

Impact of Agricultural Production Strategies on Rhizosphere Microorganisms in Field Bean Crops

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Abstract:

In 2020-21, an experiment was done on field bean crop (*Dolichos lablab*.) in a field. We studied the microbial population in the rhizosphere throughout different crop development stages using different agricultural production techniques. Beneficial microbial consortiums include biofertilizers. Beejamritha was applied externally via seed treatment, whereas Jeevamritha was given at 15-day intervals throughout crop development. The Zero Budget Natural Farming treatment had the largest number of bacteria (55×10^5), nitrogen fixers (3.4×10^5), and P solubilizers (34.8×10^5). The Organic Farming Practice (32.2×10^4) and UAS, (B) Package of Practice (23×10^3) treatments had the greatest populations of actinomycetes and fungi, respectively. Overall, ZBNF has the largest microbial population in rhizosphere soil compared to other farming methods.

Keywords: P solubilizers, N fixers, actinomycetes, bacteria, and fungi

Introduction:

The field bean, also known as *Dolichos* bean, Hyacinth bean, or Field bean, is one of the oldest cultivated crops. In Asia and Africa, it is often grown alone or in combination with other crops such as finger millet, peanuts, castor, maize, bajra and sorghum. It is a versatile crop produced for pulses, vegetables, and grazing. The crop is farmed for its green pods, and the dried seeds are utilised in different vegetable culinary recipes. It is a key protein source in southern Indian cuisine. *Lablab* is mostly grown in the peninsular area of India, particularly in Karnataka, Tamil Nadu, Andhra Pradesh, and Maharashtra. Karnataka accounts for roughly 90% of the country's land and output. Karnataka state produces around 18,000 tonnes from 85,000 hectares. Outside India, the crop is grown in East Africa for similar purposes and in Australia as a fodder crop.

Lablab is very adaptable to many climatic situations, including dry, semi-arid, subtropical, and humid climates with temperatures ranging from 22°C to 35°C, lowlands and uplands, various soil types, and pH levels from 4.4 to 7.8. Legumes can fix atmospheric nitrogen at a rate of 170 kg/ha and leave crop leftovers to enhance soils with organic matter. There is potential for developing organic nutrition management strategies for field beans grown in rainy conditions. Excessive use of chemical fertilisers in intensive cropping systems causes nitrogen imbalances, negatively impacting soil health and crop

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production. To ensure sustainable agricultural output, it is crucial to grow main crops, particularly field beans. Organic farming is a holistic system that optimises the productivity and fitness of diverse communities in the agro-ecosystem, including soil organisms, plants, livestock, and humans. It helps maintain biological diversity, reduce soil and ground water contamination, optimise biological productivity, and maintain long-term soil fertility by optimising conditions for biological activity (Watson et al., 2002). Production technology includes three management practices: efficient crop management, adequate nutrient management, and effective plant protection measures. Nutrient control is a key factor. To meet the nutrient requirements of field beans, it's important to identify the best source of nutrients, including organic manures like FYM, composting, green manures, vermicompost, and bio-fertilizers. (Ramesh et al., 2005).

Beans' capacity to create nodules allows them to fix nitrogen from the atmosphere, making them home to many microbes. Beans with Rhizobium can fix 25 to 120 kg of nitrogen per hectare. Compared to other legumes, it has low nitrogen fixation (Nleya et al., 2001). Small amounts of nitrogen fertiliser must be applied in the early growing season until lumps emerge (George and Singleton, 1992). Legumes fix nitrogen throughout growth and development, resulting in yields ranging from 10-95% (20-400 kg N/ha). Microorganism activity and abundance are important indicators of soil fertility. Increasing the number of microorganisms in soil that undertake nitrogen fixation, ammonification, and nitrification has been shown to increase plant output (Neeru and Vasudeva, 2007). Iswandi (1987) found that crop development requires a minimum inoculum level for optimal results.

A combination of 30 or 45 kg P2O₅/faddan and Rhizobium + Nitroben inoculums may increase production and quality of faba beans in sandy soils. Habasha et al. (2007).

Rhizobia strains had a substantial favourable influence on fresh and dry weight of leaves and stems, root/shoot ratio, pods/flowers ratio, and nodule number/weight when compared to NPK fertiliser plots.

The treatment with rhizobia and mycorrhiza or pseudomonas produced the most pods. We advocate employing mixed inoculant strains as a commercial inoculant to increase faba bean yield. Wakeli et al., 2007.

Rhizosphere:

The rhizosphere is the zone of soil where microorganisms and the root system interact. The soil surrounding plant roots, which extends a few millimetres beyond the root surface, plays a crucial role in plant-microorganism interactions (Grey and Smith, 2005). Plant roots release compounds that attract organisms, which can be beneficial, neutral, or detrimental to plants (Badri and Vivanco, 2009). Plant growth-promoting bacteria are a diverse collection of microorganisms present in the rhizosphere, root surface, and related areas. They promote plant development and defend against

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disease and abiotic stress (Glick, 2012).

Root exudates are chemical molecules that healthy roots release, containing over 100,000 distinct low-molecularweight secondary metabolites (Feth et al., 2014). Root exudates are carbonaceous compounds rich in amino acids, water-soluble sugars, organic acids, inorganic ions, gaseous molecules, vitamins, and enzymes. Microbes in the rhizosphere may promote plant development, defend against phytopathogens, and improve plant tolerance to abiotic stressors under suboptimal settings (Penrose and Glick 2003).

According to Adesemoye et al. (2009), chemical fertilisers are widely used in agriculture. Fertilisers offer plant nutrients including nitrogen, phosphorus, and potassium, making them vital in contemporary agriculture. To maximise fertiliser savings and growth, employ PGPB-based inoculation technology in conjunction with suitable fertilisation levels to avoid unintended environmental consequences. Using effective inoculants may promote sustainable management and reduce environmental impact by reducing the need for chemical fertilisers (Hungria et al., 2013).

This research examines how exogenous plant growth boosting microorganisms affect beneficial microflora in the rhizosphere of field bean crops grown in different production regimes.

Materials and methods:

A field research was conducted at RIOF, UAS, and GKVK during Rabi 2020-21, using the following treatments:

Field bean variety HA4 (Hebbal avare-4) was seeded on December 27, 2020, with a common spacing of 30 cm X 10 cm and gross plot sizes of 9.0 m X 30.0 m. The treatment combinations were repeated five times in RCBD.

Seed Treatment:

Seed treatment is a cost-effective and widely used approach for various inoculants. To inoculate 10 kg of normal-sized seeds, use 200 g, while bigger seeds need 400-500 g. The bag is opened, and the seed is dried in the shade for 20 to 30 minutes. To combine the inoculant with seeds, add 200 g of jaggery solution to a bucket. The microbial inoculant may then be blended by hand. Treated seeds must be shade-dried before additional planting. Seed treatment options include Rhizobium, Azotobacter, Azospirillum, and PSMs. Microorganisms that are compatible may be used to treat seeds. Coat the seeds first with Rhizobium, Azotobacter, or Azospirillum. A PSM inoculant may be put on top of another layer of bacteria. This approach maintains more of each bacteria, leading to better outcomes.

Jeevamrutha:

To make Jeevamrutha, combine 10 kg of local cow dung, 10 litres of cow urine, 2 kg of local jaggery, 2

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kg of pulse flour, and a pinch of garden soil to make a 200-liter mixture. To incubate, place the drum in the shade and cover it with a damp gunny bag. Stir the contents clockwise three times daily. Palekar, S. (2006).

Beejamrutha:

Beejamrutha was made with these ingredients: cow dung, cow urine, water, and lime. Cowdung (5 kilogramme) wrapped in a cloth was immersed in a bucket of 50 litres of water overnight. The next morning, the knotted dung is pressed and dunked in water. This extract included 5 litres of cow pee, a handful of dirt, and 50g of calcium chloride. Palekar, S. (2006).

The isolation of microorganisms

Soil samples from fields were analysed for microorganisms before planting, throughout crop growth, and at harvest. Microbial groups examined included total bacteria, fungi, actinomycetes, nitrogen fixers, and phosphate solubilizing microorganisms (PSMs). The soil microorganisms were counted using the standard plate count (SPC) method at various dilutions. The microbial groups studied were total bacteria (105 cfu/g soil), fungi (104 cfu/g soil), actinomycetes (103 cfu/g soil), free-living N fixers (105 cfu/g soil), and phosphate-solubilizing microorganisms (105 cfu/g soil). Plates were incubated at $28 \pm 2^\circ\text{C}$ for bacteria (24-48 hours) and 48-72 hours for fungus and actinomycetes. Colonies were counted for each soil sample and represented as cfu/g soil.

Details of microbial group, dilution and media

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

Treatment Details:

T1: Only seeds were sown. All other inputs and processes are zero.

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T2: Rhizobium seed treatment with FYM at 25 kg N ha⁻¹, weeding at 30 DAS, earthing at 45 DAS, and mulching (4t ha⁻¹). Organic plant protection that meets needs.

T3 involves applying Ghanajeevamrutha at 1000 kg ha⁻¹, treating seeds with beejamrutha, applying jeevamrutha at 500 litres ha⁻¹ every 15 days, and mulching at 30 DAS (4t ha⁻¹). Plant protection strategies depending on need, including preparations such as neemasthra, agniasthra, and shuntiastrha.

T4: Rhizobium seed treatment, FYM application at 7.5 t ha⁻¹, NPK (25:50:25 kg ha⁻¹), post-emergence herbicide spray (imazethapyr 10% SL 1000 ml ha⁻¹) at 30DAS, and earthing at 45 DAS.

Results and Discussion:

An experiment was done at UAS, GKVK to investigate the population of rhizosphere microorganisms (Table 1). Significant changes were identified in bacterial populations between agricultural techniques and crop stages. Treatment T2 (Zero Budget Natural Farming) had greater bacterial populations at 30 DAS (48.6), 45 DAS (36.5), 60 DAS (55), 75 DAS (44.4), and harvest (44.7), compared to package of practice and organic farming production. The absolute control group had the lowest population.

Beneficial microorganisms may be present in jeevamrutha and Beejamritha formulations due to their constituents, including cow dung, urine, legume flour, and jaggery, which contain essential nutrients, vitamins, amino acids, and growth-promoting substances like indole acetic acid (IAA) and gibberlic acid (GA) (Palekar, 2006; Sreenivasa et al., 2010; Neelima and Sreenivasa, 2011). Papen et al. (2002) and Sreenivasa et al. (2010) found beneficial microorganisms, including bacteria, yeast, actinomycetes, and fungi, in organic liquid manures. These formulations might enhance biofertilizers and biocontrol agents commonly employed in rural agricultural production. Devakumar et al. (2011) found that jeevamrutha and panchagavya promote nitrogen fixation in local substrates such FYM, pressmud, compost, and digested biogas slurry.

Table 1 shows the bacterial population (10⁵ cfu/g soil) in soil samples of field beans at different phases of development.

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)	25.0	37.0	49.0	37.0	55.5	45.0	45.0

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T3 (Organic Farming System)	24.5	32.5	34.5	30.5	39.5	36.0	35.5
T4 (Package Of Practice)	21.3	31.5	36.5	17.5	38.8	37.2	35.8
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

Table 2 shows that the population of fungus is considerably greater in T4 (Package of Practice) at 15 DAS (11), 45 DAS (23), 60 DAS (20), and 75 DAS (19), compared to Organic Farming Practice and Zero Budget Natural Farming. Fungi populations increased in all treatments at 45 DAS, while the absolute control treatment had the lowest population throughout all crop stages.

The presence of pathogenic and symbiotic fungi in the rhizosphere is influenced by plant and soil characteristics. Buée et al. (2009) found that root exudates play a crucial role in identifying rhizosphere fungus. Rhizosphere fungi play important roles in plant health and development by preventing infections, decomposing plant wastes, and delivering nutrients. Plants emit organic substances that contribute to a distinct rhizosphere nutrient pool that soil microorganisms may access, leading to variation in the fungal community (Han et al., 20167). Changes in the rhizosphere fungal community have a strong correlation with soil physical and chemical parameters. Wang et al. (2009) found that soil texture significantly impacts organic carbon concentration and microbial populations in the rhizosphere of plants.

Table 2 shows the fungal population (104 cfu/g soil) in soil samples of field beans at different phases of development.

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	9.0	7.2	15.5	18.3	10.9	6.7	8.1
T2 (Zero Budget Natural Farming)	14.0	10.5	13.9	14.2	12.8	12.0	9.3
T3 (Organic Farming)	12.8	10.6	15.5	16.5	14.0	10.7	9.2

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System)							
T4 (Package Of Practice)	10.5	11.2	13.9	23.3	20.3	19.3	6.2
SEm	1.56	0.57	2.16	1.82	1.83	1.26	0.68

Significant changes (Table 3) were seen at all crop stages between the different treatments.

T4 (Package of Practice) had the highest significant count of actinomycetes at 45 DAS (32.2), followed by T2 (26.8) (Zero Budget Natural Farming). The treatment T1 (3) absolute control had the lowest number of actinomycetes during the early stage.

Soil nutrients, such as total organic carbon and nitrogen, may impact the density of actinomycetes. *Streptomyces* sp. isolates thrive in rich soils with appropriate carbon and nitrogen sources, leading to faster breakdown rates (Stackebrandt et al., 1981; Tien et al., 1987).

Table 3 shows the population of Actinomycetes (10³ cfu/g soil) in field bean soil samples throughout different phases of development.

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	3.2	8.6	6.0	16.8	13.7	10.4	10.2
T2 (Zero Budget Natural Farming)	10.0	8.0	23.0	27.0	14.4	12.4	11.4
T3 (Organic Farming System)	10.2	10.8	12.4	22.6	16.6	10.8	8.8
T4 (Package Of Practice)	6.6	7.0	12.4	32.4	14.0	10.8	7.2
SEm	0.96	0.66	1.80	4.38	1.85	1.15	0.78
CD 5%	2.94	2.01	5.52	NS	5.68	3.51	2.37

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Table 4 shows that treatment T2 (Zero Budget Natural Farming) had a greater population of free-living nitrogen fixers, comparable to treatment T3 (Organic Farming Practice), throughout all crop stages. However, no significant differences were seen between treatments during the crop's development.

Biological nitrogen fixation (BNF) is the process by which diazotrophic microorganisms, such as bacteria and archaea, convert N_2 to NH_3 (Franche et al., 2009; Dixon and Kahn, 2004). Wood and Cooper (1988) found that increasing inoculum densities led to an increase in *Rhizobium leguminosarum* populations. In the *Rhizobium*-legume symbiosis, N_2 -fixation depends on the host plant's physiological condition. Legume nodules and their symbiotic partner (*Rhizobium*) may experience environmental challenges such as photosynthesis deficiency, water stress, salinity, soil nitrate, temperature, heavy metals, and biocides.

Table 4 shows the population of free-living nitrogen fixers (105 cfu/g soil) in soil samples of field beans at different stages of development.

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	1.4	1.7	2.1	2.3	1.3	1.3	1.5
T2 (Zero Budget Natural Farming)	2.7	1.7	2.5	3.5	2.5	1.7	1.3
T3 (Organic Farming System)	1.3	1.7	1.5	3.5	1.9	1.3	1.3
T4 (Package Of Practice)	1.3	1.5	1.7	2.9	1.3	1.5	1.3
SEm	0.36	0.39	0.75	0.69	0.32	0.21	0.21
CD 5%	NS	NS	NS	NS	NS	NS	NS

Table 5 shows that treatment T2 (Zero Budget Natural Farming) had the highest P solubilizer population, followed by T3 (Organic Farming Practice) at initial (18, 17), 45 DAS (34.4, 34.8), 60 DAS (32.6, 32.2), and harvest (24.6, 25.4). The absolute control treatment had the lowest population in the

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early stage (12). Factors affecting microbial phosphate solubilization include interactions with other microorganisms, vegetation, ecological conditions, plant types, agronomic practices, land use systems, and soil properties like organic matter and pH (Seshachala and Tallapragada, 2012).

Table 5 shows the population of phosphate-solubilizing microorganisms (105 cfu/g soil) in soil samples of field beans at different stages of development.

Treatments	Initial	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Harvest
T1 (Absolute Control)	12.2	14.2	21.8	23.0	24.0	21.6	15.6
T2 (Zero Budget Natural Farming)	18.2	19.6	29.8	34.6	32.8	29.0	24.8
T3 (Organic Farming System)	17.2	14.0	21.5	35.0	32.4	27.0	25.6
T4 (Package Of Practice)	16.2	18.2	20.8	26.6	22.6	25.4	19.0
SEm	1.25	1.86	4.06	2.58	1.68	3.76	1.67
CD 5%	3.86	NS	NS	7.94	5.17	NS	5.14

Zhang et al. (2014) found that adding tiny quantities of inorganic phosphorus to the rhizosphere promotes phytic acid mineralization by bacteria, improving plant phosphorus feeding. Soil improvers like lime and compost have been shown to improve phosphate solubility. Phosphorus Solubilizing

Azziz et al. (2012) found that crop rotation led to increased bacterial population variety and abundance. Rich organic matter in soil promotes microbial development and phosphorus solubilization.

Conclusion:

Various biotic and abiotic variables impact the growth and development of rhizosphere microorganisms. Temperature, moisture, soil pH, and aeration are important abiotic elements that sustain soil qualities, including physical, chemical, and biological. Nutrient availability is crucial for the growth and development of rhizosphere bacteria.

Plant growth-promoting rhizosphere microorganisms (PGPR) are mostly bacteria. PGPR has been hypothesised to promote plant development by many methods, including the generation of

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siderophores, phytohormones, and volatile chemicals. It also has the ability to suppress plant diseases.

Some research suggest that fertilisers may have a favourable impact on microbial soil and rhizosphere populations over time, whereas others show the reverse. Research indicates that bacterial communities in the rhizosphere vary in richness and activity based on plant species' developmental stages. Plant species choose specialised microbial communities that adapt to plant growth and inputs. Population densities in the rhizosphere vary depending on factors such as rhizodeposition, soil moisture content, and root design. Microorganisms in the rhizosphere of young plants prefer simple amino acids, while bacteria from mature plants prefer complex carbohydrates. This suggests a shift in the quality of plant root exudates and population response to competition.

The zero-budget natural farming treatment had the greatest bacteria count among the treatments studied. The application of fermented jeevamrutha at regular intervals, which has a huge population of microorganisms, may have initiated microbial activity at crop roots. Following the implementation of organic farming practices, a significant increase in microorganisms was seen near to ZBNF. This might be attributed to the availability of nitrogen and carbon nutrients at the rhizosphere. Long-term trials are needed to determine the constancy of microbial load in crop rhizospheres across different production systems.

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