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NATIONAL CENTRE OF ORGANIC FARMING
KAMLA NEHRU NAGAR, GHAZIABAD

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संपादकीय कार्यालय ***Editorial Office***

राष्ट्रीय जैविक खेती केन्द्र, गाजियाबाद

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From the desk of Chief Editor………

In view of established and well documented significance of Integrated Plant Nutrient Supply system in sustainable agriculture and increased environment concerns, the role of biofertilizers is indispensable. In recent pasts, biofertilizer production in the country witnessed ups and down and finally multiple increase but the production capacity remained under utilized due to low demand of biofertilizers and no market development assistance as in case of fertilisers to the Biofertilizer marketers. Inconsistent crop response to biofertilizer due to sub-standard quality can be considered as one of the major reasons for poor quality of these products, resulting into low demand. Once poor quality biofertilizer material reaches to the farmer, its ineffectiveness not only prevents using any more biofertiliser on his farm but the peer group of farmers also lose faith in biofertilizer. The quality assurance at each & every step of biofertiliser production and marketing starting from screening of microbial strains to the sale of finished products to the end-user, therefore, is of great significance. The need for quality control has to very systematic ever before because the biofertilizer production at present hovers around 2.0 lakh MT (solid and liquid) with more than 500 biofertilizer production units belonging to fertilizer industry, cooperatives, NGOs and private entrepreneurs, which are engaged in biofertilizer production. The role of biofertilizers has now been clearly understood and established under varied agro-climatic conditions. Therefore, a lot of emphasis is being given on production, promotion and increased popularity of biofertilizers amongst the farming community by all the sectors of the society ranging from government to private/public agencies.

Since biofertilizer contains living micro-organisms, their quality control needs great care. The biofertilizer formulations contains living micro-organisms which are very much influenced by different biotic and abiotic agro climatic conditions. The quality control aspects need to be strictly adhered to in order to ensure that all aspects are covered. The primary concern has been maintaining the availability of biofertilizers upto the field level conditions and let farmers understand proper usage of biofertilizers.

I, therefore, request all the stakeholders to suggest measures which could be taken up to make biofertilizer as a reliable eco-friendly and cost effective source of organic input. I also place on record my sincere thanks to the contributors of the paper and request all stakeholders including biofertilizer industry to send articles for inclusion in this Newsletter. Creating awareness on usage of Biofertilisers and benefits among farmers and extension Officers, understanding the difficulties of distribution and selling to small and marginal farmers who purchase few inputs, improved quality control including NABL accreditation, training, enforced quality standard and continuous supply of effective locally tested strains and research on inoculant carriers and latest technology are pre-requisites to improve biofertilisers incoculant quality in the market. Finally, I record my appreciation to the editors and team for their pains-taking efforts to publish this Biofertilizer Newsletter.

Dr. Gagnesh Sharma
Chief Editor

Biofertilizers Production Technology and Their Application

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Abstract

The increasing dependency of modern agriculture on synthetic fertilizers severely affecting our ecosystem related to water, air, and soil and thus raises many environmental hazards such as soil deterioration and the greenhouse effect. Consequently, to meet the urgency of food demand for the increasing population, there is a vital necessity for more viable options. Hence, Microbial biofertilizers such as algae, fungi, and bacteria can be considered economical and viable options for increasing agricultural production while maintaining natural biodiversity. And Biofertilizers can also enhance plant growth by easily availing plant nutrients to them and suppressing plant pathogens. Microbial biofertilizers can provide nutrients to plants mainly by phosphorus solubilization, nitrogen fixation, and enhancing plant growth activities. For better production of legumes and other crops, nitrogen-fixing bacteria of the rhizobia and other groups can be used. Furthermore, Azolla and blue-green algae (BGA) also significantly contribute to the nitrogen fixation. Another important group of microbes, Arbuscular mycorrhizal fungi are responsible for the uptake of phosphorus and other minerals to plants. Azotobacter and Azospirillum can also contribute to fixing atmospheric nitrogen and as well as in phosphorus mobilization and solubilization to plants and hence in better crop production. Also, Azospirillum helps provide plant growth-promoting substances, suppress pathogens, and stress tolerance. Hence, biofertilizer applications can be considered a viable strategy for sustainable agriculture production while maintaining the ecosystem and so reducing synthetic fertilizers use.

Keywords Microbial biofertilizers, synthetic fertilizers, biofertilizers application, biofertilizers production, Types of Biofertilizers

1. Introduction

As synthetic fertilizers have numerous environmental hazards, it is important to reduce their usage in agriculture without affecting crop yield. The introduction of Microbial biofertilizers is a viable and economical option for better crop production in sustainable agriculture. Biofertilizers are the microorganisms in single or consortia that increase the nutrient uptake of plants by rhizospheric interactions. Plant growth-promoting rhizobacteria (PGPR); enhance the growth of plants and their products through various mechanisms such as phosphorus solubilization, production of plant growth hormones, nutrient and mineral uptake and fixing of atmospheric nitrogen **(Fig1)** (Bashan et al. 1990; Okon and Labandera-Gonzalez 1994; De Freitas et al. 1997; Bashan 1998; Goldstein et al. 1999). In a study conducted by Bertrand et al. 2000, it was found that Achromobacter, a PGPR, can improve root length and hairs and enhances potassium and nitrate uptake in Brassica napus (oilseed rape), later this study was evidenced by increased shoot and root dry weight up to 33% and 21% respectively. Hence, Microbial biofertilizers can enhance nutrient uptake to plants without damaging the ecosystem while maintaining soil biodiversity.

Their usage is predicted to be increased in the nearby future due to numerous reasons such as more fertilizer demand because of increasing food demand, limited feedstock/ fossil fuels, high fertilizer cost, soil depletion, and many environmental hazards posing threat to agriculture. It is estimated that biofertilizers market share will reach US$1.66 billion by 2022 and will be compounding the annual growth rate of 13.2% during the years of 2015–2022 (Timmusk et al. 2017).

2. Biofertilizers

Microbial biofertilizers are the microorganisms either in single or in consortia when applies to the soil, plant surfaces, and seed, cause various rhizospheric interactions which result in better supply, uptake, and availability of nutrients to plant. They are easily accessible to small and marginal farmers. Biofertilizers involves a group of microorganisms such as algae, fungi, cyanobacteria, and bacteria having symbiotic relationship with host plants. Moreover, most importantly and studied groups of microbial biofertilizers are those who solubilized phosphorus and fixed nitrogen.

2.1 Nitrogen-Fixing Microorganisms

Though nitrogen is present abundantly in the atmospheric air, still it is one of the limiting nutrients because plants can't fix and uptake nitrogen themselves. However, some microbes are capable of fixing atmospheric nitrogen. Thus, the plant can only uptake atmospheric nitrogen through microbes and thereby results in the reduction of nitrogen losses through volatilization, denitrification, and leaching. These microorganisms can be in different associations such as:

a) Free-living Nitrogen-fixing bacteria

The process of nitrogen fixation assessment by free-living bacteria is challenging, however, it is to be calculated about 3 kg N ha^{1} to 10 kg N ha^{1} in plants such as Medicago sativa (Roper et al. 1995). In liquid media, Azotobacter chroococcum can fix 2–15 mg N g^{1} of carbon source in arable soils, also releases abundant slime which results in soil aggregation. Though, it has been found that some of the free-living nodulating bacterial symbionts such as Frankia fix atmospheric nitrogen in the rhizosphere of their host plant and as well as in some of the non-host plants (Smolander and Sarsa 1990). Whereas, in others, free-living bacterial strains such as Clostridium spp and Beijerinckia mobilis, the leaf spray, and seed soaking methods can enhance growth in barley and cucumber through various mechanisms such as nitrogen fixation and synthesis of plant growth hormone (Polyanskaya et al. 2002). For the cultivation of rice, in India, most commonly Free-living cyanobacteria (blue-green algae) have been used that can fix 20–30 kg N ha^{1} under optimum conditions (Kannaiyan 2002).

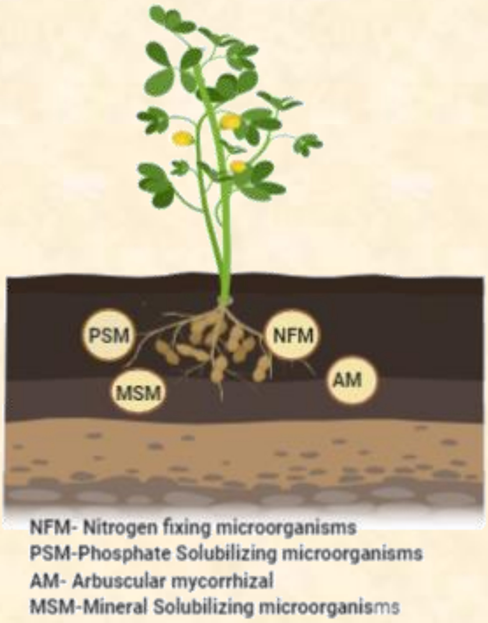

Fig1: Interaction between Plant and microorganisms

b) Symbiotic and endophytic associations

The microbes such as rhizobia, Frankia, and cyanobacteria show the symbiotic associations with host plants. For nitrogen fixation, the most important biofertilizers group (rhizobia) comprises Sinorhizobium, Mesorhizobium, Rhizobium, Allorhizobium, Azorhizobium, and Bradyrhizobium can fix up to 450 kg N ha^1 with different host legume species by root nodulation (Unkovich et al. 1997; Vance 1998; Graham and Vance 2000; Unkovich and Pate 2000). These bacterial strains can be used in different formulations such as liquid, granular, and powder with different carrier combinations such as charcoal, perlite, peat, and mineral soil (Stephens and Ranask 2000). An actinomycete, Frankia can also form nodulation with the root of many woody plants and hence contribute to nitrogen fixation (Huss-Danell 1997; Wall 2000). The mycelium of actinomycetes helps in the formation of symbiotic relationships with many other non-legume plants such as Alnus (Alder) Myrica, Casuarina, Rubus, etc. These plants have many beneficial roles in fuelwood production and fuel production, revegetation, land reclamation, and mixed plantations (Diagne et al. 2013; Schwencke and Carù 2001).

Generally, Frankia inoculated in nurseries or arid environments has a positive effect (Sprent and Parsons 2000). However, some plant leaves such as Ardisia have special internal cavities having symbiotic nitrogen-fixing bacteria e.g. Xanthomonas and Mycobacterium, which finally contribute as a nitrogen fertilizer in the soil (Miller 1990). Also, cyanobacteria (blue-green algae) is considered to be an important group as Anabaena and Nostoc can contribute up to 36% in the global nitrogen fixation and thus found to be effective in increasing rice field fertility for better production of rice around the world (Irisarri et al. 2001). Other than that, cyanobacteria has also many other advantages such as it can be used in eco-restoration and reclamation of land affected to flood (Malam Issa et al. 2001). However, the development of production and application of BGA is poor, but it should be considered as a potent microbial fertilizer for better crop production in sustainable agriculture (Hashem 2001). Besides this, BGA in the aquatic environment also produces growth hormones, vitamins, minerals, and proteins to the soil.

c) Associative/associated microbes in the rhizosphere

Nitrogen-fixing microbes have less deep interaction with plant roots as compared to endophytic symbionts. These associations interaction involves plants such as maize sugarcane, and sorghum with Herbaspirillum spp and Acetobacter diazotrophicus (Triplett 1996; James et al. 1997; Boddey et al. 2000); Leptochloa fusca (kallar grass) with Azoarcus spp. (Malik et al. 1997); maize and rice with species of Azospirillum, Alcaligenes, Bacillus, Pseudomonas, and Enterobacter (James 2000); whereas Azospirillum shows great host specificity with many perennial and annual plants (Bashan and Holguin 1997). There are numerous reports which clearly showed that Azospirillum can enhance growth and crop yield of carrot, wheat, sunflower, sugar beet, rice, tomato, oak, pepper, cotton, and eggplant by increasing nitrogen fixation and producing growth-promoting substances (Okon 1985; Bashan et al. 1989; Okon and Labandera-Gonzalez 1994). And the process of production of Azospirillum inoculum is also inexpensive and can be used in combination with peat as a carrier (Vande Broek et al. 2000). The microbial fertilizer Acetobacter diazotrophicus has been found to fix about 70% of the nitrogen in the sugarcane crop (Boddey et al. 1995). Hence, the potential of these microbes to fix atmospheric nitrogen in abundant quantities and production of plant growth substances makes them suitable applicants in the field of biofertilizers for sustainable agriculture.

2.2 Phosphorus - Solubilizing Microorganisms

The phosphorus has been found in abundant quantity in the soil but most of it is in the unavailable forms, after nitrogen, thus it is the second most limiting nutrient in the soil (Schachtman et al. 1998). And the microorganisms such as Pseudomonas and Bacillus have been known to be commonly found phosphorus-solubilizing bacteria which can enhance the availability of phosphorus in the soil by mobilizing the unavailable forms to the available ones (Richardson 2001). Some of the fungi such as Aspergillus and Penicillium can dissociate the bound phosphates in the soil through the secretion of organic acids and by lowering the pH. It has been found that when PSB, Bacillus megaterium var. phosphatic along with inexpensive rock phosphate is applied to sugarcane, it enhances sugar yield and juice quality by 12.6%, whereas the requirement of phosphorus is reduced by 25%, and thus furthermore results in the reduction of costly superphosphate usage by 50% (Sundara et al. 2002).

2.3 Mycorrhizal Biofertilizers

They are commonly known as phosphate absorbers or phosphorus-mobilizing biofertilizers. The mycorrhizal fungi can form a facultative or obligate symbiotic association with almost 80% of the land plants, where mycorrhizal fungi depend on the host plant for photosynthesis and energy whereas plant in return gets several benefits from the fungi (Thakur and Singh 2018). The fungi mycelium from the host plant roots extends deeper into the soil rhizosphere, thus increases the availability of more mineral and nutrients such as zinc, calcium, and copper to the host plant (Singh and Giri 2017). Furthermore, the mycorrhizal fungi also help in enhancing the quality of soil, water dynamics, soil aeration, and drought tolerance of plants. It also makes host plants less prone to root pathogens (Rillig et al. 2002; Thakur and Singh 2018). Thus it makes the mycorhizal fungi having huge potential in the field of biofertilizers for sustainable agriculture such as inland and vegetation restoration (Menge 1983; Sylvia 1990). The Ectomycorrhiza (of Basidiomycetes) makes a mantle on the root surface of many trees e.g. peach, Eucalyptus, Pine, Quercus,) and thus enters within the intercellular spaces of the cortical region from where it can get the host plant sugar and other forms of nutrition. The mycorrhizae fungi have many benefits such as increasing roots surface area and thus helps in water and mineral absorption, humus organic matter solubilization to secretes many inorganic nutrients and antimicrobial substances for plants protection against several pathogens. The ectomycorrhizal association has a huge role in tree plantations by increasing nutrient acquisition, particularly in forestry cultivated land (Smith and Read 1997).

Arbuscular mycorrhizal (AM) fungi e.g. Glomus are intercellular, nonspecific obligate endosymbionts that form special structures of vesicles and arbuscules in roots hence extends root system, harvest various micronutrients and moisture from down deep into the soil, furthermore, phosphorus availability and mobility increased which contribute in the plant growth. Though, because of the obligate nature of AM fungi and unculturability have made inoculation incompatible for large-scale agriculture, and therefore it requires extensive research (Wood and Cummings 1992; Ryan and Graham 2002). In agriculture, the AM Fungi can colonize differently in different host based on inoculum source (Klironomos and Hart 2002).

The only viable option for the production of infective propagules is by growing inoculum in symbiosis with living host plants or in root organ cultures, but it also has many limitations such as slow turnover time, difficulty in excluding root pathogens and high production cost. The inoculum of

AM can be used as spores (most reliable), fragments of colonized roots (effective for some taxa), or with a combination of these and incorporated soil mycelium mixed with carrier substrate like sand, pumice or clay, vermiculite, perlite, soil rite, and soil or glass pellets (Mallesha et al. 1992; Redecker et al. 1995; Gaur and Adholeya 2000; Klironomos and Hart 2002).

2.4 Other Mineral-Solubilizing Biofertilizers

Soil-living microbes can also be used to provide various other nutrients such as iron, copper, zinc, and potassium. In a study reported by Jakobsen et al. 2005. Another important nutrient, potassium (insoluble forms) can be solubilized by some of the rhizobacteria for better plant growth. When Bacillus is applied (for wheat), Paenibacillus glucanolyticus (for black pepper), Bacillus mucilaginosus in co-inoculation with the phosphate-solubilizing Bacillus megaterium (for pepper, eggplant, and cucumber), plant biomass yield is increased due to high potassium uptake (Meena et al. 2014; Etesami et al. 2017). Zinc, another important mineral is available at a low concentration in the Earth's crust, hence to overcome its deficiencies in the plant; it should be applied externally as the costlier soluble zinc sulfate. Though, some microorganisms e.g. Thiobacillus thiooxidans, Saccharomyces spp. And Bacillus subtilis can solubilize insoluble cheaper zinc compounds like zinc oxide, zinc sulfide, and zinc carbonate in the soil (Ansori and Gholami 2015). Likewise, some microbes can hydrolyze silicates and aluminum silicates through protons supplying which further causes hydrolysis and organic acids which form complexes with cations and retain them in a dissolved state to the medium while metabolizing, that can be beneficial to the plants. For example, it has been found that silicate-solubilizing Bacillus sp. combined with siliceous residues of rice straw, rice husk, and black ash results in increased rice growth and grain yield due to increased dissolution of silica and nutrients from the soil (Cakmakci et al. 2007).

2.5 Plant Growth-Promoting Microorganisms

Other than nitrogen fixer and phosphate solubilizers, certain microorganisms can also be used as microbial fertilizers as they can produce certain growth hormones which enhance plant growth (Bashan 1998). For instance, Bacillus licheniformis and Bacillus pumilus were found in the rhizosphere and releases physiologically functional plant growth promoter hormones (Gutierez-Mañero et al. 2001). Though, other bacteria such as Paenibacillus polymyxa exhibits certain other beneficial functions to host plants e.g. phosphorus solubilization, nitrogen fixation, production of chitinase, cytokinins, hydrolytic enzymes and antibiotics and soil porosity enhancement (Timmusk et al. 1999). Certain azospirillum spp. have also reported to synthesis plant promoting growth hormones (Bashan and Holguin 1997). Hence, it indicates that microorganisms have diverse potential as microbial fertilizers which need extensive research. These plant growth-promoting bacteria's protects plants from phytopathogenic microbes through various mechanisms such as the production of antimicrobial metabolites like antibiotics and siderophores, gaseous products e.g. ammonia, and fungal cell wall-degrading enzymes which cause cytolysis, leakage of ions, membrane disruption, and inhibition of mycelial growth and protein biosynthesis (Idris et al. 2007; Lugtenberg and Kamilova 2009). Certain strains of Pseudomonas release antifungal metabolites like pyrrolnitrin, phenazines, pyoluteorin, and cyclic lipopeptides of viscosinamide, which can avoid Pythium ultimum infection in sugar beet. Iron-chelating siderophores like pyoverdin and pseudobactin secreted by Pseudomonas fluorescens which can bind and take up ferric ions, and thus makes them better competitors for iron, and preventing the growth and proliferation of phytopathogens like Rhizoctonia batatticola, Pythium ultimum, and Fusarium oxysporum (Leeman et al. 1996; Hultberg et al. 2000). Pseudomonas aeruginosa also releases siderophores pyochelin, pyoverdine, and salicylic acid and which results in induced resistance against Colletotrichum lindemuthianum (on the bean) and Botrytis cinerea (on bean and tomato) (De Meyer and Höfte 1997; Audenaert et al. 2002). Though, certain Pseudomonas species secretes extracellular chitinase and laminase which can lyse mycelia of Fusarium solani. Furthermore, microbial biofertilizers also protect plants from other soilborne diseases, plant diseases, and insect pests such as Azotobacter can enter deep into the soil with antibiotics and thus stop the soilborne pathogens (Phytophthora and Pythium) spread (Wani et al. 2013).

3. Steps involved in the biofertilizer production

3.1 Strain Selection Criteria:

The microbial strain having better efficiency isolated in the laboratory, purified and multiplied on a suitable culture medium. After the application of microbial strain into seed or soil, it has to survive and fight against the normal microbial flora for showing better activity and efficiency in actual field conditions.

3.2 Preparation of Biofertilizer:

For the inoculum production, the selected microbial strain is inoculated in small flasks. The starter culture volume should be at least 1% for obtaining 1×10^9 cells/ml. After achieving the desired cell population, the culture is added to the sterilized carrier for the preparation of biofertilizer. Finally, it is the carrier that carries the efficient microbial strain to the actual field condition. Whereas, in some cases, the carrier is firstly sterilized and then inoculated, while in other cases, it is firstly inoculated and then sterilized by UV irradiation. In the final product, the cell population should be 10^9-10^{10} cells per gram and the moisture content should be around 40-60%. And ultimately, the large scale production of inoculum should take place in culture fermenters.

3.2.1. Biofertilizers Production through Fermentation

Biofertilizers are the formulation of microbes that can provide nutrients to the host plants in an environmentally friendly nature. There are many types of biofertilizers such as nitrogen-fixing microorganisms, phosphate solubilizing microbes, and plant growth-promoting microbes which are commonly used by farmers to enhance the fertility of the soil and for better crop production (Baby 2002; Jayaraj et al. 2004). Many renowned scientists have contributed in the establishment of the basis of biofertilizers application, in 1886 by Hellriegel and Wilfarth (1888) discovered N2 fixation in root nodules of legumes); Beijerinck (1888) isolated and cultivated Rhizobium from legumes root; Nobbe and Hiltner (1895) launched "Nitragin, a pure culture of rhizobia," for N2 fixation in the market; Pikovskaya (1948) introduced phosphate solubilizers; Stewart (1969) discovered N2 fixation in blue-green algae and Dobereiner and Day (1976) identified N2-fixing Azospirillum. Later, several scientists have developed biofertilizers consists of various microbes with many benefits (Podile and Kishore 2006; Abdel Ghany et al. 2013).

For the production of biofertilizer, fermentation is one of the important processes because of the user-friendly and economic benefits. The type of substrate largely affects the main product of the fermentation process. For large-scale production of biofertilizers, SSF and SMF are the commonly used substrate-based types of fermentations. Bagasse, wheat bran, paper pulp, vegetable and fruit waste, synthetic media, and rice and rice straw are the commonly used Substrates for SSF (Pandey et al. 1999). whereas, for SMF fermentation, liquid synthetic media, soluble sugars, dairy by-products, fruit and vegetable extracts, and wastewater are the commonly used substrates (Subramaniyam and Vimala 2012). Microbes are the bioagents that are responsible for the substrate conversion and product formation. Fermentation: is the microbial process for the formation of a product of interest. Therefore, for a specific biofertilizer production, there is a specific microorganism. The two main key factors for the selection of specific microbial strain for biofertilizer production for the specific crop are the environmental field conditions and the type of crop (Dodd and Ruiz-Lozano 2012).

The fermentation process should be done in a specific manner depending upon the type of microbe's aerobic and anaerobic nature. The fermenter must have aeration facility in case of aerobic fermentation, and the fermenter design must be in such a way that it should maintain anaerobic conditions for the growth of microorganisms in case of anaerobic fermentation (Rosenberger and Elsdens 1960). For the production of biofertilizer, other parameters such as temperature, sterile environment, incubation period, and pH also play an important role in product formation in the fermentation process. Biofertilizer production involves three main steps such as strain development, biomass upscale, and inoculants preparation (Sethi and Adhikary 2012).

The success of the fermentation process for the large scale production of biofertilizer mainly depends on certain sub-steps, such as the selection of efficient microorganisms, optimum growth conditions, suitable nutrient medium, large-scale production, a specific method of propagation, quality testing at each level and pilot-scale study. Further, the selection of suitable carriers for biofertilizer formulation, packaging, storage, and transport are also important factors (Biederbeck and Geissler 1993; Albareda et al. 2008; Atieno et al. 2012). The ideal carrier material should be able to maintain high viable count, high water-holding capacity, low soluble salt content, non-toxicity, biodegradability, and cost-effectiveness (Gomare et al. 2013). The government of India has recommended certain quality standards, under the ambit of FCO 1985 (amended in the year 2009) to maintain the overall quality of produced biofertilizers, that must be followed by the manufacturer and for commercialization of the biofertilizers such as Rhizobium, Azotobacter, Azospirillum, phosphate-solubilizing, and phosphate-mobilizing biofertilizers (Yadav and Chandra 2014). This part of the chapter covers the information on fermentation processes used for the production of the above-mentioned biofertilizers.

Production of N2-Fixing Biofertilizers

N2-fixing biofertilizers are the microorganisms that can fix atmospheric N2 into plant usable form of N in the soil such as Azotobacter Azospirillum and Rhizobium are the most effective and widely used N2 fixers.

Production of Rhizobium Biofertilizers

The first step for Rhizobium biofertilizer production is the selection of strain. The selection of strain must be host-specific, and strain should be able to survive in host-specific environmental conditions. It has been observed that each host plant requires a specific Rhizobium species for effective nodules formulation. The formation of effective nodulation plays an important role in host plant growth (Abi-Ghanem et al. 2012).

Additionally, the suitable C and N sources and mineral nutrients needed for the higher growth rate of bacteria helps to decide the selection of culture medium for the better cultivation of microbes for large scale production of biofertilizer. For the cultivation of Rhizobium species, Yeast extract mannitol (YEM) is the commonly used medium. The YEM medium contains (g/l): mannitol, 10.0; yeast extract, 1.0; K2HPO4, 0.5; MgSO4.2H2O, 0.2; NaCl, 0.1; CaCO3, 1.0; and pH, 6.8 ± 0.2 (Allen and Allen 1950; Subba Rao 1977). However, the use of YEM for large-scale production of Rhizobium is costly. Therefore, commercial producers have a preference for inexpensive and easily available media for production purposes (Ben Rebah et al. 2002). Hence, several scientists have developed media comprises of agricultural waste and industrial by-products such as corn steep liquor, deproteinized leaf extracts, molasses, jaggery solution, wastewater sludge and cheese whey for Rhizobium species cultivation (Jain et al. 2000; Estrella et al. 2004; Ben Rebah et al. 2007; Singh et al. 2011). These ingredients provide essential nutrients needed for Rhizobium cultivation and as well as develop inexpensive and readily available medium in comparison of YEM medium. Besides this, before using these kinds of media for large-scale production, it is important to optimize the growth parameters such as temperature, aeration, pH, and agitation. The optimum pH is between 6 and 8, aerobic conditions and temperature of 28 °C, are needed for better results for the N2-fixing Rhizobium species (Agarwal and Ahmad 2010; Parthiban et al. 2011). The sequence of events is like suitable strain selection, suitable medium, and optimum growth conditions at the laboratory level, and then the final step will be scaleup.

Generally, it is carried out in first pilot-scale production and then large-scale production using different sizes fermenters (Bissonnette et al. 1986). Lastly, the obtained culture is used for either carrier-based formulation or liquid formulation. The pure culture of required Rhizobium species is upscaled and mixed with a suitable carrier material (e.g., lignite, vermiculite, peat, charcoal, kaolin, etc.) for carrier-based biofertilizers (Singh et al. 2012). Later, under aseptic conditions, the formulation is packed in polythene bags and provided to farmers. For liquid-based biofertilizers, materials such as oil, water, or solvents are used as carriers. It has been studied by Leo Daniel et al. (2013) that liquid biofertilizers (Azospirillum, Azotobacter, and Bacillus,) formulated with 0.1% carboxymethylcellulose (CMC), 2% polyvinylpyrrolidone (PVP), and 0.025% polysorbate enhances the growth and survival of the cells for a longer period. The final product is analyzed for the quality after formulation, and it must fulfill the standard specifications as per FCO 1985, for the production of Rhizobium biofertilizer production in India.

Production of Azotobacter Biofertilizers

The most commonly used medium for the cultivation of Azotobacter species is Ashby's N-free medium. It contains the following (g/l): sucrose, 20.0; K2HPO4, 0.2; MgSO4.2H2O, 0.2; NaCl, 0.2; K2SO4, 0.1; CaCO3, 5.0; and pH, 7.4 ± 0.2 (Subba Rao 1977). In this medium, sucrose is used as a C source and atmospheric N2 as an N source.

Production of Azospirillum Biofertilizers

The medium commonly used for the Azospirillum growth is OAB medium. It contains Solution A and B. Solution A (g/l): malic acid, 5; NaOH, 3; MgSO4.7H2O, 0.2; CaCl2, 0.02; NaCl, 0.1; NH4Cl, 1.0; yeast extract, 0.1; FeCl3, 0.01; (mg/l):

Production of Phosphate-Solubilizing Biofertilizers

For the production of Phosphate-Solubilizing Biofertilizers, P-solubilizing ability and their application on the field are the two important factors responsible for the selection of suitable strain. The growth and activity of phosphate-solubilizing strains under field conditions depend on different soil properties such as organic matter, P content, and physical and chemical nature (Kim et al. 1998). The selection of a suitable production medium is the next step, where strain grows and increases cell numbers. The most commonly used medium for PSB such as Bacillus and Pseudomonas is Pikovskaya's medium (Pikovskaya 1948; Roychowdhury et al. 2015). It contains the following (g/l): glucose, 10; tricalcium phosphate (TCP), 5; (NH4)2SO4, 0.5; NaCl, 0.2; MgSO4.7H2O, 0.1; KCl, 0.2; yeast extract, 0.5; MnSO4.H2O, 0.002; FeSO4.7H2O, 0.002; and pH, 7.2 ± 0.2. The next step after P-solubilizing strain selection and production medium in the preparation of mother culture by inoculating pure bacterial culture in the sterile medium and then incubated under shaking condition till the cell population reaches to ~10^9 CFU/ml. Then the scale-up the mother culture is transferred to a pilot-scale fermenter and later on to larger fermenter for bulk production. The required final volume of biofertilizer for application decides the capacity of a fermenter used. The fermentation process would be carried out to the population of selected strain cells reach to 10^9 CFU/ml with continuous agitation and aeration for ~7 days (Pindi and Satyanarayana, 2012). The purity and growth of the selected strain would be checking each day. Finally, the broth would be harvested, stored under cool temperature, and then mixed with suitable carrier material under aseptic condition. The cell count of the final formulation should be $\geq 10^7$ CFU/g for carrier-based formulation. To sustain the culture viability, the carrier-based biofertilizers are usually stored at a cool temperature. The Phosphate solubilizing microbial biofertilizer can be liquid base also ($\geq 10^8$ CFU/ml). Generally, the liquid-based biofertilizers have higher viability, higher activity in the field, and better stability at high temperatures (Leo Daniel et al. 2013; Nehra and Choudhary 2015). For carrier-based and liquid-based formulations of P-solubilizing biofertilizer in India, the recommended quality standards should be as per FCO 1985 (amended in the year 2009).

AM fungus Production -Phosphate-Mobilizing Biofertilizers

It has been studied extensively that AM fungus has a huge potential to be used as a P-mobilizing biofertilizer (Berruti et al. 2016; Rillig et al. 2015). But its application is less because of its obligate symbiotic nature. The AM Fungus cannot be produced in the laboratory at a large scale using synthetic media. Another major limitation of using AM Fungus, its role as P-mobilizing biofertilizer depends on plant genotypes and type of soil. Therefore, the AM Fungus production is generally occurred in control conditions in pot cultures to avoid contaminants. To provide AM fungi massive colonization, the most commonly used host plants such as Zea mays and Brachiaria for large-scale crop inoculum development. The preparation of inoculum using this method involves a concentrated set of the same kind of propagules usually found in natural soil inocula (Berruti et al. 2016). It has been found that the application of AM fungus increased the growth, yield, and nutrient content of host plants. Besides this, the success of the application of AM fungus depends on many factors such as application method (single species or consortium), inoculum origin (native or foreign), and growing conditions (greenhouse or open field) (Secilia and Bagyaraj 1987; Herrmann and Lesueur 2013). The standard specification of P-mobilizing biofertilizer should be as per FCO 1985 in India (amended in the year 2009).

4. For Biofertilizer Production - Past and Present Fermentation Technologies

The present population of the world is around 7.4 billion, and it is going to reach around 9.9 million in the year 2050 (Population Reference Bureau, USA). According to the Food and Agriculture

Organization (FAO) of the United Nations, the average demand for agricultural commodities will be 60% higher in the year 2030 than today (Mia and Shamsuddin 2010). To meet the food demand of such a huge population is going to be a serious concern in the nearby future. In India, the two major limiting factors are a reduction in the availability of agricultural land and poor soil fertility.

Earlier, the agricultural production is increased by using synthetic fertilizers, however, it has caused much ecological damage (Bhardwaj et al. 2014). Therefore, the present time demands to develop technologies that can sustainably increase agricultural production without affecting the environment. To achieve this target, there are currently two technologies having huge potential Organic farming and microbial fermentation. The production and application of microbial fertilizers such as phosphate solubilizing, N2 fixing, plant growth promoters, and phosphate mobilizing) by using microbial fermentation technology for increasing plant productivity and fertility of the soil for sustainable agriculture (Armada et al. 2014). Earlier, the farmers didn't have much knowledge about the benefits of biofertilizers in the field of agriculture, however from the last decade; they understood the biofertilizers' advantages for better crop production.

The government of different countries has conducted several training programs and provided subsidies to the farmers which play an important role in creating awareness and knowledge for biofertilizer usage. Also, the major lacuna about the availability of limited information regarding microbial strains, cultivation conditions, nutrient media, storage, packaging, formulation, application, and microbe's behavior in the real field condition was due to limited research, but nowadays, a lot of information is available because of extensive research on biofertilizers. Presently, vast information is available in the form of published research articles about soil properties, cultivation media, host specificity, a better understanding of incubation conditions, advancement in bioprocessing, and up-gradation in fermenter designs (Zohar-Perez et al. 2005; Malusa et al. 2012).

For nitrogen fixers, the survival and efficiency of nitrogen-fixing strains depend on host plant variety and host soil (Morgan et al. 2005). Earlier, all the nodulating bacteria come under one genus, Rhizobium, and then the results of the application of Rhizobium biofertilizers do not come as expected. However, recently, due to the advancement of the latest molecular biology techniques, the classification of nodulating bacteria was done again and they were kept in different new genera. The introduction of polyphasic taxonomy helps to identify the new genera and species based on their symbiotic performance with selected hosts and also identify new cultural and morphological characteristics due to rRNA-DNA hybridization, DNA-DNA relatedness, 16S rRNA analysis, multilocus enzyme electrophoresis, and RFLP (van Rossum et al.1995). Therefore, the development and application of host-specific strain have started for obtaining better results in the form of crop yield and nitrogen fixation (Hameed et al. 2004).

There is a huge potential in the development of new microbial strains having better efficiency due to advancements in technologies like recombinant DNA technology and genetic engineering. There has been a report regarding the successful transfer of nitrogenase activity into a variety of non-diazotrophic bacteria (e.g., Pseudomonas protegens Pf-5 X940) through the application of genetic engineering. In a case studies of Fox et al. 2016; Li et al. 2016, It has been found out that when wheat and maize are inoculated with a good rhizosphere colonizer, P.

protegens Pf-5 X940 carrying nif genes from P. stutzeri A1501, it has improved host plant growth and N content. The endogenous regulation for both N2 fixation and assimilation in Azotobacter vinelandii has been manipulated by Rafael et al. (2017). They have developed A. vinelandii recombinant strains by a single mutation (substitution of the native promoter with exogenously inducible promoter) and double mutation (by a deletion in the nifLgene). Both the recombinant strains secrete very high levels of ammonium (>20 mM) under special growth conditions into the growth medium and enhance the growth of cucumber plants without adding any nitrogen fertilizer.

Earlier, a lot of time was spent for the formulation of suitable biofertilizer media but due to the development of new statistical software such as Design-Expert, SPSS, Origin, etc., and statistical media optimization techniques, it is quite easy and fast to develop the best media for the better production of biofertilizer. There are numerous studies whereby using agricultural waste and industrial by-products, it is possible to make a cost-effective and strain-specific medium (Peng et al. 2014). There is a need for a better understanding of cultivation conditions. For the development of efficient biofertilizers, it is necessary to simulate the field conditions in the laboratory. In India, since 1970, the biofertilizer production has started commercially. Before that, suppliers from the other countries provided imported cultures, with defined medium and growing conditions.

However, sometimes microbial strains giving good results in the laboratory failed to perform in field conditions. The activity of biofertilizers varies from soil to soil due to different soil properties such as alkalinity, salinity, acidity, moisture, the population of native microbes, and availability of nutrients. It has been found that survivability and activity of Mycorrhizae, Rhizobia, and other microorganisms can vary significantly due to soil alkalinity (Paul and Nair 2008). It has been found that pH above 9.0 limits nutrients such as Ca, P, K, and Mg availability (Duraraj et al. 2016). Earlier, this kind of literature was not available, due to which biofertilizers failed miserably under real field conditions. However, in recent times, because of the available literature, microbial strains can be isolated from the local areas and can be cultivated using a simulation of real field conditions. Then, several field trials can be conducted at the actual field, after that potent microbial strain can be used for the large scale production of biofertilizer (Trivedi and Pandey 2007). Recently, the development of fermenter designs, automatic control systems for maintaining parameters (aeration, pH, foaming, agitation, etc.), and mathematical modeling results in the betterment of fermentation technology in form of precision, and userfriendly (Saithi et al. 2016). The earlier single microbial strain was used for biofertilizer production but nowadays; consortium (more than one strain) has been used successfully in actual field conditions. Chang and Yang (2009) reported the development of thermo-tolerant multifunctional phosphate-solubilizing biofertilizers by using six different thermo-tolerant phosphate-solubilizing microbes into agricultural and animal wastes. Zaiadan et al. (2014) found that among two developed consortia ZOB-1 (Chlorella Vulgaris, Anabaena variabilis, and Azotobacter sp.) and ZBOB-2 (Chlorella Vulgaris, Nostoc calcicola, and Azotobacter sp.), ZOB-1 displayed better germination and rice plants growth.

There are two types of commercially available Biofertilizers such as liquid-based and solid based. Solid-based biofertilizers, also known as carrier-based biofertilizers, and synthesized with the help of carrier materials e.g. peat, activated charcoal, soil, humus, lignite, etc. These materials can act as a carrier for microbes. Earlier, carrier-based biofertilizers were used commonly. But there are some drawbacks associated with carrier-based biofertilizers such as low shelf life, contamination, being bulky, temperature sensitivity, the problem of packaging, and high transport cost (Shanware et al. 2014; Trivedi et al. 2016). Generally in conventional carrier-based methods, carriers such as charcoal and lignite (100 mesh size) are sterilized using open-topped stainless steel trays. But, recently, this method has been improved by FAO; where the sterilized carrier is sealed in packages. To prevent contamination while sterilization, gamma radiation, and autoclaving the carrier material at 121 °C for 1 h is found to be one of the effective methods (Senoo et al. 2002; Abd El-Fattah et al. 2013). Nowadays, liquid biofertilizers consist of oil, water, emulsion, etc. The liquid biofertilizers have a shelf life of ~2 years (longer survival in the field which fulfills the nutrient demand of crops for the entire life cycle), UV radiation tolerant, high temperature(up to 55°C) stability for storage, easy packaging, fewer chances of contamination, user-friendly field application, higher potential to fight against the native population and required 10 times lower dosage as compared to carrier-based biofertilizers (Verma et al. 2011; Nehra and Choudhary 2015). Recently, "nanoencapsulation technology" has been introduced based on the nanotechnology approach. The conventional used PGPR biofertilizer is less effective as most of it gets lost during the application of PGPR biofertilizer. While the PGPR synthesized using nanoencapsulation technology overcome this problem and gave better results (Pindi and Satyanarayana 2012).

Recent developments in the area of molecular biology, microbial taxonomy, metabolic engineering, genetic engineering, nanotechnology, and computer science have played an important role for the betterment of fermentation technology and help to understand the biofertilizer production process. Therefore, the process of large scale production of biofertilizer became cost-effective and user-friendly and cost-effective. In this way, novel research and development in the field of biofertilizer development needed to replace synthetic fertilizers and ultimately for better crop productivity for sustainable agriculture to meet the need for increasing food demand.

5. Biofertilizers Application

The production of carrier-based biofertilizers is the most effective and inexpensive method for better crop production. The large scale production involves major steps such as isolation of efficient microbial strains, preparation of carrier material, mixing of culture with sterilized carrier material, and packing. The carrier material should ideally have the following properties such as locally available, inexpensive, readily available, non-toxic, easy to process, high water-holding capacity, and organic and can carry high cell population and as well as should be able to support microbial survivability for a long time. The commonly used carrier material such as vermiculite, peat soil/lignite, press mud, charcoal, soil mixture, and farmyard manure is generally neutralized. But, they have certain limitations such as temperature sensitivity, possessing lower shelf-life, becoming less effective by low cell counts, and being contamination prone. Thus, there was a need for the development of liquid formulations for Azospirillum, Rhizobium, Acetobacter, and Azotobacter, though it is a costlier method, but has advantages of having higher cell counts, easier production, longer shelf-life, no contamination, storage up to 45 C and greater competence in soil(Ngampimol and Kunathigan 2008). However, the application method of biofertilizers involves seedling root dipping, seed treatment, and soil application.

5.1 Seed Treatment

Seed treatment is the most common, effective, and cost-effective method for all types of microbial strains (Sethi et al. 2014). The seeds are uniformly mixed and coated in a slurry (having inoculants and 200 mL of rice kanji), shade-dried and then sown within 24 h. To obtain fruitful results, direct seed coating is one of the methods of application of biofertilizer using gum arable or sugary syrup and efficient microbial strain especially coating of microbes over specific host plant seeds Firstly, the prepared inoculum are directly coated or used by the slurry method. The CaCO3 is added to sticky seeds immediately after seed coating. In 1896, Voucher used this technique of seed inoculation. Sometimes, under actual field conditions, the nodulating bacteria are not present in sufficient quantity to fix atmospheric nitrogen. Under such conditions, it is necessary to inoculate seeds directing with the efficient microbial strain. Depending upon seed quantity, if quantity is small, the coating can be done in a plastic bag; if quantity is large the coating can be done in the bucket in case of liquid biofertilizers. The seed treatment can be done by using two or more bacteria together for example; nitrogen fixer such as Azotobacter, Rhizobium, and Azospirillum can be taken along with phosphorus-solubilizing microbes without any antagonistic effect, and provide the maximum quantity of each bacterium on individual seed needed for better results (Chen 2006). The seed treatment can be done for many plants by using Rhizobium in case of pulses like pea, chickpea, groundnut, soybean, lentil, beans, berseem, lucern, black gram, green gram, pigeon pea, and cowpea,) for, Azotobacter (cereals like oat, wheat, barley; seasum, oilseeds like mustard, linseeds, sunflower, castor; millets like finger millets, pearl millets, Kodo millet; forage crops and grasses like sudangrass, bermudagrass, para grass, Napier grass, star grass, etc.), and Azospirillum or phosphorus-solubilizing bacteria (maize, rice, and sorghum) (Taylor and Harman 1990).

5.2 Seedling Root Dipping

This application is used for plantation crops such as vegetables, cereals, trees, fruits, sugarcane, grapes, cotton, banana, and tobacco where seedling roots are dipped in a water suspension of microbial inoculants (nitrogen-fixing Azotobacter or Azospirillum and phosphorus-solubilizing microbial biofertilizer) for sufficient time. The treatment time varies for different crops, for example, 20–30 min for vegetable crops and 8–12 h for paddy before transplantation (Barea and Brown 1974).

5.3 Soil Application

In soil application, the biofertilizer is applied directly to the soil either alone or in combination. The mixture of phosphate solubilizer, rock phosphate, and cow dung is kept in shade overnight while maintaining its moisture content at 50% and then applied to the soil (Pindi and Satyanarayana 2012). For example Azotobacter (for coffee, tea, rubber, all fruit/agroforestry plants for fuelwood, fruits coconuts, gum, fodder, leaves, spice, nuts, flowers, and seeds) and Rhizobium (for leguminous plants or trees) (Zahran 1999; Hayat et al. 2010).

Mostly, the final inoculant is the mixture of the broth and finely sterilized carrier material. A good carrier should have the following properties such as good moisture absorption capacity, non-toxicity, easy to sterilize, free of lump forming material, easily available, good buffering capacity, and inexpensive for maintaining the survivability of selected microbial strain. For the preparation of inoculum, the most commonly used carrier is peat. But in India, peat is not easily available. While there is a readily available wide range of carriers such as coal, charcoal, lignite, filter mud, vermiculite, mineral soils, polyacrylamide, vegetable oils, and bagasse, etc. The carrier processing such as drying, mining, and milling is the most capital intensive aspect for the production of bio-fertilizer. Firstly, the carrier such as peat is mined, drained, and cleared off stones, roots, etc. Then it is shredded and dried to pass through heavy mills. For seed coating, the material with a particle size of 10-40 µm is collected. Whereas, for soil implant inoculant, Peat with a particle size of 500-1500 µm is used. And it is really important to neutralize carrier material by adding precipitated calcium carbonate (pH 6.5-7.0). Lastly, it is sterilized to use with an inoculant.

6. Microbial Biofertilizers available in the market

In the market, there are numerous microbial biofertilizers available in the form of dried or liquid cultures under different trade names, that can be used for various purposes such as soil fertility and plant growth enhancement). For example, the rhizobium biofertilizers can maintains soil fertility, fix 50–300 kg N ha1 which increases crop yield by 10–35%, and leaves residual nitrogen for subsequent crops (Davis 1996; Chen 2006). Similarly, he Azotobacter biofertilizer can maintains soil fertility, fix 20–40 mg N g1 of carbon source that causes up to crop 15% increase in yield and produces plants growth-promoting substances such as indole acetic acid, vitamin B complexes, and gibberellic acid; and also control plant diseases by killing some of the plant pathogens (Abd El-Lattief 2016; Kurrey et al. 2018). The phosphorus-solubilizing bacterial biofertilizers are nonspecific and can be used for all crops, produce enzymes that mineralize the insoluble organic phosphorus into a soluble form, thereby increasing crop yield by 10–30% (Sharma et al. 2013).

7. Limitations of Microbial Biofertilizers

Though biofertilizer technology is environmentally friendly and has many advantages, there are certain limitations associated with this technology that causes doubt among stakeholders about its application. The main disadvantages related with biofertilizers which need immediate attention by extensive research as well as proper planning involves lower nutrient density (thus, are required in bulk to be made available for most crops), plant specificity, the difficulty of storage, requirement of separate machinery and skill for production and application than that used for chemical fertilizers, and more importantly insufficient awareness about their use, application and benefits among farmers (Malusà et al. 2016). Also, there are certain limitations about biofertilizers application which affect the technology at stages of production, marketing, or usage (Jangid et al. 2012).

8. Conclusion

Microbial biofertilizers are the most important part of sustainable organic farming in the current agricultural practices as compare to synthetic fertilizers which cause numerous environmental damage. They can fix atmospheric nitrogen in the soil and host pant root nodules, solubilize phosphate (from insoluble forms to soluble forms), help in the decomposition of organic matter for soil mineralization, and also produce plant growth promoter hormones and antimetabolites to enhance plant growth. Thus, biofertilizers can results in better crop production and yield, sustain soil structure (by maintaining soil aggregation for better water relations), and made plants drought tolerance (by maintaining stomatal functioning, enhancing leaf water and turgor potential, and increasing root development). Though, due to the increasing awareness and demand about the biofertilizers use among farmers and planters may encourage the young entrepreneurs to explore biofertilizer manufacturing, but this requires reassurance and support from the government also. The production of microbial biofertilizer technology is an integral part of modern-day agriculture for sustainability. It should be proper for the social and infrastructural situations of the users, viable and cost-effective, renewable, acceptable by different societal segments, adaptable to existing local conditions and various cultural patterns of society, stable in a long-term perspective, productive and practically implementable. Therefore, it is obvious that awareness of the importance and economic feasibility of biofertilizer application technology has to be improved by the proper practical training of farmers and dealers.

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Research Abstract

Improving Crop Yield and Nutrient Use Efficiency via Biofertilization—A Global Meta-analysis-

The application of microbial inoculants (biofertilizers) is a promising technology for future sustainable farming systems in view of rapidly decreasing phosphorus stocks and the need to more efficiently use available nitrogen (N). Various microbial taxa are currently used as biofertilizers, based on their capacity to access nutrients from fertilizers and soil stocks, to fix atmospheric nitrogen, to improve water uptake or to act as biocontrol agents. Despite the existence of a considerable knowledge on effects of specific taxa of biofertilizers, a comprehensive quantitative assessment of the performance of biofertilizers with different traits such as phosphorus solubilization and N fixation applied to various crops at a global scale is missing. We conducted a meta-analysis to quantify benefits of biofertilizers in terms of yield increase, nitrogen and phosphorus use efficiency, based on 171 peer reviewed publications that met eligibility criteria. Major findings are: (i) the superiority of biofertilizer performance in dry climates over other climatic regions (yield response: dry climate +20.0 ± 1.7%, tropical climate +14.9 ± 1.2%, oceanic climate +10.0 ± 3.7%, continental climate +8.5 ± 2.4%); (ii) meta-regression analyses revealed that yield response due to biofertilizer application was generally small at low soil P levels; efficacy increased along higher soil P levels in the order arbuscular mycorrhizal fungi (AMF), P solubilizers, and N fixers; (iii) meta-regressions showed that the success of inoculation with AMF was greater at low organic matter content and at neutral pH. Our comprehensive analysis provides a basis and guidance for proper choice and application of biofertilizers.

Lukas, Schutz, Andreas Gattinger, Matthias Meier, Andrian Muller, Thomas Boller, Paul Mader and Natarajan Mathimaran (2018) Front. Plant Sci., 12.

Contrasting Impacts of Long-Term Application of Biofertilizers and Organic Manure on Grain Yield of Winter Wheat in North China Plain

The effects of long-term incorporation of organic manure and biofertilizers have been investigated on winter wheat in the North China Plain (NCP). The five-year field experiment (2013–2018) has illustrated the responses of grain yield and yield components. Seven fertilization approaches, included pig farm-yard-manure and biofertilizers amendments combined with five NPK% drop levels of chemical fertilizer ratio + organic fertilizer + biofertilizer (0, C+O+B) 25%, CL4; 50%, CL3; 75%, CL1; and 100%, CL0), without fertilizer as control (CK), in NCP during the years 2013–2018. Results showed that the grain yields of CL1 and CL2 were equivalent to CL0 in all growing seasons except 2014/2015. The grain yields of CL4 were 29.9% to 46.6% lower than that of CL0 during 2014/2015, 2016/2017, and 2017/2018. The valuable spike-number, grain number per-spike, and 1000-grain weight showed significant variations among different growing periods.

Regression analysis of grain yield and yield components indicated that number grains per-spike showed significant increase in seed yield formation. The 1000-grain weight was the major parameter that influenced yield of moderate and low yielding periods, respectively. The results revealed that application of 30 m3 ha pig farm-yard-manure and 20 kg ha biofertilizers has reduced at least 50% of the NPK fertilization without dropping grain yields in the North China Plain.

Keywords : biofertilizers; organic manuring; grain yield; winter wheat; sustainable agriculture.

Amara Cisse, Adnan Arshad, Xiaofen Wang, Fanta yattara and Yuengao Hu (2019) : Agronomy, 9:312.

Effects of Biofertilizer Produced from Bradyrhizobium and Streptomyces griseoflavus on Plant Growth, Nodulation, Nitrogen Fixation, Nutrient Uptake, and Seed Yield of Mung Bean, Cowpea, and Soybean

Abstract: The use of biofertilizers is important for sustainable agriculture, and the use of nodule bacteria and endophytic actinomycetes is an attractive way to enhance plant growth and yield. This study tested the effects of a biofertilizer produced from Bradyrhizobium strains and Streptomyces griseoflavus on leguminous, cereal, and vegetable crops. Nitrogen fixation was measured using the acetylene reduction assay. Under N-limited or N-supplemented conditions, the biofertilizer significantly promoted the shoot and root growth of mung bean, cowpea, and soybean compared with the control. Therefore, the biofertilizer used in this study was effective in mung bean, cowpea, and soybean regardless of N application. In this study, significant increments in plant growth, nodulation, nitrogen fixation, nitrogen, phosphorus, and potassium (NPK) uptake, and seed yield were found in mung beans and soybeans. Therefore, Bradyrhizobium japonicum SAY3-7 plus Bradyrhizobium elkanii BLY3-8 and Streptomyces griseoflavus are effective bacteria that can be used together as biofertilizer for the production of economically important leguminous crops, especially soybean and mung bean. The biofertilizer produced from Bradyrhizobium and S. griseoflavus P4 will be useful for both soybean and mung bean production.

Keywords : biofertilizer; nitrogen fixation; nodulation; plant growth; seed yield

National Events

National Conference on "Quality Control of Microbial Products and Botanicals of Agricultural Importance"

One day National conference on "Quality Control of Microbial Products and Botanicals of Agricultural Importance" was organized by DAC & FW and NCOF Ghaziabad in the auditorium of this office on 20.12.2019, wherein delegates from Ministry of Agriculture & Farmers Welfare, scientists and officers from IARI, ICAR, Government of India, State Government, State Agriculture Universities, organic farming experts, entrepreneurs from Biofertilizer units, renowned farmers from various states, speakers, participants, media representatives, officers of National and Regional Centres of Organic Farming where present. The Programme was inaugurated by the delegates of Ministry of Agriculture & Farmers Welfare -Dr. Alka Bhargava Additional Secretary, Dr. S.K. Malhotra Agriculture Commisioner, Smt Neeraja Adidam Joint Secretary INM and Sri Vipin Kumar Bansal, Director NCOF. The conference comprised of four technical sessions covering different aspects of microbials and botanicals. Several recommendations came out after panel discussion. The conference served as a common platform for knowledge dissemination, recent advancements and sharing of the technology on different aspects of organic inputs.

Refresher Training Course on Quality Control of Biofertilizers and Organic Fertilizers conducted from 06 January 2020 to 12 January 2020, at NCOF Ghaziabad

Refresher Training Course on Quality control of Biofertilizers and Organic Fertilizers was conducted from 06 January to 12 January 2020 at this office in which all technical officers of NCOF & RCOF participated. The course comprised of both lectures as well as hands on training covering all the aspects of quality testing of organic inputs. The training was imparted by several technical experts, scientists and officers from IARI, CFQCTI, NBAIM etc.

BIOFACH INDIA : 7 to 9 November 2019 at India Expo Centre (IEML) in Greater Noida.

11th BIOFACH INDIA, an organic platform in India to connect and network with audience interested in organic farming was organized at India Expo Centre, IEML, Greater Noida, Delhi-NCR, from 7th to 9th November 2019.

This event provided the perfect business platform to organic stakeholders, retailers, exporters / importers, Govt. boards, state pavilions, certification bodies, consultants and associations from India and all over the world to congregate for networking. Organic is more than a label or certification: organic stands for quality and conviction – for the responsible use of nature's resources. BIOFACH INDIA serve as the meeting place in India where anyone and everyone who is connected to organic converge annually to share their passionate interest for organic products, network at a common platform and educate themselves about the latest developments in the organic sector.

STATE-WISE PRODUCTION OF BIOFERTILISERS IN INDIA -2018-19 (in MT)

S N.	*State*	*Solid Carrier Based (MT)*	*Liquid Carrier Based (KL)*
South Zone			
1	And & Nicobar	-	-
2	Andhra Pradesh	263.88	-
3	Daman & Diu	-	-
4	Karnataka	3253.7	758.19
5	Kerala	108.24	2.098
6	Lakshadweep	-	-
7	Pondicherry	121.9	6.192
8	Tamil Nadu	4187.243	536.784

9	Telangana	2556.29	12711.74
West Zone			
1	Chhattisgarh	172.029	133.74
2	Gujarat	10596	430.529
3	Goa	2044	-
4	Madhya Pradesh	7426.517	327.193
5	Maharashtra	15049.75	4193.78
6	Rajasthan	791.81	1.12
7	D & N Haveli	-	-
North Zone			
1	Delhi	394	-
2	Chandigarh	-	-
3	Haryana	2128.7	246.54
4	Himachal Pradesh	135.112	12.05
5	Jammu & Kashmir	-	0.04
6	Punjab	7167.384	220.785
7	Uttar Pradesh	2451.8	2444.55
8	Uttarakhand	3359.79	281.406
East Zone			
1	Bihar	130.83	-
2	Jharkhand	20.96	-
3	Odisha	8166.89	149.692
4	West Bengal	2050	37.74
North East Zone			
1	Arunachal Pradesh	0	0
2	Assam	617.197	-
3	Manipur	81.5	100
4	Meghalaya	-	-
5	Mizoram	2.5	-
6	Nagaland	17.452	-
7	Sikkim	-	51.5
8	Tripura	81.79	-
	Total	**73377.264**	**22645.669**

STATE-WISE PRODUCTION OF BIOFERTILISERS IN INDIA (2019-20)

	State	Carrier based (MT)	Liquid based (KL)
South Zone			
1	Andaman & Nicobar Island	-	-
2	Andhra Pradesh	228.27	-
3	Daman & Diu	-	-
4	Karnataka	3606.72	1218.00
5	Kerala	91.03	5512.14
6	Lakshadweep	-	-
7	Puducherry	121.90	7.57
8	Tamil Nadu	11611.00	1481.59
9	Telengana	1536.33	174.75
West Zone			
1	Chhattisgarh	26.77	189.64
2	Gujarat	20788.00	9444.00
3	Goa	50.00	-
4	Madhya Pradesh	1330.01	315.64
5	Maharashtra	15897.00	237.14

6	Rajasthan	2142.78	
7	Dadar & Nagar Haveli	-	-
North Zone			
1	Delhi	345.00	-
2	Chandigarh	-	-
3	Haryana	2794.77	-
4	Himachal Pradesh	320.00	137.90
5	Jammu & Kashmir	-	-
6	Punjab	9252.19	192.49
7	Uttar Pradesh	2142.78	2539.89
8	Uttarakhand	3118.98	4980.00
East Zone			
1	Bihar	375.00	1900.00
2	Jharkhand	5.15	-
3	Odisha	449.20	1719.00
4	West Bengal	2200.00	-
North East Zone			
1	Arunachal Pradesh	-	-
2	Assam	640.33	4.90
3	Manipur	13.20	11.51
4	Meghalaya	-	-
5	Mizoram	1.40	-
6	Nagaland	18.75	-
7	Sikkim	-	-
8	Tripura	340.06	39.78
	Total	79446.61	30105.94
* Source : Data received from States			

STATE WISE PRODUCTION OF ORGANIC MANURES IN INDIA -2018-19 (in MT)

Sl. No.	State	City Compost (A)	Organic manure (B)	Vermi-compost (C)	PROM (D)	Bioenriched Organic Manure (MT) (E)	Rural Compost (F)	Farm Yard Manure (G)	Total Manure MT (A+B+C+D+E+F+G)	Deoiled Cake MT
1	Andhra Pradesh	-	787.45	390	0	70	16000	62000	79247.45	8000
2	Arunachal Pradesh	-	-	-	-	-	-	-	-	-
3	Assam	-	-	-	-	-	-	-		-
4	Bihar	0	26965	110090	0	0	-	-	137055.00	-
5	Chhattisgarh	1000	1484	1050	2662.2	0	-	-	6196.20	-
6	Delhi	3686.91	27.21	293	0	0	-	-	4007.12	-
7	Goa	13.82	0	44.03	0	0	-	-	57.85	-
8	Gujarat	52261	44077.89	690	21250.05	5000	-	-	123278.940	-
9	Haryana	0	34.1	0	3915.64	0	-	-	3949.74	-
10	Himachal Pradesh	-	0.5	5145	0.5	0	0.00	29	5175.00	0.1

11	Jammu & Kashmir	-	-	-	-	-	-	-	-	-
12	Jharkhand	714.00	-	-	-	-	-	-	-	-
13	Karnataka	71081	136116	33007	13454	14531	1011000	37481000	38760189.00	2035
14	Kerala	-	-	77750.00	-	-	-	-	-	-
15	Madhya Pradesh	4071.00	8941.41	10089.28	17056.39	-	-	-	40158.08	-
16	Maharashtra	17638	3457	796.907	1494.2	0	-	-	23386.11	1004.28
17	Manipur	50.00		-	-			-	50.00	
18	Meghalaya	-	-	-	-	-	-	-	-	-
19	Mizoram	-	-	-	-	-	-	-	-	-
20	Nagaland	-	-	-	-	-	-	-	-	-
21	Odisha	32500	7860.66	30800	122.35	35	-	-	71318.01	
22	Punjab	27230.59	10980.85	164.99	6247.2	121.38	-	-	44745.01	209.57
23	Rajasthan	19500	2800	12200	11100	50	-	-	45650.00	
24	Sikkim	-	-	-	-	-	-	-	-	-
25	Tamil Nadu	-	-	34109.00	-	-	8875	1067348	1110332.00	13302
26	Telengana	35652.5	1029.16	427885	1023.35	0	-	-	465590.01	
27	Tripura	-	-	-	-		-	-	-	-
28	Uttar Pradesh		977.4	0	4210.25	11941	-	-	17128.65	-
29	Uttarakhand	917.86	666.18	63.95	346.63	2.365	30000	-	31996.99	11852
30	West Bengal	45000	3449	3330	0.2	0	-	-	51779.20	-
31	Chandigarh	-	-	-	-	-	-	-	-	-
32	Puducherry	150	-	70	-	-	500.00	500.00	1220.00	60
	Total	**311466.68**	**249653.81**	**747968.16**	**82882.96**	**31750.75**	**1066375.00**	**38610877.00**	**41100974.35**	**36462.950**

BOOK REVIEW

Biofertilizers for Sustainable Agriculture and Environment, 1st ed. 2019 Edition by Bhoopander Giri (Editor), Ram Prasad (Editor, Qiang-Sheng Wu (Editor), Ajit Varma (Editor)

This book provides a comprehensive overview of the benefits of biofertilizers as an alternative to chemical fertilizers and pesticides. The book covers the latest research on biofertilizers, ranging from beneficial fungal, bacterial and algal inoculants; to microbes for bioremediation, wastewater treatment; and recycling of biodegradable municipal, agricultural and industrial waste; as well as biocontrol agents and bio-pesticides. As such, it offers a valuable resource for researchers, academics and students in the broad fields of microbiology and agriculture.

Biofertilizers and Biopesticides – 29 June 2019 by Krishnendu Acharya (Author, Surjit Sen (Author, Manjula Rai (Author)

Biofertilizers and biopesticides have emerged as potential environment – friendly agricultural inputs and they are gaining priority over additives of inorganic system of cultivation which are shown to have many negative effects upon ecosystem and human health. Biofertilizers and biopesticides contain living microorganisms or their products which, when applied to seeds, plant surfaces, or soil, promotes growth and proliferation. The book was written primarily as text book for skill enhancement courses (SEC) of undergraduate botany and microbiology students but this book would also be useful for researchers, farmers, social workders, extension people and inductrialists to get an idea to adopt new strategies to increase agricultural production sustainably.

राष्ट्रीय जैविक खेती परियोजना के अंतर्गत राष्ट्रीय एवं क्षेत्रीय जैविक खेती केन्द्रों के पते और उनके कार्यक्षेत्र राज्य List and Address of National and Regional Organic Farming Centres with states of their jurisdiction	
निदेशक राष्ट्रीय जैविक खेती केन्द्र सेक्टर 19, हापुड़ रोड, कमला नेहरू नगर, गाजियाबाद–201 002 (उ.प्र.) 0120-2764906, 2764212; Fax:0120-2764901 वेबसाइट : http://ncof.dacnet.nic.in ईमेल : nbdc@nic.in	Director National Centre of Organic Farming, Sector 19, Hapur Road, Kamla Nehru Nagar, GHAZIABAD - 201 002 (UP). 0120-2764906, 2764212; Fax:0120-2764901 Web: http://ncof.dacnet.nic.in Email: nbdc@nic.in
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र (मुख्यालय) सेक्टर 19, हापुड़ रोड, कमला नेहरू नगर, गाजियाबाद–201 002 (उ.प्र.) 0120-2764212; Fax:0120-2764901 ईमेल : rcofhq.gzb-agri@gov.in उत्तर प्रदेश (आजमगढ़, बलिया, बस्ती, चंदौली, देवरिया, फैजाबाद, अयोध्या, गाजीपुर एवं गोरखपुर जिलों का छोड़कर), उत्तराखंड, दिल्ली एवं राजस्थान	Regional Director Regional Centre of Organic Farming (HQ), Sector 19, Hapur Road, Kamla Nehru Nagar, GHAZIABAD - 201 002 (UP). 0120-2764212; Fax:0120-2764901 Email: rcofhq.gzb-agri@gov.in Delhi, Rajasthan, Uttarakhand, and Uttar Pradesh (Except Districts Of Azamgarh, Ballia, Basti, Chandauli, Deoria, Faizabad, Ayodhya, Ghazipur & Gorakhpur).
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र कन्नामन्गला क्रास, व्हाइट फील्ड – होसकोटे रोड, काडुगोडी पोस्ट, बेंगलूरू–560 067 (कर्नाटक) 080-28450503 ईमेल : biofkk06@nic.in, कर्नाटक, केरल, तमिलनाडु, पांडिचेरी तथा लक्षद्वीप	Regional Director Regional Centre of Organic Farming, Kannamangala Cross, Whitefield – Hosekote Road, Kadugodi Post, BENGALURU-560067 (Karnataka). 080-28450503 Email: biofkk06@nic.in, Karnataka, Kerala, Tamilnadu, Pondicherry and Lakshdweep
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र प्लाट 23–पी, खण्डागिरी–चंदका रोड, कलिंगा स्टूडियो चौक के पास, घटीकिया, भुवनेश्वर–751 003 (उड़ीसा) 0674-22954958 ईमेल : biofor04.or@nic.in उड़ीसा, पश्चिम बंगाल एवं अंडमान निकोबार	Regional Director Regional Centre of Organic Farming, Plot No.23(P), Khandagiri-Chandka Road, Near Kalinga Studio Chowk, Ghatikia, BHUBANESHWAR-751003 (Orissa). 0674-2954958, Email: biofor04.or@nic.in Orissa, West Bengal and Andman & Nicobar
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र किसान भवन, सेक्टर 14, पंचकुला–134 109 (हरियाणा) 0172-2971718 ईमेल : biofhr05@nic.in हरियाणा, हिमाचल प्रदेश, पंजाव, जम्मू एण्ड कश्मीर एवं चंडीगढ़।	Regional Director Regional Centre of Organic Farming, Kisan Bhawan, Sector 14, Panchkula-134 109 (Haryana). 0172-2971718, Email: biofhr05@nic.in Haryana, Himachal Pradesh, Punjab, Jammu & Kashmir and Chandigarh (UT).

क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र लांगोल रोड, लैम्फलपेट, इंफाल–795 004 (मनिपुर) 0385-2413239 ईमेल : biofmn01@nic.in आसाम, अरुणाचल प्रदेश, मेघालय, मिजोरम, मणिपुर, नागालैंड, त्रिपुरा तथा सिक्किम	Regional Director Regional Centre of Organic Farming, Langol Road, Lamphelpat, IMPHAL-795 004 (Manipur). 0385-2413239 Email: biofmn01@nic.in Assam, Arunachal Pradesh, Meghalaya, Mizoram, Manipur, Nagaland, Tripura and Sikkim
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र 67/1, केशव स्मृति, लक्ष्मीपुर, शताब्दीपुरम, मुस्कान प्लाजा के पीछे, जबलपुर–482 002 (मध्य प्रदेश) 0761-2971234, ईमेल : biofmp06@nic.in मध्य प्रदेश एवं छत्तीसगढ़।	Regional Director Regional Centre of Organic Farming, 67/1, Keshav Smriti, Lakshmipur, Shatabdipuram, Behind Muskan Plaza, JABALPUR-482 002 (Madhya Pradesh). 0761-2971234, Email: biofmp06@nic.in Madhya Pradesh and Chhattisgarh.
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र अमरावती रोड, राष्ट्रीय राजमार्ग 6, ग्राम – गोंडखेरी, पोस्ट – वाडी, कलमेश्वर, नागपुर–440 023 (महाराष्ट्र) 07118-297054 ईमेल : biofmh10@nic.in महाराष्ट्र, आन्ध्र प्रदेश, तेलंगाना	Regional Director Regional Centre of Organic Farming, Amravati Road, NH6, Village – Gondkhairy, Post – Wadi, Kalmeshwer, NAGPUR-440 023 (Maharashtra). 07118-297054, Email: biofmh10@nic.in Maharashtra, Andhra Pradesh and Telengana
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र केन्द्रीय आलू अनुसंधान केन्द्र परिसर, सहायनगर, जगदेव पथ पटना–801 506 (बिहार). 0612-2225024, ईमेल : rcof.pat-agri@gov.in बिहार, झारखंड एवं पूर्वी उत्तर प्रदेश (आजमगढ़, बलिया, बस्ती, चंदौली, देवरिया, फैजाबाद, अयोध्या, गाजीपुर एवं गोरखपुर जिले)	Regional Director Regional Centre of Organic Farming, Central Potato Research Station Campus, Sahaynagar, Jagdev Path, Patna-801 506 (Bihar). 0612-2225024, Email: rcof.pat-agri@gov.in Bihar, Jharkhand and Eastern Uttar Pradesh (Azamgarh, Ballia, Basti, Chandauli, Deoria, Faizabad, Ayodhya, Ghazipur & Gorakhpur Districts)
क्षेत्रीय निदेशक क्षेत्रीय जैविक खेती केन्द्र एपीआईसी, पोडियम लेवल कृषि भवन, सेक्टर 10 ए, गांधीनगर – 382 010 (गुजरात). 079–23257465, ईमेल : ad-gnagar@ncof.dacnet.nic.in गुजरात, गोआ, दमन एवं दीव, दादर और नगर हवेली	Regional Director Regional Centre of Organic Farming, APIC, Podium Level Krishi Bhawan, Sector 10-A Gandhinagar-382 010 (Gujrat). 079-23257465, Email : ad-gnagar@ncof.dacnet.nic.in Gujrat, Goa, Damn & Diu, Dadra and Nagar Haveli
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