

Role of Bio-fertilizer for Maximizing Productivity of Selected Horticultural Crops

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ABSTRACT

This review was aimed to discuss the role of bio-fertilizer for maximizing productivity of selected horticultural crops. Data of previous researches were analysed to justify the objective. It was concluded that transfer of technology by the research institutions and industry is essential in the management of natural resources in sustainable agriculture as the microbial fertilizers hold vast potential for the future. The crop- microbial soil ecosystem energized in sustainable agriculture with considerable ecological stability and environmental quality improves the potential of bio-fertilizers in vegetable production.

Keywords: Agriculture, bio-fertilizer, horticultural crops.

Indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, has polluted water basins, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility [1]. Therefore, solutions are required to maintain crop productivity and to simultaneously reduce chemical inputs in terms of chemical fertilizers and pesticides. Another resource that limits plant productivity is water. According to global-change predictions, several areas of the globe could become arid or semi-arid regions due to a reduction in precipitation [2]. Therefore, any strategy that reduces the amount of water added to crops without a loss in yield, is desirable. To fulfill the above desired practices, one possibility is the use of soil microorganisms that increase the nutrient- and water-use efficiency and uptake capacity [3].

The rhizosphere is a thin zone of soil surrounding the root zone that is immensely influenced by the root system [4]. Compared to the neighboring bulk soil, this zone is rich in nutrients, due to the accumulation of a variety of organic compounds released by the roots through exudation, secretion and rhizodeposition. These organic compounds can be used as carbon and energy sources by microorganisms and microbial activity is particularly intense in the rhizosphere. The rhizosphere is therefore home to a variety of root associated bacteria commonly referred to as

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rhizobacteria. Such beneficial rhizobacteria that positively influence plant growth are referred to as plant growth promoting rhizobacteria (PGPR).

In Africa, research into bio-fertilizers for sorghum is ongoing in Ethiopia, Kenya and Zimbabwe, for cowpea in Cameroon, for groundnut and bambara groundnut in Madagascar, and for rice in Rwanda, with unspecified work in Burkina Faso, Cote d'Ivoire, the Democratic Republic of the Congo, Kenya, Rwanda and Senegal. The UNESCO Microbiological Resources Centre (MIRCEN) project at the University of Nairobi in Kenya has, since 1981, developed a Rhizobium inoculant known as BIOFIX, currently the main inoculant available on the local market. In Eastern Europe, Armenia and the Republic of Moldova are testing Azotobacter and Rhizobium [5].

Teaching of microbiology in the senior universities may have started in early 80s. By Prof. Brihanu Abegaz Research in Biofertilizer started as early as 1982 at MARC by Amare Abebe on Haricot Bean. 1984-86 transferred to HARC by Desta Beyene continued by his disciple Angaw Tsige. Focused mainly on BNF of highland pulses till these days 8 - 1990 NSSL / NSRC / NSTC started the research in 1988 - 1990 NSSL / NSRC / NSTC started the research under MoA and continued under EAIR till 2008. BNF, a) Rhizobium: released inoculant for six pulses (FB, FP, CP, HB, Ln, SB). b) Cyanobacteria: Anabinaazolea PSM (two bacteria, *Bacillus* and *Pseudomonas* three fungi: *Aspegillus*, *Trichoderma*, *Mucor*)

Plants have a number of relationships with fungi, bacteria, and algae, the most common of

which are with mycorrhize, rhizobium, and cyanophyceae. These are known to deliver a number of benefits including plant nutrition, disease resistance, and tolerance to adverse soil and climatic conditions. These techniques have proved to be successful bio fertilizers that form a healthy relationship with the roots [6].

Therefore, the objective of the review to discuss the role of bio fertilizers in the productivity of selected horticultural crops.

The Role of Bio-Fertilizers in Horticultural Crop Production

The different kinds of soil microorganisms colonizing the rhizosphere or the plant tissues can have a tremendous impact on the nutrient uptake capacity of the plants, and in increasing the efficiency of the applied fertilizers. Indeed, only 30–50% of applied N fertilizers and 10–45% of P fertilizers are taken up by crops [7]. Their contribution can be limited to a single nutrient element, as in case of N-fixing bacteria, or to a variety of elements, such as for arbuscular mycorrhizal fungi (AMF) [8].

The microorganisms that can be utilized for the production of biofertilizers belong to several taxa of the fungi, bacteria and, possibly, protozoa kingdoms [9]. For practical purposes, they are normally grouped in functional groups depending on features related to the kind of interaction they establish with the plants: rhizosphere or endophytic species, either symbionts, associated or free living. Rhizosphere is the interface where the soil, plant roots, microbes and fauna strongly interact [10]. This “space” is further classified into ectorhizosphere, the portion outside the root, and end rhizosphere, which includes the root epidermis and cortex, where endophytic microorganisms can be present. In rhizosphere relationships, plant growth promotion microorganisms (PGPM) may colonize the rhizosphere, the surface of the root, or even superficial intercellular spaces [11]. In endophytic relationships, PGPM actually reside within apoplectic spaces inside the host plant [12].

A part from the different mechanisms that directly affect plant nutrient uptake, other PGPM-mediated mechanisms may indirectly reverberate on plant nutrition: enhanced resistance to drought, salinity and water logging [13], increasing root growth through production of ACC deaminase [14], or of hormones like auxins (IAA), abscisic acid (ABA) [15], gibberellic

acid (GA) and cytokinin's [16], enhanced resistance to oxidative stress, production of water-soluble B group vitamins [17]. In this section, a brief review is presented on the role in nutrient uptake for the PGPM that can be potentially, or are already, used to produce biofertilizers, according to the prevalent nutrient element provided to the plant microbiology in the senior universities may have started in early 80s.

Horticultural Crops Production through Organic Farming

1. Concept and Importance

Microbial inoculants or Biofertilizer are important components of organic farming, which help to nourish the crops through required nutrients. These microbes help to fix atmospheric nitrogen, solubilize and mobilize phosphorus, translocated minor elements like zinc, copper, etc., to the plants, produce plant growth promoting hormones, vitamins and amino acids and control plant pathogenic fungi, thus helping to improve the soil health and increase crop production. Biofertilizers like Rhizabium, azotobacter, Azospirillum and blue green algae (BGA) are in use since long. These organisms fix atmospheric nitrogen and supply it to plants. Hence, bio fertilizers are to be of some extent. The bacterial biofertilizers contribute 20-30 kg N/ha/season. Rhizobium inoculants are used for leguminous crops. Azotobacter can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. Azospirillum inoculants are recommended mainly for sorghum, millets, maize, sugarcane and wheat. Blue green algae belonging to general Nostoc, Anabaena, tolypothrix and Aulosira fix atmospheric nitrogen and are used as inoculants for paddy crop grown both under upland and low land conditions. However, the inoculants are most effective under low land rice cultivation and contribute 20-30 kg N per ha per season with better quality of grains. Anabaena in association with water fern Azolla contributes nitrogen up to 60 Kg/ha/season and also enriches soils with organic matter. Freelifving in soil and symbiotic with plants and directly or indirectly contribute towards the nitrogen nutrition of the plants. There are following advantages in using bio fertilizers in horticultural (vegetable) crops [18].

- They help in the establishments and growth of crop plants and trees.

- They enhance biomass production and grain yields by 10-20 percent.
- They are useful in sustainable agriculture.
- They are suitable in organic farming.
- They play an important role in agro forestry survival system.

The benefits of bio- fertilizers [6] are

- Increase crop yield by 20-30%.
- Replace chemical nitrogen and phosphorus by 25 %.
- Stimulate plant growth.
- Activate the soil biologically.
- Restore natural soil fertility.
- Provide protection against drought and some soil borne diseases.
- Cost effective.
- Supplement to fertilizers.
- Eco-friendly (friendly with nature).
- Reduces the costs fertilizers use, especially regarding nitrogen and phosphorus.
- Bio fertilizers will help solve such problems as increased salinity of the soil and chemical run-offs from the agriculture fields.

2. Nutrient recycling

Bio-fertilizers facilitate the continuous and long-term soil improvement, recycling and availability of nutrients and minerals essential for the survival, growth and bearing of fruits of a wide variety of plants and trees.

3. Environmental Friendly

Instead of polluting the environment, agriculture and industrial wastes are processed into biodegradable fertilizers. These fertilizers are enhanced with microorganisms that continuously grow and act upon the soil to increase the availability and uptake of water, nutrients and minerals.

4. Cheaper Supplement to Inorganic Fertilizers

Generally, these bio-fertilizers can supply the nutrients requirement of the plants from as low as 30% to as high as 100%. This would imply significant savings and additional profit.

The Main Bio-Fertilizers used in Horticultural Crop Production

A. <i>Rhizobium</i> spp.:	B. <i>Azospirillum</i> :
C. Blue Green Algae:	D. <i>Azolla</i> :
E. Phosphate Solubilising Biofertilizer:	F. <i>Azotobacter</i>
G. Mycorrhizal fungi	Ectomycorrhiza, Endomycorrhiza: Vesicular Arbuscular Mycorrhiza:
H. Genetically Engineered Microbes:	

Phosphate-Solubilizing Microbial Inoculants

Application of *Azospirillum* + Phosphate-Solubilizing Bacteria + 5% Cow Urine + 50% recommended dose of "N" through Vermicompost + 50% recommended dose of NPK fertilizer was most effective in increasing vegetative growth parameters, such as plant height, number of branches, plant spread, as well as flower yield parameters like number of flowers, flower diameter, fresh and dry weight of flowers, flower yield, flowering duration, shelf life, and it also had the maximum benefit: cost ratio.

Thus, use of inorganic fertilizers conjointly with bio-fertilizers and organic manures resulted in excellent vegetative growth and flower yield attributes in African marigold [19].

Biofertilizers exploitation and nutrients profile of crops, A key advantage of beneficial microorganisms is to assimilate phosphorus for their own requirement, which in turn available as its soluble form in sufficient quantities in the soil. *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Fusarium*, *Sclerotium*, *Aspergillus* and *Penicillium* have been reported to be active in the solubilization process [20]. A phosphate-solubilizing bacterial strain NII-0909 of *Micrococcus* sp. has polyvalent properties including phosphate solubilization and siderophore production [21]. Similarly, two fungi *Aspergillus fumigatus* and *A. Niger* were isolated from decaying cassava peels were found to convert cassava wastes by the semi-solid fermentation technique to phosphate biofertilizers [21]. *Burkholderia vietnamiensis*, stress tolerant bacteria, produces gluconic and 2-keto-gluconic acids, which involved in phosphate solubilization [22]. *Enterobacter* and *Burkholderia* that were isolated from the rhizosphere of sunflower were found to produce siderophores and indole compounds (ICs) which can solubilize phosphate.

Role of Bio Fertilizers in Some Horticultural Crops

An experiment conducted by Tomato cultivars (Sultana-7 and Super Strain-B) were germinated with various concentrations (0–200 mM) of NaCl. Seed germination in the Super Strain-B was promoted by 25 mM NaCl. However, the germination of both cultivars was progressively inhibited by 50 and 100 mM NaCl and obstructed at 200 mM NaCl, and this response was more pronounced for Sultana-7.

Therefore, SuperStrain-B was selected for further investigation, such as growth under NaCl stress (50 and 100 mM) and inoculation with vesicular-arbuscular mycorrhizal fungus (*Glomus fasciculatum*, VAMF). The leaves of Super Strain-B showed reduced mineral (N, P, K and Mg) uptake and K/Na ratio as well as increased Na uptake and N/P ratio in response to salinity. Moreover, salinity decreased the chlorophyll (Chl) contents coupled with an increase in Chl a/b, Hill-reaction activity, and quenched Chl a fluorescence emission. These changes reflect a disturbance in the structure, composition and function of the photosynthetic apparatus as well as the activity of photosystem 2. The superoxide dismutase and peroxidase activities of leaves were enhanced by salinity, whereas the catalase activity was decreased. Leaf polysaccharides and proteins, as well as shoot biomass, also decreased as a result of salinity, but the total soluble sugars and root to shoot ratio improved. VAMF enhanced both the photosynthesis and productivity of plants; thus, VAMF may alleviate the adverse effects of salinity in plants by increasing their salt tolerance. Although mycorrhizal infection showed a negative correlation with salinity, it remained relatively high (21 and 25%) at 100 mM NaCl.

Mycorrhizae are mutually beneficial (symbiotic) relationships between fungi and plant roots [23]. VAM fungi infect and spread inside the root. They possess special structures known as vesicles and arbuscules. The plant roots transmit substances (some supplied by exudation) to the fungi and the fungi aid in transmitting nutrients and water to the plant roots. The fungal hyphae may extend the root lengths 100-fold.

The hyphae reach into additional and wetter soil areas and help plants absorb many nutrients, particularly the less available mineral nutrients such as phosphorus, zinc, molybdenum and copper. Some VAM fungi form a kind of sheath around the root, sometimes giving it a hairy, cottony appearance. Because they provide a protective cover, mycorrhizae increase seedling tolerance to drought, to high temperatures, to infection by disease fungi and even to extreme soil acidity.

Application of VAM produces better root systems which combat root rotting and soil-borne pathogens. The greatest growth response to Mycorrhizal fungi is probably in plants in highly weathered tropical acid soils that are low in basic cations and P and may have toxic levels of aluminum. Plants that have coarse or limited root systems should benefit the Most.

Table 1. Effects of plant growth-promoting rhizobacteria (PGPR) application on vegetable crops.

Crop	PGPR (species/strain)	Application mode	Experimental conditions	Effects	References
Broccoli	Brevibacillus uszeria	Root-dipping of seedlings for 60 min (108 CFU ml ⁻¹)	Field	Increased yield, plant weight, head diameter, chlorophyll content, macronutrient and micronutrient uptake	Yildirim <i>et al.</i> (2011)
	<i>Pseudomonas fluorescens</i> strain MTCC103d	Root-dipping of seedlings for 5 min Pots, greenhouse conditions	Pots, greenhouse conditions	Enhanced plant growth, nutrient uptake, and broccoli's yield when combined with the recommended dose of superphosphate fertilizer	Tanwar <i>et al.</i> (2014)
Cabbage	<i>Pantoea agglom</i> erans strain RK-92	Seed-dipping (108 CFU mL ⁻¹) before planting	Pots, greenhouse conditions	Enhancement of growth, nutrient, and hormone content	Turan <i>et al.</i> (2014)
Carrot	<i>Rhizobium leguminosarum</i> strain PEPV16b	Seed-dipping (1.5 10 ⁹ CFU per seed) before planting	Pots, greenhouse conditions	Increased dry matter of shoots and roots, increased root length and root hair number	Flores-Félix <i>et al.</i> (2013)
Cucumber	<i>P. agglomerans</i> strain FFd	Foliar spray (108 CFU mL ⁻¹), at ten days interval, for three times during seedling development	Greenhouse conditions (unheated)	Increased plant length, dry matter and mineral content of fruits, as well as number, weight and width of fruits	Dursun <i>et al.</i> (2010)

Lettuce	Serratia plymuthica strain RR-2-5-10d, Stenotrophomonas rhizophila strain e-p10d, Pseudomonas extremorientalis strain TSAU20d, P. fluorescens strain PCL1751d and SPB2145d	Seed-dipping for 15 min (108 CFU mL ⁻¹)	Pots, greenhouse under semi-controlled environmental conditions	Increased plant height (up to 15%), dry weight (up to 62%) and fruit yield (up to 32%) in a soil amended with 3% NaCl	Egamberdieva <i>et al.</i> (2011)
	Microbial consortium (Bacillus cereus strain AR156a, Bacillus subtilis strain SM21a, and Serratia sp. strain XY21d)	Soil drench (1011 CFU per plant)	Pots, greenhouse conditions	Induced systemic tolerance to drought stress, by maintaining photosynthetic efficiency and root vigor and increasing some of antioxidant activities (i.e. superoxide dismutase activity)	Wang <i>et al.</i> (2012)
	Azospirillum brasilense strain Sp7b and Sp245b, Herbaspirillum seropedicae	Seed-dipping for 60 min (1011 CFU per seed)	Petri dishes, temperature-controlled growing cabinet	Increased germination, increased length and weight of roots, improved vigor index of germinating seeds	Mangmang <i>et al.</i> (2015a)
	Rhizobium leguminosarum biovar phaseoli strain P31b	Seed-dipping for 60 min low fertility	Field	The beneficial effect on growth in a soil with low fertility	Chabot <i>et al.</i> (1996)
	Beneficial effect on growth in a soil with low fertility				
	Rhizobium leguminosarum strain VF39SMB	Seed-dipping for 60 min	Petri dishes, controlled growth chamber	The beneficial effect on early seedling growth and increase in root length	Noel <i>et al.</i> (1996)
	Agrobacterium sp.b, Alcaligenes piechaudi, Comamonas dovorans strain 26c	Direct inoculation of axenic seedlings	Petri dishes, controlled growth chamber	Root promotion or inhibition is correlated to indole-3-acetic acid levels produced by rhizobacteria (indole-3-acetic acid overproduction is deleterious to plants)	Barazani and Friedman (1999, 2000)
	Serratia proteamaculans strain ATCC35475d, Rhizobium leguminosarum bv. viciae strain 128C56Gb	Root inoculation	Greenhouse conditions	Alleviated the negative effects of salinity on the plant, increased photosynthesis and total chlorophyll content, stomatal conductance, fresh weight, leaf area, N, P, and K uptake, and activity of some antioxidant enzymes	Han and Lee (2004)
	Rhizobium leguminosarum bv. viciae strain 128C56Gb A. brasilense strain Sp245b Pseudomonas	Seed-dipping for 180 min (107 CFU per seed) Soil drench (1010 CFU per plant; two treatments)	Pots, greenhouse conditions	severely saline conditions and increase in the antioxidant enzyme activities in response to severe salinity	Kohler <i>et al.</i> (2009)

	mendocinaPall eronistraind				
	A. brasilense strain Sp245b	Seed-dipping for 90 min (109 CFU per seed)	Pots, greenhouse conditions (under natural light)	Promoted early germination, seedling settlement of seeds and increased leaf dry weight, leaf area and chlorophyll content when plants were grown at 40 mmol-3 NaCl	Fasciglione <i>et al.</i> (2012)
	A. brasilense strain Sp7-Sb	Seed-dipping for 60 min and re-inoculation by drenching 7 days later	Petri dishes, temperature-controlled growing cabinet	Increased in the number of leaves, seedling height, and root length	Mangmang <i>et al.</i> (2015b,c)
Pepper	Bacillus strains P. fluorescens strain IISR-6d, IISR-11d and IISR-51	Seed-dipping for 30 min (108 CFU mL ⁻¹)	Pots, greenhouse conditions	Increased fresh root and shoot weights, stem diameter, dry root, and dry shoot weights increased	Kokalis-Burelle <i>et al.</i> (2002) Paul and Sarma (2006)
	Bacillus licheniformis strain K11a	Soil drench (7 108 CFU mL ⁻¹ per plant; one treatment)	Pots, greenhouse conditions	root length, total root area, and the number of root tips Increased in the root and shoot length and dry weight under drought stress	Lim and Kim (2013)

a. Bacilli. b. Alpha proteobacteria. c. Betaproteobacteria, d. Gammaproteobacteria e. Actinobacteria

Source: (Maurizio and, Ricardo, 2015).

Vegetable Crops

I. Tomato (*Solanum lycopersicum*)

Tomato requires higher amounts of NPK, the deficiency of any one of these nutrients restrict its growth and yields severely. Like other horticultural crops, tomato growth is also influenced by PGPR application [24]. For instance, the results have shown that seedling inoculation with Burkholderia tropic resulted in effective root colonization of tomato plants which further spreads to aerial tissues [25]. Also, the significant colonization led to a consistent increase in tomato production in two different crop seasons. Moreover, the PGPR recovered from tomato rhizosphere facilitated the growth and enhanced shoot length, root length, fresh weight, dry weight and P content of tomato plants. Additionally, the IAA positive strains of Pseudomonas when used as inoculant, enhanced seedlings and fruit yields of tomato. In a recent study conducted both in pots and fields.

The results indicated significant differences among the kinds of biofertilizer and their combination with inorganic fertilizers [26]. The plant height, considered to be an important factor to judge the vigor was found increased to a significant level with the application of organic manures in both the seasons. The treatment T (Azospirillum + 75% N + 100% PK) recorded the tallest plants (72.6 cm) and the highest number of

branches plant⁻¹ (8.80). The increase in growth characters might be due to the fact that the Azospirillum inoculated plants were able to absorb nutrients from solution at faster rates than uninoculated plants resulting in accumulation of more dry matter, N, P and K in the stems and leaves [26].

Similar to plant height, the branching was increased due to the application of biofertilizer. The number of branches plant⁻¹ is of considerable importance and it has a positive association with yield. Azospirillum + 75% N + 100% PK registered the highest number of branches plant⁻¹ (8.80). Greater the number of secondary branches more will be the number of flowers produced which is ultimately going to reflect on the total number of flowers produced [26].

In tomato, large size fruits are preferred. In the present study also, application of Azospirillum has recorded maximum fruit size including more number of fruits. Azospirillum + 75% N + 100% PK recorded 33.7 fruits per plant with an average fruit weight of 35.63 g. Azospirillum inoculation benefits plant growth and increases the yield of crops by improving root development, mineral uptake and plant water relationship. In addition to nitrogen fixation, Azospirillum also produces growth promoting substances like IAA and GA and these hormones go a long way in enhancing the crop

growth. The source of IAA from Azospirillum might have increased the various endogenous hormonal levels in plant tissue, that was responsible for the enhanced pollen germination and tube growth, which ultimately increased the fruit set.

The higher fruit set may also be due to a higher percentage of productive flowers [26]. The highest yield was also recorded with the application of Azospirillum + 75% N + 100% PK (43.85 t ha⁻¹) due to the high yield contributing characters like a number of fruits plant⁻¹ and average fruit weight. The N availability and N content of the plants were enhanced due to the application of Azospirillum. The increased

uptake of available N influences the growth characters since N is the chief constituent of a protein essential for the formation of protoplasm, which leads to cell division, cell enlargement and ultimately resulting in increased plant growth and yield. Azospirillum fixes the atmospheric nitrogen in the soil enhances the production of phytohormones like substances and increased uptake of nutrients such as phosphorus and potassium. The biological activity of the microorganisms would have helped the soil status to become ready to serve zone for essential nutrients to plant's root system [26].

Table 2. Effect of Biofertilizer on the performance of tomato (Mean data for two years)

S.No	Treatments	Plant height (cm)	No. of branches plant ⁻¹	No. fruits per plant ⁻¹	Fruit weig ht(g)	Yield(t ha ⁻¹)	BCR (o Bri)	TSS
T ₁	Recommended NPK	63.2	7.72	31	32.57	41.55	3.56	4.37
T ₂	Azospirim 75%N+100%NPK	72.6	8.8	33.7	35.63	43.85	3.76,	4.45
T ₃	Azospirim+							
T ₄	Recommended NPK Azotobacter	70.4	8.59	32.3	34.23	41.53	3.51	4.4
T ₅	+75%N+100%PK Azotobacter+	59.2	7.95	27.1	30.13	35.19	3.02	4.36,
T ₆	Recommended NPK	63.9	7.02	25.8	28.63	31.6,	2.67	4.41
T ₇	PSB+75%P+100%NK	64.1	7.25	26.8	28.67	31.9,	2.73	4.35
T ₈	PSB+ Recommended NPK	70.5	7.43	27.8	30.1	37.08,	3.13	4.41
T ₉	VAM+75%P+100%NK	64.2	7.17	26	29.43	31.49	2.7	4.34
T ₉	VAM+Recommended NPK	65.8	7.07	26.9	29.37	33.93	2.83	4.41
SED		1.47	0.19	0.63	0.53	0.57	-	0.24
CD(5%)		3.12	0.4	1.32.	1.11	1.21	-	Ns

2. Chili

The response of chili was studied to VAM inoculation in the black clayey soil [18]. Seedlings were inoculated in nursery beds with four different VAM fungi *G. fasciculatum*, *G. albidum*, *G. macrocarpum* and isolate 1-14. They found that *G. fasciculatum* caused maximum increase in growth, P, Zn content, flowering and yield. The yield of *G. fasciculatum* inoculated plants at 37.5kg pha⁻¹ was more than the un-inoculated at 75kgp/ha. also studied similar response of cultivars 'Pusa Jwala' and X-235 to *G. fasciculatum* inoculation and recorded an increase in mycorrhizal colonization, plant height and dry matter in 69 days old inoculated plants. In

AICVIP trials, it has also been recommended that application of Azospirillum at 2kg/ha as basal application in combination with 75 percent recommended a dose of nitrogen (i.e. 56kg/ha) under Tamil Nadu conditions increased yield.

It was reported that among the combination of organic and inorganic fertilizers, the highest mean available phosphorus content recorded with 200 percent recommended NPK +FYM at 10t/ha followed by vermin compost at 2.5t/ha at the same level of inorganic fertilizers [18]. The available potassium content in the soil was maximized with 200 percent recommended a dose of NPK+FYM at 10t/ha followed by Azospirillum at 5kg/ha along with 200 percent

recommended a dose of NPK. When we compared the yield of chili treated by bio-fertilizers is completely different from those of untreated one (Table.1).

3. Cucumber and Sugar Beet

The germination of sugar beet seeds on moist filter paper was very weak. The treatments of soils with bio-fertilizers had increasing effects. The effects of bio-fertilizers on germination intensity were examined. The final germination of plants was recorded after 6 days. The energy of germination increased when bio-fertilizers were applied. The results are shown in the (Table 3) [27].

Regarding cucumber, both Al concentrations showed a toxic effect on plant growth. The effects were stronger with higher Al concentration. The tissue of plants with Al was looser, the leaves were thinner. It shows the results (Table 3) and (Figure 5). When the bio-fertilizers were used the toxic effect reduced, which is presented in (Tables 5- 6). In some cases, chlorosis was observed. Chlorosis is one of the symptoms of Al toxicity (Figure 3-5). The chlorophyll contents of the plants were higher when the bio-fertilizers were added to the nutrient solution (Table 5). The bio-fertilizers had an increasing effect on the photosynthetic pigments level [27].

Table 3. Yield increase in vegetables at farmer's fields at different locations of India

Place	Treatment	Crops	Yield		Increase in yield over untreated (%)
			Control (q/ha)	Treated (q/ha)	
Umri Nagpur	Azotobacter	Okra	24.8	26.0	8.3
AmbadaNarkhed	PSM	Brinjal	125.0	137.5	10.0
Tivara Amravati	Azotobacter	Brinjal	190.0	220.0	15.8
NagapurSweagram	Azotobacter	Chilli	14.5	16.0	10.3
Bopapur Nagpur	PSM	Cauliflower	34.0	36.5	7.35
ChikhaliKatol	Azotobacter	Cauliflower	32.5	34.5	6.2
ChicholiParshivani	Azotobacter	Okra	23.4	25.5	8.97

Table 4. The energy of germination of sugar beet seeds germinated in acidic soil, treated with Al₂(SO₄)₃, Phylazonit MC® and BioNitroPhos® and Bioplasma®

	3. day	4. day	5. day	6. day
Control	27%	41%	79%	100%
Phylazonit	69%	92%	97%	100%
BioNitroPhos	67%	88%	97%	100%
Bioplasma	42%	71%	91%	100%

Table 5. Effects of different Al- concentrations on the weight of shoot and root of sugar beet (n=3 ± s.e.) (g plant-1)

Treatment	Shoot weight	Shoot dry weight	Root dry weight
Control	2.05±0.37	0.17±0.07	0.15±0.01
10-4 Al ₂ (SO ₄) ₃	1.94±0.16	0.16±0.06	0.13±0.05
10-3 Al ₂ (SO ₄) ₃	1.13±0.08	0.10±0.007	0.07±0.006

Source :(Marianna et al., 2009)

Table 6. Effects of bio-fertilizers on the root weight of cucumber under different Al- stress condition (n=3 ± s.e.) (g plant-1)

	Root Dry Weight	
	10-4 M Al ₂ (SO ₄) ₃	10-3 M Al ₂ (SO ₄) ₃
Controll	0.9±0.60	0.10±0.006
Phylazonit	1.04±0.09	0.21±0.01
BioNitroPhos	0.97±0.65	0.16±0.09
Bioplasma	0.95±0.68	0.15±0.12

Table 7. Effects of different biofertilizers days after the germination

	Days after the germination		
	17. day	20. day	23. day
Control	48,9±0,70	44.2±0.92	38.8±1.80
10 ⁻⁴ Al ₂ (SO ₄) ₃	44,8±1,55	44.3±1.60	36.6±1.05
10 ⁻⁴ Al ₂ (SO ₄) ₃ +Phylazonit	42,3±0,81	40.8±1.19	41.0±1.49
10 ⁻⁴ Al ₂ (SO ₄) ₃ +BioNitroPhos	41,5±0,45	40.2±2.15	38.0±0.47
10 ⁻⁴ Al ₂ (SO ₄) ₃ +Bioplasma	42,8±0,19	41.9±0.13	40.7±0.33

Source:(Marianna et al., 2009)

Both Al concentrations showed a toxic effect on plant growth. The effects were stronger with higher Al concentration. As the tissue of plants

grown in solution with Al was looser, the leaves were thinner. The bio-fertilizers increased leaves

weight only under low Al concentration, but not under high Al concentration [27].

4. Spinach (*Spinacia oleracea* L.)

Microbiological changes

Data show that although Azotobacter, phosphate dissolving bacteria and total bacterial population occurred in high densities in the rhizosphere of un-inoculated spinach plants growing in the fertile clay loam soil (Table 8). Inoculating spinach seeds with Azotobacter chroococum and phosphate dissolving bacteria enriched the rhizosphere of spinach plants with such bacteria during the first 45 days from sowing in the two successive cultivation seasons. The highest increase in the counts of free-living nitrogen fixers (Azotobacter chroococum) was recorded in T7, T6, T3 and T2, and T7, T6, T2 and T3 in descending order in the first and second seasons, respectively. The increase in Azotobacter chroococum counts in the above-mentioned treatments could be attributed to the presence of adequate amounts of available phosphorous resulting from either super-phosphate applied or inoculation with phosphorein, as well as from the stimulative effect of the plant rhizosphere on the adjacent microorganisms. For phosphate dissolving bacteria (*Bacillus megatherium* var *phosphaticum*) the highest counts of such organism were recorded in T4, T5, T6, and T7, and T4, T5, T6 and T7 in descending order in the first and second season, respectively. In this regard, on soybean, on legume crops, on okra yield, on pigeon pea mentioned that seed inoculation with PDB increased PDB density in the plant rhizosphere [28].

Growth parameters and yield

The effect of both bio (i.e. Azotobacter chroococum & phosphoric) and N and P chemical fertilizers (singly or in combination) on some growth parameters of spinach plants expressed as plant height, number of leaves, leaf area, fresh and dry weight of plant as well as total yield is shown in (Table 9). The results indicated that seed inoculation with phosphorein (300 g) and fertilizing the plants with nitrogen at the rate of 40 kg/ fed + P₂O₅ at 15 or 7.5 kg/ fed (i.e., T4 & T5) followed by T2 (20 kg N/ fed + 22.5 kg P₂O₅ / fed + 300 g Azoto) induced significant increase in plant growth and yield in the two successive growth seasons, in comparison with the control treatment (received mineral fertilizer alone at the

rate of 40 kg N/ fed + 22.5 kg P₂O₅ / fed) and other treatments (Assiouty and Abo-sedera, 2007). Similar results were obtained on other vegetable crops, cabbage, Azotobacter, faba bean, tomato and on beans for phosphorein [28]. The stimulative effect of these microorganisms (Azotobacter chroococum and Phosphoric) might be attributed to its efficiency in supplying the growing plants with biologically fixed nitrogen, dissolved immobilized phosphorus and produced phytohormones, which could stimulate nutrients absorption as well as photosynthesis process which subsequently increased plant growth and yield [29]. The results recorded in (Table 9) confirmed this conclusion. Although it is obvious also from the same table that the plants produced from seeds inoculated with 300 g/ fed Azotobacter + 300 g/ fed phosphor in inocula alone without N and P fertilization (T7) were the lowest in plant growth and yield. This could indicate that bio-fertilizers can be partially, but not completely, substitute chemical fertilizers [28].

Pigments content of spinach leaves

Results in (Table 10) indicate the effect of inoculation with Azotobacter and Phosphorein bio-fertilizers singly or in combination with different rates of the mineral fertilizers (N and P) on chlorophyll and carotenoids content. It is evident from the obtained data that the plant leaves treated with 300 g/ fed phosphorein and fertilized with 40 kg N and 15.0 or 7.5 kg P₂O₅/fed (Treatments 4 and 5) or 300 g/ fed Azotobacter inoculum plus 20 kg N/ fed + 22.5 P₂O₅ / fed (T2) contained more chlorophyll (b) and carotenoids in the two growing seasons compared with the control treatment (T1) as well as the other treatments. On the other hand, chlorophyll (a) and total chlorophyll (a and b) contents did not show a significant response to the studied treatments. In this regard, on sugar beet and cauliflower had been reported that the highest chlorophyll and carotenoid content in the plant leaves were achieved by inoculation with Azotobacter) [28].

Table 8. Effect of bio-and chemical fertilizers on total bacterial counts (T.C), Azotobacter (Azoto) and of phosphate-dissolving bacterial (PDB) counts after 45 days from sowing in 2002-2003 and 2003-2004 seasons (mean of four replicates)

Treatments	2002-2003 (season)			2003-2004 (season)		
	Tot. count x10 ⁸	Azotox10 ⁴	PDBx10 ⁴	Tot. count x10 ⁸	Azotox10 ⁴	PDBx10 ⁴
T1-40N+22.5 P ₂ O ₅ ...control	35	18	26	33	18	25
T2-20N+22.5 P ₂ O ₅ +300(g) Azotobacter	45	250	35	44	240	36
T3-10N+22.5 P ₂ O ₅ +300(g) Azoto.	40	280	30	41	200	31
T4-40N+15 P ₂ O ₅ +300(g) phosphorein	52	30	400	45	31	400
T5-40N+7.5 P ₂ O ₅ +300(g)phospho	55	35	350	50	34	350
T6-20N+15 P ₂ O ₅ +300(g)Azoto+300(g)phospho	58	320	300	51	370	320
T7without(NP0fertilizer+300Azoto.+300phospho	48	400	300	46	380	250

Table 9. Effect of bio-and chemical fertilizers on some growth parameters and yield of spinach in 2002-2003 and 2003-2004 seasons at harvest (mean of four replicates)

Treatments	2002-2003 (season)						2003-2004 (season)					
	Plant heig-ht cm.	No. of leaves/ plant	Leaf area cm ²	F.W/ Plant (g)	D.W/ Plant (g)	Total yield Ton/fed	Plant height cm.	No. of leaves/ plant	Leaf area cm ²	F.W/ Plant (g)	D.W/ plant (g)	Total yield Ton/ fed
T1 - 40 N + 22.5 P ₂ O ₅ Control	54.7	42.7	132.4	336.3	31.6	7.398	67.7	40.3	119.5	352.3	32.9	6.694
T2 - 20 N + 22.5 P ₂ O ₅ + 300 (g) Azotobacter	55.0	47.8	181.9	420.0	38.3	9.240	71.3	47.8	162.8	491.6	41.6	8.849
T3 - 10 N + 22.5 P ₂ O ₅ + 300 (g) Azto.	55.0	45.7	149.8	395.0	34.0	7.898	68.0	45.7	140.2	391.1	33.6	7.430
T4 - 40 N + 15.0 P ₂ O ₅ + 300 (g) Phosphorein	59.7	55.5	205.5	427.7	51.3	9.409	73.3	65.0	186.7	518.9	56.5	9.859
T5 - 40 N + 7.5 P ₂ O ₅ + 300 (g) Phospho.	57.0	53.5	185.1	423.3	41.0	9.312	73.3	52.0	173.8	494.5	52.9	9.394
T6-20 N+15.0 P ₂ O ₅ + 300 (g) Azto. + 300 (g) Phospho.	53.7	41.3	130.9	300.0	29.7	6.900	67.3	39.5	114.1	335.9	32.3	6.382
T7-Without (NP) fertilizer+ 300 Azto.+ 300 Phospho.	50.7	30.7	88.1	183.0	15.2	5.132	57.3	34.3	102.6	201.1	21.8	4.625
L.S.D. 5%	1.5	1.7	4.4	1.1	3.6	0.161	1.7	1.4	3.7	1.9	3.6	0.158

Table 10. Effect of bio-and chemical fertilization on photosynthetic pigments contents of spinachleaves (mg/100 g fresh wt) in 2002-2003 and 2003-2004 seasons at harvest (mean of four replicates)

Treatments	2002-2003 (season)				2003-2004 (season)			
	Chlorophyll a	Chlorophyll b	Total (a+b)	Carotene	Chlorophyll a	Chlorophyll b	Total (a+b)	Carotene
T1-40 N + 22.5 P ₂ O ₅ Control	51.5	23.3	74.8	33.5	52.0	23.2	75.2	24.0
T2-20 N + 22.5 P ₂ O ₅ + 300 (g) Azotobacter	53.1	25.9	79.0	35.2	53.0	25.1	78.1	25.5
T3-10 N + 22.5 P ₂ O ₅ + 300 (g) Azoto.	52.0	23.5	75.5	34.5	52.5	23.6	76.1	25.0
T4-40 N + 15.0 P ₂ O ₅ + 300 (g) Phosphorein	58.0	26.5	84.5	36.6	54.2	29.8	84.1	26.5
T5-40 N + 7.5 P ₂ O ₅ + 300 (g) Phospho.	53.6	26.4	80.0	36.4	54.1	25.5	79.6	26.3
T6-20 N+15.0 P ₂ O ₅ +300 (g) Azoto. +300 (g) Phospho.	51.5	22.8	74.3	33.0	51.0	22.9	73.9	23.6
T7-Without (NP) fertilizer+300Azoto. +300 Phospho.	45.7	21.6	67.3	29.5	45.0	21.0	66.0	21.5
L.S.D. 5%	NS	2.1	NS	1.7	NS	1.8	NS	1.7

5. Cabbage (*Brassica oleracea*)

Brassica oleracea (cabbage) is yet another important vegetable that has greatly been influenced by PGPR application [30]. For instance, it was evaluated [31] the impact of *B.megaterium* strain TV-91C, *P. agglomerans* strain RK-92, and *B. subtilis* strain TV-17C on

growth, nutrient, and hormone content of cabbage seedlings. The PGPR application in general increased fresh and dry shoot and root weight, stem diameter, seedling height, chlorophyll reading values, and leaf area of cabbage seedlings compared with the control plants. Among PGPR, *B. megaterium* resulted in highest seedling nutrients and enhanced all

growth parameters, but the maximum increase in leaf area, gibberellic acid, salicylic acid, and indole IAA were obtained with *P. agglomerans*. Seed inoculation with *B. megaterium* increased fresh and dry shoot and root weight by 32.9, 22.6, 16, and 35.69%, respectively. Inoculations also increased stem diameter, seedling height, and SPAD chlorophyll values by 47.5, 27.2, and 5.8%, respectively.

The application of biofertilizers like *Azospirillum* and *Azotobacter* has given favorable response for enhancing yield. It was observed 8 to 15 per cent increased the yield of 'Golden Acre' by inoculation with *Azotobacter* and *Azospirillum* alone and in the combination. Inoculation of Bio-fertilizers like *Azotobacter* and *Azospirillum* increased seed yield in cabbage cv. Golden Acre was also reported [18].

Under All India Coordinated Vegetable Improvement Project trials, it was observed that for getting optimum yield (526q/ha) and C: B ratio (1:2.09), seed treatments with *Azospirillum* (500q/ha) + soil application (5kg/ha) + seedling dipping (1.0kg/ha) + application of 60kg N/ha have been recommended for variety 'Pride of India' under Sloan condition. However, some experiment was conducted at Kaymore Plateau and Satpura hills of Madhyapradesh on variety 'Pride of India' revealed that application of *Azospirillum* seedling at 1.0 kg/10-liter water + soil application at 5kg/ha supplemented with 75 percent of the recommended dose of N (i.e., 180kg/ha), [18].

6. Eggplant (*Solanum melongena*)

Plant height, number of branches per plant and yield attributes were found maximum when eggplants were grown in soils treated jointly with varying level of fertilizers, PSB, *Azospirillum* and *Azotobacter*. Physiological functions like photosynthesis and transpiration rate, stomatal resistance, internal CO₂ concentration and leaf temperature at the flowering stage were highest at 100% RDF applied together with PSB, *Azospirillum* and *Azotobacter*. The PGPR have also been reported to enhance the yield and quality of eggplants under stressed environment. As an example, the inoculation effect of PGPR, *Pseudomonas* sp. DW1 on growth, mineral uptake and physiological activities of the antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of eggplant plants grown under salinity

stress was found variable. *Pseudomonas*-inoculation increased the germination percentage and also enhanced the growth relative to uninoculated eggplants. While comparing the impact of *Pseudomonas* inoculation and salinity on eggplants, it was found that salinity significantly decreased K⁺ concentration and increased Na⁺ concentration but did not significantly decrease Ca²⁺ content in shoots of eggplants. In contrast, *Pseudomonas* sp. inoculated plants had higher shoot Ca²⁺ but no increase in shoot Na⁺ concentration compared to non-inoculated plants grown under salinity stress. Instead, *Pseudomonas* application reduced NaCl in plants grown in soils treated even with 2 and 3 g kg soil⁻¹ NaCl.

The effects of *Azotobacter chroococum* and *Glomus fasciculatum* on the growth of brinjal (*Solanum melongena* L.) were observed [18]. The seeds were soaked in bacterial suspension for 12 hours and then sown in nursery beds. Mycorrhizal inoculums containing 250 spores per 100 ml of culture were placed on top layers of nursery soil. The treated plants were transplanted after 30 days and 50 percent recommended fertilizers were used. The result indicated that mixed inoculums showed a significant response to plant growth compared to the individual inoculation.

It was reported that 25 per cent of recommended phosphorus can be saved if brinjal plant is inoculated both with *Glomus fasciculatum* and *Azospirillum* [18].

7. Pumpkin

The studies on the effect of nitrogen and phosphorus with *Azospirillum* and phosphobacteria in pumpkin revealed that application of 9 kg nitrogen and 18 kg phosphorus per hectare along with bio-fertilizers recorded the highest fruit yield of 16.90 and 17.79 kg per plant [18].

Fruit crops

1. Banana

Banana is always considered as a gross feeder and requires large amounts of nitrogen (N) and potassium (K) followed by phosphorus (P), calcium (Ca) and magnesium (Mg) to maintain high yields [32,33]. The physiological limitation in N-storage capacity is also a constraint for commercial cultivation of this crop. The deficiency symptoms quickly develop and extra N must be frequently applied even on fertile soil

(Robinson, 1996). The excess use of chemical fertilizer is undesirable, because (1) production of chemical fertilizers is a costly process, (2) most of the energy is provided by the consumption of non-renewable fossil fuels, and (3) considerable pollution is caused through both the production and use of mineral N-fertilizers, and this is exacerbated by the relatively low efficiency of their uptake by the plants [34].

Fluorescent pseudomonas bacteria which added as biofertilizer (i.e. 0, 1, 2, 3 L/plant/year) and FYM application (i.e. 50, 75, 100 kg/plant/year) significantly increased bunch weight, number of finger/bunch, finger weight, finger length, finger diameter, total sugars, starch, acidity and T.S.S [35]. The best treatment was 100 kg FYM + 3L Bio. As well as it was indicated that inoculation of banana plants cv. Gaint with Azospirillum twice (sucker + soil) produced the highest number of hands/bunch and maximized the yield (69.15 t/ha). However, inoculation with Azotobacter increased T.S.S. and reducing sugar contents, while both total sugar and acidity contents were not constant in banana fruits.

It was suggested that bacterial spp Azotobacter and Asospirillum inoculation alleviates drought stress through improving vegetative growth, photosynthetic pigments and chemical composition in Eggezi and Picual olive cvs. In this situation it could be concluded that, using biofertilizer can solve the problem of soil pollution partial as a result of using excess of chemical fertilizers and produce safety foods with high quality.

Table 11. Effect of farmyard manure (FYM) and biofertilizer rates on fruit chemical characters of "Grand Nain" banana plants during two seasons

Treatment	Total sugars %		Starch %		Acidity %		T.S.S %	
	2002	2003	2002	2003	2002	2003	2002	2003
50 Kg FYM/plant/year	15.23	14.76	1.32	1.37	0.29	0.30	15.30	15.70
50 Kg FYM+1L Bio	16.27	16.67	1.41	1.52	0.31	0.32	16.00	16.70
75 Kg FYM+1L Bio	16.67	17.00	1.47	1.58	0.32	0.34	16.70	17.00
100 Kg FYM+1L Bio	16.83	17.22	1.57	1.65	0.34	0.35	18.00	17.00
50 Kg FYM+2L Bio	17.50	18.20	1.69	1.80	0.34	0.36	19.00	20.00
75 Kg FYM+2L Bio	18.26	19.43	1.76	1.87	0.35	0.37	20.00	21.00
100 Kg FYM+2L Bio	18.73	19.73	1.84	2.00	0.36	0.39	20.70	21.00
50 Kg FYM+3L Bio	19.73	20.20	1.89	2.33	0.40	0.42	21.20	22.00
75 Kg FYM+3L Bio	21.97	22.10	1.97	2.43	0.42	0.43	22.30	23.00
100 Kg FYM+3L Bio	22.90	23.73	2.03	2.63	0.44	0.45	23.00	23.70
L.S.D. at 0.5%	0.59	0.57	0.12	0.94	0.016	0.017	0.26	1.26

Source: (El-Shenawi and El-Sayed, 2005)

2. Citrus

For producing good citrus transplants, we need to get a good growth of seedling rootstocks this

commonly by giving them the optimum cultural practices such as fertilization. Also supplying the rootstocks with their nutrient requirements help to shortage the period for getting the transplant. Bio fertilization is considered an important factor in reducing the used rates of chemical fertilizers which appear to be safely for environment, improving soil fertility and increasing soil productivity. Phosphorine is a bio fertilizer which contains a phosphate dissolving bacteria *Baccillus megaterium* which hydrolyze the insoluble phosphorus into soluble one. Nitobine is a bio fertilizer which contains fixing bacteria *Azospirillum* spp. they are known to fix atmospheric nitrogen and benefit host plants by supplying growth hormones and vitamins. *Azotobacter* spp., *Azospirillum* spp. and *pseudomonas* spp., phosphate dissolving bacteria *Baccillus megaterium* and vascular mycorrhiza. The benefit of using bio fertilization as chemical fertilizer substitution is due to the activities of *Azospirillum* and *Azotobacter* to fix nitrogen gas from soil atmosphere (non symbiotic nitrogen fixer) to become ammonium N and due to the effect of phosphorylase as an enzyme produced by *aeromonas* to dissolve fixed P in the soil and also due to the increase in particle soil aggregation and soil aeration done by *Aspergillus*. *Azospirillum* is one of the non symbiotic N fixer which increases root number 15-20% enzyme activities at root zone, concentrations of IAA and soil aeration. *Azobacter*, a non symbiotic N fixer increased crop yield up to 30%, and produced plant growth promoting substances such as IAA, gibberellins and cytokinins [36].

Spices crops

Black gram

The dry matter accumulation and the uptake of the examined elements were higher when bio fertilizers were used. The percentage and the vigor of germination were 10-30% higher than control values. The uptake of Al decreased when bacteria containing fertilizers were applied. Supposedly, this effect is due to the release of organic anions by the bacteria. The applied Al concentrations had different toxicity. It makes it necessary to investigate the tolerance and sensitivity of cultivated plants to the different heavy metal-forms. The experiments have proved the beneficial effect of bacteria containing fertilizers.

The use of these bio-fertilizers is recommended in the agricultural practice as well as in heavy metal polluted circumstances [27]. Application of bio fertilizers is an acceptable approach for higher yield with good quality and safe for human consumption. The results showed that either single or mixed inocula gave positive response to the studied parameters [37]. This response was accompanied by significance increase in fresh and dry weight and other parameters. Growth parameters increased due to the mixed bio-fertilizers treatments. This primitive effect of bio- fertilizers treatments is the same line with those obtained by the researcher who stated that vegetative growth parameters increased in the bio- fertilizes treatments compared with the control.

In the investigation, the single strain inoculants was not always as good as the mixed inoculants strings in terms of biomass accumulation and N₂ fixation as compared that single inoculation of rizobia performed lower in terms of N₂ fixation and N accumulation [37].

Treatments with plant growth promoting rhizobacteria increase germination percentage, seedling vigor, emergence, root and shoot growth, total biomass of plants, seed weight, early flowering grains and fodder and fruit yields.

Single and combined inoculation promoted early day's dry weight and shoot length in blackgarm when compared to control [37]. The inoculated plants both root and shoot length increased significantly than the control. Treatment with bio- fertilizers enhances the chlorophyll content of *Vigna mungo*.

The chlorophyll content is maximum in mixed inoculation of *Rizobium* with phosphobacteria and minimum in control (single bio- fertilizers) [37]. The beneficial effect of bacterial inoculation as an increased chlorophyll content might have been due to the supply of high amount of nitrogen to the growing tissue and organs supplied by N₂ fixing *Azospirillum* and *Azotobacter*. The effect of *Azospirillum* on various growth characters where the treatments with *Azospirillum* resulted in significant increase in total chlorophyll content. The increased amount of chlorophyll content in leaves indicated the photosynthetic rate. The chlorophyll content might be due to synergetic interaction of bio-fertilizers. Protein content was increased at all the bio-fertilizers inoculation [37].

Prospects and Limitation

It is a low cost and easy technique. The biofertilizers increase 15-35% additional yield in most of the vegetable crops. Besides fixing atmospheric nitrogen, cyanobacteria synthesize and excrete several growths hormones (auxins and ascorbic acid) and vitamins which enhance seed germination and growth of crop plants. They do not cause atmospheric pollution and increase soil fertility. Some biofertilizers excrete antibiotics and thus act as pesticides [38,39,40].

They improve physical and chemical properties of soil such as water holding capacity, buffer capacity etc. Some of the biofertilizers enhance crop yield even under ill irrigated conditions where chemical fertilizers are of not much advantage. They are ecofriendly and pose no danger to the environment [41,42,43].

The limited self-life, particularly of bacterial bio fertilizers dictates that product streams must be handled with a quick delivery system at low temperatures. A strong extension and training program actively supported by research and industry. We should have recognized that in adopting a rational approach the use and management of natural resources in sustainable agriculture, the microbial fertilizers hold vast potential for the future [44,45,46].

In developing countries, the most important challenge is to produce sufficient food for the growing population from the inelastic land area. The product of biological origin can be advantageously blended to replace a part of the energy-intensive inputs. It is in this context; biofertilizers can provide to the small and marginal farmers on economically viable lover for realizing the ultimate goal of increasing productivity. These microbial systems siphon out an appreciable amount of nitrogen from the atmospheric reservoir and enrich the soil with this important and scarce nutrient [47,48,49].

The crop- microbial soil ecosystem can, therefore, be energized in sustainable agriculture with considerable ecological stability and environmental quality. Although the potential of biofertilizers in vegetable production system has been well documented and sustained, the major reason for the shifting fortunes of these biological inputs lies in lack of an organized industrial back-up, an effective quality control system, powerful extension machinery and a broad research base [50,51,52].

A. Production Constraints

Despite significant improvement/refinement in BF technology over the years, the progress in the field of BF production technology is below satisfaction due to the followings Unavailability of appropriate and efficient strains: Lack of region-specific strains is one of the major constraints as bio-fertilizers are not only crop specific but soil specific too. Moreover, the selected strains should have the competitive ability over other strains, N fixing ability over a range of environmental conditions, ability to survive in broth and in inoculants carrier. Unavailability of suitable carrier: Unavailability of the suitable carrier (media in which bacteria are allowed to multiply) due to which shelf life of bio-fertilizers is short is a major constraint [53,54].

Peat of a good quality (more than 75% carbon) is a rare commodity in India. Nilgiri peat is of poor quality (below 50% carbon). According to the availability and cost at the production site, the choice is only with lignite and charcoal in India. As per the suitability the order is peat >lignite > charcoal > FYM > soil >rice husk. The good quality carrier must have good moisture holding capacity, free from toxic substances, sterilizable and readily adjustable PH to 6.5-7.0. Under Indian conditions where extremes of soil and weather conditions prevail, there is yet no suitable carrier material identified capable of supporting the growth of bio-fertilizers. Better growth of bacteria is obtained in the sterile carrier and the best method is Gamma irradiation of sterilization (while using an autoclave, lime mixed lignite is filled up to two third capacity of steel trays for 1-2 hours for three days and sterilized at 121 0C) for carrier material. Mutation during fermentation: Bio-fertilizers tend to mutate during fermentation and thereby raising production and quality control cost. Extensive research work on this aspect is urgently needed to eliminate such undesirable changes [55,56].

B. Market level constraint

Lack of awareness of farmers: In spite of considerable efforts in recent years, the majority of farmers in India are not aware of bio-fertilizers, their usefulness in increasing crop yields sustainably. Inadequate and Inexperienced staff: Because of inadequate staff and that too not technically qualified who can attend to technical problems. Farmers are not given proper instructions about the application aspects. Lack

of quality assurance: The sale of poor quality bio-fertilizers through corrupt marketing practices results in loss of faith among farmers, to regain the faith once is very difficult and challenging. Seasonal and unassured demand: The bio-fertilizer use is seasonal and both production and distribution are done only in few months of the year, as such production units particularly private sectors are not sure of their demand [57,58].

C. Resource constraint

Limited resource generation for BF production: The investment in bio-fertilizer the production unit is very low. But keeping in view of the risk involved largely because of short shelf life and no guarantee of off-take of bio-fertilizers, the resource generation is very limited [57,59].

D. Field level constraints

Soil and climatic factors: Among soil and climatic conditions, high soil fertility status, unfavorable PH, high nitrate level, high temperature, drought, deficiency of P, Cu, Co, Mo or presence of toxic elements affect the microbial growth and crop response. Native microbial population: Antagonistic microorganism already present in soil competes with microbial inoculants and many times do not allow their effective establishment by out-competing the inoculated population. Faulty inoculation techniques: Majority of the marketing sales personals do not know proper inoculation techniques. Bio-fertilizers being living organisms required proper handling, transport and storage facilities [60,61].

Summary

The world population is increasing in geometric progression, while food production grows in arithmetic progression. The demand and supply of food are not proportional in many countries in the world. Hence; solving food problems is not easy, and all the tools and knowledge at our disposal are needed. Food production has grown, in some countries dramatically. However; at present, the population grows year to year even if the food grains production increase. Increasing food grains cannot be produced unless we carefully make use of biological nitrogen. Biological nitrogen fixation is the key to sustain agricultural productivity through the application of biofertilizers in the field. There is an urgent need to transfer this technology to the field of

farmers and into an industry by producing these fertilizers on large scale.

Biofertilizers are ready to use live formulates of such beneficial microorganisms and helps build up the micro-flora and in turn the soil health in general. The use of bio-fertilizers, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of fixing atmospheric nitrogen, solubilizing phosphorous, and stimulating plant growth through the synthesis of growth promoting substances.

Bio-fertilizers are organisms that enrich the nutrient quality of the soil that form a healthy relationship with the roots. Bio-fertilizer as a substance contains living micro-organisms and is known to help with the expansion of the root system and better seed germination. They differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi.

This soil microorganism plays an important role in improving soil fertility and crop productivity due to their capacity to fix atmospheric nitrogen, insoluble phosphate and decompose farm wasters resulting in the release of plant nutrient. Hence; biofertilizer is used to hasten biological activity to improve the availability of plant nutrients. The biofertilizers containing biological nitrogen-fixing organisms are very effective in vegetable production. It enhances biomass production and benefits of biofertilizers that may increase crop yield by 20-30% and replace chemical nitrogen and phosphorus by 25 %. Utilization of bio-fertilizers offers the following advantages: Nutrient recycling, environmentally friendly and cheaper Supplement to Inorganic Fertilizers. Bio-Fertilizers are used in Vegetable Production are Rhizobial Inoculants, Azotobacter inoculants and Azospirillum Inoculant.

The role of Biofertilizers in some vegetable crops is significantly identified by the researchers. For instance; in tomato plants subjected to inoculation with *Glomus fasciculatum* in the nursery and subsequently transplanted to field produced increased dry matter, leaf area, phosphorus content and yield as compared to untreated control in variety.

Application bio-fertilizer in plants compared to untreated plants, increased 48 and 40% of plant yield in a greenhouse and field experiment, respectively. Chilies provide a yield of *G. fasciculatum* inoculated plants at 37.5kg pha-1 was more than the un-inoculated at 75kgp/ha. In Onion production co-inoculation of bio-fertilizers supplemented with 50 percent reduced doses of N and P, gave maximum bulb yield (217.3q/ha). Similarly; Research outputs indicated that using bio-fertilizers in different vegetables at farmers' fields increase yield in the range of 6.2 -15.8%. The vegetable crops like Cucumber and Sugar Beet enhanced the energy of germination increased when bio-fertilizers were applied and also when the bio-fertilizers were used the toxic effect of Al (chlorosis) reduced. Furthermore; the bio-fertilizers had an increasing effect on the photosynthetic pigments level.

Generally, Biofertilizers have for long witnessed shifting fortunes in agriculture. A transfer of technology by the research institutions and industry is essential in the management of natural resources in sustainable agriculture as the microbial fertilizers hold vast potential for the future. The crop- microbial soil ecosystem energized in sustainable agriculture with considerable ecological stability and environmental quality improves the potential of biofertilizers in vegetable production. Biofertilizers can provide to the small and marginal farmers on economically viable lover for realizing the ultimate goal of increasing productivity.

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