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Effect of breeds of cow, locations and different days after preparation of Jeevamrutham on its microbial characteristics

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Abstract
An experiment was conducted on Jeevamrutham to study the effect of cow dung collected from different locations and different days after preparation on the microbial population. The changes in physico-chemical and chemical characteristics during the storage period were also analyzed. Ten cows were selected from 9 different districts of Telangana for the preparation of Jeevamrutham**.** Jeevamrutham was prepared and characterized for microbial population (total bacteria, total fungi, actinomycetes) at different days of preparation. The maximum microbial population was recorded in between 8th to 12th day of preparation of Jeevamrutham and among the breeds Gir breed selected from IFS unit, PJTSAU and Jersey breed from Mahbubnagar recorded higher population of bacteria, whereas in case of total fungi, actinomycetes, Jeevamrutham obtained from Jersey and Lal khandhari selected from Adilabad recorded higher population at all the stages as compare to other cows. pH and EC of Jeevamrutham was increased significantly and OC, total N, total P, total K, Total S and total micronutrients (Zn, Cu and Mn) were decreased due the consumption by micro-organisms for their growth and development.

Keywords: Jeevamrutham, Jersey, Lal khandhari, Gir, bacteria, fungi, actinomycetes

Introduction
With the advent of green revolution there has been a substantial increase in production of food grains through the use of improved crop varieties, increased use of high levels of fertilizers and pesticides. The indiscriminate and unbalanced use of these chemicals has led to the multi nutrient deficiencies in soils and crop plants, reduction in natural fertility levels of soils, diminishing return on inputs, pollution with toxic chemicals and destruction of soil biological health. Under these conditions, to improve the soil health and sustain soil productivity, it is important that the conjunctive use of organic manures, biofertilizers and inorganic fertilizers under field conditions and possibility of advocating organic farming assumes importance. Among the organic sources, the use of cow dung gains importance as a main source of organic manure.

India accounts for the largest population of livestock with about 535.78 million that contributes 4.5% of GDP and 25.6% of total agriculture GDP (Source: 20th Livestock Census, 2019). Among the livestock, cow has a prominent place in our country and generates 9–15 kg dung/day (Werner *et al*. 1989) [14]. Among the states, Telangana has a largest cattle population of 90.5 lakhs and generating quite enough cow dung per day.

A number of agricultural products have been developed based on the fermentation of cow dung and this has been resultant in various number of formulation like Jeevamrutham, Beejamrutham, Vermicompost, Vermiwash, Panchagavya, etc. Among this in the present study Jeevamrutham which is considered as essential for crop growth and which acts as most effecting culture for enhancing microbial activity in soil (Sreenivasa *et al.* 2010; Devakumar *et al.*, 2014; Kulkarni and Gargelwar, 2019) [12, 3, 15]. Van Fassen and Van Dijk, (1987) reported that many factors like cattle species, type and age of the animal, nutrient composition of the feed and climate of the region influence the chemical composition of dung and urine. Devakumar *et al.* (2014) [3] and Kulkarni and Gargelwar (2019) [15] also reported the higher microbial population in Jeevamrutham in between 9th and 12th days of preparation. Hence, this experiment was conducted to study the interaction between cow breed and time of storage and their effect on microbial population of Jeevamrutham.

Material and Method

Ten cows were selected from 9 different districts of Telangana for the preparation of Jeevamrutham. Details of collection of samples are given in Table 1.

Table 1: Details of location and breed of cow and type of fodder for the collection of cow dung, cow urine and soil

S. No	Given serial no.	District (locations)	Breed of cows	Fodder fed to them
01	C_1	IFS unit, College farm, PJTSAU, Rajendranagar, Rangareddy	Gir	Hybrid napier grass (fresh as well as dried and crushed)
02	C_2	Dairy farm, PVNRTVU, Rajendranagar, Rangareddy	Sahiwal	Super napier and hybrid napier
03	C_3	Nagarkurnool	Sahiwal	Paddy straw
04	C_4	Nalgonda	Gir	Paddy straw
05	C_5	Mahabubnagar	Jersey	Paddy straw along with napier grass, green gram haulm and concentrate
06	C_6	Adilabad	Lal khandhari	Soybean, redgram, Bengal gram haulm and jowar straw
07	C_7	Nizamabad	Ongle	Paddy straw and super napier grass
08	C_8	Karimnagar	Ongle	Super napier, paddy straw and jowar
09	C_9	Siddipet	Deoni	Paddy straw and fodder jowar
10	C_{10}	Warangal (urban)	Ongle	Paddy straw

Collection of constituents

The cow dung and cow urine sample were collected during early morning hours. The cow dung was collected immediately after removing the surface layer and approximately about 3-5 kg per day. Cow urine was collected after wiping away faecal matter from the vulva, the cows were stimulate to urinate by stroking the side of the vulva for approximately 15-30 seconds and approximately 5L of main stream urine was collected from cow in a clean container and then transferred in to a labeled bottle and stored in refrigerator at 4 ºC until use and further analysis, as per the procedure given by (Rawat *et al.*, 2019) [10]. This process repeated for 3 days, once in the morning at each day to obtain about 20 kg of cow dung and 20 liters of urine for the preparation of Jeevamrutham, a small amount of soil was also collected from the location from where the cow dung and cow urine were collected.

Preparation of Jeevamrutham

A big plastic drum with a capacity of 200 L was taken and 10 kg of cow dung was added. To this 10 L of cow urine was added and it was mixed well. Then 2 kg of jaggery, 2 kg of bengal gram flour and 100 g of ant hill soil was added and finally all the ingredients were mixed well with water. The volume was then made up to 200 L. The drum was covered with a lid and kept in shade / dry place**.** The mixture was stirred well in a clock wise direction twice or thrice in a day (morning, afternoon & evening) for about 1 minute regularly (Palekar, 2006) [4]. Microbial population was enumerated in Jeevamrutham formulation at the intervals of 1st, 2nd, 3rd, 4th, 8th, 10th, 12th and 15th day after preparation (DAP) of Jeevamrutham i.e. S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9 and S_{10}.

Total microbial population of total bacteria, total fungi and actinomycetes, were analyzed. The method advocated for the enumeration was serial dilution and spread plate count technique with appropriate selective medium. Enumeration of microbial population was carried out using Nutrient agar media for bacteria, Potato Dextrose Agar media for fungi and Starch Casein Agar media for actinomycetes at 10^6 dilution for bacteria and 10^5 dilutions for fungi, actinomycetes, and the plates were incubated at 28 ± 2 ºC.

The pH and EC of Jeevamrutham was read directly using glass electrode pH meter (Elico LI-610) (Rawat *et al.*, 2019) [10]. Organic Carbon content was determined by wet chromic acid digestion method as outlined by Walkley and Black (1934) [7]. Total N by Microkjeldhal method (Piper, 1966) [6]. Total P was determined by Vanado-molybdo phosphroric yellow colour method, Total K was determined by flame photometer, Total S by turbidimetric method using spectrophotometer and Total micronutrients using Atomic Absorption Spectrophotometer after digestion of sample with Diacid.

Results and Discussion

Total bacteria

The data obtained on the effect of cow dung obtained from different locations used in the preparation of Jeevamrutham on bacteria population was analyzed at different days of preparation i.e. 1st, 2nd, 3rd, 4th, 8th, 10th, 12th and 15th and is depicted in Fig 1. There was a significant increase in total bacterial population from S_1 (23.80 × 10^6 CFU/mL) to S_5 (66.00× 10^6 CFU/mL) later decreased up to S_8 (33.70 × 10^6 CFU/mL). The total bacterial population was highest in the Jeevamrutham obtained from C_1 (86.20 × 10^6 CFU/mL) and it was significantly higher and followed by C_5 (73.10× 10^6 CFU/mL) while the lowest bacteria population was recorded in C_{10} (9.00 × 10^6 CFU/mL). The Jeevamrutham obtained from C_2 (46.90× 10^6 CFU/mL), C_7 (45.60× 10^6 CFU/mL) and C_3 (43.90 × 10^6 CFU/mL) were on par with each other and followed C_1 and C_5 in recording higher bacterial population. At S_5 interval, the Jeevamrutham obtained from C_4 (111× 10^6 CFU/mL), C_6 (31.50× 10^6 CFU/mL), C_9 (42.5× 10^6 CFU/mL) and C_{10} (28.50 × 10^6 CFU/mL) recorded significantly higher bacterial population and there was a significant different between the bacterial population in Jeevamrutham obtained from different cows at this stage. At S_6 interval, the Jeevamrutham obtained from C_5 (107× 10^6 CFU/mL) was significantly higher in bacterial population and this was followed by C_7 (71 × 10^6 CFU/mL), C_2 (70 × 10^6 CFU/mL) which were on par with each other in recording higher bacteria population at this stage. At S_7 interval, the Jeevamrutham obtained from C_1 (117× 10^6 CFU/mL) was significantly higher in bacterial population followed by C_3 (75 × 10^6 CFU/mL).

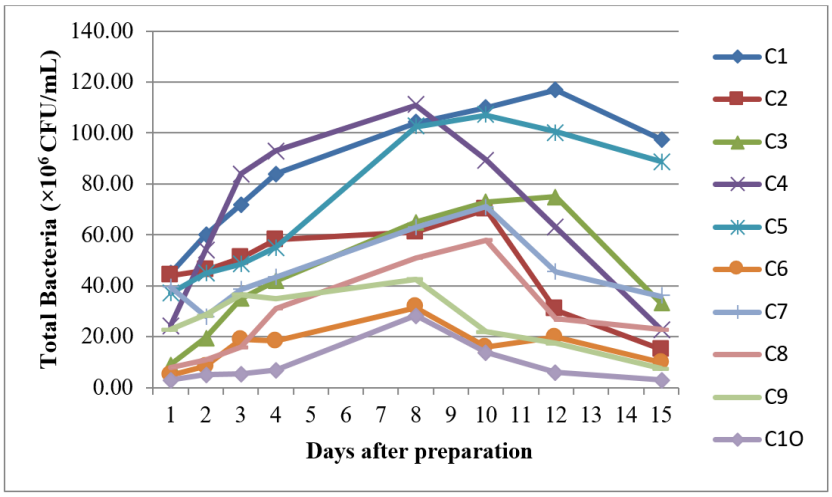

Fig 1: Effect of cow dung obtained from different locations and days of preparation of Jeevamrutham on bacterial population (× 10^6 CFU mL^{-1})

Total fungi

The data obtained on the changes in total fungi population as influenced by time intervals and cow species is graphically presented in Fig 3.There was a significant increase in total fungal population from S_1 (2.60 × 10^5 CFU/mL) to S_7 (35.50× 10^5 CFU/mL) and decreased on S_8 (16.10 × 10^5 CFU/mL). The total fungal population was highest in the Jeevamrutham obtained from C_5 (61.30 × 10^5 CFU/mL) and it was significantly higher and was followed by C_6 (30.90 × 10^5 CFU/mL) while the lowest fungal population was recorded in C9 (5.30 × 10^5 CFU/mL). The Jeevamrutham obtained from C_1 (22.50× 10^5 CFU/mL) and C_2 (20.50 × 10^5 CFU/mL) were on par with each other in recording higher fungal population and followed C_5 and C_6. These were followed by C_3 (12× 10^5 CFU/mL) and C_7 (11.10× 10^5 CFU/mL) which were on par with each other. The Jeevamrutham obtained from C_{10} (5.80 × 10^5 CFU/mL), C_4 (5.50 × 10^5 CFU/mL) and C_9 were on par with each other in recording the lower fungal population followed C_3 and C_7. At S_5 interval, the Jeevamrutham obtained from C_6 (93 × 10^5 CFU/mL) was significantly higher in fungal population and it was followed by C_{10} (11.50 × 10^5 CFU/mL). At S_6 interval, the Jeevamrutham obtained from C_1 (61.50 × 10^5 CFU/mL) recorded significantly higher fungal population followed by C_3 (31 × 10^5 CFU/mL). The population of fungi in these cows decreased from S_6 days onwards, reaching lowest at S_8. At S_7 interval the Jeevamrutham obtained from C_5 (115× 10^5 CFU/mL) was significantly higher in fungal population and this was followed by C_2 (90.50 × 10^5 CFU/mL) and C_7 (28 × 10^5 CFU/mL) in recording higher fungal population. These were followed by C_8, C_4, and C_9 which were on par with each other. In all the cows the fungal population decreased to the lower level at S_8.

Actinomycetes

The data obtained on the changes in total actinomycetes population as influenced by time intervals and cow species is graphically presented in Fig 3. There was a significant increase in total actinomycetes population from S_1 (8.00 × 10^5 CFU/mL) to S_7 (41.70 × 10^5 CFU/mL) and decreased on S_8 (32.75 × 10^5 CFU/mL). However the actinomycetes population at S_5 (40.45× 10^5 CFU/mL), S_6 (40.70 × 10^5 CFU/mL) and S_7 were on par with each other. The total actinomycetes population was highest in the Jeevamrutham obtained from C_6 (65 × 10^5 CFU/mL) and it was followed by C_5 (64.90 × 10^5 CFU/mL) which are on par with each other in recording higher actinomycetes population while the lowest bacteria population was recorded in C_3 (2.40 × 10^5 CFU/mL). The Jeevamrutham obtained from C_1 (27.60× 10^5 CFU/mL) and C_7 (26.70 × 10^5 CFU/mL) were on par with each other and was followed by C_9 (18.30 × 10^5 CFU/mL) and C_2 (16.50 × 10^5 CFU/mL) which were on par with each other in recording the lower actinomycetes population. At S_4 interval, the Jeevamrutham obtained from C_1 (79 × 10^5 CFU/mL) recorded significantly higher in actinomycetes population followed by C_{10} (13.50 × 10^5 CFU/mL). At S_5 interval, the Jeevamrutham obtained from C_7 (78.00 × 10^5 CFU/mL) and C_8 (68.50× 10^5 CFU/mL) were on par with each other in recording the higher actinomycetes population. At S_6 interval the Jeevamrutham obtained from C9 (31.50× 10^5 CFU/mL) was significantly higher in actinomycetes population and this was followed by C_3 (5.50 × 10^5 CFU/mL). At S_7 interval, the Jeevamrutham obtained from C_5 (140 × 10^5 CFU/mL) and C_6 (134 × 10^5 CFU/mL) were on par with each other in recording the higher actinomycets population followed by C_2 (35.50 × 10^5 CFU/mL) and in all the cows the actinomycetes population decreased to the lower level at S_8.

Thus, the result obtained on the bacteria population indicates that the Jeevamrutham obtained from C1 (Gir breed from IFS unit, College farm, PJTSAU) and C_5 (Jersey from Mahabubnagar) recorded higher population of bacteria, whereas in case of total fungi and actinomycetes, Jeevamrutham obtained from C_5 and C_6 (Lal Khadhari from Adilabad) recorded higher population at all the stages as compare to other cows. The maximum microbial population was recorded in between 8th to 12th day of preparation of Jeevamrutham. Devakumar *et al.* (2014) [3] and Kulkarni and Gargelwar (2019) [15] also reported the higher microbial population in Jeevamrutham in between 9th and 12th days of preparation.

The Jeevamrutham obtained from jersey breed recorded higher microbial load which could be attributed to the feed and fodder given to them along with the concentrate whereas the lower values of microbial and manurial properties could be attributed in other cow breeds due to the poor quality of fodder given to them.

Paired t-test was used to compare the means of the physico-chemical and chemical parameter of Jeevamrutham obtained on 15th day of preparation (15th DAP) with the fresh Jeevamrutham (obtained on 1st DAP) and given in Table 2. Freshly prepared Jeevamrutham found to be highly acidic with the pH of 3.91 and there was an increasing trend in pH

due to storage. The EC of the Jeevamrutham at the final stage indicated a significant change and the initial EC of 0.78 dSm^{-1} was observed for the fresh preparation of Jeevamrutham. The organic carbon content of Jeevamrutham decreased significantly with ageing from 0.76% to 0.38%. The decrease in the organic carbon content could be attributed to the utilization of carbon as a source of energy for microorganisms, thus causing a reduction in organic carbon content Rameeza and Usha (2016) [9], Reddy *et al.* (2021) [11] and Chakraborty and Sarkar (2019) [2]. The significant decrease in N content in Jeevamrutham with storage could be attributed to the volatilization loss during fermentation and subsequent biochemical changes. The decrease in total P, K and S content during the storage also has been reported by Pillei (2012), Radha and Rao (2014) [8]. This could be due to the utilization of phosphorus for the body building activities of microorganisms during the storage period. There was a significant reduction in Zn and Mn during the storage in Jeevamrutham whereas in regard of total Cu, difference was not significant though there was a decrease in copper content.

Table 2: Comparison of physico-chemical and chemical characteristics of fresh Jeevamrutham and Jeevamrutham obtained on 15th DAP (mean values)

Parameter	Jeevamrutham (1st DAP)		Jeevamrutham (15th DAP)		Mean difference	t value	p value
	Mean	SD	Mean	SD			
Ph	3.91	0.73	4.86	0.80	-0.95	-5.86	(0.0002)**
EC (dSm^{-1})	0.78	0.52	1.76	0.66	-0.98	-7.42	(0.0000)**
OC %	0.76	0.18	0.38	0.17	0.37	6.54	(0.0001)**
TN (%)	0.08	0.02	0.06	0.02	0.02	3.62	(0.0056)**
TP (%)	0.06	0.02	0.04	0.02	0.01	2.42	(0.0386)*
TK (%)	0.07	0.02	0.06	0.02	0.01	2.76	(0.0220)*
TS (ppm)	361.12	219.20	250.41	175.65	110.71	4.44	(0.0016)**
TZn (ppm)	16.08	8.97	10.70	9.78	5.38	3.11	(0.0125)*
TCu (ppm)	3.26	4.24	1.89	2.63	1.37	2.26	(0.0504)
TMn (ppm)	2.92	3.65	0.76	1.41	2.16	2.67	(0.0255)*

Not statistically (ns) significant (≥p 0.05); * statistically significant (p<0.05); **statistical significance (p<0.01)

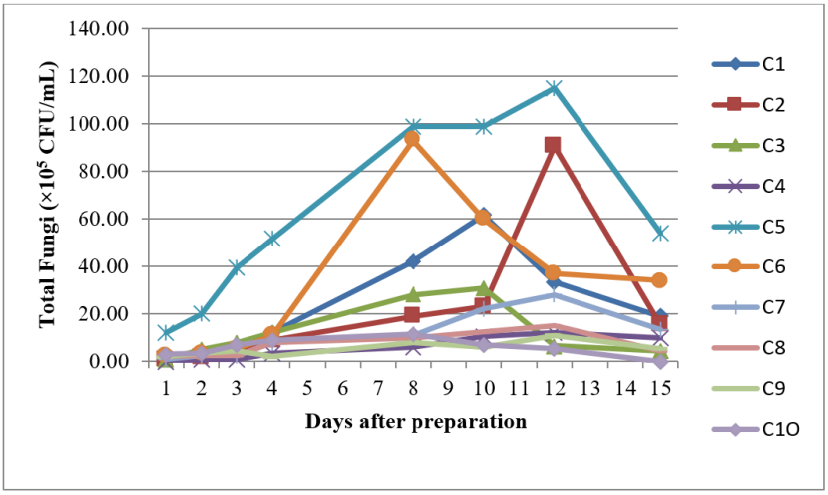

Fig 2: Effect of cow dung obtained from different locations and days of preparation of Jeevamrutham on total fungi population (× 10^5 CFU mL^{-1})

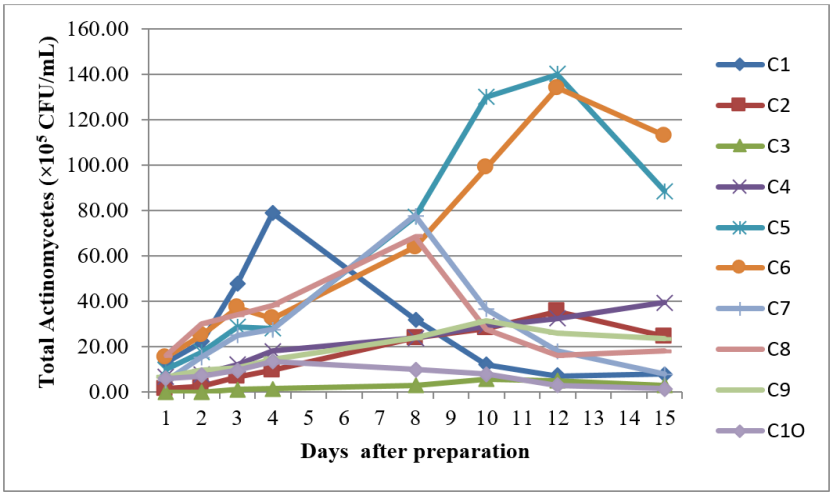

Fig 3: Effect of cow dung obtained from different locations and days of preparation of Jeevamrutham on actinomycetes population (× 10^5 CFU mL^{-1})

Conclusions

Overall, the results of the study showed that the Jeevamrutham obtained (in between 8th to 12th days of preparation) from Jersey breed could be utilized for field application. These results are based on the significantly higher microbial population and higher contents of nutrient compare to other breed. The Jeevamrutham obtained from jersey breed recorded higher properties which could be attributed to the feed and fodder given to them along with the concentrate whereas the lower values of microbial and manurial properties in Ongle, Sahiwal, Deoni breeds could be attributed due to the poor quality of fodder given to them. The Jeevamrutham could also be used to supplement the nutrient requirements of crop grown in Telangana state particularly during the time of drought. Since the split application of nitrogen may not be possible in dry spell and study on the quantity of Jeevamrutham to be applied as foliar spray/soil application needs to be standardized.

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Conflict of interest

The authors declare that there is no conflict of interest.

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