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Boosting Agricultural Production - Beneficial Biofertilizers

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A B S T R A C T

By 2050, the world population is expected to reach nine billion, so doubling the production of food in order to meet human daily needs will be a great challenge for the global agricultural system. Soil is the most complex natural entity with broad range of microbial biomass; however the soil's natural fertility gradually decreases to the use of synthetic chemicals, fertilizers and pesticides. Global food production has therefore declined, and its production needs to be boosted in a sustainable way in order to introduce the idea of biofertilizer. A significant part of Integrated Nutrient Management is biofertilizer. In soil fertility and sustainability the potential biofertilizers play a key role. The three main forms of biofertilizers used are *Rhizobium, Arbuscular Mycorrhizal Fungi* and *Azolla*. These are organic substances which use different strains of specific micro-organisms in order to increase soil fertility and boost agricultural productivity in a sustainable manner for the future.

Introduction

The soil is a diverse natural entity having diverse diversities of microbial population and with enormous and critical agricultural functions. The microbial biomass is made of various species of bacteria, fungi, algae and other living micro-organisms. In order for a plant growth to grow at its optimum level, essential as well as accessory nutrients; macronutrients and micronutrients must be readily available in sufficient and in balanced proportions (1). Soil infertility is the major contributing factor in reducing crop yields in different countries worldwide. The use of improved varieties and other agricultural practices would not help farmers until the soil fertility is restored. The loss of the soil fertility gradient in its entirety can be recuperated and regenerated by following certain protocols and principles such as integrated soil fertility management (ISFM), integrated nutrient management (INM), nutrient conservation management techniques, biological nitrogen fixation (BNF) and an improved input efficiency (2). Biofertilizer is an important component of INM. Economic and environmentally sustainable inputs for farmers are the potential biofertilizers that play a key role in productivity and soil sustainability. A Biofertilizer is a substation with live micro-organisms that colonizes the rhizosphere or the inside of plant cells when applied to seeds, plant surfaces or soil, and promotes plant growth by increasing the availability and supply of primary nutrients to the host plant(3).Bacteria, fungi, algae, cyanobacteria etc. are the primary sources of biofertilizers. Biofertilizers are organic substances using different microorganism organisms and stains to improve soil fertility. These are biological preparations with enough density that have an advantageous function in the rhizosphere for plant growth. These biofertilizers include microorganisms to restore the natural cycle of the soil nutrients and produce organic soil. Healthy plants can be produced through the use of biofertilizers, thus improving soil health and sustainability. By natural nitrogen fixation processes, the solubility of phosphorus, and the synthesis of growth-promoting substances stimulating growth, they add various essential nutrients. These fertilizers are not like chemical fertilizers that have intrinsically harmful effects on plants and crops. The use of synthetic fertilizers and pesticides can be minimized by using biofertilizers. In reality, the natural biological system of mobilization of the nutrients is promoted through the use of biofertilizers (4).

What are Biofertilizers

Biofertilizer may also be defined as a preparation of a live or latent cell containing efficient nitrogen (N) fixation, phosphate (P) solubilization, or cellulite microorganisms used in seed, soil applications or composting areas in order that the number of these micro-organisms may be increased and that certain microbial processes may be accelerate to augment the extent of the availability of nutrients in a form which can assimilated by plant. The bio-fertilizer idea started traditionally with the small-scale processing of compost that is now obviously a proof of bio-fertilizer abilities. This is understood by the accelerating of crops by different methods to ensure successful crop production the total decomposition of bio residues and by-products (5). The substrates used for inoculating these microbes are mine sands and other agricultural wastes hence production cost is cheaper and environmentally safe.

Need of Biofertilizers

In general the soils fertility gradient in most countries are depleting due to indiscriminate and judicious use of chemically synthetized fertilizers which is substantially causing pollution, contamination and weakening the soil, run-off leading to polluted water catchment areas and basins, destruction of microbial biomass by creating unfavorable environment for them to survive, leading crops and plants being more attracted towards disease infestations with less yield per capital and reduced soil fertility status. The long-term application of chemical fertilizers often degrades the soil and affects the growth of crops. The world population is projected to hit about 8 billion by 2025 (6).Meaning that there will be a need to feed more individuals than the present thus a more holistic approach is needed. An estimate by 2020 a target of 322 million tonnes production of food grain, required nutrient will be approximately 28.8 million tonnes but its availability will only be 21.6 million having a deficit of nearly 7.2 million tones (7). The farmers are at present unable to afford increased cost of synthetic fertilizers. Depletion of natural soil fertility due to the removal of nutrients, which is integrated by environmental risks, poses a greater risk to sustainable farming (8). In these circumstances, the use of biofertilizer in comparison to synthetic fertilizers is economically environment-friendly, more effective, profitable and long-term accessible for farmers (9).Thus applications of various biofertilizers restore the natural fertility of soil. It also on the other hand, enhances water holding capacity of the soil and adds essential nutrients such as nitrogen, vitamins and proteins to the soil. They are the natural form of fertilizers and hence, widely used and is applicable in agriculture.

Important types of Biofertilizers

There are various biofertilizers which are classified and grouped into different types according to microorganisms that are contained in. The following table (Table 1) presents an overview of biofertilizers used on the bases of various microorganisms (10). Biofertilizers include various types:

Nitrogen fixing biofertilizer (NFB) - *Rhizobium*

Nitrogen is one of the primary nutrients necessary for crop growth and productivity. Around 78 percent of N on volumetric basis is found in the atmosphere. The largest part of the elemental N that recycles the earth through the N-cycle process is solely due to its fixation by a certain group of unique microorganisms. A significant mechanism for establishing the N balance in the soil ecosystem is known as biological nitrogen fixation (BNF). Nitrogen inputs through BNF helps in sustainable agricultural production that is ecologically friendly. Through the use of biofertilizers the benefit of nitrogen fixation by legumes to boost and increase the yield of legumes and other species can be achieved(11).Natural N fixing bacteria *Rhizobium* belongs to family *Rhizobiaceae.* It is symbiotic in nature, a diazotrophic gram-negative bacteria having the potential to fix N approximately 50-100 Kg/ ha^{-1} in association with legumes and amount fixed is highly variable with different crop species. This bacterium is especially important to leguminous plants like beans, alfalfa, chickpea, red-gram, lentil, members of the pea family etc. The efficient nodulation by rhizobium in leguminous plants depends largely on the availability of a strain compatible with a specific legume. It colonizes tumor-like growths known as the root nodules of the specific legumes (Figure 1) that function as ammonia production facilities. *Rhizobium* can be classified on the basis of the types of plant they are associated (Table 2) with and also the rate of growth (12). *Rhizobium* has ability to fix atmospheric N in symbiotic association with legumes and certain non-legumes like Parasponia. The population of rhizobium in the soil is dependent on the existence of leguminous crops and other host plants having different strains of this particular specie. The population declines in the absence of legumes. In order to speed up N-fixation, the population of Rhizobium successful strains near the rhizosphere also requires artificial seed inoculation. Each legume requires a specific species of *Rhozobium* to form effective nodules (13). Inoculation with rhizobium is a well-known agricultural procedure in order to ensure the adequacy of legume nitrogen rather than N-fertilizer (14). The oxygen (O_2) level is stimulated by special haemoglobin called leg-hemoglobin found in the root nodules. This globin is a protein that is coded by plant genes but symbiotic bacteria are the heme cofactor. This is only achieved if the plant is rhizobially infected. This is only produced when the plant is infected with *Rhizobium*. The root cells of the plant transform sugar into organic acids, which the bacteria supply. So instead free ammonia, the plant can receive amino acids.

Root nodule formation, Nitrogenase and N-fixation

In bacteria, there are numerous sets of genes that regulate various aspects of the nodulation process. A single *Rhizobium* strain can infect certain species of legumes but not others e.g. *R. leguminosarum* biovar*viciae*to *R. leguminosarum* biovar*trifolii*. The specificity genes specify what strain of Rhizobium infects the particular legume. Effective rhizobial strains develop N-fixing nodules. The "nod genes" are the ones that direct various stages of nodulation. The first operation is the communication of the host plant with the free rhizobia through the distribution of such substances like acids into the soil by root cells. Rhizobia are responsible for the reactions between certain compounds on the cell wall and root surface that detect their decent host plant and attach it to the ends of the root hairs. The root cells secrete flavonoids which activate the nod genes in bacteria, inducing formation of the nodules. The bacteria are bound to the root hair releasing the nodal factors "nod" that cause root hair curling. Through the root hair tips the rhizobia invade leading to the formation of an infection thread. The infection thread grows through the root hair cells and penetrates other root cells nearby often with branching of the thread. The bacteria multiply within the expanding network of tubes, continuing to produce nod factors which stimulate the root cells to proliferate, eventually forming a root nodule. These plant compounds induce the expression of nodulation (nod) genes in rhizobia, which in turn produce lipo-chitooligiosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (15) (16) (17).The nodule is divided into four main parts; the cortex, meristem, vascular bundle and bacteroid. Thousands of live rhizobial bacteria are present in each root nodule. The cortex and meristem supply nutrients, vascular bundle releases nitrogenase enzyme and bacteroid is the place where N-fixation occurs. The enzyme nitrogenase is responsible for conversion of nitrogen gas (N_2) to ammonia (NH_3) in nitrogen-fixing organisms. The reaction requires hydrogen as well as energy from ATP. The nitrogenase complex is sensitive to oxygen, becoming inactivated when exposed to it. *Rhizobium* controls oxygen levels in the nodule with leghaemoglobin. To convert one molecule of N_2twelve (12) energy (ATP) molecules are required.

Rhizobium activity and yield increment

Different findings with Rhizobium inoculation in different cereal grains have to some degree increased yield (Table 3).

The inoculation of *Rhizobium leguminosarum* bv. *trifolii*E11, *Rhizobium* sp. IRBG74 and *Bradyrhizobium* sp. IRBG271 increased rice grain and straw yields by 8 to 22 and 4 to 19%, at different N rates respectively (23). Also N, P and K uptake were increased by 10 to 28 percent due to rhizobial inoculation which also increased Fe uptake in rice by 64 percent. In order to increase shoots and return under drought stress, it is suggested that promoting effect of Rhizobium and Bradyrhizobium inoculation should not only be regarded as symbiotic N2 fixators for legumes and non-legume as plant growth promoting rhizobacteria (PGPR).In the rice field experiment carried out in Vietnam, Inoculation of R. legunisarum increased vegetative biomass phase (24).*Rhizobium* forms white, translucent, glistening elevated and comparatively small colonies on selective medium whereas on optimized medium bacteria utilizes Congo red slowly slightly pinkish colonies(25)(Figure 2).

Furthermore the productivity and quality of fenugreek than gram seed can significantly be increased through rhizobial inoculation seed coating for legume crops. While the growth of subsequent crops was lower by inoculated controls and non-leguminous, this biofertilizer type shows more potential to benefit more from rhizobial symbiosis during legume crops growth. The physical and chemical characteristics (Table 4) profile of soil at start of experiment inadequate NPK for plantation and after the use of appropriate biofertilizer profile was potentially improved.

Mycorrhizae

Mycorrhiza (fungus root) is the mutualistic association between plant roots and fungal mycelia. The special association between tree roots and ectomycorrhizal fungi was given the name "mycorrhiza" by Frank in 1885.The mycorrhizae association exists among 95 percent of plant species. It may act as an important connection between soil and plant roots. Figure 3 (26) is an example is of pine seedlings that are with and without mycorrhizas highlighting some of the mycorrhizal properties. The rhizosphere and mycorrhizosphere of ectomycorrhizal (EM) pine seedlings differ dramatically from one another in plant and soil attributes. The association is characterized by the movement of plant produced carbon (C) to fungus and fungal acquired nutrients to plants. Mycorrhizal fungi are the key components of the rhizosphere are considered to have important roles in natural and managed ecosystems. Mycorrhiza provides the key benefit of increased soil exploration with the increased availability of the host roots to N, P, K, Zn, Cu, S, Fe, Ca, Mg and Mn (27).

Main groups of Mycorrhizae

The structure and function of mycorrhizal associations is very different. The ectomycorrhizae and the endomycorrhizae, while an unusual intermediate group of mycorrhizae, are recognized as being two principal mycorrhizal groups. Fungal hyphae form in Ectomycorrhiza both outside the root and within the root of the epidermis and cortex intercellular spaces. There is no intracellular penetration into epidermal or cortical cells, but between these cells there is a large network called a Hartignet. Sheath or Mantle raises the absorbing root surface and provides root defense.Hartignet can serve as P transport and storage organ. The pinaceaes (Pin, Fir, Spruce, Larch and Semlock) and the Fagaceae (Willows, Poplars, Chesnuts), the Birchs (Birch, Alderes), the Salicaceae (Willows and Poplars), and the Myrtaceaes; ectomycorrhizae are popular on wooded trees. The fungi forming ectomycorrhizal association are coming under basidiomycotina and ascomycotina. eg: *Laccarialaccata, Suillus, Rhizopogan, Amanita*. There are 3 sub-groups of endomycorrhizae; but the Arbuscular Mycorrhizal fungi are by far the most common ones. The AM fungi are Endogonaceae members and create an intern network of hyphae, which spreads into the ground and absorbs mineral salts and water. This fungus does not form an external mantle but lives within the root. In all forms, hyphae run between and inside the root cells which includes, Ericoid mycorrhiza (Associated with some species of Ericaceous plants), Orchid mycorrhiza is associated with orchid plants and Arbuscular mycorrhiza that is associated with most of the plant families.

Arbuscular Mycorrhizal Fungi (AMF/AM)

The term Mycorrhiza denotes “fungus roots”. It is a symbiotic relationship between host plants and certain groups of fungi at the root interphase, in which the fungal partner benefits from obtaining the photosynthesis of the host and in turn the host benefits from obtaining the required nutrients in particular P, calcium, copper, zinc etc. AM fungi are one of the essential components of the soil micro biome and obviously associate with other microorganisms in the rhizosphere. AMF has a significant connection between plants and soil mineral nutrients. They occur in soil environments and establish beneficial symbiosis with angiosperm roots and other plants (28). Thus, they are collecting growing interest as biofertilizers. AM is an endomorphic mycorrhizal fungi produced by aseptate phycomycetous, with the majority of crops grown under a broad ecological range, is associated with. It is of the Endogonaceae family, the Muccorales order belonging to the Zygomycetes class (29).The AM forming genera of the family includes *Acaulospora ,Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis*and *Scutellospora*. They are obligate symbionts in nature that belong to Glomeromycota phylum (30), which form mutualistic symbioses with about 80 percent of plants including agricultural crops. AMF nourishes the host plants with basic nutrients like P and in exchange they utilize C as a source of food (31). Attachment to the plant roots allowsmycelia to emerge that are linked to the root system that acquires nutrients from soil that are unavailable to the plants(32). The hyphae of AM are quite thin as compared to the plant roots and thus they are able to reach small pore spaces of soils i.e. micro-pores (33). The interface between the plant and fungus is then exchangeable within the roots with carbon and mineral nutrients. AM fungal hyphae colonizes the cortex exclusively and form highly ramified structures within the cells i.e. arbuscules that are considered a functional nutrient exchange site (34).Due to their hyphae network, AMF causes greater absorption of P and also allows in the absorption of other micronutrients such as zinc and copper. There are more than 180 species of AMF (35).AM fungi can increase the efficiency of the surface root absorption by up to 10 times(36). The discovery of two ancestral classes of AMF species from divergent ribosomal DNA sequences and classified into two new families known as the Archaeosporaceae and Paraglomaceae. The skeletal structure of macro aggregates is formed by the physical entanglement of soil particles and organic materials. AM fungus plays an important role in soil aggregation. Such aggregates improve the storage of C and nutrients and establish a favorable environment for soil microorganisms to thrive and grow. In organic or sustainable farming systems, VAM fungi are especially important for controlling plant diseases, depending on biological processes rather than agricultural chemicals. Root colonization by VAM fungi can provide protection from parasitic fungi and nematodes (37). Figure 4 illustrates some species of mycorrhizal fungi isolated from the rhizosphere of *Ceratoniasiliqua*10 months afterinoculation (38).

Colonization process

AMF are probably the most common fungi present in agricultural soils, making up between 5 and 50% of the total microbial biomass of the soil (39) though their actual diversity is low (40).There are no visual morphologic changes due to AM colonization in the plant roots. Host infection occurs through spore germination, hyphal growth to the host roots in the soil complex occurs by the host root penetration and spread of infection at inter and intracellularly in the root cortex. There are two phases of colonization: extra matric and intra-radical phases. In extra matric after the germination of chlamydospores, the events occur outside the root. Mycelium explores larger soil volume. Fungal growth can be 80-130 times the length of root. Extra matric hyphae (EMH) are bigger than inner hyphae in diameter. When the fungus recognizes this plant, the host roots form the appresorium and the appresorium is used to penetrate the plant. EMH finishes in the soil with restful spores. Events taking place within the root cortex is known as the intra-radical phase. The fungus may develop both intercellular and intracellular hyphae in the cortical cells after penetration of the cortex. Two morphological structures are developed in the cortical cells, namely arbuscules and vesicles.After the hyphal entry into cortical cells, the arbuscules are the first structures developed. The arbuscules are the fine hyphatic filaments with a dichotomous branch look like small trees. About 2 days after penetration, the arbuscules begin to form.

The fungus and host root are known to be the major site of exchange. They are short-lived and degenerate (4-13 days).Some fungal species form vesicles at the roots following the formation of arbuscules. Terminal or intercallery hyphal swellings of the hyphae called vesicles. Lipids and cytoplasms are found in the vesicles. They act as a P-storage organ and are often at the root. The vesicles are approximately 30-100 μm in size. In vesicles P can be accumulated as polyphosphates. In particular in phosphorous mobilization, EMH, the vesicles and Arbuscules play a key role in nutrient transfer. AMF's hyphae network extends to depleted areas of soil which acquire a larger area of P soil. Other beneficiary nutrients also have enhanced assimilation in AM-roots include nitrogen as ammonium (NH_4^+) and zinc (Zn) (41)(Figure 5).

Azolla biofertilizer

Azolla is a small and floating free aquatic fern that has its origins in Asia, Africa and America and is commonly called mosquito ferns, duckweed fern and water fern.The name *Azolla*is derived from the two Greek words, Azo(to dry) and Ollyo(to kill) thus reflecting that the fern is killed by drought. Lamarck coined the term Azolla in 1783 for the first time. Pteridophyta, the Polypodiopsida and Salviniales divisions belongs to Azolla. This is made up of also two subgéneras and six living species and also belongs to the Salviniaceae family.*Azolla*is placed in the monotypic family *Azollaceae*and there are seven extant species of *Azolla*(42)(43).The subgenus *EuAzolla*, characterized by three megaspore floats and septateglochidia, included four species: A. filiculoides Lain.A. caroliniana WiUd. A. microphyllaKaulf.,and A. Mexicana Presl. Table 5 presents the last classification proposed by Saunders and Fowler (44).

Azolla is found generally in tropical, subtropical and warm temperate areas around the world and is normally grown in freshwater, swamps and in rivers and lakes where the water is not turbulent (45).Three *Azolla*sp. *i.e. A. caroliniana*, *A. microphylla*, and A. *pinnata*(Figure 6) are commonlyfound all over the Indian subcontinent. The macrophyte of *Azolla* is called frond which ranges from 1 cm to 2.5 cm in length in species such as A. pinnata and 15 cm or more in the largest species like *A. nilotica*(46). Azolla is grown as monocrop or intercrop in the paddy field and is mixed in the mud/sol to increase the soil's content of humus and nutrients. This practice of *Azolla* cultivation is widely popular in the countries of south-east Asia like India, China, Phillipines, Indonesia etc.*Azolla* has various applications such as feed to animals, food to humans, medicines, biogas processing, hydrogen fuel, water purifier, weed control, ammonia volatilization reduction and is known as the green gold mine(47).Peters (48) reported that the use of *Azolla* increased rice yields by 112 percent over unfertilized controls when applied as a monocrop during the fallow season, by 23 percent when applied as an intercrop with rice, and by 216 percent when applied both as a mono-crop and an intercrop. *Azolla* produces approximately 300 tonnes of green bio-hectares a year in a typical subtropical climate, when used in paddy-growing as a bio-fertilizer, which is equivalent to 800 kg of N. Basal usage @ 10-12 tones/ha^{-1}, increases soil nitrogen by 50-60 kg/ha, decreasing the rice's requirements for nitrogen fertilizers 30-35 kg.

The advantages of *Azolla* application in rice are as follows. Use of *Azolla* increases rice yield by 20 to 30 percent. Seultrope (49) conducted an experiment and reported that *Azolla* can be utilized as fodder for cattle and pigs. Das *et al.*, (50) found that digested *Azolla* slurry remaining after biogas production was suitable as fish pond fertilizer.

Common Azolla species

S.NO	Azollaspecies
1	*A. pinnata*
2	*A. caroliniana*
3	*A. microphylla*

Role of Azolla in N-fixation, enhancement of crop productivity and as Livestock Feed

The ability to fix atmospheric N at significantly higher rates has contributed to the biofertilizer exploitation of different microorganisms. In order to increase the soil fertility index, the application of *Azolla* on rice paddy fields plays a positive role. The ability of N fixation is due to the presence of the symbiotic cyanobacterium *Anabaena* that occurs in the dorsal leaf cavities of the fronds (52).

Table.1 Types of Biofertilizers and Microorganisms (Ritika and Uptal, 2014)

Groups	Examples
	Nitrogen fixing bio-fertilizers
Free-living	*Azotobacter, Bejerinkia, Clostridium, Klebsiella, Anabaena, Nostoc*
Symbiotic	*Rhizobium, Frankia, Anabaena, Azollae*
Associative symbiotic	*Azospirillum*
	Phosphate solubilizing bio-fertilizer
Bacteria	*Bacillus megateriumvar, Phosphaticum, Bacillus subtilis, Bacillus circulans*
Fungi	*Penicillum Spp. Aspergillusawamori*
	Phosphate mobilizing bio-fertilizers
Arbuscular Mycorrhiza	*Glomus Spp., Gigaspora Spp., Acaulospora Spp. Scutellospora Spp. and Sclerocystis Spp.*
Ectomycorrhiza	*Laccaria Spp. PisolithusSpp, Boletus Spp. and Amanita Spp.*
Ericoid Mycorrhiza	*Pezizellaericae*
Orchid Mycorrhiza	*Rhizoctoniasolani*
	Bio-fertilizers for micronutrients
Bacillus spp	Silicate and zinc solubilizers
	Plant growth promoting Rhizobacteria
Pseudomonas	*Pseudomonas fluorescens*

Table.2 Few *Rhizobium* species and their corresponding hosts (KavyaDashora, 2011)

S.No.	Rhizobium species	Host plants
1	*Bradyrhizobium japanicum*	*Glycine max* (soybean)
2	*Rhizobium fredii*	*Glycine max* (soybean)
3	*R. phaseoli*	*Phaseolus vulgaris* (common bean)
4	*R. meliloti*	*Medicago sativa* (alfalfa)
5	*"Cowpea rhizobia" group or Rhizobium sp.*	*Vigna unguiculata* (cowpea)
6	*R. trifolii*	*Trifolium* sp. (clovers)
7	*R. Ieguminosarum*	*Pisum sativum* (peas)

Table.3 Yield and other beneficial effects of *Rhizobium* with cereals

Host plants	*Rhizobia*	*Colonization*	*Percent increase*	*References*
Rice	*Bradyrhizobium*	*Rhizosphere*	*20 (total biomass)*	*(18)*
Rice	*R. leguminosarum*	*Roots*	*15 - 22, 8 - 22 (grain yield)*	*(19)*
Rice	*R. leguminosarum*	*Rhizosphere*	*43 (yield)*	*(20)*
Wheat	*R. trifolii*	*Roots*	*24 (wheat shoot dry matter and grain yield*	*(21)*
Maize	*R. trifolii*	*Roots*	*34 (yield), 11 (yield)*	*(22)*
Maize	*Sinorhizobium* spp.	*Roots*	*49 - 82 (yield)*	*(22)*

Table.4 Physical and chemical characteristics of experimental soils (Gomare *et al*., 2013)

a. Before inoculation of Biofertilizers		*b. After 60 days inoculation of Biofertilizers*			
Soil parameter	***Used for set preparation***	***Soil parameter***	***Selective***	***Optimized***	***Control***
pH	*4.1*	***pH***	*5.4*	*5.7*	*4.3*
Available N	*18.55*	***Available N***	*32.55*	*42.22*	*20.55*
Available P	*5.12*	***Available P***	*7.88*	*8.52*	*6.20*
Available K	*60.58*	***Available K***	*68.80*	*106.00*	*64.80*
Organic matter (%)	*1.424*	***Organic matter (%)***	*1.880*	*2.292*	*1.698*

Table.5 Few Mycorrhizal species and corresponding hosts

S.No.	Mycorrhizal species	Host plant
1	*Acaulospora. laevis*	*Solanum lycopersicum* (tomato)
2	*Acaulospora. koreana*	*Lindera obtusiloba* (spice bush)
3	*Glomus. aggregatum*	*Medicago truncatula* (barrelclover)
4	*Glomus. deserticola*	*Lactuca sativa L* (lettuce)
5	*Scutellospora. nigra*	*Allium cepa* (onion)
6	*Glomus. etunicatum*	*Zea mays* (corn)

Table.6 Classification of Azolla (Saunders and Fowler, 1993

DIVISION	Pteridophyta		
CLASS	Filicopsida		
ORDER	Salviniales		
FAMILY	Azollaceae		
GENUS	*Azolla*		
SUBGENERA	*Azolla*		*Tetrasporocarpia*
SECTIONS	*Azolla*	*Rhizosperma*	
SPECIES	*A. caroliniana* *A. filiculoides* *A. mexicana* *A. microphylla* *A. rubra*	*A. pinnata*	*A. nilotica*
SUBSPECIES		*A. pinnata* subsp. *africana* *A. pinnata* subsp. *asiatica* *A. pinnata* subsp. *pinnata*	

Table.7 Chemical composition of Azolla (Singh and Subudhi, 1978

Constituents	Dry matter (%)
Crude protein	24-30
Crude fat	3.3-3.6
Nitrogen	4-5
Phosphorous	0.5-0.9
Calcium	0.4-1.0
Potassium	2-.4.5
Magnesium	0.5-0.65
Manganese	0.11-0.16
Iron	0.06-0.26
Soluble sugars	3.5
Crude fire	9.1
Starch	6.54

Fig.1 Nodules on leguminous plant roots

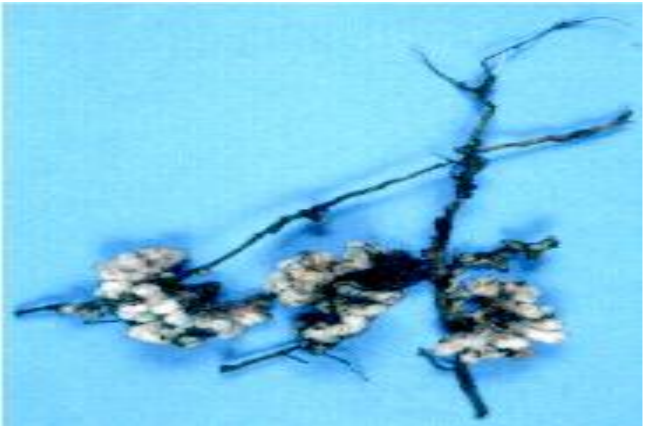


Fig.2 Rhizobium bacterial colonies under selective and optimized medium (Gomare *et al*., 2013)

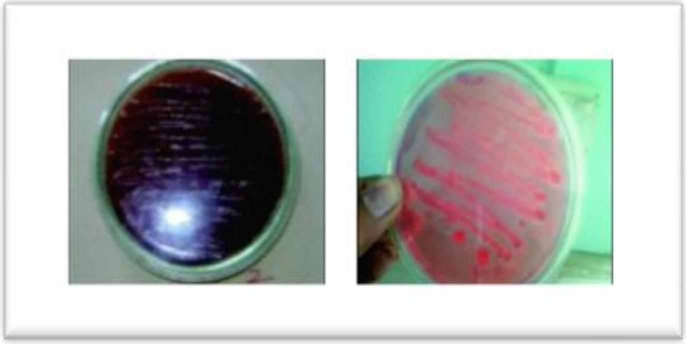


Fig.3 Rhizosphere versus Mycorrhizosphere (Cardon and Whitbeck, 2007)

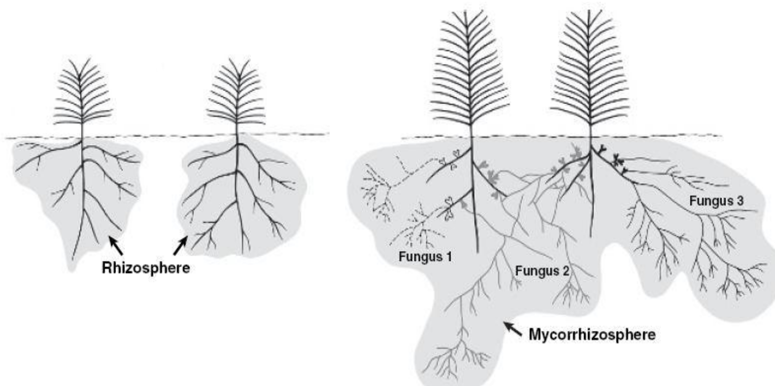
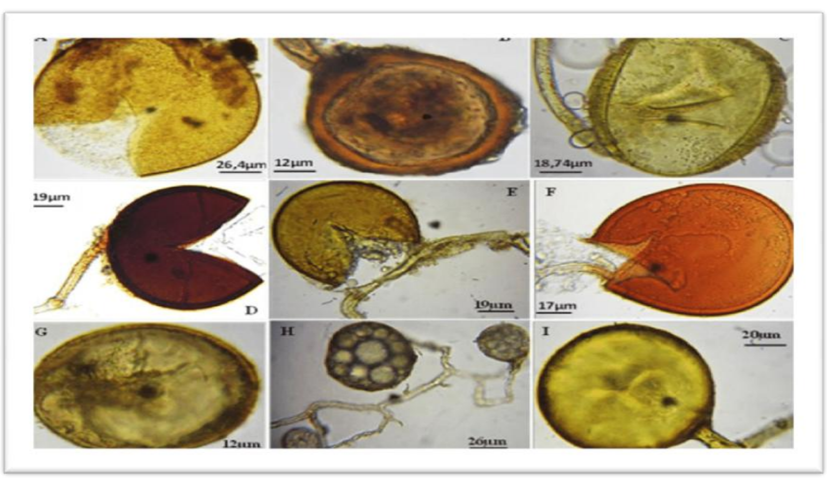


Fig.4 Some species of Mycorrhizal fungi isolated from the rhizosphere of *Ceratonia siliqua* (Zouheir *et al*., 2016)



(A) *Glomus. fecundisporum*, (B) *Glomus. macrocarpum*, (C) *Glomus. calospora*, (D) *Glomus. deserticola*, (E) *Glomus.fasciculatum*, (F) *Glomus. aggregatum*, (G) *Aucaulospora. gedanensis*, (H)*Glomus. clarum*and (I) *Glomus. Etunicatum*

Fig.5 Effects of Arbuscular Mycorrhizal colonization (Jacott *et al*. 2017)

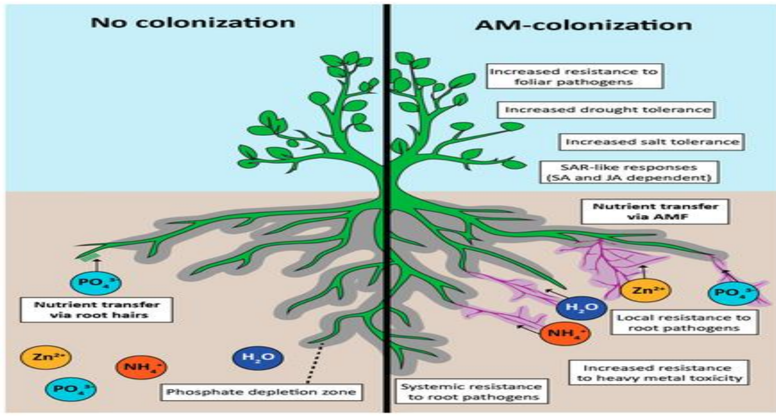
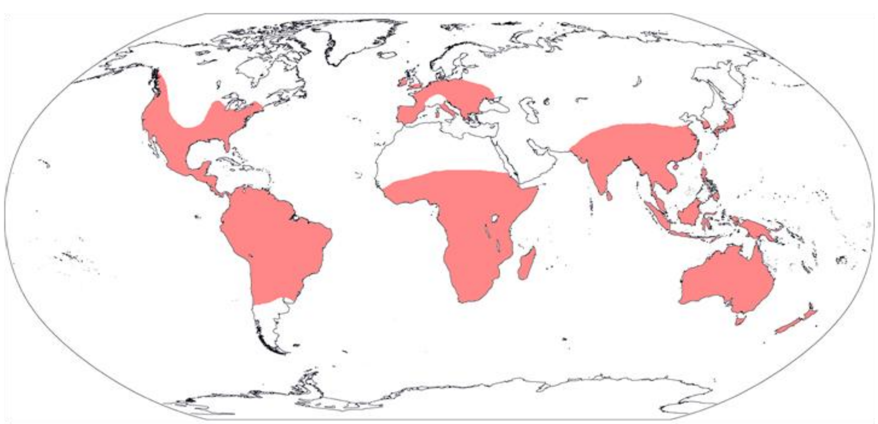


Fig.6 Azolla species (Roy *et al*., 2016)(51)

A. caroliniana *A. microphylla* *A. pinnata*

Fig.7 World distribution of Azolla species



As an effective biofertilizer for rice paddy farms, *azolla* strains were successfully exploited due to the N fixing ability. *Azolla* inoculation has been reported in order to improve soil biological health, in addition to maintaining rice production. There has also been growing activity of soil urease and phosphatase as *Azolla* is added (53).Field experimental studies have been carried out at the Centre for Conservation and Utilization of Blue Green Algae, Indian Agricultural Research Institute, New Delhi. Optimum yields were reported and improved grain and soil quality was seen in these studies in organic Basmati rice. The inputs used in this study included *Azolla*, blue green algae, farmyard manure and vermi-compost as organic amendments (54). Experiments at the Banaras Hindu University in Varanasi have shown that *Azolla* has beneficial effects in rice production (55). *Azolla* application has been found to substantially enhance the physical and chemical properties; N, organic material and other cations (Mg, Ca, and Na) released into the soil(56). *Azolla pinnata* has also been recorded as biofertilizer on Kerala acidic soils (57). Weeds are suppressed and ammonia volatilisation is decreased in rice areas due to the development of a dense rice mat in *Azolla* rice fields (58).A recent study of *Azolla's* effectiveness as a sustainable feed for animals and poultry has been reviewed (59). Azolla is a suitable source of feed for animals; cattle, goats, pigs and fisheries. it can be used as a fodder for cattle, fish and poultry feed because of the high nutrient content (Proteins, Vitamins, Calcium, Phosphorus, Iron, Copper, Magnesium, Beta carotene and Amino acids) (Table 6) (66). Azolla is proposed for use in animal feed as an advantage for improved livestock production that is part of agriculture (60). Use of *Azolla* protein supplement for the fish *Tilapia mossambica* and observed increase in feeding, absorption and growth rate (61). Almazan *et al.,* (62) conducted a study where *A. pinnata*was fed to Nile tilapia (*Oreochromisniloticus*) fingerlings and adult males. Feeding studies on Buffalo calves indicate that *Azolla* feed is a possible and unconventional protein source (63). Poultry fed on Azolla show significant increase in the body weights and consequently have resulted in an increase in the net return (64). Kannaiyan, and Kumar (65) screened azolla hybrids AH-Cl, AH-C2, AH-C3 and AH-C4 with wild cultures (*A.filiculoides* and *A. microphylla)* to produce a better biomass. Finding indicated that *Azolla* hybrids produce a greater biomass than wild cultures.

Geographic distribution of Azolla

The map of *Azolla* (below) of the modern distribution by Small and Darbyshire (67) is clearly illustrated.

In conclusion it is projected that by 2050 the world's population will reach nine billion, so the global agricultural system will face the task of doubling the production of food in order to meet humanity's everyday needs. Keeping the idea in mind of reducing the dependence on synthetic chemicals and fertilizers to safeguard human, environmental health, maintaining and regaining soils natural fertility is a priority. The existing global capacity to produce food currently exceeds the planned yield demand, highlighting the need to adopt and revitalize environmentally-friendly technologies such as the use of biofertilizers. Biofertilizers are fertilizers that contain living microorganisms which accelerate microbial biomass functioning in the soil system. Through the use of biofertilizer like *Rhizobium*, *AMF*, *Azolla* and other beneficial species it aids in increasing crop productivity by way of supplying plant, crops and livestock's with essential nutrients through fixation, solubilization/mobilizing, feed and by decomposition of organic residues like straw and plant debris. Biofertilizer are gaining popularity due the various advantages they have over chemicals as it reduces cost and is eco-friendly. A low input scheme can be introduced by using organic and biological fertilizers and the sustainability of farms can be helped. Through the use biofertilizers there is a huge potential to boost the agricultural production in a sound manner on a long term basis without harming our natural diversity.

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