prepare Jeevamrutha (Palekar 2006)^[4]; 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.

Microbial analysis

The biological properties such as total microbial population of bacteria, fungi, actinomycetes, phosphate solubilizing microorganisms (PSM), nitrogen fixers, *Pseudomonas*, potassium solubilizing microorganisms (KSM), Zinc solubilizing microorganisms (ZnSM) and *Trichoderma* were analysed. The method adapted 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, Pikovskayas Media for PSM, Walksman No.72 for nitrogen fixers, Kings B media for *Pseudomonas*, Aleksandrow media for KSM, Zinc solubilising selective media for ZnSM and *Trichoderma* selective media for *Trichoderma* at 10^6 , 10^4 , 10^3 , 10^3 , 10^4 , 10^4 , 10^3 , 10^2 and 10^2 dilutions respectively and the plates were incubated at 28 ± 2 °C.

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) and mean were separated by Duncan Multiple Range Test (DMRT).

Results and Discussion

The total microbial population viz., bacteria, fungi, actinomycetes, phosphate solubilizing microorganisms (PSM), nitrogen fixers, *Pseudomonas*, potassium solubilizing microorganisms (KSM), Zinc solubilizing microorganisms (ZnSM) and *Trichoderma* were significantly influenced by different storage days (1st day after preparation to 15th days after preparation). The pronounced increase in microbial population during ageing is clearly evident from Table 1, 2(a) and 2(b).

The highest bacterial population was noticed in malnad gidda breed jeevamrutha (90.33×10^6 / ml of jeevamrutha) and the next best was Gir breed jeevamrutha (79.33×10^6 / ml of jeevamrutha) at 7th DAP of jeevamrutha. The population

gradually increased in the middle of storage (1st DAP to 7th DAP) and further decreased gradually (8th DAP to 15th DAP). Similar trend was observed in fungal population. Actinomycetes population was maximum on 9th DAP of jeevamrutha in all the breeds. However, jeevamrutha prepared with Malnad gidda (20.33×10^3 / ml of jeevamrutha) recorded the highest population of Actinomycetes compared to all other desi breeds of jeevamrutha at 9th DAP (Table 01). Radha and Rao (2014) also reported the slow growth of actinomycetes in freshly prepared fermented liquid organic formulation compared to bacterial and fungi population. Devakumar *et al.*, (2014) observed higher colony forming units of bacteria, actinomycetes, fungi and nitrogen fixers in Jeevamrutham at 7th DAP.

The highest population of PSM was recorded in malnad gidda and gir jeevamrutha at 7th DAP. Jeevamrutha prepared with sahiwal recorded lower population of PSM and out of 15 days, higher population was observed at 9th DAP. The population of Nitrogen fixers were highest at 8th DAP in Jeevamrutha of malnad gidda and sahiwal breeds, whereas, gir breed jeevamrutha showed maximum population at 10th DAP.

Pseudomonas population in different desi breeds jeevamrutha were analysed, the highest population of *Pseudomonas* were observed at 7th DAP of jeevamrutha in all three desi breeds (malnad gidda; 20.66×10^4 / ml of jeevamrutha, gir; 18.33×10^4 / ml of jeevamrutha and Sahiwal; 16.33×10^4 / ml of jeevamrutha), similar trend was observed in KSM population (Malnad gidda; 10.00×10^3 / ml of jeevamrutha, gir; 9.66×10^3 / ml of jeevamrutha and Sahiwal; 9.66×10^3 / ml of jeevamrutha). Zinc solubilizing microorganisms were highest at 8th DAP in malnad gidda jeevamrutha (17.00×10^2 / ml of jeevamrutha) in case of gir breed jeevamrutha it was at 10th DAP (19.00×10^2 / ml of jeevamrutha) and sahiwal breed jeevamrutha at 9th DAP (15.66×10^2 / ml of jeevamrutha). The highest ZnSM population was observed in gir breed jeevamrutha compared to all other desi breeds. Maximum *Trichoderma* population was noticed at 8th DAP of jeevamrutha in all desi breeds (malnad gidda; 20.00×10^3 / ml of jeevamrutha, gir; 18.00×10^3 / ml of jeevamrutha and Sahiwal; 16.00×10^3 / ml of jeevamrutha). Babu (2011)^[1] reported that uncountable rate of *Bacillus* were found in the Jeevamrutha of desi cows, 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.

The over-all results revealed that; the highest microbial population was observed between 7th to 9th days after preparation of jeevamrutha. hence, it's considered as a best time for the application of jeevamrutha. Out of three desi breeds, jeevamrutha prepared with malnad gidda showed maximum microbial population compared to other desi 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 increasing soil organic carbon.

Table 1: Enumeration of general microflora of jeevamrutha prepared from different cow dung and cow urine of desi cow breeds.

Days After Preparation	Bacterial population of Jeevamrutha (CFU=No. X 10 ⁶ per ml of Jeevamrutha)			Fungi population of Jeevamrutha (CFU=No. X 10 ⁴ per ml of Jeevamrutha)			Actinomycetes population of Jeevamrutha (CFU=No. X 10 ³ per ml of Jeevamrutha)		
	Malnad Gidda	GIR	Sahiwal	Malnad Gidda	GIR	Sahiwal	Malnad Gidda	GIR	Sahiwal
1	40.33 ^h	35.66 ⁱ	32.00 ^{jk}	10.00 ^j	9.66 ^h	7.33 ^h	6.66 ⁱ	4.33 ^k	1.00 ^k
2	48.33 ^g	38.00 ⁱ	33.00 ^{ji}	16.33 ⁱ	14.66 ^f	12.00 ^g	8.66 ^h	6.33 ^j	1.66 ^j
3	57.33 ^f	48.66 ^h	35.66 ^{hi}	20.66 ^{ef}	19.66 ^d	17.66 ^e	10.33 ^g	8.33 ^h	5.66 ^g
4	61.66 ^e	56.33 ^g	38.33 ^h	25.66 ^c	23.66 ^c	22.66 ^c	12.33 ^f	11.33 ^f	6.66 ^f
5	77.33 ^{bc}	62.33 ^{ef}	55.66 ^d	27.66 ^b	26.66 ^b	25.66 ^b	13.33 ^e	12.66 ^e	9.00 ^e
6	86.66 ^a	72.33 ^b	68.00 ^b	29.33 ^a	28.33 ^a	27.33 ^a	14.00 ^e	13.66 ^d	11.66 ^c
7	90.33 ^a	79.33 ^a	77.33 ^a	30.66 ^a	29.33 ^a	26.33 ^{ab}	16.66 ^c	15.33 ^c	11.33 ^c
8	81.33 ^b	69.33 ^{bc}	62.33 ^c	25.66 ^c	24.66 ^c	22.33 ^c	19.33 ^b	18.33 ^b	13.66 ^b
9	73.33 ^{cd}	67.33 ^{cd}	51.00 ^e	23.66 ^d	20.00 ^d	19.66 ^d	20.33 ^a	19.33 ^a	15.00 ^a
10	70.00 ^d	64.33 ^{de}	49.33 ^{ef}	22.00 ^e	18.33 ^e	17.66 ^e	15.66 ^d	13.66 ^d	10.00 ^d
11	65.33 ^e	59.66 ^{fg}	47.00 ^{fg}	19.00 ^{gh}	18.00 ^e	17.33 ^e	13.66 ^e	13.33 ^{de}	6.33 ^f
12	51.00 ^g	47.00 ^h	46.00 ^g	19.33 ^{fg}	15.66 ^f	15.66 ^f	10.33 ^g	10.00 ^g	5.33 ^g
13	49.33 ^g	35.00 ⁱ	35.33 ^{hi}	17.66 ^{hi}	12.33 ^g	11.66 ^g	8.66 ^h	7.33 ⁱ	4.33 ^h
14	29.33 ⁱ	29.66 ^j	29.00 ^k	11.00 ^j	7.66 ⁱ	7.33 ^h	8.33 ^h	6.66 ^{ij}	4.66 ^h
15	19.66 ^j	13.00 ^k	13.00 ^l	9.66 ^j	5.33 ^j	4.66 ⁱ	5.33 ^j	4.33 ^k	3.66 ⁱ

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

Table 02(a): Enumeration of beneficial microflora of jeevamrutha prepared from different cow dung and cow urine of desi cow breeds

Days After Preparation	PSM population in Jeevamrutha (CFU=No. X 10 ³ per ml of Jeevamrutha)			Nitrogen fixers population in Jeevamrutha (CFU=No. X 10 ⁴ per ml of Jeevamrutha)			Pseudomonas population in Jeevamrutha (CFU=No. X 10 ⁴ per ml of Jeevamrutha)		
	Malnad Gidda	GIR	Sahiwal	Malnad Gidda	GIR	Sahiwal	Malnad Gidda	GIR	Sahiwal
1	7.00 ^{ij}	5.00 ^j	4.33 ^j	5.00 ^j	5.00 ^k	5.00 ^k	10.33 ^h	9.66 ⁱ	7.66 ^h
2	10.00 ^h	7.33 ^h	8.33 ⁱ	7.33 ⁱ	6.33 ^j	6.33 ^j	12.66 ^g	11.33 ^h	8.66 ^g
3	11.33 ^g	9.66 ^g	9.33 ^h	7.66 ⁱ	7.33 ⁱ	7.33 ⁱ	13.66 ^f	12.66 ^{ef}	9.66 ^e
4	14.33 ^e	10.33 ^g	12.66 ^f	9.33 ^h	8.00 ⁱ	8.33 ^h	14.66 ^e	13.66 ^d	10.33 ^d
5	16.66 ^d	12.33 ^e	13.33 ^{ef}	10.66 ^g	10.00 ^g	10.00 ^g	16.33 ^d	15.33 ^c	12.33 ^c
6	19.66 ^b	15.66 ^{ab}	14.33 ^d	14.33 ^d	11.00 ^f	11.66 ^e	18.66 ^b	16.33 ^b	14.33 ^b
7	20.66 ^a	16.00 ^a	16.33 ^{bc}	18.33 ^{bc}	13.00 ^d	14.33 ^c	20.66 ^a	18.33 ^a	16.33 ^a
8	19.33 ^b	15.33 ^{abc}	17.00 ^{ab}	20.33 ^a	17.66 ^b	19.66 ^a	18.66 ^b	15.33 ^c	12.33 ^c
9	17.66 ^c	15.00 ^{bc}	17.33 ^a	20.00 ^a	19.00 ^a	19.33 ^a	17.66 ^c	13.33 ^{de}	10.33 ^d
10	16.66 ^d	14.66 ^c	15.66 ^c	18.66 ^b	19.33 ^a	17.00 ^b	15.33 ^e	12.33 ^{fg}	9.33 ^{ef}
11	16.33 ^d	13.33 ^d	13.66 ^{de}	17.66 ^c	17.33 ^b	15.00 ^c	13.66 ^f	11.66 ^{gh}	9.00 ^{fg}
12	13.33 ^f	11.33 ^f	13.00 ^{ef}	13.33 ^e	14.33 ^c	14.33 ^c	12.00 ^g	11.00 ^h	7.66 ^h
13	7.33 ⁱ	6.33 ⁱ	12.66 ^f	11.33 ^f	12.00 ^e	13.00 ^d	10.66 ^h	9.66 ⁱ	6.33 ⁱ
14	6.66 ^{ij}	3.33 ^k	11.00 ^g	10.00 ^{gh}	9.33 ^{gh}	11.00 ^{ef}	10.33 ^h	9.33 ⁱ	6.00 ^{ij}
15	6.33 ^j	2.66 ^k	9.66 ^h	9.66 ^h	9.00 ^h	10.33 ^{fg}	10.00 ^h	9.00 ⁱ	5.66 ^j

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

Table 02(b): Enumeration of beneficial microflora of jeevamrutha prepared from different cow dung and cow urine of desi cow breeds

Days After Preparation	KSM population in Jeevamrutha (CFU=No. X 10 ³ per ml of Jeevamrutha)			ZnSM population in Jeevamrutha (CFU=No. X 10 ² per ml of Jeevamrutha)			Trichoderma population in Jeevamrutha (CFU=No. X 10 ³ per ml of Jeevamrutha)		
	Malnad Gidda	GIR	Sahiwal	Malnad Gidda	GIR	Sahiwal	Malnad Gidda	GIR	Sahiwal
1	4.00 ^g	4.00 ^{hi}	4.00 ⁱ	5.66 ^j	6.00 ^h	5.00 ^j	5.66 ⁱ	4.00 ^k	4.00 ^j
2	5.00 ^f	4.66 ^{fg}	4.66 ^h	6.33 ^{ij}	7.66 ^g	6.33 ^k	7.00 ^h	5.33 ^j	5.66 ⁱ
3	5.66 ^e	5.00 ^f	5.33 ^g	7.00 ^{hi}	8.00 ^g	7.00 ^j	8.66 ^g	6.33 ⁱ	6.00 ^j
4	7.33 ^d	6.00 ^e	6.66 ^{de}	8.00 ^g	10.00 ^f	7.66 ⁱ	9.00 ^g	7.66 ^h	7.00 ^h
5	8.00 ^c	7.66 ^c	7.00 ^d	9.66 ^f	11.33 ^e	8.33 ^h	11.00 ^e	9.00 ^g	10.00 ^e
6	9.66 ^a	9.33 ^{ab}	9.00 ^b	10.00 ^f	12.00 ^e	10.00 ^f	13.66 ^d	9.66 ^f	13.00 ^c
7	10.00 ^a	9.66 ^a	9.66 ^a	15.33 ^b	15.66 ^c	13.33 ^c	16.33 ^c	11.33 ^e	15.33 ^a
8	8.66 ^b	9.00 ^b	9.33 ^{ab}	17.00 ^a	18.00 ^b	15.00 ^b	20.00 ^a	18.00 ^a	16.00 ^a
9	7.00 ^d	7.00 ^d	7.66 ^c	14.66 ^b	18.66 ^{ab}	15.66 ^a	18.33 ^b	16.00 ^b	14.33 ^b
10	6.00 ^e	6.66 ^d	6.33 ^{ef}	13.00 ^c	19.00 ^a	13.66 ^c	16.66 ^c	14.00 ^c	13.33 ^c
11	5.66 ^e	6.00 ^e	6.00 ^f	12.66 ^{cd}	15.66 ^c	12.00 ^d	13.00 ^d	12.66 ^d	11.00 ^d
12	4.00 ^g	4.33 ^{gh}	5.00 ^{gh}	12.00 ^d	14.66 ^d	11.33 ^e	11.33 ^e	11.00 ^e	9.66 ^{ef}
13	2.66 ^h	3.66 ^{ij}	4.66 ^h	11.00 ^e	11.66 ^e	9.00 ^g	10.00 ^f	10.66 ^e	9.33 ^{ef}
14	2.00 ⁱ	3.33 ^j	3.00 ^j	8.00 ^g	10.00 ^f	7.66 ⁱ	9.00 ^g	9.66 ^f	9.00 ^f
15	1.66 ⁱ	2.00 ^k	1.66 ^k	7.66 ^{gh}	9.66 ^f	5.00 ^l	8.66 ^g	8.66 ^g	8.00 ^g

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

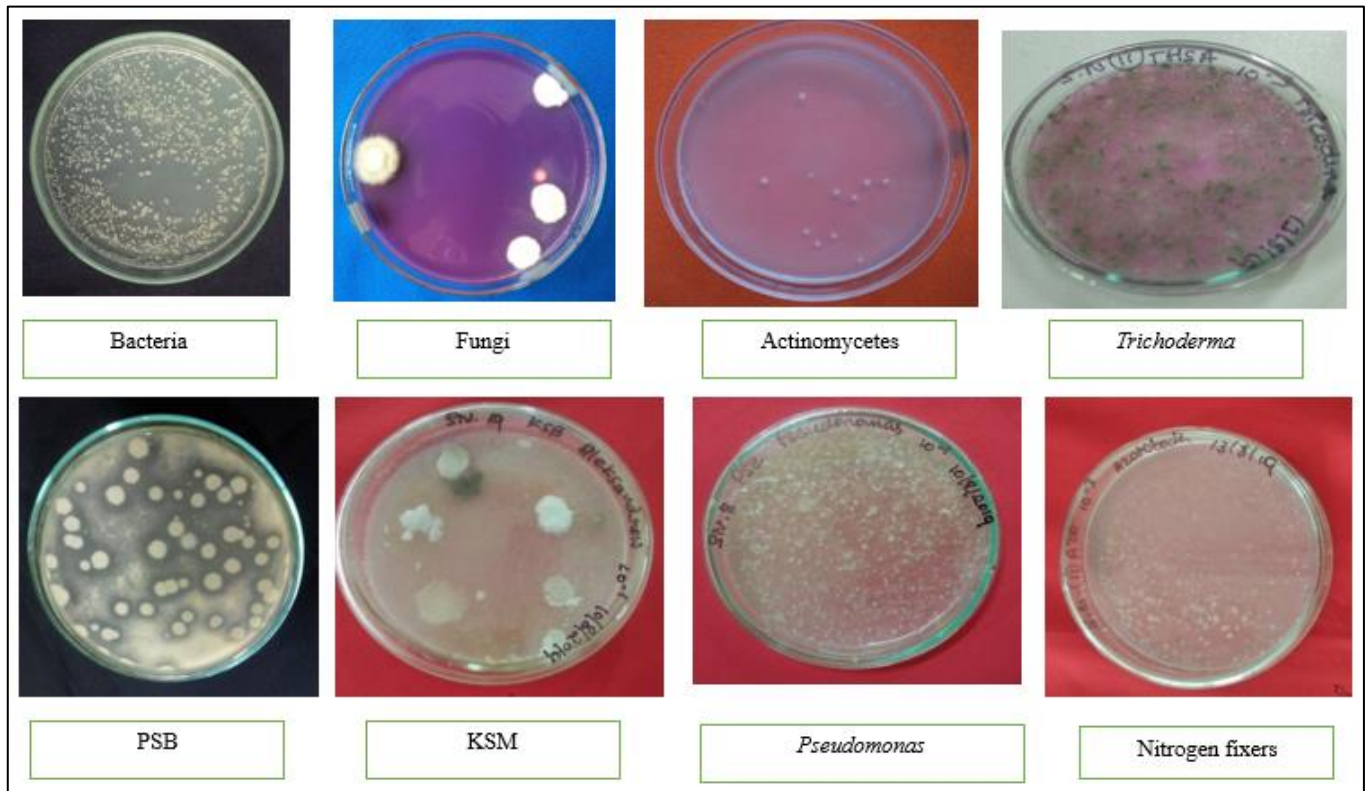


Plate 1: Micro-organisms observed in desi cow breeds Jeevamrutha

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