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REVIEW ARTICLE

BIO-FERTILIZERS IN ORGANIC AGRICULTURE

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SUMMARY

Experiencing the adverse effects of synthetic input dependent agriculture the concept of organic agriculture is gaining momentum. Almost 31 million hectares of land are currently managed organically by more than 6, 00, 000 farmers worldwide, constitutes 0.7 per cent of agriculture land. India had brought more than 2.5 m ha land under certification of organics. In these systems production is based in synergism with nature, which makes systems of unending life i.e. sustainable. Deteriorative effects of synthetic chemical inputs are obvious, but, at the same time we need to revive soil health and living which support to sustainable production system. Soil environment needs to be made congenial for living of useful microbial population, responsible for continuous availability of nutrients from natural sources.

Bio-fertilizers being essential components of organic farming play vital role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients or convert insoluble P in the soil into forms available to plants, there by increases their efficiency and availability. Currently there is a gap of ten million tonnes of plant nutrients between removal of crops and supply through chemical fertilizers. In context of both the cost and environmental impact of chemical fertilizers, excessive reliance on the chemical fertilizers is not viable strategy in long run because of the cost, both in domestic resources and foreign exchange, involved in setting up of fertilizer plants and sustaining the production. In this context, organic manures (bio-fertilizers) would be the viable option for farmers to increase productivity per unit area.

The mycorrhizal associations (VAM) in alleviating Al toxicity, increasing N, P and micronutrient uptake, maintaining soil structure by the production specific protein called "Glomulin" has been repeatedly demonstrated. Liquid bio-fertilizer technology now, shares more advantage over conventional carrier based bio-fertilizers and can be considered as a breakthrough in field of Bio-fertilizer technology and should find greater acceptance by farmers, extension workers and commercial bio-fertilizer manufactures. In this review, the established facts observed and the work carried out by many researchers on bio-fertilizers is discussed.

Key words: Bio-fertilizers, Crop growth, Sustainability, VA-mycorrhizae

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1. Introduction

Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agro-chemicals. Though the use of chemical inputs in agriculture is inevitable to meet the growing demand for food in world, there are opportunities in selected crops and niche areas where organic production can be encouraged to tape the domestic export market.

Bio-fertilizers are being essential component of organic farming are the preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms used for application to seed, soil or composting areas with the objective of increasing number of such micro-organisms and accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by plants. Bio-fertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both, in association with plant roots and without it, solubilise insoluble soil phosphates and produces plant growth substances in the soil. They are in fact being promoted to harvest the naturally available, biological system of nutrient mobilization (Venkatashwarlu, 2008a). The role and importance of biofertilizers in sustainable crop production has been reviewed by several authors (Biswas *et al*. 1985; Wani and Lee, 1995; Katyal *et al*. 1994). But the progress in the field of BF production technology remained always below satisfaction in Asia because of various constraints.

It may be noted, only 30 % of India's total cultivable area is covered with fertilizers where irrigation facilities are available and the remaining 70 % of the arable land, which is mainly rain fed, very negligible amount of fertilizers are being used. Farmers in these areas often use organic manures as a source of nutrients that are readily available either in their own farm or in their locality. The North- Eastern (NE) region of India provides considerable opportunity for organic farming due to least utilization of chemical inputs. It is estimated that 18 million hectare of such land is available in the NE that can be exploited for organic production. With the sizable acreage under naturally organic/default organic cultivation, India has tremendous potential to grow crops organically and emerge as a major supplier of organic products in world's organic market (Venkatashwarlu. 2008a) The report of Task Force on Organic Farming appointed by the Government of India also observed that in vast areas of the country, where limited amount of chemicals are used and have low productivity could be exploited as potential areas to develop into organic agriculture. Arresting the decline of soil organic matter is the most potent weapon in fighting against unabated soil degradation and imperiled sustainability of agriculture in tropical regions of India, particularly those under the influence of arid, semiarid and sub-humid climate. Application of organic manures particularly bio-fertilizers is the only option to improve the soil organic carbon for sustenance of soil quality and future agricultural productivity (Ramesh, 2008).

Why to explore bio-fertilizers:-

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.

- Demand is much higher than the availability. It is estimated that by 2020, to achieve the targeted production of 321 million tonnes of food grain, the requirement of nutrient will be 28.8 million tonnes, while their availability will be only 21.6 million tones being a deficit of about 7.2 million tones.
- Depleting feedstock/fossil fuels (energy crisis) and increasing cost of fertilizers. This is becoming unaffordable by small and marginal farmers.

- Depleting soil fertility due to widening gap between nutrient removal and supplies.
- Growing concern about environmental hazards.
- Increasing threat to sustainable agriculture.

Besides above facts, the long term use of bio-fertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers (Venkataraman and Shanmugasundaram, 1992).

Estimated demand and supply of some important bio-fertilizers in India

The annual requirement and production of different bio-fertilizers have clearly shown tremendous gap in this area. Thus a strategy for judicious combination of chemical fertilizers and biofertilizers will be economically viable and ecological useful. It should be recommended that biofertilizers are not a substitute, but a supplement to chemical fertilizers for maximizing not only the yield but also agro system stability.

2. Potential characteristic features of some bio-fertilizers

Nitrogen fixers

Rhizobium: belongs to family ***Rhizobiaceae,*** symbiotic in nature, fix nitrogen 50-100 kg/ ha. with legumes only. It is useful for pulse legumes like chickpea, red-gram, pea, lentil, black gram, etc., oil-seed legumes like soybean and groundnut and forage legumes like berseem and lucerne. Successful nodulation of leguminous crops by *Rhizobium* largely depends on the availability of compatible strain for a particular legume. It colonizes the roots of specific legumes to form tumour like growths called root nodules, which acts as factories of ammonia production. *Rhizobium* has ability to fix atmospheric nitrogen in symbiotic association with legumes and certain non-legumes like ***Parasponia***. *Rhizobium* population in the soil depends on the presence of legume crops in the field. In absence of legumes, the population decreases. Artificial seed inoculation is often needed to restore the population of effective strains of the *Rhizobium* near the rhizosphere to hasten N-fixation. Each legume requires a specific species of *Rhozobium* to form effective nodules. Many legumes may be modulated by diverse strains of *Rhizobia*, but growth is enhanced only when nodules are produced by effective strains of *Rhizobia*. It is thus extremely important to match microsymbionts prudently for maximum nitrogen fixation. A strain of *Rhizobia* that nodulates and fixes a large amount of nitrogen in association with one legume species may also do the same in association with certain other legume species. This must be verified by testing. Leguminous plants that demonstrate this tendency to respond similarly to particular strains of *Rhizobia* are considered "effectiveness" group (Wani and Lee 2002).

Azospirillum: belongs to family ***Spirilaceae***, heterotrophic and associative in nature. In addition to their nitrogen fixing ability of about 20-40 kg/ha, they also produce growth regulating substances. Although there are many species under this genus like, *A.amazonense, A.halopraeferens, A.brasilense*, but, worldwide distribution and benefits of inoculation have been proved mainly with the *A.lipoferum and A.brasilense.* The *Azospirillum* form associative symbiosis with many plants particularly with those having the C_4-dicarboxyliac path way of photosynthesis (Hatch and Slack pathway), because they grow and fix nitrogen on salts of organic acids such as malic, aspartic acid (Arun, 2007a). Thus it is mainly recommended for maize, sugarcane, sorghum, pearl millet etc. The Azotobacter colonizing the roots not only remains on the root surface but also a sizable proportion of them penetrates into the root tissues and lives in harmony with the plants. They do not, however, produce any visible nodules or out growth on root tissue.

Azotobacter: belongs to family ***Azotobacteriaceae***, aerobic, free living, and heterotrophic in nature. *Azotobacter*s are present in neutral or alkaline soils and *A. chroococcum* is the most commonly occurring species in arable soils. *A. vinelandii, A. beijerinckii, A. insignis* and *A. macrocytogenes* are other reported species. The number of *Azotobacter* rarely exceeds of 10^4 to 10^5 g^{-1} of soil due to lack of organic matter and presence of antagonistic microorganisms in soil. The bacterium produces anti-fungal antibiotics which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent (Subba Rao, 2001a). The isolated culture of *Azotobacter* fixes about 10 mg nitrogen g^{-1} of carbon source under *in vitro* conditions. *Azotobacter* also to known to synthesize biologically active growth promoting substances such as vitamins of B-group, indole acetic acid (IAA) and gibberellins. Many strains of *Azotobacter* also exhibited fungi static properties against plant pathogens such as *Fusarium, Alternaria and Helminthosporium.* The population of *Azotobacter* is generally low in the rhizosphere of the crop plants and in uncultivated soils. The occurrence of this organism has been reported from the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops, (Arun, 2007a).

Blue Green Algae (Cyanobacteria) and Azolla: These belongs to eight different families, phototrophic in nature and produce Auxin, Indole acetic acid and Gibberllic acid, fix 20-30 kg N/ha in submerged rice fields as they are abundant in paddy, so also referred as ‘paddy organisms’. N is the key input required in large quantities for low land rice production. Soil N and BNF by associated organisms are major sources of N for low land rice. The 50-60% N requirement is met through the combination of mineralization of soil organic N and BNF by free living and rice plant associated bacteria (Roger and Ladha, 1992). To achieve food security through sustainable agriculture, the requirement for fixed nitrogen must be increasingly met by BNF rather than by industrial nitrogen fixation. Most N fixing BGA are filamentous, consisting of chain of vegetative cells including specialized cells called heterocyst which function as micro nodule for synthesis and N fixing machinery. BGA forms symbiotic association capable of fixing nitrogen with fungi, liverworts, ferns and flowering plants, but the most common symbiotic association has been found between a free floating aquatic fern, the Azolla and *Anabaena azollae* (BGA). *Azolla* contains 4-5% N on dry basis and 0.2-0.4% on wet basis and can be the potential source of organic manure and nitrogen in rice production. The important factor in using *Azolla* as biofertilizer for rice crop is its quick decomposition in the soil and efficient availability of its nitrogen to rice plants (Kannaiyan, 1990). Besides N-fixation, these biofertilizers or biomanures also contribute significant amounts of P, K, S, Zn, Fe, Mb and other micronutrient. The fern forms a green mat over water with a branched stem, deeply bilobed leaves and roots. The dorsal fleshy lobe of the leaf contains the algal symbiont within the central cavity. Azolla can be applied as green manure by incorporating in the fields prior to rice planting. The most common species occurring in India is *A. pinnata* and same can be propagated on commercial scale by vegetative means. It may yield on average about 1.5 kg per square meter in a week. India has recently introduced some species of Azolla for their large biomass production, which are *A.caroliniana, A. microphylla, A. filiculoides and A. mexicana.*

Phosphate solubilizers

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. Among the bacterial genera with this capacity are *pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium* and *Erwinia.* There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres. These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non rhizosphere soil (Raghu and Macrae, 2000). The soil bacteria belonging to the genera *Pseudomonas* and *Bacillus* and *Fungi* are more common. The major microbiological means by which insoluble-P compounds are mobilized is by the production of organic acids, accompanied by acidification of the medium. The organic and inorganic acids convert tricalcium phosphate to di- and- monobasic phosphates with the net result of an enhanced availability of the element to the plant. The type of organic acid produced and their amounts differ with different organisms. Tri- and di-carboxylic acids are more effective as compared to mono basic and aromatic acids. Aliphatic acids are also found to be more effective in P-solubilization compared to phenolic, citric and fumaric acids. The analysis of culture filtrates of PSMs has shown the presence of number of organic acids including citric, fumaric, lactic, 2-ketogluconic, gluconic, glyoxylic and ketobutyric acids.

Phosphate absorbers

Mycorrhiza (an ancient symbiosis in organic agriculture)

The term Mycorrhiza denotes "fungus roots". It is a symbiotic association between host plants and certain group of fungi at the root system, in which the fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host and the host in turn is benefited by obtaining the much needed nutrients especially phosphorus, calcium, copper, zinc etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. These fungi are associated with majority of agricultural crops, except with those crops/plants belonging to families of *Chenopodiaceae, Amaranthaceae, Caryophyllaceae, Polygonaceae, Brassicaceae, Commelinaceae, Juncaceae and Cyperaceae.* They are ubiquitous in geographic distribution occurring with plants growing in artic, temperate and tropical regions alike. VAM occur over a broad ecological range from aquatic to desert environments (Mosse *et al.* 1981). Of 150 species of fungi that have been described in order *Glomales* of class *Zygomycetes,* only small proportions are presumed to be *mycorrhizal*. There are six genera of fungi that contain species, which are known to produce *Arbuscular mycorrhizal fungi* (AMF) with plants. Two of these genera, *Glomus and Sclerocytis*, produce chlamydospores only. Four genera form spores that are similar to azygospores: *Gigaspora, Scutellospora, Acaulospora and Entrophospora.* The oldest and most prevalent of these associations are the arbuscular mycorrhizal (AM) symbioses that first evolved 400 million years ago, coinciding with the appearance of the first land plants. Crop domestication, in comparison, is a relatively recent event, beginning 10, 000 years ago (Sawers *et al*. 2007).

Zinc solubilizers

The nitrogen fixers like *Rhizobium, Azospirillum, Azotobacter, BGA* and Phosphate solubilizing bacteria like *B. magaterium, Pseudomonas striata,* and phosphate mobilizing Mycorrhiza have been widely accepted as bio-fertilizers (Subba Roa, 2001a). However these supply only major nutrients but a host of microorganism that can transform micronutrients are there in soil that can be used as bio-fertilizers to supply micronutrients like zinc, iron, copper etc., zinc being utmost important is found in the earth's crust to the tune of 0.008 per cent but more than 50 per cent of Indian soils exhibit deficiency of zinc with content must below the critical level of 1.5 ppm of available zinc (Katyal and Rattan, 1993). The plant constraints in absorbing zinc from the soil are overcome by external application of soluble zinc sulphate ($ZnSO_4$). But the fate of applied zinc in the submerged soil conditions is pathetic and only 1-4% of total available zinc is utilized by the crop and 75% of applied zinc is transformed into different mineral fractions (Zn-fixation) which are not available for plant absorption (crystalline iron oxide bound and residual zinc). There appears to be two main mechanisms of zinc-fixation, one operates in acidic soils and is closely related with cat ion exchange and other operates in alkaline conditions where fixation takes by means of chemisorptions, (chemisorptions of zinc on calcium carbonate formed a solid-solution of $ZnCaCO_3$), and by complexation by organic ligands (Alloway, 2008).

The zinc can be solubilized by microorganisms viz., *B. subtilis, Thiobacillus thioxidans and Saccharomyces sp.* These microorganisms can be used as bio-fertilizers for solubilization of fixed micronutrients like zinc (Raj, 2007). The results have shown that a *Bacillus sp.* (Zn solubilizing bacteria) can be used as bio-fertilizer for zinc or in soils where native zinc is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc sulphide (ZnS) instead of costly zinc sulphate (Mahdi *et al*. 2010).

3. Potential role of bio-fertilizers in agriculture

Nitrogen-fixers (NF) and Phosphate solubilizers (PSBs)

The incorporation of bio-fertilizers (N-fixers) plays major role in improving soil fertility, yield attributing characters and thereby final yield has been reported by many workers (Subashini *et al*. 2007a; Kachroo and Razdan, 2006; Son *et al.* 2007). In addition, their application in soil improves soil biota and minimizes the sole use of chemical fertilizers (Subashini *et al.* 2007a).

Under temperate conditions, inoculation of *Rhizobium* improved number of pods plant^{-1}, number of seed pod^{-1} and 1000-seed weight (g) and thereby yield over the control. The number of pods plant^{-1}, number of seed pod^{-1} and 1000-seed weight (g) recorded were 25.5, 17.1 and 4.7 per cent more over the control, respectively which was statistically significant Bhat *et al.* (2009). In rice under low land conditions, the application of BGA+ *Azospirillum* proved significantly beneficial in improving LAI and all yield attributing aspects. Grain yield and harvest index also exhibit a discernable increase with use of bio-fertilizers (Dar and Bali, 2007). Afzal, (2006) found that seed and straw yield of green gram increased significantly up to single inoculation with *Rhizobium* under 20 kg N + 45 kg P_2O_5 ha^{-1} fertility level. Field trials carried out in different locations have demonstrated that under certain environmental and soil conditions inoculation with azotobacteria has beneficial effects on plant yields. The effect of *Azotobacter chroococcum* on vegetative growth and yields of maize has been studied by numerous authors (Hussain *et al.*, 1987; Martinez Toledo *et al.*, 1988; Nieto and Frankenberger, 1991; Mishra *et al.*, 1995; Pandey *et al.*, 1998; Radwan, 1998), as well as the effect of inoculation with this bacterium on wheat (Emam *et al.*, 1986; Rai and Gaur, 1988; Tippanavar and Reddy, 1993, Elshanshoury, 1995; Pati *et al.*, 1995; Fares, 1997a).

Alkaline phosphatase activity in the peach roots was highest with *Azotobacter chroococcum* + P fertilizer (Godara *et al.,* 1995). Results of a greenhouse pot experiments with onion showed that application of *G. fasciculatum* + *A. chrooccocum* + 50% of the recommended P rate resulted in the greatest root length, plant height, bulb girth, bulb fresh weight, root colonization and P uptake (Mandhare *et al.* 1998). Inoculation with *Azotobacter* + *Rhizobium* + VAM gave the highest increase in straw and grain yield of wheat plants with rock phosphate as a P-fertilizer (Fares, 1997a). Elgala *et al.* (1995) concluded that with microbial inoculation rock phosphate could be used as cheap source of P in alkaline soils and that combined inoculation could reduce the rate of fertilizer required to maintain high productivity.

It is an established fact that the efficiency of phosphatic fertilizers is very low (15-20%) due to its fixation in acidic and alkaline soils and unfortunately both soil types are predominating in India accounting more than 34% acidity affected and more than seven million hectares of productive land salinity/alkaline affected (Yawalkar *et al.*, 2000). Therefore, the inoculations with PSB and other useful microbial inoculants in these soils become mandatory to restore and maintain the effective microbial populations for solubilization of chemically fixed phosphorus and availability of other macro and micronutrients to harvest good sustainable yield of various crops. Commercial exploitation of phosphatic microbial inoculants can play an important role particularly in making the direct use of abundantly available low grade phosphate possible. Among the bacterial genera with this capacity are *pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium* and *Erwinia.*

Beside N-fixation and P-solubilization, the incorporation of nitrogen fixing bacteria (*Azotobacter* spp.) under the commercial name 'cerealien' and phosphate dissolving bacteria (*Bacillus megaterium*) 'phosphorien' has shown the highest degree in inducing the degree of the physiological tolerance to salinity which enables the stressed plants of the Seets cultivar of wheat to be adapted and keep better performance against all applied levels of salinity (3000, 6000 and 9000 ppm). This performance was reflected by the increase in growth, dry matter accumulation, yield as well as chemical constituents. All chemicals constituents including N, P, K+, sugars, proline and were increased as compared to their control treatments in the cultivar Seets. Mohmoud and Mohamad, 2008.

Mycorrhizae

The fungi that are probably most abundant in agricultural soils are arbuscular mycorrhizal (AM) fungi. They account for 5–50% of the biomass of soil microbes (Olsson *et al.*, 1999). Biomass of hyphae of AM fungi may amount to 54–900 kg ha^{-1} (Zhu and Miller, 2003), and some products formed by them may account for another 3000 kg (Lovelock *et al.*, 2004). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10–20 (Rillig *et al.*, 2001). The external mycelium attains as much as 3% of root weight (Jakobsen and Rosendahl, 1990). Approximately 10–100 m mycorrhizal mycelium can be found per cm root (McGonigle and Miller, 1999).

The mineral acquisition from soil is considered to be the primary role of mycorrhizae, but they play various other roles as well which are of utmost important:

Improved nutrient uptake (Macro and micronutrients)

The improvement of P nutrition of plants has been the most recognized beneficial effect of mycorrhizas. The mechanism that is generally accepted for this mycorrhizal role consists of a wider physical exploration of the soil by mycorrhizal fungi (hyphae) than by roots. A speculative mechanism to explain P uptake by mycorrhizal fungi involves the production of glomalin. Glomalin contains very substantial amounts of iron (up to 5% of the glomalin pool, Lovelock *et al.*, 2004). Assuming 0.5 mg glomalin g^{-1} soil with 1% iron, and assuming that this iron was derived initially from unavailable Fe–P forms in the NaOH-Pi fraction, the destabilization of this bond could have released 1.75 mg P per pot, comparable to the 2.01 mg NaOH-Pi that was taken up. Bolan *et al.* (1987) had already proposed that mycorrhizal fungi may break the bond between Fe and P, but they did not suggest a mechanism. Further research into the physiological and ecological roles of glomalin is needed to address this question. AM plants have been reported to improve nutrition of the other macronutrients N and K. In acid soils, AM fungi may be important for the uptake of ammonium (NH_4^+), which is less mobile than nitrate (NO_3^-) and where diffusion may limit its uptake rate. Although nitrate is much more mobile than ammonium (uptake is regulated through mass flow). Because of their small size, AM fungal hyphae are better able than plant roots to penetrate decomposing organic material and are therefore better competitors for recently mineralized N (Hodge, 2003). By capturing simple organic nitrogen compounds, AM fungi can short-circuit the N-cycle.

It is also reported that the AM- fungi also increases the uptake of K, and concentration of K has been found more in mycorrhizal than non-mycorrhizal plants (Bressan *et al.*, 2001). Apart from this, the AM-fungi also increases the uptake and efficiency of micronutrients like Zn, Cu, Fe etc. by secreting the enzymes, organic acids which makes fixed macro and micronutrients mobile and as such are available for the plant.

Better water relation and drought tolerance

AM fungi play an important role in the water economy of plants. Their association improves the hydraulic conductivity of the root at lower soil water potentials and this improvement is one of the factors contributing towards better uptake of water by plants. Also, leaf wilting after soil drying, did not occur in mycorrhizal plants until soil water potential was considerably lowered (approx. 1.0 M. Pa). Leaflets of Leucaena plants inoculated with VA mycorrhizae did not wilt at a xylem pressure potential as low as -2.0 MPa. Mycorrhiza induced drought tolerance can be related to factors associated with AM colonization such as improved leaf water and turgor potentials and maintenance of stomatal functioning and transpiration, greater hydraulic conductivities and increased root length and development.

Soil structure (A physical quality)

Whereas the role of mycorrhizal associations in enhancing nutrient uptake will mainly be relevant in lower input agro-ecosystems, the mycorrhizal role in maintaining soil structure is important in all ecosystems (Ryan and Graham, 2002). Formation and maintenance of soil structure will be influenced by soil properties, root architecture and management practices.

The use of machines and fertilizers are considered to be responsible for soil degradation. The specific adsorption of P by functional groups can affect the charge balance and cause dispersion of particles (Lima *et al*., 2000). Soil aggregation is one component of soil structure. Mycorrhizal fungi contribute to soil structure by (1) growth of external hyphae into the soil to create a skeletal structure that holds soil particles together; (2) creation by external hyphae of conditions that are conducive for the formation of micro-aggregates; (3) enmeshment of micro aggregates by external hyphae and roots to form macro aggregates; and (4) directly tapping carbon resources of the plant to the soils (Miller and Jastrow, 1990, 2000). This direct access will influence the formation of soil aggregates, because soil carbon is crucial to form organic materials necessary to cement soil particles. Hyphae of AM fungi may be more important in this regard than hyphae of saprotrophic fungi due to their longer residence time in soil, because fungivorous soil fauna prefers hyphae of the latter over those of AM fungi (Klironomos and Kendrick, 1996; Gange, 2000). In addition, AM fungi produce glomalin (12-45 mg/cm^3), a specific soil-protein, whose biochemical nature is still unknown. Glomalin is quantified by measuring several glomalin related soil-protein (GRSP) pools (Rillig, 2004). Glomalin has a longer residence time in soil than hyphae, allowing for a long persistent contribution to soil aggregate stabilization. The residence time for hyphae is considered to vary from days to months (Staddon *et al.*, 2003) and for glomalin from 6 to 42 years (Rillig *et al.,* 2001). Steinberg and Rillig (2003) demonstrated that even under relatively favorable conditions for decomposition, 40% of AM fungal hyphae and 75% of total glomalin could be extracted from the soil 150 days after being separated from their host. Glomalin is considered to stably glue hyphae to soil. The mechanism is the formation of a 'sticky' string-bag of hyphae which leads to the stability of aggregates.

Enhanced phytohormone activity

The activity of phytohormones like cytokinin and indole acetic acid is significantly higher in plants inoculated with AM. Higher hormone production results in better growth and development of the plant.

Crop protection (Interaction with soil pathogens)

AM fungi have the potential to reduce damage caused by soil-borne pathogenic fungi, nematodes, and bacteria. Meta-analysis showed that AM fungi generally decreased the effects of fungal pathogens. A variety of mechanisms have been proposed to explain the protective role of mycorrhizal fungi. A major mechanism is nutritional, because plants with a good phosphorus status are less sensitive to pathogen damage. Non-nutritional mechanisms are also important, because mycorrhizal and non-mycorrhizal plants with the same internal phosphorus concentration may still be differentially affected by pathogens. Such non-nutritional mechanisms include activation of plant defense systems, changes in exudation patterns and concomitant changes in mycorrhizosphere populations, increased lignification of cell walls, and competition for space for colonization and infection sites (Kaisamdar, *et al.* 2001). It is also reported that increased production and activity of phenolic and phytoalexien compounds with due to AM-inoculation considerably increases the defense mechanism there by imparts the resistance to plants.

4. Constraints in bio-fertilizer use

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 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 suitable carrier (media in which bacteria are allowed to multiply) due to which shelf life of bio-fertilizers is short is a major constraint. 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 production site, choice is only with lignite and charcoal in India. As per the suitability the order is peat >lignite > charcoal > FYM > soil >rice husk. Good quality carrier must have good moisture holding capacity, free from toxic substances, sterilisable 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 sterile carrier and the best method is Gamma irradiation of sterilization (while using 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.

Market level constraints

Lack of awareness of farmers

Inspite of considerable efforts in recent years, 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 is done only in few months of year, as such production units particularly private sectors are not sure of their demand.

Resource constraint

Limited resource generation for BF production

The investment in bio-fertilizer 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.

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.

Liquid Bio-fertilizers (Break through in BF-Technology)

Liquid bio-fertilizers are special liquid formulation containing not only the desired microorganisms and their nutrients but also special cell protectants or chemicals that promote formation of resting spores or cysts for longer shelf life and tolerance to adverse conditions. (Hegde, 2008).

Bhattacharyya and Kumar (2000), states that, bio-fertilizers manufactured in India are mostly carrier based and in the carrier-based (solid) bio-fertilizers, the microorganisms have a shelf life of only six months. They are not tolerant to UV rays and temperatures more than 30 ^{0}C. The population density of these microbes is only 10^8 (10 crores) c.f.u/ml at the time of production. This count reduces day by day. In the fourth month it reduces to 10^6 (10 lakhs) c.f.u/ml and at the end of 6 months the count is almost nil. That's why the carrier-based bio-fertilizers were not effective and did not become popular among the farmers. These defects are rectified and fulfilled in the case of Liquid bio-fertilizers. The shelf life of the microbes in these liquid bio-fertilizers is two years. They are tolerant to high temperatures (55 ^{0}C) and ultra violet radiations. The count is as high as 10^9 c.f.u/ml, which is maintained constant up to two years. So, the application of 1 ml of liquid bio-fertilizers is equivalent to the application of 1 Kg of 5 months old carrier based bio-fertilizers (1000 times). Since these are liquid formulations the application in the field is also very simple and easy. They are applied using hand sprayers, power sprayers, fertigation tanks and as basal manure mixed along with FYM etc.

5. Conclusion and future strategies

- Identification/ selection of efficient location/ crop/soil specific strains for N-fixing, P, Zn- solubilizing and absorbing (mycorrhizal) to suit different agro climatic conditions.
- Strain improvement through biotechnological methods.
- Exchanging the cultures between countries of similar climatic conditions and evaluating their performance for better strain for particular crop. Checking the activity of cultures during storage to avoid natural mutants.
- Use of sterile carriers and installing centralized unit of gamma chamber facilities at different locations to use by private and government manufacturers in the case of use of carrier based inoculants till the development of alternate formulation. Identifying two or three common carrier materials in different countries based on availability and recommends them to the producers.
- Developing suitable alternate formulations viz., liquid inoculants / granular formulations for all bioinoculants, to carrier based inoculants. Standardizing the media, method of inoculation etc., for the new formulations.
- Employing microbiologists in production units to monitor the production. Developing cold storage facilities in production centers.
- Technical training on the production and quality control to the producers and rendering technical advice and projects to manufacturers. Organizational training to the extension workers and farmers to popularize the technology. Dissemination of information

through mass media, publications and bulletins.

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