



Effect of various crop production systems on population of rhizosphere microorganisms in field bean crop

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Abstract:

An experiment was conducted during 2018-19, in field bean crop (*Dolichos lablab.*) at research experimental plot, RIOF and NSP field, UAS, Bengaluru. Where we have imposed various farming production systems to study the status of rhizosphere microbial population at various growth stages of the crop. The beneficial microbial consortia includes biofertilizers, and Beejamritha was provided externally through the seed treatment application and jeevamritha was provided throughout the crop growth period at 15 days interval. Among the results obtained, the Zero Budget Natural Farming treatment was recorded highest population of bacteria (55×10^5), N fixers (3.4×10^5) and P solubilizers (34.8×10^5). With respect to the population of actinomycetes and fungi it was observed that the highest count was in the treatment of Organic Farming Practice (32.2×10^4) and UAS, (B) Package of Practice (23×10^3) respectively. But in the overall scenario ZBNF has shown the highest count of microbial population in the rhizosphere soil, compare to rest of the farming production systems.

Key words : Bacteria, Fungi, actinomycetes, N fixers and P solubilizers

Introduction:

Field bean usually known as Dolichos bean, Hyacinth bean or Field bean is one of the most ancient crops among cultivated plants. It is mainly cultivated either as a pure crop or mixed with finger millet, groundnut, castor, corn, bajra or sorghum in Asia and Africa. It is a multipurpose crop grown for pulse, vegetable and forage. The crop is grown for its green pods, while dry seeds are used in various vegetable food preparations. It is one of the major sources of protein in the diets in southern states of India. Within India, Lablab is a field crop mostly confined to the peninsular region and cultivated to a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra. Karnataka contributes a major share, accounting for nearly 90 per cent in terms of both area and production in the country. Karnataka state records production of about 18,000 tonnes from an area of 85,000 hectares. Outside India, the crop is cultivated in East Africa, with similar uses, and in Australia as a fodder crop.

Lablab is remarkably adaptable to wide areas under diverse climatic conditions such as arid, semi-arid, sub-tropical and humid regions where temperatures vary between 22°C–35°C, low lands and uplands and many types of soils and the pH varying from 4.4 to 7.8. Being a legume, it can fix atmospheric nitrogen to the extent of 170 kg/ha besides leaving enough crop residues to enrich the soils with organic matter. Hence, there is a scope to develop the organic nutrient management practices for field bean under rain fed condition. Continuous use of only chemical fertilizers in intensive cropping system is leads to imbalance of nutrients in soil, which has an adverse effect on soil health and also on sustainable crop yields. Hence, In order to achieve the sustainability in crop production development for major crops in general and field bean in particular is the need of the hour. Organic farming is a holistic system designed to optimize the productivity and fitness of diverse communities in the agro-ecosystem including living organisms viz., soil organisms, plants, livestock and human being etc., organic farming plays a vital role in maintaining biological diversity, decrease soil and ground water contamination, optimize biological productivity (Watson et al., 2002), maintain long-term soil fertility by optimizing conditions for biological activity in the soil. Production technology involves three management practices viz., efficient crop management, appropriate nutrient management and effective plant protection measures. Among them nutrient management plays important role. In addition to organic manures such as FYM, recycling of organic wastes through composting, green manures and biological inputs like vermicompost and bio-fertilizers etc., constitute important components for plant nutrient management and it is indispensable to identify the better source of nutrient and quantity to meet the nutrient requirement of field bean. (Ramesh et al., 2005)

Beans, due to the ability of forming nodules, can fix nitrogen from the atmosphere and thus they contain one or more types of microorganisms. Beans in association with *Rhizobium* can fix from 25 to 120 kg N / ha Compared to other legumes it is considered poor nitrogen fixation (Nleya *et al.*, 2001), in the beginning of the growing season, until appearing lumps, application of small quantities of nitrogen fertilizers is necessary (George and Singleton, 1992). As a result of nitrogen fixation during the growth and development of legumes, the share of fixed nitrogen in yield is 10-95 % or 20-400 kg N / ha. The number and activity of microorganisms can be considered as a significant indicator of potential and effective soil fertility. Changing the number of microorganisms that perform nitrogen fixation processes, ammonification, nitrification and other processes in the soil, it is often positively correlated with the yield of plants (Neeru and Vasudeva, 2007). For crop growth minimum inoculum level is necessary to obtain beneficial effects (Iswandi, 1987).

The combined of 30 or 45 kg P₂O₅/ faddan associated with *Rhizobium* + *Nitrobein* mixed inoculums could be applied to get high quantity and quality yield of faba bean in cultivated sandy soils. (Habasha *et al.*, 2007). There was a significant positive effect of rhizobia strains as evident from fresh and dry weight of leaves and stems, root/shoot ratio, pods/ flowers ratio as well as the number and weight of nodules compared to NPK fertilizer plots. The highest number of pods was achieved in treatment of rhizobia mixed with mycorrhiza or pseudomonas. Therefore, we recommend using the mixed inoculants strains as commercial inoculant for improving production of faba bean. (Wakeli *et al.*, 2007)

Rhizosphere :

The rhizosphere can be defined as the soil region where processes mediated by microorganisms are specifically influenced by the root system. This area includes the soil connected to the plant roots and often extends a few millimeters off the root surface, being an important environment for the plant and microorganism interactions (Gray and Smith, 2005), because plant roots release a wide range of compounds involved in attracting organisms which may be beneficial, neutral or detrimental to plants (Badri and Vivanco, 2009). The plant growth-promoting bacteria belong to a beneficial and heterogeneous group of microorganisms that can be found in the rhizosphere, on the root surface or associated to it, and are capable of enhancing the growth of plants and protecting them from disease and abiotic stress (Glick, 2012).

Healthy roots exude various organic compounds (including more than 100,000 different low-molecular-weight secondary metabolites (Feth et al. 2014), called root exudates. Root exudates are carbonaceous substances containing a wide range of amino acids, water-soluble sugars, organic acids, inorganic ions/gaseous molecules, and various vitamins and enzymes. The beneficial role of microbes in the rhizosphere can be manifested by direct plant growth promotion, indirectly providing protection from phytopathogens and fortifying the plant's tolerance to certain abiotic stresses under sub-optimal environmental conditions (Penrose and Glick 2003).

Agricultural production currently depends on the large-scale use of chemical fertilizers (Adesemoye et al., 2009). These fertilizers have become essential components of modern agriculture because they provide essential plant nutrients such as nitrogen, phosphorus and potassium. However, the overuse of fertilizers can cause unanticipated environmental impacts to achieve maximum benefits in terms of fertilizer savings and better growth, the PGPB-based inoculation technology should be utilized along with appropriate levels of fertilization. Moreover, the use of efficient inoculants can be considered an important strategy for sustainable management and for reducing environmental problems by decreasing the use of chemical fertilizers (Hungria et al., 2013).

Hence the present study was undertaken to know the impact of externally provided plant growth promoting microorganisms on rhizosphere beneficial microflora in various production systems of the field bean crop.

Materials and Methods:

A field investigation has been undertaken at RIOF, UAS, GKVK during *Rabi* 2018-19 with the following treatments:

Field bean crop variety HA4 (Hebbal avare-4) was sown on 27-12-2018 at a common spacing of 30 cm X 10 cm and gross plot sizes were 9.0 m X 30.0 m, respectively. These treatment combinations were replicated five times in RCBD.

Seed Treatment:

Seed treatment is the most commonly used method for different types of inoculants, and is an effective and economic method. For 10 kg of normal-size seeds, 200 g of inoculant is used, and for larger size seeds, 400–500 g of inoculants is used. The bag is opened and the seed is dried in shade for 20–30 min. The inoculant is mixed with seeds by adding a sticking agent jaggery (200 g) solution in a bucket and the microbial inoculant can be mixed directly by hand. Treated seeds have to be shade-dried and should be used for further sowing. Seed treatment can be carried out using *Rhizobium*, *Azotobacter*, *Azospirillum*, and with PSMs. Seed treatment can be accomplished using a consortium of compatible microorganisms. The seeds should be coated first with *Rhizobium*, *Azotobacter*, or *Azospirillum*. A PSM inoculant can be coated as the outer layer after a layer of other bacteria. This method will maintain a higher number of each bacterium, which is needed for better results.

Jeevamrutha:

Jeevamrutha is prepared by mixing 10 kg local cow dung with 10 litres cow urine, add 2 kg local jaggery, 2 kg pulse flour and handful of garden soil and the volume made up to 200 litres. Keep the drum in shade covering with wet gunny bag and stir the mixture clockwise thrice a day and incubate. (Palekar, S., 2006)

Beejamrutha:

Beejamrutha was prepared using the ingredients viz cow dung, cow urine, water and lime. Cowdung (5 kg) tied in a cloth was dipped in a bucket containing 50 liters of water overnight. Next day morning, the tied dung is frequently squeezed and dipped in the water. Five liters of cow urine, a handful of soil and 50g of calcium chloride was added to this extract. (Palekar, S., 2006)

Isolation of microorganism's:

The soil samples from research experiment at RIOF and NSP field were analysed for soil microorganisms at initial stage (before sowing) and different stages of crop growth till harvest of the crop. The microbial groups considered for analysis were total bacteria, fungi, actinomycetes, free living nitrogen fixing bacteria (N fixers) and phosphate solubilizing microorganisms (PSMs). The soil microorganisms were enumerated by following standard plate count (SPC) technique at different dilutions. The dilutions and media used for the above mentioned microbial groups were for total bacteria (10^5 cfu/ g of soil), fungi (10^4 cfu/ g of soil), actinomycetes (10^3 cfu/ g of soil), free living N fixers (10^5 cfu/g of soil) and phosphate solubilizing microorganisms (10^5 cfu/g of soil). The plates were incubated at $28\pm 2^\circ$ C for 24-48 hours for bacteria and 48-72 hours of incubation for fungi and actinomycetes. The colonies were recorded for each soil sample and expressed as colony forming units per gram of soil sample (cfu/g soil).

Details of microbial group, dilution and media

Sl. No.	Microbial group	Dilution	Media
1	Actinomycetes	10^{-3}	Kuster's agar
2	Soil fungi	10^{-4}	Martin's Rose bengal- Streptomycin sulfate agar
3	Phosphate solubilizing microorganisms (PSMs)	10^{-5}	Sperber's agar
4	Free-living nitrogen fixing bacteria	10^{-5}	Jensen's agar
5	Total bacteria	10^{-5}	Soil extract agar

Treatment details:

T₁: Only sowing of seeds. All other inputs and practices are nil.

T₂: Seed treatment with Rhizobium, FYM applied based on N equivalent (25 kg N ha^{-1}), weeding at 30 DAS, earthing up at 45 DAS, mulching (4 t ha^{-1}). Need based plant protection using organic materials.

T₃: Ghanajeevamrutha application at 1000 kg ha^{-1} , seed treatment with beejamrutha, application of jeevamrutha at 15 days interval at $500 \text{ liters ha}^{-1}$ and mulching at 30 DAS (4 t ha^{-1}). Need based plant protection measures using preparations like nemastra, agniastra, shuntiastra etc.

T₄: Seed treatment with *Rhizobium*, FYM application at 7.5 t ha^{-1} and NPK ($25:50:25 \text{ kg ha}^{-1}$), spraying of post-emergence herbicide (imazethapyr 10% SL 1000 ml ha^{-1}) at 30DAS, earthing up at 45 DAS.

Results and Discussion:

An experiment was conducted at UAS, GKVK, to study the status of population of rhizosphere microorganisms (Table 1). Where significant differences were observed among the bacterial population of various farming practices and at various stages of the crop. The treatment T2 (Zero Budget Natural Farming) was recorded higher population of bacteria at 30 DAS (48.6), 45 DAS (36.5), 60 DAS (55), 75 DAS (44.4) and harvest (44.7), followed by package of practice and organic farming production. And the least population was observed in the absolute control.

The presence of beneficial microorganisms in the jeevamritha and Beejamrita 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) and gibberlic acid (GA) (Palekar, 2006; Sreenivasa *et al.*, 2010; Neelima and Sreenivasa, 2011). Hence, the higher beneficial microorganisms found in these organic formulations are in conformity with Papen *et al.*, (2002), Sreenivasa *et al.*, (2010) who have also reported the presence of naturally occurring beneficial microorganisms predominantly bacteria, yeast, actinomycetes and certain fungi in organic liquid manures. Hence, these formulations would serve a long way in supplementing many of the biofertilizers and biocontrol agents used in crop production in the rural areas. This is also in conformity with Devakumar *et al.*, (2011) 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 1: Bacterial population ($\times 10^5$ cfu/g soil) in soil samples of Field bean at various stages of crop growth

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	21.4	25.2	29.4	15.4	40.2	25.6	30.1
T2 (Zero Budget Natural Farming)	24.6	36.4	48.6	36.5	55.0	44.4	44.7
T3 (Organic Farming System)	24.2	32.2	34.2	30.2	39.2	35.8	35.3
T4 (Package Of Practice)	21.0	31.0	36.0	17.0	38.4	36.8	35.5
SEm	1.24	2.71	3.34	4.86	4.07	3.62	2.88
CD 5%	NS	NS	10.30	14.97	12.55	11.14	8.87

* DAS = Days after sowing

With respect to the population of fungi (Table 2) it is recorded significantly higher in the treatment T4 (Package of Practice) at 15 DAS (11), 45 DAS (23), 60 DAS (20), and 75 DAS (19) followed by Organic Farming Practice and Zero Budget Natural Farming treatments. The fungi population is higher in all the treatments at the stage of 45 DAS but the least population was observed in the treatment absolute control at all the stages of the crop.

The rhizosphere contains both pathogenic and symbiotic fungi but their predominance of a specific community depends on many factors related to plants and soil. In particular, root exudates have been deemed an important factor when selecting for specific rhizosphere fungi (Buée *et al.* 2009). Rhizosphere fungi are closely linked to plant health and growth, owing to their roles in antagonizing pathogens, decomposing plant residues, and providing nutrients. Variation in the fungal community of the rhizosphere is suggested to be plant-dependent because roots release several organic compounds that contribute to a unique rhizosphere nutrient pool, which is accessible to soil microorganisms (Han *et al.*, 20167). Soil physical and chemical properties are known to be significantly correlated with changes in the rhizosphere fungal community. Soil texture highly affects the organic carbon content and consequently determines plant rhizosphere microbial communities (Wang *et al.*, 2009).

Table 2: Fungal population ($\times 10^4$ cfu/g soil) in soil samples of Field bean at various stages of crop growth

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	9	7	15.2	18	10.6	6.4	7.8
T2 (Zero Budget Natural Farming)	13.8	10.2	13.6	13.9	12.6	11.8	9
T3 (Organic Farming System)	12.6	10.4	15.2	16.2	13.8	10.4	9
T4 (Package Of Practice)	10.2	11	13.6	23	20	19	6
SEm	1.56	0.566	2.155	1.820	1.831	1.258	0.684
CD 5%	NS	1.743	NS	5.607	5.643	3.875	2.109

* DAS = Days after sowing

Significant differences (Table 3) were found at all the stages of the crop in between the various treatments. Where as the treatment T4 (Package of Practice) was recorded the highest significant count of actinomycetes at 45 DAS (32.2) followed by treatment T2 (26.8) (Zero Budget Natural Farming). And the least population of actinomycetes was recorded in the treatment T1 (3) absolute control at initial stage.

The population density of actinomycetes in the soil might be influenced by the soil nutrients like total organic carbon and nitrogen. Most of the isolates tend to grow in fertile soils, which are an important characteristic feature of *Streptomyces* sp. (Stackebrandt et al., 1981) and with adequate source of carbon and nitrogen present in it that enhance the rate of degradation (Tien et al., 1987).

Table 3: Actinomycetes population ($\times 10^3$ cfu/g soil) in soil samples of field bean at various stages of crop growth

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	3	8.4	5.8	16.6	13.5	10.2	10
T2 (Zero Budget Natural Farming)	9.8	7.8	22.8	26.8	14.2	12.2	11.2
T3 (Organic Farming System)	10	10.6	12.2	22.4	16.4	10.6	8.6
T4 (Package Of Practice)	6.4	6.8	12.2	32.2	13.8	10.6	7
SEm	0.95	0.65	1.79	4.36	1.84	1.14	0.77
CD 5%	2.93	2.00	5.50	NS	5.66	3.50	2.36

* DAS = Days after sowing

The population of free living nitrogen fixers (Table 4) were higher in the treatment T2 (Zero Budget Natural farming) which is on par with the treatment T3 (Organic Farming Practice) at all the stages of the crop. But all the treatments were differing each other non-significantly at all the stages of the crop.

Biological nitrogen fixation (BNF) is the process responsible for the reduction of N_2 to ammonia (NH_3) (Franche *et al.*, 2009) and is performed in diazotrophic microorganisms, particularly bacteria and archaea (Dixon and Kahn, 2004). Wood and Cooper (1988) showed that at increasing inoculum densities increasing numbers of *Rhizobium leguminosarum*. In the *Rhizobium*-legume symbiosis, which is a N_2 -fixing system, the process of N_2 fixation is strongly related to the physiological state of the host plant. Typical environmental stresses faced by the legume nodules and their symbiotic partner (*Rhizobium*) may include photosynthate deprivation, water stress, salinity, soil nitrate, temperature, heavy metals, and biocides.

Table 4: Population Free living nitrogen fixers ($\times 10^5$ cfu/g soil) in soil samples of field bean at various stages of crop growth

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	1.4	1.6	2	2.2	1.2	1.2	1.4
T2 (Zero Budget Natural Farming)	2.6	1.6	2.4	3.4	2.4	1.6	1.2
T3 (Organic Farming System)	1.2	1.6	1.4	3.4	1.8	1.2	1.2
T4 (Package Of Practice)	1.2	1.4	1.6	2.8	1.2	1.4	1.2
SEm	0.356	0.389	0.751	0.693	0.324	0.212	0.208
CD 5%	NS	NS	NS	NS	NS	NS	NS

* DAS = Days after sowing

With respect to population of P solubilizers (Table 5) it shows that the highest count of P solubilizers were significantly found in the treatment T2 (Zero Budget Natural Farming) followed by the treatment T3 (Organic Farming Practice) at initial (18, 17), 45 DAS (34.4, 34.8), 60 DAS (32.6, 32.2) and at harvest (24.6, 25.4). The least population was observed in the absolute control treatment at initial stage (12). Among the factors influencing microbial phosphate solubilization are interactions with other microorganisms in the soil, the extent of vegetation, ecological conditions, climatic zone soil types, plant types, agronomic practices, land use systems, and the soil's physicochemical properties such as organic matter and soil pH (Seshachala and Tallapragada, 2012).

Table 5: Population of phosphate solubilizing microorganisms ($\times 10^5$ cfu/g soil) in soil samples of field bean at various stages of crop growth

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	12	14	21.6	22.8	23.8	21.4	15.4
T2 (Zero Budget Natural Farming)	18	19.4	29.6	34.4	32.6	28.8	24.6
T3 (Organic Farming System)	17	13.8	21.3	34.8	32.2	26.8	25.4
T4 (Package Of Practice)	16	18	20.6	26.4	22.4	25.2	18.8
SEm	1.252	1.857	4.055	2.576	1.678	3.764	1.667
CD 5%	3.859	NS	NS	7.938	5.171	NS	5.136

* DAS = Days after sowing

Recently, Zhang et al. (2014) reported that adding small amounts of inorganic phosphorus to the rhizosphere could drive phytic acid mineralization by bacteria and thereby improve plant phosphorus nutrition. Lime and compost, used as a soil improver, also had positive effects on phosphate solubilizers. Phosphorus Solubilizing Bacteria population richness and diversity, according to Azziz et al. (2012), were more abundant and diverse following crop rotation. Soil rich in organic matter will favor microbial growth and therefore favors microbial phosphorus solubilisation.

Conclusion:

There are various biotic and abiotic factors influencing the growth and development of the rhizosphere microorganisms. Abiotic factors viz, temperature, moisture, soil pH, and aeration play a key role in maintaining the physical, chemical and biological properties of the soil. Apart from this, availability of nutrients will play a vital role in the growth and development of the rhizosphere microorganisms.

The rhizosphere microorganisms consists largely of bacteria that benefit plant growth, which are better known as PGPR. Multiple mechanisms of plant growth promotion have been proposed based on PGPR, whether direct or indirect, including the production of siderophores, phytohormones, volatile compounds, in addition to their capabilities of bio control and antagonism to plant pathogens.

There are studies says that, the long-term effect of fertilizers can produce positive effects on microbial soil and rhizosphere populations, while others drastically opposite to their use in agriculture. Renowned findings showed that temporal shifts in diversity and relative activity were observed in rhizosphere bacterial communities with developmental stage for all plant species studied. The plant species selected for specialized microbial communities that change in response to plant growth and plant inputs. Differences in population densities in the rhizosphere reflect differences in the quantity and quality of rhizodeposition, moisture content of the soil and the root architecture. Microorganisms present in the rhizosphere of young plants preferentially utilize simple amino acids, whereas bacteria from mature plants utilize the more complex carbohydrates, this indicates the change in the quality of plant root exudates and the ability of populations to respond under competition.

From the above observations, it is clear that zero budget natural farming treatment has recorded highest microbial count compared to rest of the treatments. It may be due to the application of fermented jeevamrutha at regular time intervals, containing large population of microorganisms. which in turn triggered the microbial population at crop rhizosphere. Thereafter package of practice and organic farming practice had shown immediate huge population next to ZBNF, it may be due to the sufficient availability of externally applied nitrogen and carbon nutrient sources to the growth and development of the microorganisms at rhizosphere. further the long term experiments are required to study the consistency of microbial load at crop rhizosphere in various production system.

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