

Production and microbial analysis of Jeevamrutham for Nitrogen fixers and Phosphate solubilizers in the rural area from Maharashtra.

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Abstract: Field experiment was conducted on the presence of beneficial bacteria present in the organic liquid formulation. The main focus was given on the presence of nitrogen fixing bacteria and phosphate solubilizing bacteria. It was found that the colony forming unit CFU/ml is higher in the initial days of preparation, DAP around 8 to 12 days. This indicates that Jeevamrutham could be effectively used in the 8-12th DAP. The breakdown of the organic waste increases the nitrogen and phosphorus content and leads to the increase in the population of nitrogen fixing and phosphate solubilizing bacteria. The physicochemical studies of Jeevamrutham suggests progressive fermentation of the ingredients.

Key words Jeevamrutham, N – fixers, Phosphate- solubilizers bacteria, DAP.

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I. Introduction

Organic liquid fertilizer is the product of fermentation process, constituting efficient living soil microorganisms and improves plant growth, productivity through supply of easily utilizable nutrients. Such fertilizers are cost effective and ecofriendly bioinoculants having great potential to enhance agricultural production in sustainable way. Biofertilizers are grouped into different types based on their function such as nitrogen fixing, phosphate solubilizing and other plant growth promoting biofertilizers by different mechanism (Suthar, H. *et al* 2017).

Excessive use of the chemical fertilizer has disadvantage over the fertility of the soil. Jeevamrutham is the best alternative that we can use in place of chemical fertilizer. Jeevamrutham is completely organic and can be used in the organic farming. Jeevamrutham serves as the rich source of the microorganism that fix nitrogen, solubilize phosphorus, also it is the rich source of carbon, nitrogen, phosphorus, potassium and many micronutrients (Devakumaret al., 2014, Sreenivasa, M.N. *et al.*, 2010).

Jeevamrutham is low cost improvised preparation that enriches the soil with indigenous microorganism required for mineralization from native cow dung, cow urine, horse gram and jaggery. (Gore, N.S. and Sreenivasa M.N., 2011). Jeevamrutham, in acidic soil when applied increases pH and in alkaline soil decreases pH, thus creates favorable condition for availability of maximum nutrients to plants, pH 6.5 to 7.8. This condition increases the crop yield, and cuts down an entire expenses of chemical fertilizer. The plant growth promoting Rhizobacteria, *Bacillus pumillus* and *B. licheniformis* produce high amount of physiologically active Gibberellins (Francisco, J., Gutierrez, M. *et al* 2008).

BIOTECHNOLOGY OF JEEVAMRUTHAM FERMENTATION

Fermentation of organic manure as in Jeevamrutham would help to remove lignin, tannic acid, oil which are present in plant based organic raw material and are harmful to roots and inhibit the growth. However, fermenting the organic raw material might eliminate the problem. For the fermentation of fertilizer comparatively dry materials are mixed with very wet materials to obtain a preferred moisture content at a mass. Aeration is very necessary factor for quality fermentation to take place. (Eric W. Ewerson, 1946).

At the early stage of fermentation, a lot of oxygen is required. The effect of oxygen shortage and excessive carbon dioxide can be very serious. The optimal period of fermentation can be 40 days according to Olivera Neto. According to him, greater availability of nutrients occurs with a longer period of fermentation, because in short period sufficient decomposition of biofertilizer does not occur to release nutrients (de Oliveira Neto HT, *et al* 2017).

Also, bad odor is one of the quality fermentations of fertilizer (Arslan Topal EI, 2016).

The grinded organic materials are inoculated and thoroughly mixed with seeds stock of aerobic soil bacteria accordingly. The method may be practiced as a batch operation but continuous operation using tank fermentation is preferred. The intake of water and nutrients are inhibited due to damage of roots. Hence it is necessary to put organic fertilizer for fermenting.

II. Materials and Methods

Since the fertilizer quality depends on the inputs with different physical and chemical characteristics, we selected following raw materials for quality fermentation.

Materials:

Cow dung, Cow urine, Gram flour, Jaggery, banana peels and soil as a bioinoculant. Cow dung is an integral component of the organic formulation and serves as a source of beneficial microorganisms. Presence of naturally occurring beneficial microorganisms predominantly, bacteria, yeast, actinomycetes, and certain fungi have been reported in cow dung based organic manures (Swain, MR., Ray RC., 2009).

Cow urine, Gomutra is proved to enhance the resistance of plants against wide range of plant pathogens like mycoplasma, viruses, bacteria, fungi, nematodes and insects causing diseases and damages. As both cow dung and cow urine are rich in amino acids, results in increasing the nitrogen percentage in organic fertilizer (Herran J, Toress, RRS, Rojo, Ge. 2008).

Jaggery contains potassium approximately 30 percent of RDI, recommended daily intake which is the quality nutrients for plants, and it is required in large amounts for growth and reproduction in plants and increased drought resistance. It is used to purify the upcoming jeevamrutham fertilizer (<http://www.indiastudychannel.com>).

Gram flour is one of the ingredients in fertilizer preparation because of good level of amino acids. Amino acids improve the beneficial microflora which helps healthier root system that can fight diseases and maintain effectiveness in adverse condition. Chick pea is good source of dietary protein exhibiting good balance of amino acid composition and high bioavailability. Moreover seeds contain a low fat level but are rich in glutathione, some vitamin like thiamine and niacin and minerals viz. calcium, magnesium, zinc, potassium, Ferrous, and phosphorus (Boye et al 2010, Esmat et al 2010).

Banana peels are good source of phosphorus, potassium as macronutrients and calcium manganese and sodium as micronutrients. Peels are most effective when composted to blend with nitrogen rich materials. Also, it was found that banana peels tend to breakdown quickly either in compost or soil (Swain, MR, Ray RC, 2009).

Soil as a bioinoculant-Soil is rich in NPK content, rich in nitrogen fixing bacteria, and is more fertile. Two handfuls of soil taken from the roots of banyan tree (or any other old tree found close to the farm which acts as the inoculum of various beneficial micro-organisms like nitrogen fixing and phosphate solubilizing bacteria. The presence of these naturally occurring bacteria serves a long (Papen, H.A. et al 2002., Sreenivasa et al 2010).

All the above contents are collected and keeping the objective in mind that fermentation of above said contents might enhance the beneficial microflora of jeevamrutham, the laboratory preparation of jeevamrutham and study for the efficient microorganism from it was conducted in Chintamani college of science Pombhurna, Gondwana University Gadchiroli, Dist Chandrapur, Maharashtra. Table 1.

METHOD FOR JEEWAMRUTHAM PREPARATION-

For the preparation of Jeevamrutham traditional method was followed which is given by (Palekar, S. 2006). Some modification was made in order to increase the fertility factor of the Jeevamrutham. Jeevamrutham was prepared by adding 2 liters of water with 250 gm. Indian /desi cow dung and 75 ml cow urine /gomutra, to this liquid formulation add 100 gm of jaggery /gul. and 100gm of gram flour /besan and banana peels. Two handfuls of soil taken from the roots of banyan tree. The above ingredients should be stored in a cool place and away from sunlight. The mixture needs to be stirred couple of times (10 min every time) for 4 days. The ingredients are fermented and Jeevamrutham is prepared for the use. This jeevamrutham can now be used for 2-3 days. The pH value of fermented jeevamrutham was studied. Table- 2.

Table 1: Composition of Jeevamrutham

Ingredients	
Local fresh cow dung	250
Urine	75ml
Gram flour	100gm
Jaggery	100gm
Banana peels	100gm
Soil as bioinoculant	100 gm

Method for isolation of efficient micro-organisms, the laboratory study to isolate efficient microorganism was made by serial dilution and colony plate count method(Cappuccino. J G and Sherman,N.2004).

Samples were drawn daily up to the 20 days for the isolation of microorganisms from jeevamrutham. Samples were studied for nitrogen fixing bacteria and phosphate solubilizing bacteria,inoculating sample on simple Nutrient media,Jensen's medium, andPikovsky'smedium. Colonies are counted and CFU per ml was recorded,(Table 3 and 4). Further the colonies are confirmed for the presence of Bacilli by Gram staining method.

III. Results

The pH value of prepared jeevamrutham is suggestive of progressive fermentation of the ingredients and are as shown in table 2.

Table 2: Studied pH value of jeevamrutham ingredient:

	PH
Jewamrutha	4.92
Local cow urine	8.16
Cow dung	8.08
Pulse flour	6.7

It was noticed that colony forming unit CFU in jeevamrutham were higher at the 8-13th days of preparation,DAP, and also found that most of the colony forming unit are of viz Azotobacterial spp. and Rhizobium spp.on Jensen's medium, Bacillus sp.on nutrient agar, Phosphate solubilizers on Pikovskysmedia. The Gram staining result of colonies on nutrient agar indicated the presence of bacilli.

Table 3 and 4- Colony forming unit of microbial population in Jeevamrutham between 1-20th days of preparation

Table 3:

CFU/ML	Microbial population									
	Days after preparation DAP									
	01	02	03	04	05	06	07	08	09	10
Bacteria 10 ⁵	186	242	244	324	336	468	645	735	768	810
N- Fixing bacteria 10 ⁵	21	16	14	26	21	16	24	30	44	56
P- Solubilizing bacteria 10 ⁵	54	39	28	34	27	36	45	51	58	72

Table 4:

CFU/ML	Microbial population									
	Days after preparation DAP									
	11	12	13	14	15	16	17	18	19	20
Bacteria 10 ⁵	794	679	398	478	514	501	354	319	291	244
N- Fixing bacteria 10 ⁵	54	45	36	42	46	44	38	28	17	16
P- Solubilizing bacteria 10 ⁵	64	68	59	46	36	44	39	34	26	21

IV. Discussion

The studied pH value of prepared Jeevamrutham is suggestive of good potential of organic manure. It was found that Jeevamrutham is the rich source of the microbial population. The maximum microbial population was found on the 8th up to 13th day after preparation (Table 3-4). Jeevamrutham also enhances the growth of nitrogen fixing bacteria in locally available substrates such as farm yard manure, compost and biogas slurry (Devkumaret al 2011).

In our study we found rise in count of nitrogen fixing bacteria on 11th day further resulting in decrease from 12th day up to 20th day. The quality of the jeevamrutham mainly depends upon the breeds of the cow, cow urine milk and type of the green gram. Addition of banana peels resulted in increase of nutritive value of jeevamrutham, also soil as bioinoculant from the roots of banyan tree when added, helped to increase various beneficial microorganisms like Nitrogen fixing and phosphate solubilizing bacteria as recorded by the rise of colony count of Nitrogen fixing bacteria and phosphate solubilizing bacteria.

Significant growth of viable microbes in seven month old Jeevamrutham would prove significance of using it as consortium of microorganism, also reported the uncountable rate of Bacillus sp. Higher microbial population of the liquid formulation made them as potent source to maintain soil fertility and to enhance the nutrient availability in faster decomposition of bulky organic manures. (Babu, S.K. 2011). In the laboratory

studies conducted on jeevamrutham prepared we found phosphate solubilizing bacteria in high titer value. Gram staining done have proved them as *Bacillus* spp. Indigenous formulation based on cow dung fermentation are commonly used in organic farming results obtained from such biodynamic preparation provide a basis for understanding the beneficial effect of biodynamic preparation. (Radha T.K. and Rao D.L.N.2014)

V. Conclusion

Jeevamrutham is the rich source of the beneficial micro-organism such as nitrogen fixing and phosphate solubilizing bacteria. From the study it was found that Jeevamrutham is efficiently used between 8th and 12th days of preparation. Addition of such organic liquid manure would help to improve efficient microbial consortia thereby increasing NPK content and plant growth promoting factors. The use of Jeevamrutham is the best alternative to chemical fertilizer and our bioenhancer could be potent source to improve soil fertility, crop productivity and quality.

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