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Microbial Characterization of Jeevamrutha and its Effect on Beneficial Soil Microorganisms

Vibha G.[1], Lingaraju H. G.[1*] and Shankramma Kalikeri[2]

[1]Department of Environmental Sciences, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagara, Mysuru 570015, Karnataka, India

[2]Division Nanoscience and Technology, School of Life Sciences, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagara, Mysuru 570 015, Karnataka, India

Corresponding Author

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Abstract: The excessive use of chemicals, fertilizers, pesticides, and weedicides in agriculture has generated issues for all life on earth, even if technology-induced farming is necessary to support the world's expanding population. As a result, strategies that are more ecologically conscious have emerged, like sustainable intensification and agroecology. Jeevamrutha is a type of organic liquid formulation, which is made with readily available ingredients like water, jaggery, pulse flour, and farm wastes like cattle dung and urine. When applied to soil, it creates a friendly microorganism environment, which supports the availability of essential nutrients for plant growth. The objectives of this experiment aimed at characterization of Jeevamrutha and the effect of Jeevamrutha on beneficial soil microorganisms. The experiment was conducted to see how storage period of Jeevamrutha affected the microorganisms present in it for a period of 14 days. The effect of application of Jeevamrutha in varying concentrations on the population of beneficial microorganisms in soil was observed. Five treatments with different combinations were laid out for the experiment. The results obtained from the experiment revealed that Jeevamrutha was effective for usage between 8th day to 10th day after preparation (DAP). Application of Jeevamrutha at 100% concentration recorded significantly higher microorganisms when compared to other treatments but such a high concentration could be toxic to the crops. As a result, Jeevamrutha at 50% and 75% concentration proved to be more effective in terms of growth and yield when applied to the crops.

Keywords: Jeevamrutha, Characterization, Nitrogen fixers, Phosphate solubilizing bacteria

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Introduction

With the Green revolution introduced in late sixties for increasing food production in the country, plant nutrition-responsive crop hybrids with greater potential in crop yields, application of synthetic agrochemicals, farm mechanization and improved cultural practices were adopted by farmers (Krishnaprabu, 2019). Countries like India are considering their options for switching to conventional agriculture due to the combined effects of satisfying food demand on a small scale and producing toxin-free agricultural products (Hans and Rao, 2018). The excessive use of chemicals, fertilizers, pesticides, and weedicides in agriculture has generated issues for all life on earth, even if technology-induced farming is necessary to support the world's expanding population (Singh, 2021). As a result, strategies that are more ecologically conscious have emerged, like sustainable intensification and agroecology. Such solutions have been promoted as alternative agricultural production approaches that are more closely aligned with the UN Sustainable Development Goals (SDGs) (Rockström *et al.,* 2017; Wezel *et al.,* 2020).

The simultaneous application of organic manures, biofertilizers, and inorganic fertilizers under field settings is crucial under these circumstances to improve the soil health and maintain soil production, and the possibility of promoting organic farming gains relevance (Naveena *et al.,* 2019). Among the organic sources, the use of cow dung gains importance as a main source of organic input and the use of cow dung is becoming more popular as a primary source of organic manure. (Naik *et al.,* 2022).

The practice of Zero Budget Natural Farming (ZBNF) has recently gained popularity in India (Shankaranna, 2018). Using the ZBNF approach instead of costly fertilizers and pesticides will lower input costs while also preserving soil health and water supplies (Behera, 2022). Crop growth and yield are improved when liquid organic manures like Jeevamrutha are used, and the physical, chemical, and biological qualities of the soil are also enhanced (Gangadhar *et al.,* 2020). They are abundant in growth-promoting substances including IAA and GA, as well as beneficial microflora, macronutrients, vital micronutrients, numerous vitamins, and important amino acids (Nitin and Purohit, 2021). It is a significant organic liquid formulation that, when added to soil, encourages the development of helpful bacteria and creates an environment that is favorable for microorganisms, making it possible for essential nutrients for plant growth, like nitrogen, phosphorus, and potassium, to be available (Chaithra and Sujith, 2021). Given this, the current objectives were created to discover if Jeevamrutha application may enhance the soil's beneficial microflora and to examine the effect of storage duration on the microbial population of Jeevamrutha.

Materials and Methods

Preparation of Jeevamrutha:

A laboratory experiment was conducted at Department of Environmental Science on the campus of Jagadguru Sri Shivarathreeshwara Academy of Higher Education and Research in Mysore district, Karnataka, India. The dung and urine of desi cow were collected to prepare Jeevamrutha. A standard procedure was used to prepare Jeevamrutha (Singh and Lal, 2019). 125 g of cow dung, 50 ml of cow urine, 25 g of pulse flour, 25 g of jaggery, one handful of soil and 1.5 liters of tap water were used to prepare 1.5 liters of Jeevamrutha. All the ingredients were mixed in a plastic container; the mouth of the container was covered with a wet jute bag and was kept in the shade for seven days. Each day the content was mixed thoroughly in a clockwise direction with a wooden stick (Sutar *et al.,* 2018).

Characterization of Jeevamrutha:

Samples were gathered in sterile polythene vials after Jeevamrutha preparation in order to analyze the microbial population. Microorganisms were isolated from Jeevamrutha using samples taken every two days. The biological properties such as total microbial population of actinomycetes, phosphate solubilizing bacteria (PSB) and nitrogen fixers were analyzed. Serial dilution and plate count technique with appropriate medium were used for enumeration. Enumeration of microbial population was carried out using nutrient agar for colony forming bacteria, Zapak Media for PSB, Walksman No.77 for nitrogen fixers and actinomycetes at 10^{-4} dilution and the plates were incubated at 28 ± 2 ºC (Gowda *et al.,* 2021; Naik *et al.,* 2022).

Influence of Jeevamrutha on beneficial soil microorganisms:

An experiment was conducted in the form of pot culture in the naturally ventilated garden of the Department of Environmental Science on the campus of Jagadguru Sri Shivarathreeshwara Academy of Higher Education and Research in Mysore district, Karnataka, India. Before transplanting tomato seedlings, the pots were filled with media in a 2:1:1 ratio of soil, vermicompost, and coco peat per pot, and this media was evenly applied to each pot by mixing. Factorial Randomized Complete Block Design (FRCBD) with three replications and five treatments was the design of choice for this study. Organic manure was used as treatment in different combinations, this included T_1- Control + Vermicompost + Cocopeat, T_2- 25% Jeevamrutha + Vermicompost + Cocopeat, T_3- 50% Jeevamrutha + Vermicompost + Cocopeat, T_4- 75% Jeevamrutha + Vermicompost + Cocopeat, T_5- 100% Jeevamrutha + Vermicompost + Cocopeat. Jeevamrutha was applied as soil application at 15, 30, 45, 60, 75, and 90 DAS (Days After Sowing). Rhizosphere soil was collected at the end of every month on 30th, 60th and 90th DAS. Microbial population in soil was determined by standard serial dilution plate count method. 10 g of soil (treatment wise) was weighed and mixed in 90 ml sterilized water blank to give 10^1 dilutions. Subsequent dilutions up to 10^{-6} for Phosphate Solubilizing Bacteria, nitrogen fixers and 10^{-9} for colony forming bacteria were made by transferring serially 1 ml of each dilution to 9 ml of sterilized water blanks. The population of Phosphate Solubilizing Bacteria (PSB), nitrogen fixers and colony forming bacteria were estimated by standard serial dilution plate count method and by taking 1 ml from selected dilution of 10^{-6}, 10^{-9} and were transferred aseptically to petri dishes and the desired media were added to the respective dilutions. Plating on appropriate media viz., W77 media for Nitrogen fixers and actinomycetes, Nutrient Agar for colony forming bacteria and Zapak media for Phosphate Solubilizing Bacteria was used. The data obtained from experimentation were statistically analyzed by using Analysis of Variance (ANOVA). Critical Difference (CD) values were calculated whenever the 'F' test was found significant at the 5 per cent level.

Results and Discussion

Characterization of Jeevamrutha:

The total microbial population viz., nitrogen fixers, phosphate solubilizing microorganisms (PSM), colony forming bacteria was significantly influenced by different storage days (2nd day after preparation to 14th days after preparation). The pronounced increase in microbial population during ageing is depicted in Table 1.

The highest population of nitrogen fixers was noticed at 8th DAP (62 X 10^4 CFU ml^{-1} of Jeevamrutha) and 10th DAP (65 x 10^4 CFU ml^{-1} of Jeevamrutha). The population gradually increased in the middle of storage (2nd DAP to 8th DAP) and further decreased gradually (10th DAP to 14th DAP). An experiment on microbial analysis of Jeevamrutha recorded the maximum microbial population on the 8th day to 13th day after preparation. A rise in the count of nitrogen fixing bacteria was recorded on 11th day (54 x 10^5 CFU ml^{-}) further resulting in decrease from 12th day up to 20th day (16 x 10^4 CFU ml^{-1}) (Kulkarni and Gargelwar, 2019). The population of colony forming bacteria was maximum on 8th DAP of Jeevamrutha (93 x 10^4 CFU ml^{-1}) and 10th DAP (91 x 10^4 CFU ml^{-1}). The population gradually decreased after 10th DAP of Jeevamrutha. A study on shelf-life period of Jeevamrutha found that the total microbial population viz., bacteria, phosphate solubilizing microorganisms (PSM), nitrogen fixers, etc. were significantly influenced by different storage days (1st day after preparation to 15th days after preparation). The over-all results revealed that the highest microbial population was

Table 1: Characterization of Jeevamrutha

Days After Preparation	Nitrogen fixers population in Jeevamrutha (CFU=No. x 10^4 ml^{-1} of Jeevamrutha)	Colony forming bacteria population in Jeevamrutha (CFU=No. x 10^4 ml^{-1} of Jeevamrutha)	PSB population in Jeevamrutha (CFU=No. x 10^4 per ml^{-1} of Jeevamrutha)
Day 2	21	50	28
Day 4	38	72	40
Day 6	50	81	50
Day 8	62	93	62
Day 10	65	91	67
Day 12	51	80	59
Day 14	45	68	43
±S.E.	5.6352	5.5842	5.2255
CD (P = 0.05)	0.092 Non-Significant	0.483 Non-Significant	0.445 Non-Significant

observed between 7th to 9th days after preparation of Jeevamrutha (Santosha *et al.,* 2021). Jeevamrutha at 10th DAP recorded the highest colonies of Phosphate Solubilizing Bacteria (67 x 10^4 CFU ml^{-1}) and out of 14 days, lowest population was observed at 2nd DAP (28 x 10^4 CFU ml^{-1}). Similarly, a study showed that because of the bio-enhancer Jeevamrutha, bacterial population increased rapidly from an initial value of 1.80 x 10^7 CFU ml^{-1} at 0 day to 324.2 x 10^7 CFU ml^{-1} at 9th day after that it rapidly decreased. The number of p-solubilizing microbes rapidly increased from 0 day (1.20 x 10^6 CFU ml^{-1} to 9th day (5.04 x 10^6 CFU ml^{-1}) (Ram *et al.,* 2018).

Influence of Jeevamrutha on beneficial soil microorganisms:

At 30 DAS, the soil application of Jeevamrutha @ 100% resulted in significantly higher number of nitrogen fixers population in the sample soil (116 x 10^6 CFU g^{-1} at 72 h). The lowest population of nitrogen fixers were found in the control sample (13 x 10^6 CFU g^{-1} at 72 h) (Table 2). Significant increase in the colony forming bacteria were recorded among all the samples i.e., control (72 x 10^9 CFU ml^{-1}), 25% Jeevamrutha (93 x 10^9 CFU g^{-1}), 50% Jeevamrutha (170 x 10^9 CFU g^{-1}), 75% Jeevamrutha (238 x 10^9 CFU g^{-1}) and 100% Jeevamrutha (300 x 10^9 CFU g^{-1}) (Table 3). Significantly higher Phosphate Solubilizing Bacteria was counted @ 100% Jeevamrutha (73 x 10^6 CFU g^{-1} at 24 h, 95 x 10^6 CFU g^{-1} at 48 h and 107 x 10^6 CFU g^{-1} at 72 h) while control had the lowest number of colonies (7 x 10^6 CFU g^{-1} at 24 h, 10 x 10^6 CFU g^{-1} at 48 h and 14 x 10^6 CFU g^{-1} at 72 h) (Table 4). In a similar study, the data on soil microbial counts for bacteria, fungi and actinomycetes harvest of chickpea were statistically significant. At harvest, significantly higher bacterial counts were recorded in combinations with Jeevamrutha (28.49) and was on par with Vermicompost + Jeevamrutha (27.22) and FYM + Jeevamrutha (26.57). Significantly higher actinomycetes counts was recorded in FYM + Jeevamrutha (21.13) and was on par with Vermicompost + Jeevamrutha (19.36) (Tirupati *et al.,* 2019). The increase in microbial counts could be due to cumulative effect of various sources of organic manures in increasing organic carbon content of soil which acted as carbon and energy source for microbes and their quick build up in the soil (Palekar, 2006).

With soil application of Jeevamrutha at 60 DAS, there was a significant difference in population of nitrogen fixers (14 x 10^6 CFU g^{-1}, 42 x 10^6 CFU g^{-1}, 56 x 10^6 CFU g^{-1}, 73 x 10^6 CFU g^{-1}, 107 x 10^6 CFU g^{-1} @ control, 25%, 50%, 75% and 100% Jeevamrutha, respectively) (Table 2). Notable changes in colony forming bacteria were recorded in all Jeevamrutha variations. Highest colonies were recorded @100% Jeevamrutha application (268 x 10^9 CFU g^{-1} at 72 h). Whereas, there was a steady growth with increase in sample variations (Table 3). For Phosphate Solubilizing Bacteria count, there was a significant increase in

Table 2: Population of Nitrogen fixers at 30, 60 and **90 DAS at different treatment variations**

Treatment	**Nitrogen fixers population (CFU=No. x 10^6 g^{-1} @ 30 DAS)**			**Nitrogen fixers population (CFU=No. x 10^6 g^{-1} @ 60 DAS)**			**Nitrogen fixers population (CFU=No. x 10^6 g^{-1} @ 90 DAS)**		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Control	6	10	13	5	8	12	7	14	20
25% Jeevamrutha	25	37	45	22	36	48	21	34	47
50% Jeevamrutha	36	63	70	34	52	67	32	48	64
75% Jeevamrutha	40	57	72	49	62	77	48	60	76
100% Jeevamrutha	82	99	116	70	84	100	72	84	98
±S.E.	12.5236	14.7255	16.9747	11.149	12.7499	14.8034	11.2294	11.8152	13.1909
CD (P = 0.05)	0.08 Significant	0.042 Significant	0.01 Significant	0.002 Significant	0.001 Significant	0.002 Significant	0.04 Significant	0.067 Non-Significant	0.04 Significant

Table 3: Number of colonies forming bacteria in different Jeevamrutha treatments at 30, 60 and 90 DAS

Treatment	**Colony forming bacteria population (CFU=No. x 10^9 g^{-1} @ 30 DAS)**			**Colony forming bacteria population (CFU=No. x 10^9 g^{-1} @ 60 DAS)**			**Colony forming bacteria population (CFU=No. x 10^9 g^{-1} @ 90 DAS)**		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Control	25	50	72	20	30	35	10	22	34
25% Jeevamrutha	67	80	93	63	76	99	66	79	91
50% Jeevamrutha	108	138	170	105	125	169	109	139	171
75% Jeevamrutha	162	200	238	152	180	208	158	185	220
100% Jeevamrutha	210	255	300	180	220	268	180	225	256
±S.E.	32.9281	37.6983	42.9833	28.9983	34.2555	40.8086	30.8182	36.3291	40.8762
CD (P = 0.05)	0.001 Significant	0.02 Significant	0.01 Significant	0.01 Significant	0.25 Non-Significant	0.02 Significant	0.01 Significant	0.018 Significant	0.001 Significant

all the samples, while 100% application of Jeevamrutha recording the highest population (80 x 10^6 CFU g^{-1} at 24 h, 96 x 10^6 CFU g^{-1} at 48 h, 110 x 10^6 CFU g^{-1} at 72 h). Control had the lowest number of Phosphate Solubilizing Bacteria (6 x 10^6 CFU g^{-1} at 24 h, 10 x 10^6 CFU g^{-1} at 48 h and 15 x 10^6 CFU g^{-1} at 72 h) (Table 4). A study conducted on effect of Jeevamrutha on beneficial microorganisms recorded significant difference observed in P-solubilizers due to the application of Jeevamrutha. Higher P-solubilizers were observed with Jeevamrutha (27.90, 31.50 at 60 DAT and 26.68, 30.43 at harvest during kharif and summer, respectively) and lower was recorded in without Jeevamrutha (25.35, 29.72 at 60 DAT and 24.05, 28.37 at harvest during kharif and summer, respectively) (Boraiah *et al.,* 2017) .

At 90 DAS, treatment with 100% Jeevamrutha resulted in maximum nitrogen fixers population (72 x 10^6, 84 x 10^6, 98 x 10^6 CFU g^{-1} at 24, 48 and 72 h) (Table 2), colony forming bacteria population (180 x 10^9 , 225 x 10^9, 256 x 10^9 CFU g^{-1}) (Table 3) as well as Phosphate Solubilizing Bacteria (76 x 10^6, 90 x 10^6, 104 x 10^6 CFU g^{-1}),

Table 4: Phosphate Solubilizing Bacteria count in soil samples for different variations of Jeevamrutha

Treatment	PSB population (CFU=No. x 10^6 g^{-1} @ 30 DAS)			PSB population (CFU=No. x 10^6 g^{-1} @ 60 DAS)			PSB population (CFU=No. x 10^6 CFU g^{-1} @ 90 DAS)		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	7	10	14	6	10	15	5	10	16
25% Jeevamrutha	22	34	42	22	36	50	20	36	52
50% Jeevamrutha	34	51	56	34	47	59	32	46	58
75% Jeevamrutha	41	58	73	51	58	70	50	60	72
100% Jeevamrutha	73	95	107	80	96	110	76	90	104
± S.E.	11.03	14.0449	15.5261	12.7028	14.1124	15.3668	12.3028	13.2272	14.2885
CD (P = 0.05)	0.26 Non-Significant	0.118 Non-Significant	0.02 Significant	0.02 Significant	0.27 Non-Significant	0.01 Significant	0.03 Significant	0.043 Significant	0.002 Significant

respectively whereas, all these microbial population were recorded minimum in control (Table 4). However, application of Jeevamrutha @ 75% recorded second highest microbial parameters after Jeevamrutha @ 100%. Data pertaining to application of biodynamic manures and biofertilizers on soil microbial population of chrysanthemum cv. Thai Chen Queen clearly reflect that soil microbial population were significantly influenced by the various treatments. Treatment combination resulted in maximum bacterial population (290 x 105 , 288 x 105 CFU g^{-1}), actinomycetes population (147 x 105 , 146 x 105 CFU g^{-1}) as well as more N-fixers (74 x 104 , 71 x 104 CFU g^{-1}) and P-solubilizers (163 x 103 ,160 x 103 CFU g^{-1}), respectively. Whereas, all these microbial population were recorded minimum in control (Khudus *et al.,* 2021).

Conclusion

To conclude, Jeevamrutha is a rich source of beneficial micro-organisms such as nitrogen fixers, Phosphate Solubilizing Bacteria and colony forming bacteria. By means of a variety of methods, including the fixation of atmospheric nitrogen and the solubilization of phosphorus in its inaccessible form, these bacteria contribute to better plant growth. Based on the results, Jeevamrutha's potent was maximum between 8th to 10th days after preparation. Although Jeevamrutha @ 100% recorded highest microbial content, it could be toxic to the crops as it did not record any significant difference in the growth and yield parameters. Hence, Jeevamrutha @ 50% and 75% proved to be ideal dosages to the crops. The findings also suggested that Jeevamrutha might be a significant means of beneficial bacteria that could raise soil fertility, increase crop yield, and enhance the quality of products. The use of organic manures like Jeevamrutha can help address a variety of issues facing modern agriculture and will lay the foundation for sustainable production using natural resources.

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