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Effect of different farming practices on total beneficial microorganisms in direct seeded rice

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Abstract
The present study was conducted to study the biological properties of soil under natural farming system, where only indigenous cow dung based on-farm produced formulations *viz*. jeevamrutha, beejamrutha and ghanajeevamrutha were used in comparison to conventional farming (CF) practices with recommended fertilizer and pesticide doses in direct seeded rice. ZBNF resulted in higher population of beneficial microorganisms like bacteria, fungi, actinobacteria, phosphate solubilizing microorganisms and N-fixers. Highest population of the total bacteria (54.13 x106 CFU/g soil), fungi (25.86 x104 CFU/g soil), actinobacteria (7.13 x103 CFU/g soil) and free-living N-fixers (76.83 x105 CFU/g soil) was observed in the natural farming treatment as given by Shri. Subhash Palekar and was significantly higher compared to all the other treatments. Least soil microbial population *viz*., total bacteria (37.40 $x10^6$ CFU/g soil), fungi (20.13 $x10^4$ CFU/g soil), actinobacteria (2.97 $x10^3$ CFU/g soil) and N-fixers (36.43 $x10^5$ CFU/g soil) was observed in treatment absolute control.

Keywords: Natural farming, organic production system, rhizosphere, direct seeded rice

Introduction

Rice is the world's most important crop and is a staple food for more than half of the world's population and cultivated globally being one of the most important cereal crops worldwide. USDA Global rice production in 2021-22 is forecast at a record 510.8 million tons (milled basis), down 0.9 million tons from the previous forecast. Production forecasts for 2021-22 were lowered for Bangladesh, China, EU, Peru, and Thailand, but raised for Australia, Guyana, South Korea, and Russia. Global rice consumption and residual use in 2021-22 is projected to be a record 510.9 million tons, down almost 0.4 million tons from the previous forecast. The 2021-22 global ending rice stocks forecast was lowered 1.1 million tons to 186.8 million tons, 0.1 million tons below the year-earlier record (Agricultural Market Intelligence Centre, PJTSAU).

Direct seeded rice (DSR) systems have been considered a sustainable strategy for sustainable rice (*Oryza sativa* L.) production and resilience under adverse climatic conditions. Providing essential nutrition for more than 50% of the global population, there has been a significant decline in rice productivity due to climate change (Raj *et al*., 2022) [19]. The DSR option has the advantages of directed seeding in low residue pressure, using the zero-tillage and DSR option has been known to sustain soil systems and increase productivity and profitability (Kumar *et al*., 2021) [12]. One of the ways to further improve rice production is to enhance the associations between rice plants and the microbiome that exists around, on, and inside the plant (Raio and Puopolo, 2021) [18]. A multitude of microorganisms stimulate plant growth and productivity through a variety of mechanisms that include improved nutrient acquisition, altered gene expression, enhanced physiological and biochemical traits, and inhibition of phytopathogens (Abdullah *et al*., 2021) [1]. In current agriculture scenario, due to heavy dependence on chemical fertilizers and application of harmful pesticides on the crops, sustainability of the agriculture systems is distorted, income of farmers stagnated and food security and safety became a daunting challenge. Our all modern agricultural practices widely carry on a very different heavy range of agrochemicals including different types of organic and inorganic fertilizers (Tapke *et al*., 2017) [25]. Farming monocultures, such as rice, wheat and cotton etc., repeated on the same land results in the depletion of top soil, soil productivity, groundwater purity and beneficial microbes. It is finally making the crop plants vulnerable to parasites and pathogens. Environmental pollution by chemical fertilizers and pesticides is posing a serious threat worldwide. Their continuous usage may destroy the beneficial soil micro flora (Shaikh and Gachande, 2015) [22].

Growing inclination towards Zero Budget Natural farming (ZBNF) in India to reduce expenditure on external input and to reduce indebtness of farmers, study was conducted to study the biological properties of soil under natural farming system, where only indigenous cow urine and dung based on-farm produced formulations *viz*. jeevamrutha, beejamrutha and ghanajeevamrutha were used in comparison to conventional farming (CF) practices with recommended fertilizer and pesticide doses in direct seeded rice. Natural farming is “the use of holistic production management systems which promote and enhance agro-ecosystem health, including biodiversity, biological cycles and soil biological activity”. This sustainable way of farming is also known as ‘Do-nothing farming’ or ‘No-tillage farming’. After this a new agriculture technique was introduced among farmers is Zero Budget Natural farming (ZBNF). First it was popularized and gained a huge success in southern India, especially Karnataka where it was firstly practiced (Kumar, 2012) [12].

This method of farming was introduced by Shri Subhash Palekar, for which he was honored with Padma Shri in 2016 (Anonymous, 2016) [4]. Various indigenous formulations such as cow urine and dung based Beejamrit, Jeevamrit and Panchgavya have shown their beneficial effects on soil biological properties (Sreenivasa *et al*., 2009 and Gangadhar *et al*., 2020) [23, 11], and are getting popular among farmers. Beejamrit, ghanjeevamrit and jeevamrit contain a lot of microbial properties and increase the soil micro flora with drastic increase in different soil enzymes.

Material and Methods

Experiments were initiated in cropping system mode at experimental plots of Zonal Agricultural Research Station (ZARS), V. C. Farm, Mandya during *Kharif* 2019 and 2020 with the following treatments:

Treatment details

- T_1: Absolute control
- T_2: Organic production system
- T_3: Natural Farming (Natural farming protocol as given by Shri. Subhash Palekar)
- T_4: Recommended package of practices (UAS, GKVK, Bengaluru)

Direct seeded rice crop variety Thanu (KMP-101) was sown on Kharif 2019 and 2020 at a spacing of 30 cm X 10 cm and gross plot sizes were 9 m X 12 m respectively. The above treatment combinations were replicated five times in RCBD.

In the treatment T_1, only sowing of seeds was done and all other inputs and practices are nil. In the treatment T_2, seed treatment with *Azospirillum* and PSB, FYM applied based on N equivalent (100 kg N ha^{-1}), mulching with paddy straw (4 t ha^{-1}) and weeding at 30 DAS and neem based plant protection measures using organic materials. In the treatment T_3, ghanajeevamrutha application at 1000 kg ha^{-1}, seed treatment with beejamrutha, application of jeevamrutha at 15 days interval at 500 liters ha^{-1} and mulching with paddy straw (4 t ha^{-1}) at 30 DAS. Neem based plant protection measures using preparations like neemastra, agniastra, shuntiastra etc. In treatment T_4, seed treatment with carbendazim, FYM application at 10 t ha^{-1} and NPK (100:50:50 kg ha^{-1}) recommended dose of nitrogen applied as 50% basal + 25% tillering + 25% panicle initiation stage, potassium was applied as 50% basal + 50% tillering and 100% phosphorus applied as a basal dose. Spraying of pre-emergence herbicide (bensulfuron methyl 0.6% + pretilachlor 6% GR @ 10 kg /ha) at 2-3 DAS and post emergence herbicide (bispyribac sodium 10% SC @ 200 ml / ha) at 30 DAS and one hand weeding.

Preparation of Beejamrutha (Palekar, 2016)

Components

- 20 liters of water
- 5 kg desi cow dung
- 5 litres of desi cow urine
- One handful soil from the surface of boundary or field
- Lime 50gms

Add desi cow urine and handful of undisturbed soil from bund to water. Tie the cow dung in a cloth and hang it inside the bucket containing water and cow urine. Let it stand for overnight (for 12 hours). Take one litre water and add 50 gm lime in it, let it soak for a night. The next morning, squeeze the bundle of cow dung and dunk the cow dung in the water three times continuously so that all essence of cow dung will enter the water and add the lime water which was prepared last night. Beejamrutha is ready to treat the seeds. The prepared beejamrutha can be used to treat seeds for upto 1 acre land.

Preparation of Jeevamrutha (Palekar, 2016) [14]

Jeevamrutha was prepared from natural as well as easily found materials such as fresh cow dung, cow urine, jaggery, flour of any kind (such as green gram, Bengal gram, black gram), soil that is free from chemicals, clean water which is free from chlorine and other chemicals under shade and mix well the mixture two times a day until 7 days complete. Mix 10 kg of desi cow dung, 10 litres of desi cow urine, 2 kg of organic jaggery, 2 kg of pulse flour and one handful of soil with 200 litres of water mix well two times a day and applied after 7 days. Jeevamrutha was applied at an interval of 15 days.

Preparation Ghana jeevamrutha (Palekar, 2016) [14]

Ghana jeevamrutha is dry or solid Jeevamrutha. Ghana Jeevamrutha is as effective as jeevamrutha to the soil. The number of microbes in the soil increases. Microbes are the main constituent for increasing soil fertility. Mix 400 kg of desi cow dung with 2 kg of organic jaggery, 2 kg of pulse flour spread uniformly on the ground to form a layer and add required amount of desi cow urine on the layer, mix completely. Now make a heap of the treated cow dung and cover it again using jute bag for 48 hours. Allow it for fermentation. Then spread on the floor, after shade drying is completed, store it in jute bags in the room. Ghana jeevamrutha is stored for six months and it will be applied before sowing in powder form.

Microbial analysis of Beejamrutha, Jeevamrutha and Ghana Jeevamrutha

The cow based natural products like beejamrutha, jeevamrutha and Ghana jeevamrutha were analyzed for total bacteria, fungi, actinomycetes, P solubilizers and free living nitrogen fixers by using pour plate method. The bacteria, P solubilizers, *Pseudomonas* and free living nitrogen fixers were serially diluted and incubated at 28 °C. The actinomycetes were isolated by using Kuster’s agar media, incubated at 28 °C to 30° C and the samples were analyzed for fungi on PDA media with incubation temperature of 26 °C to 28 °C.

Collection of samples and isolation of beneficial microflora

The soil samples collected from experimental plots of ZARS, V.C. Farm Mandya were analyzed for soil microorganisms at initial stage (before sowing) and different stages of crop growth till harvest of the crop. The soil microorganisms were enumerated by following standard serial dilution plate count method at different dilutions. For microbial enumeration 10 gm of freshly collected soil samples were serially diluted in sterile water blanks. The details of dilutions and the culture media used for the enumeration of microbial groups are presented in the table 1.

Table 1: Details of microbial group, dilution and media used for the isolation of microorganisms

Microbial group	Dilution	Media
Total bacteria	10^{-6}	Soil extract agar
Fungi	10^{-4}	Potato dextrose agar and MRBA
Actinomycetes	10^{-3}	Kuster's agar
Phosphate solubilizing microorganisms (PSMs)	10^{-5}	Sperber's agar
Free-living nitrogen fixing bacteria	10^{-5}	Jensen's agar

Results and Discussion

Microbial analysis of Beejamrutha, Jeevamrutha and Ghana jeevamrutha

An experiment was conducted on liquid formulations to study microbial diversity and know the best period of its use in crop production. Higher colony forming units (CFU) were observed on the day of preparation of beejamrutha and in jeevamrutha it was between 7th to 13th days after preparation (DAP). Higher number of bacteria, different fungi and N-fixers clearly indicate that the jeevamrutha is enriched consortia of native soil microorganisms. It was found that beejamrutha would give best result if it is used on the day of preparation and Jeevamrutha between 7th to 13th days after preparation. The microbial studies revealed that higher bacterial population was recorded followed by N-fixers, P-solubilizers, fungi and actinomycetes. Due to the higher beneficial microbial load would mobilise more of plant nutrients and provide plant growth promoting substances and also other micro nutrients required by the plants

Table 2: Microbial load in beejamrutha at different days after preparation

Microbial populations (CFU/ ml)						
Different hours	**Bacteria (10^5)**	**Fungi (10^4)**	**Actinomycetes (10^3)**	**N-fixers (10^4)**	**P-solubilizers (10^4)**	***Pseudomonas* (10^5)**
Initial	330	05	-	110	62	79
2	322	05	-	120	53	75
3	300	05	-	130	54	65
4	290	05	-	160	53	65
6	280	05	-	120	53	60
8	260	04	-	115	51	59

Beejamrutha is an ancient organic formulation commonly used as a seed treatment in organic and natural farming in India. This low-cost input is primarily composed of cow dung, cow urine, and forest soil, which is often supplemented with limestone. In organic agriculture, it is a traditional practice among the farming community to incubate the seeds in beejamrutha prepared overnight before sowing in the field. On the other hand, growing data suggests that cow-based formulations are mostly enriched with microbial sources, including several plant growth-promoting rhizobacteria that are capable of synthesizing plant growth regulators (Sreenivasa *et al*., 2009) [23]. However, the microbiological properties of beejamrutha and their temporal changes over different periods of decomposition are largely unexplored.

In this study, we aim to analyze the microbial load of beejamrutha at different days of preparation. This study further elucidates the microbial niche and their dynamics in Beejamrutha, including the plant-beneficial bacteria. It was observed that the population of plant-beneficial bacteria such as bacteria, fungi, P solubilizers and *Pseudomonas* was highest initially after preparation. The free-living nitrogen fixers proliferate progressively up to 4th day of preparation.

The presence of beneficial microorganisms in these liquid formulations might be mainly due to their constituents such as: cow dung, cow urine, legume flour and jaggery containing both macro and essential micro nutrients, many vitamins, essential amino acids, growth promoting substances like indole acetic acid (IAA), gibberallic acid (GA) and beneficial microorganisms (Palekar, 2016; Sreenivasa *et al*., 2009; Neelima and Sreenivasa, 2011) [14, 13, 23].

Table 3: Microbial load in Jeevamrutha at different days after preparation

Microbial populations (CFU/ ml)						
Days after preparation	**Bacteria (10^5)**	**Fungi (10^4)**	**Actinomycetes (10^3)**	**N-fixers (10^4)**	**P-solubilizers (10^4)**	***Pseudomonas* (10^5)**
1	30	6	8	46	30	10
2	200	13	10	80	44	22
3	48	20	14	70	54	32
4	70	21	16	93	61	100
5	120	24	12	113	55	80
6	180	28	10	122	58	113

7	300	30	8	136	60	146
8	320	28	7	143	63	153
9	200	26	-	149	70	103
10	150	24	1	100	80	80
11	120	20	-	77	62	42
12	103	18	-	80	59	33
13	121	10	-	69	40	26
14	114	6	-	45	26	20

It was found that Jeevamrutha is the rich source of the microbial population. The maximum microbial population was found on the 7th up to 13th day after preparation (Table 3). Jeevamrutha also enhances the growth of nitrogen fixing bacteria in locally available substrates such as farm yard manure, compost and biogas slurry (Devkumar *et al*., 2011) [8]. In our study we found rise in count of nitrogen fixing bacteria on 2nd day further resulting in decrease from 10th day up to 14th day. The quality of the jeevamrutham mainly depends upon the breeds of the cow, cow urine milk and pulse flour. Presence of naturally occurring beneficial microorganisms predominantly, bacteria, yeast, actinomycetes and certain fungi have been reported in cow dung based organic manures (Swain and Ray, 2009[24]). As both cow dung and cow urine are rich in amino acids, results in increasing the nitrogen percentage in organic fertilizer (Herran *et al*., 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. 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 (Boye *et al*., 2010 and Esmat *et al*., 2010) [7, 10].

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, 2011) [5].

In the laboratory studies conducted on jeevamrutham prepared we found phosphate solubilizing bacteria in high titer value. The total bacterial count was increased upto 320 CFU/ml on 7th day and population decreased 14th day onwards. 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 and Rao, 2014) [17]. The higher beneficial microorganisms found in these organic formulations are in confirmity with Papen *et al.,* 2002 and Sreenivasa *et al*., 2009 who have also reported the presence of naturally occurring beneficial microorganisms predominantly bacteria, yeast, actinomycetes and certain fungi in organic liquid manures. This is also in conformity with Devakumar *et al*., (2011) [8] who have reported that both jeevamrutha and panchagavya have enhanced the growth of nitrogen fixers in locally available substrates such as FYM, pressmud, compost and digested biogas slurry.

Table 4: Microbial load in Ghanajeevamurtha

Microorganisms	Total population (CFU/g of sample)
Bacteria (10^5)	183
Fungi (10^4)	20
Actinomycetes (10^3)	56
N-fixers (10^4)	42
P-solubilizers (10^4)	28

Ghana jeevamrutham, the solid form of jeevamrutham was analysed for the total beneficial microflora. Microbes in cow dung supplies the enough nutrients such as nitrogen and carbon and increases the microbial activity in soil. Traditionally, the jeevamrutham was prepared by using cow dung and urine of desi cows, organic jaggery and water whereas ghana jeevamrutham is the solid form jeevamrutham that acts as a natural fertilizer for the crop plants.

The Ghana jeevamrutha sample which was used for experimental studies was analysed for the total beneficial microorganisms. The total bacteria was found to be 183 CFU/g, the fungi 20 CFU/g, actinomycetes 56 CFU/g, N-fixers 42 CFU/g and P solubilizers 28 CFU/g. So the application of Ghana jeevamrutha could supplement the application of biofertilizers and they can be prepared easily by locally available materials by the farmers, in rural areas. From this study it is evident that Ghana jeevamrutha can be applied to the soil before sowing to increase the soil beneficial microflora.

Table 5: Effect of farming practices on total population of bacteria (×10^6CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Treatments	Initial			30 DAS			60 DAS			90 DAS			Harvest		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	12.8	13	12.9	15.4	16.6	16.0	22.6	23.2	22.9	25.8	26.2	26.0	18.8	19.4	19.1
T_2	16.4	17.2	16.8	28.0	30.0	29.0	49.6	52.3	50.9	32.2	33.1	32.6	18.6	20.6	19.6
T_3	15.2	19.5	17.3	25.4	27.5	26.4	36.4	40.5	38.4	29.4	30.2	29.8	17.2	20.4	18.8
T_4	9.8	11.2	10.5	19.0	19.7	19.3	25.8	27.6	26.7	21.0	21.5	21.2	10.4	11.8	11.1
S Em ±	2.17	2.54		2.70	2.73		2.43	2.60		2.40	2.45		1.40	1.50	
CD @5%	NS	NS		8.00	9.15		7.30	7.90		7.10	7.90		4.10	4.70	

Note: T_1: Absolute control T_2: Organic production system T_3: Natural farming (NF protocol as given by Shri. Subhash Palekar) T_4: Recommended package of practices

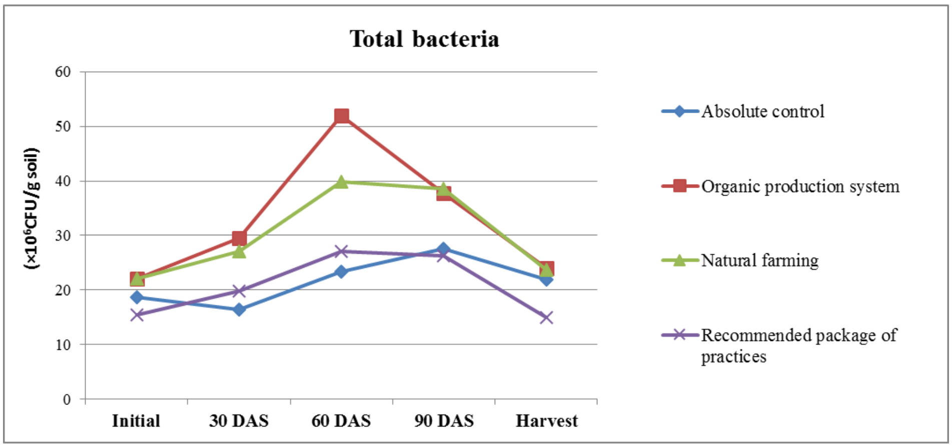

Fig 1: Population of total bacteria (×10^6 CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

The crop production systems and growth stages of crop have influence on microbial groups considered for the study. It is strictly clear that the population of all microbial groups are on par during initial stage and at harvest stages of the crop. The maximum population can be found at active stage of the crop growth i.e., 60 DAS, later it started declining towards the maturity of the crop.

The pooled data (2019-20 and 2020-21) presented in the table 5 indicated that, After 30 DAS, the highest population of total bacteria (29 x10^6 CFU/g soil) was observed in the treatment T_2: Organic production system, followed by T_3: natural farming (NF protocol as given by Shri. Subhash Palekar) and least soil bacterial population (22.9 x10^6 CFU/g soil) was observed in treatment T_1: absolute control (16 x10^6 CFU/g soil). The total bacterial count after 60 DAS was observed to be highest in T_2: Organic production system (50.9 x10^6 CFU/g soil), followed by T_3: natural farming (38.4 x10^6 CFU/g soil). Similarly 90 DAS and at harvest same trend was observed but lowest population of bacteria was observed in treatment T4: Recommened package of practice (11.1 x10^6 CFU/g soil)) due to the toxicity of chemical fertilizers bacterial load was decreased compare to other treatments.

The population of the bacteria at par during initial and at harvest stages of the crop, significantly higher population of bacteria was observed at active stage of the crop growth. Later on their population started declining towards the maturity of the crop. Among the treatments, T_2 (organic production system) treatment has recorded highest bacterial count compared to rest of the treatments, it may be due to the sufficient availability of organic matter content and better nutrient availability for proliferation of microorganisms.

Limited research has been conducted on the effect of cow based formulations on field crops, but it has shown positive results in improved crop yield in rice (Amareswari and Sujathamma, 2014) [3], peanut, pomegranate (Patel *et al*., 2021) [16], and pest management.

Table 6: Effect of farming practices on total population of fungi (×10^{4}CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Treatments	Initial			30 DAS			60 DAS			90 DAS			Harvest		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	8.0	9.0	8.5	13.8	15.5	14.6	17.6	20.0	18.8	18.4	19.1	18.7	10.0	11.0	10.5
T_2	11.2	11.5	11.3	16.4	19.6	18.0	31.2	33.1	32.1	28.2	27.6	27.9	15.4	16.4	15.9
T_3	12.0	14.6	13.3	18.2	20.4	19.3	26.2	32.4	29.3	21.2	20.1	20.6	10.4	12.4	11.4
T_4	7.6	10.3	8.9	15.4	17.5	16.4	27.2	29.7	28.4	19.6	21.3	20.4	11.6	11.4	11.5
S Em ±	1.02	1.23		2.02	2.40		2.32	2.63		1.84			1.54	1.90	
CD @5%	3.06	3.54		NS	NS		7.00	7.94		5.50			NS	NS	

Note: T_1: Absolute control T_2: Organic production system T_3: Natural farming (NF protocol as given by Shri. Subhash Palekar) T_4: Recommended package of practices

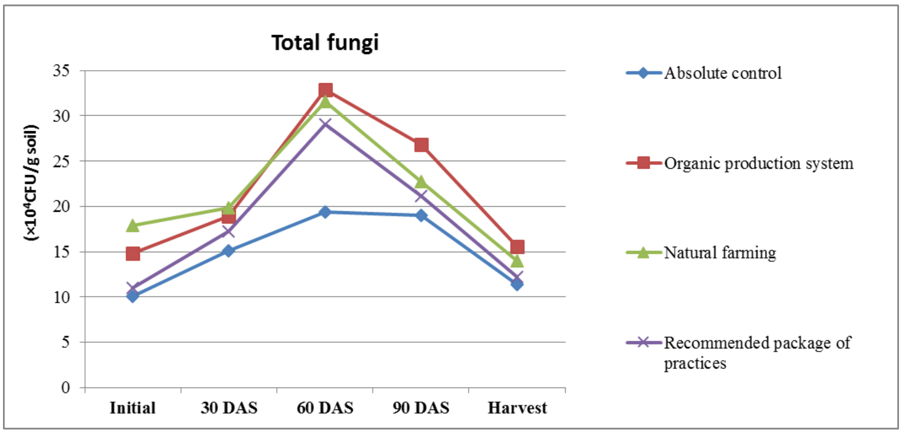

Fig 2: Fungal population (×10^{4}CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Cropping practices have a great potential to improve soil quality through changes in soil biota. Yet the effects of these soil-improving cropping systems on soil fungal communities are not well known. We analyzed soil fungi from different treatments by using serial dilution technique. Fungal communities responded to the cropping practices differently.

The pooled data (2019-20 and 2020-21) presented in the table 6 indicated that, After 30 DAS, the highest population of total fungi (19.3 x10^4 CFU/g soil) was observed in the treatment T_3: natural farming (NF protocol as given by Shri. Subhash Palekar) and least soil fungal population (14.6 x10^4 CFU/g soil) was observed in treatment T_1: absolute control. The total fungal count after 60 DAS was observed to be highest in T_2: Organic production system (32.1 x10^4 CFU/g soil), followed by T_3: natural farming (29.3 x10^4 CFU/g soil). Similarly 90 DAS and at harvest same trend was observed but lowest population of fungi was observed in treatment T_1: Control due to the less availability of nutrients.

Among the treatments, T_3: natural farming (NF protocol as given by Shri. Subhash Palekar) treatment has recorded highest fungal population count compared to all the other treatments. It may be due to the application of fermented jeevamrutha at regular time intervals that contains microorganisms which plays an important role in the conversion of unavailable forms of nutrients to available form in the plant root zone. The microbes present in jeevamrutha helps non-available form to dissolved form when it is inoculated into the soil.

Various authors reported different fungi from cow based organic formulations. For example, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus rapens*, *Aspergillus fumigatus*, Rhizopus stolonifer, *Mucor mucedo*, *Fusarium* sp. and *Vericosporium* spp. were reported in CD (Adegunloye *et al*., 2007) [2]; saprophytic fungi (yeast and molds) such as *Alternaria* sp., *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Geotrichum* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Sporotrichum* sp., *Thamnidum* sp., *Candida* sp.,. *Rhodotorula* sp., *Saccharomyces*, *Sporobolomyces*, *Trichosporon*, and *Torulopsis* sp.

Table 7: Effect of farming practices on total population of actinobacteria (×10^3CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Treatments	Initial			30 DAS			60 DAS			90 DAS			Harvest		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	6.2	5.4	5.8	7.4	8.8	8.1	8.6	9.5	9.0	7.2	10.4	8.8	5.2	6.7	5.9
T_2	8.4	9.8	9.1	9.6	11.6	10.6	13.4	14.2	13.8	15.2	16.4	15.8	7.4	8.5	7.9
T_3	6.4	8.6	7.5	11.4	13.4	12.4	11.4	11.5	11.4	13.4	14.8	14.1	6.2	8.9	7.5
T_4	5.4	6.4	5.9	9.6	12.6	11.1	10.2	14.3	12.2	8.2	9.2	8.7	4.4	5.5	4.9
S Em ±	0.72	0.82		1.13	1.62		1.16	1.60		1.36	1.53		0.87	0.94	
CD @5%	2.15	2.56		NS	NS		NS	NS		4.07	4.63		NS	NS	

Note: T_1: Absolute control T_2: Organic production system T_3: Natural farming (NF protocol as given by Shri. Subhash Palekar) T_4: Recommended package of practices

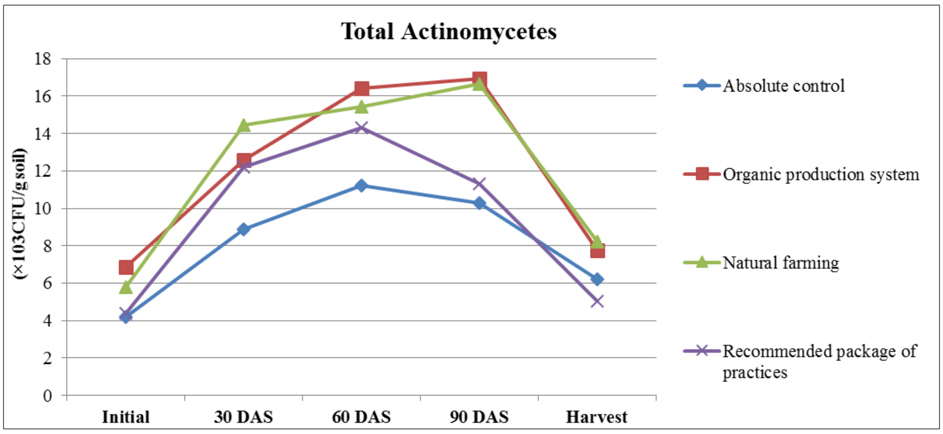

Fig 3: Actinobacterial population (×10^3CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Actinomycetes are members of a heterogenous group of Gram-positive, anaerobic bacteria accounted for a filamentous and branching growth pattern (Berkowitz, 1994) [6]. These actinomycetes are an integral part of cow dung those have been implicated in the production of unpleasant flavors, odors, and colors. Of the specific types of actinomycetes, *Nocardia* spp. are predominately present among cow dung microflora (Radha and Rao, 2014) [17]. Moreover, a very high number of nocardioform, *Rhadococcus coprophillus* have been isolated from the cow based organic formulations of domesticated herbivores (Rowbotham and Cross, 1977) [21].

Actinomycetes require neutral pH for their growth were isolated by following pour plate method with an incubation temperature of 28 °C to 30 °C. After 30 DAS the highest actinomycetes count was observed in the natural farming treatment (12.4 ×10^3CFU/g soil) followed by organic production system (11.1 ×10^3CFU/g soil). The lowest population was observed in the treatment with no other inputs T_1: control due to absence of nutrients.

Similarly, After 60 DAS, 90 DAS and at harvest the highest actinomycetes count was observed in the treatment with organic production system (13.8 ×10^3CFU/g soil, 15.8 ×10^3CFU/g soil and 7.9×10^3CFU/g soil) followed by natural farming system (11.4 ×10^3CFU/g soil, 14.1 ×10^3CFU/g soil and 7.5 ×10^3CFU/g soil). The lowest population was observed in the treatment with no other inputs T_1: control due to absence of nutrients. The treatment T_3 and T_4 are on par with each other for total actinomycetes count.

Table 8: Effect of farming practices on total population of free-living nitrogen fixing bacteria (×10^5CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Treatments	Initial			30 DAS			60 DAS			90 DAS			Harvest		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	8.4	9.0	8.7	14.6	15.4	15.0	16.4	18.5	17.4	15.2	17.3	16.2	11.4	12.4	11.9
T_2	11.2	13.4	12.3	20.8	21.3	21.0	31.2	33.4	32.3	27.4	29.2	28.3	14.6	15.4	15.0
T_3	13.6	16.4	15.0	24.2	26.1	25.1	28.2	30.4	29.3	23.2	24.3	23.7	16.4	18.2	17.3
T_4	12.6	11.9	12.2	25.6	24.6	25.1	39.2	40.3	39.7	20.6	21.4	21.0	18.4	16.7	17.5
S Em ±	1.15	1.24		1.90	2.31		1.66	1.87		1.90	2.13		1.64	1.90	
CD @5%	3.45	4.00		5.70	6.34		5.00	5.60		5.70	6.40		NS	NS	

Note: T_1: Absolute control T_2: Organic production system T_3: Natural farming (NF protocol as given by Shri. Subhash Palekar) T_4: Recommended package of practices

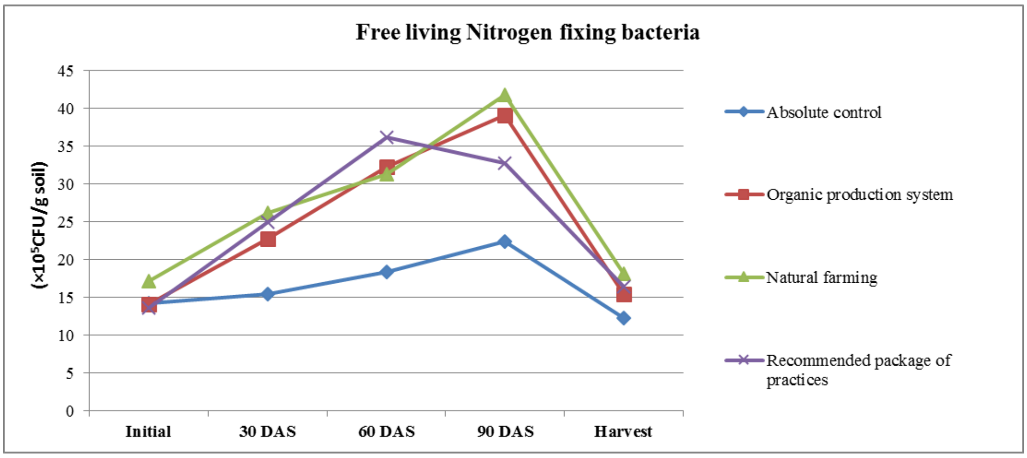

Fig 4: Population of free-living nitrogen fixing bacteria (×10^5CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Presence of naturally occurring beneficial microorganisms, predominantly bacteria, yeast, actinomycetes, and certain fungi have been reported in cow dung (Swain and Ray, 2009) [24]. Research related to isolation and characterization of the beneficial attributes of the bacteria present in biodynamic preparations are few. Adding nitrogen in the form of synthetic fertilizers can have negative environmental impacts since inorganic N, particularly nitrate; can be dispersed into surface and groundwater, leading to eutrophication.

N cycling in natural ecosystems and traditional agricultural production relies on biological N fixation primarily by diazotrophic bacteria. Diazotrophs are highly diverse and are widely distributed across bacterial and archael taxa. Most of biological nitrogen fixation (BNF) is carried out by diazotrophs in symbiosis with legumes (Dixon and Khan, 2004) [9]. However, under specific conditions bacteria which are free-living in soil (e.g., cyanobacteria, *Pseudomonas*, *Azospirillum*, and *Azotobacter*) may fix significant amounts of nitrogen (0 to 60 kg N ha^{-1} $year^{-1}$). This may be particularly important in organically managed soils, which typically have a lower proportion of nitrogen in available forms (Rosen and Allan, 2007) [20].

The soils samples from different treatments were analyzed for the total N fixers, the highest number of N fixers was observed in the treatment T_2 which was imposed with organic production system followed by the treatment T_3 with natural farming. Since microorganisms requires carbon source as their energy source, it was supplied in the form of cow based formulations like jeevamrutha, beejamrutha and ghanajeevamrutha in the natural farming treatment there was an higher population of N fixers.

Table 9: Effect of farming practices on total population of phosphate solubilizing microorganisms (×10^5CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Treatments	Initial			30 DAS			60 DAS			90 DAS			Harvest		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	6.8	7.5	7.1	11.4	12.4	11.9	14.6	13.1	13.8	14.6	15.1	14.8	12.4	13.1	12.7
T_2	8.0	10.5	9.2	18.6	23.2	20.9	31.2	35.2	33.2	27.4	29.2	28.3	12.8	13.4	13.1
T_3	10.4	11.4	10.9	20.6	24.1	22.3	34.8	33.2	34.0	26.2	27.3	26.7	13.2	16.2	14.7
T_4	3.0	4.5	3.7	12.6	15.2	13.9	18.6	19.5	19.0	19.2	20.3	19.7	10.2	13.6	11.9
S Em ±	0.82	1.23		1.85	2.31		1.94	1.90		2.02	2.65		0.85	1.56	
CD @5%	2.50	3.24		5.55	7.24		5.82	6.21		6.07	6.13		NS	NS	

Note: T_1: Absolute control T_2: Organic production system T_3: Natural farming (NF protocol as given by Shri. Subhash Palekar) T_4: Recommended package of practices

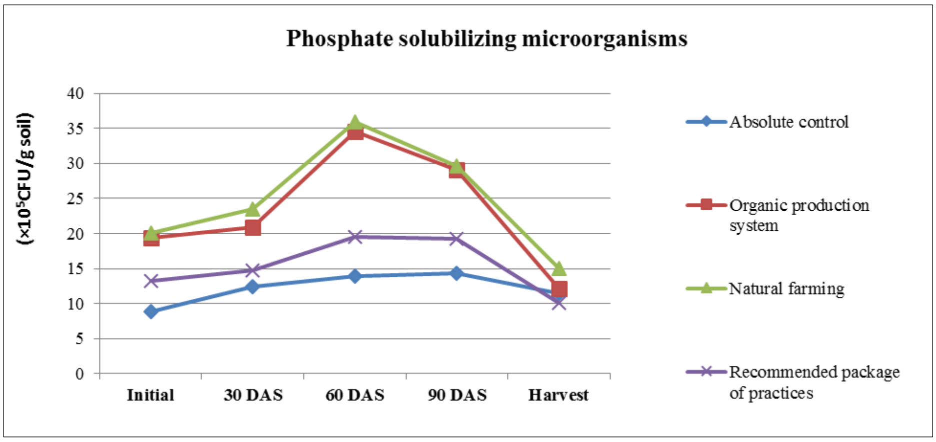

Fig 5: Population of phosphate solubilizing microorganisms ($\times 10^5$CFU/g soil) in rhizosphere of direct seeded rice at various stages of crop growth

Phosphorus (P) is a macronutrient required for the proper functioning of plants. Because P plays a vital role in every aspect of plant growth and development, deficiencies can reduce plant growth and development. Though soil possesses total P in the form of organic and inorganic compounds, most of them remain inactive and thus unavailable to plants. Since many farmers cannot afford to use P fertilizers to reduce P deficits, alternative techniques to provide P are needed. Phosphate solubilizing microbes (PSMs) are a group of beneficial microorganisms capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble P form that can easily be assimilated by plants. PSM provides an ecofriendly and economically sound approach to overcome the P scarcity and its subsequent uptake by plants.

The highest population of P solubilizers was observed in the treatment T_3: natural farming (NF protocol as given by Shri. Subhash Palekar) after 30 DAS (22.3 $x10^5$ CFU/g soil), 60DAS (34.00 $x10^5$ CFU/g soil) and at harvest (14.7 $x10^5$ CFU/g soil). It may be due to the application of fermented jeevamrutha at regular time intervals containing large population of microorganisms which helps in development of the microorganisms at the vicinity of rhizosphere.

Conclusion

Natural farming (NF), an environmentally friendly agricultural practice similar to organic farming, unlike conventional farming, little is known about the influence of NF on soil microbial communities, especially the surface soil. Many agricultural products based on indigenous fermentation technologies are used in organic farming like biodynamic preparations and liquid manures. Cow dung is an integral component of all these preparations and serves as a source of inoculum of beneficial microorganisms. We therefore compared the effect of two years conventional practice (CP), conventional practice without chemicals (CF), and NF on soil properties and microbial community structure, presence of naturally occurring beneficial microorganisms, predominantly bacteria, actinomycetes, N fixers, P solubilizers and certain fungi was observed in the treatment supplemented with cow based formulations like jeevamrutha, ghanajeevamrutha and seed treatment with beejamrutha.

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