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A Review: Biofertilizers that fix nitrogen in liquid form

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Abstract : Biofertilizers are biologically derived nutrients that are given to the soil to improve soil fertility. Biofertilizers are substance that contains biological organisms. Microorganisms have arisen as promising alternatives for increasing the productivity, reliability and long term viability of agriculture. There are different kinds of biofertilizers for plant growth and crop production such as Nitrogen fixing, Phosphate solubilizing, compost, Mycorrhizal and plant growth promoting biofertilizers etc. Liquid biofertilizer is more effective and shelf life is higher than solid biofertilizers. By nitrogen fixing bacteria atmospheric nitrogen convert into ammonium which utilizes the plants as nutrients. This bacteria live with plants in a symbiotic relationship. This ammonium used by plants for their growth and production increases. It is available in various form like powder form, liquid form, solid form. This method is easy for production and inexpensive for nitrogen fixation. It is easy to apply in field by various methods either fungal or bacterial biofertilizer in liquid form. It's limitation is it can't be mix up with chemical fertilizers otherwise it's reverse effects observed and kept under the low temperature for storage. This biofertilizers ale lower cost and ten times effective than chemical fertilizers. By using biofertilizers instead of chemical fertilizers reduce environmental and land pollution.

Keyword: *Azotobacter* spp, Bacteria, liquid biofertilizer, nitrogen fixation, *Rhizobium spp*,

I. Introduction

Biofertilizers are living cells or effective microorganisms that assist plants in absorbing nutrients for growth. Biofertilizers are bacteria based fertilizers that boost soil fertility (Abdullahi *et. al.*, 2012 ; Devi *et al.*,2017; Itelima *et al.*,2018). Selective microorganisms such as bacteria, fungus and algae are referred to as biofertilizer. Biofertilizers are low cost, environmentally, friendly and renewable source of land nutrients that are critical for long term soil fertility and sustainability. Biofertilizers cannot completely replace chemical fertilizers, but they can help minimise their use and support more sustainable agriculture system (Mahdi *et al.*, 2010; Okur,. *et al.*, 2018). Biofertilizers are crucial components in developing an organic farming system and in supporting the crop increasing Productivity and boosting soil fertility (Govindan *et al.*,2005; ko latt *et al.*,2018). Despite the fact that nitrogen is the most abundant gas in the atmosphere, it is absent from soil parent material. As a result, soil nitrogen intake for plant nutrition and crop yield is heavily influenced by organic matter break down and synthetic fertilizers application and biological nitrogen fixation through the activity of nitrogenase enzymes (Galloway *et al.*,2008; Hedin *et al.*,2009; Vitousek *et al.*,1991; Mahmud *et al.*, 2020). The states with the most production are Madhya Pradesh, Maharashtra , Karnataka and Tamilnadu. Several thousand tonnes of biofertilizers are needed in India however, there aren't enough production facilities (Gehlot *et al.*,2021; Mohod *et al.*, 2015).

II. Biofertilizers

Biofertilizer is a type of fertiliser that contains live microorganisms, whose activities are believed to have an impact on the soil ecosystem and provide additional nutrients for plants. These microbes, as well as the minerals derived from the raw materials, are employed to increase the health and nutrition of the soil. There are various types of biofertilizers available, with the primary distinctions being the raw materials used, the methods of usage, and the microorganism sources (Ngampimol *et al.*,2008). The use of biofertilizer is consequently necessitated largely for two reasons. First, since increasing fertiliser use leads to higher crop output, and second, because increased chemical fertiliser use damages soil texture and causes other environmental issues(Al Abboud *et al.*,2014). Plant development is known to be enhanced by the provision of plant nutrients, and bio-fertilizers containing helpful microorganisms instead of synthetic chemicals may help to maintain environmental health and soil production. Furthermore, the application of bio-fertilizers can boost productivity per unit area in a short length of time, consume less energy, reduce soil and water contamination, increase soil fertility, and encourage phytopathogenic organism antagonism and biological control (Sneha *et al.*,2018; Yasin *et al.*,2012)

III. Classification of biofertilizers

3.1 Nitrogen fixing biofertilizers

- Free living bacteria:- It's difficult to quantify nitrogen fixation by free-living bacteria, although it's been estimated to range from 3 kilogramme N ha1 to 10 kg N ha1 in some plants, such as Medicago sativa.
- Symbiotic:- Rhizobial biofertilizers are available in powder, liquid, and granular forms, and can be delivered in a variety of sterilised carriers such as peat, perlite, mineral soil, and charcoal. Frankia, a nitrogen-fixing *Actinomycetes*, may develop root nodules in a variety of plants, just as rhizobia.
- Associated:- Endophytic symbioses are absent from the rhizosphere (associative/associated). These nitrogen-fixing microorganisms have a more distant relationship with roots than endophytic symbionts (Thomas *et al.*,2019).

3.2 Phosphate solubilizing biofertilizers

Several soil bacteria and fungus, including *Pseudomonas*, *Bacillus*, *Penicillium*, *Aspergillus*, and others, release organic acids that lower the pH in their surroundings, allowing bound phosphates in soil to dissolve. Inoculation of *Bacillus polymyxa* and *Pseudomonas striata* peat-based cultures resulted in increased wheat and potato yields (Al Abboud *et al.*,2014).

3.3 Plant growth promoting biofertilizers

Rhizobacteria that promote plant growth have the potential to fix atmospheric nitrogen and produce specific metabolites such as auxin, cytokinin, gibberellins, hydrogen cyanide (HCN), phytohormones, and unstable compounds. PGPR also produces mineral dissolving chemicals, such as phosphorus solubilization, as well as internal resistances (Sneha *et al.*,2018; Satyaprakash *et al.*,2017).

3.4 Mycorrhizal biofertilizers

Phosphate absorbers or phosphorus-mobilizing biofertilizers are what they're called. Mycorrhizal fungi create obligatory or facultative functional mutualistic symbioses with more than 80% of all land plants, in which the fungus is reliant on the host for photosynthates and energy in exchange for a variety of benefit (Gomare *et al.*,2018).

3.5 Compost biofertilizers

Compost is a brittle, murky decaying material that forms a symbiotic food web in the soil and contains around 2% (w/w) nitrogen, phosphate, and potassium, as well as bacteria, earthworms, and dung beetles. Compost can be made from a wide range of resources, including straw, leaves, cow bedding, fruit and vegetable wastes, biogas plant slurry, industrial wastes, city garbage, sewage sludge, industry waste, and so on. Different degrading microorganisms such as *Trichoderma viridae*, *Aspergillus niger*, *Aspergillus terreus*, *Bacillus spp.*, and many Gram-negative bacteria generate compost from these materials (Gomare *et al.*,2018).

IV. Nitrogen fixation

Nitrogen fixing organisms *Azotobacter spp, Rhizobium spp, Acetobacter spp*. *Azotobacter* is a free living bacteria that can fix atmospheric nitrogen into the soil, making it a valuable source of natural biofertilizer for a wide range of crops (Gehlot *et al.*,2021; Mohod *et al.*, 2015). It is said to create polysaccharides, which protect against desiccation, mechanical stress, phagocytosis and page attack as well as engage in the updating of metal ions as sticky agents (Sethi *et al.*, 1974; Chaube *et al.*, 2018 ; Homaeigohar *et al.*, 2020). *Azotobacter, Rhizobium* and *Acetobacter* are significant players in the nitrogen cycle in nature, binding atmospheric nitrogen that is unavailable to plants and releasing it as ammonium ions into the soil (Roumani *et al.*,2015; Gomare *et al.*, 2013). In oligotrophic conditions when nitrogen is scarce, the capacity to fix nitrogen provides a distinct ecological benefit. This comes at the expense of less efficient growth than an organism that used fix nitrogen (Heijnen *et al.*,1981; Inomura *et al.*, 2018).When *Rhizobium* bacteria in the soil bind to root hairs, a symbiotic relationship between plant and bacteria develops. Plant protein called lectins, bind bacteria to the surface of root hairs, which are then pierced by germs, resulting in highly specialized attachment process. The infected root cells divide to create a nitrogen fixing nodule, which offers the essential anaerobic environment for nitrogen fixation (Beham *et al.*,2021; Gomare *et al.*, 2013).

V. Form of biofertilizers

The physical form of the biofertilizer is determined by the type of carrier used. Different types of soil materials (peat, coal, clays, inorganic soil), organic materials (composts, soybean meal, wheat bran, sawdust, etc.), and inert materials can all be used to make dry inoculants (e.g., vermiculite, perlite, kaolin, bentonite, silicates). Solid carriers are typically in the shape of powder, granules, or beads. Powder material sizes might range from 75 m to 0.25 mm in standard sizes. Granules and beads range in size from 100–200 mm in diameter to 3–4 mm.

Powdered inoculants can be used to coat seeds, or they can be suspended in a liquid and applied directly to the furrow, or the seeds/plants can be dipped in it right before sowing/planting. After 130 days of storage at 25°C, the sludge-based carrier maintained target rhizobia populations (107-108 cells g1), pH around neutral, and a good water holding capacity. Coal, clays, and inorganic soils (such as lapillus, volcanic pumice, or diatomite earths) are all available in various places and can be utilised as carriers. Their microbial load varies depending on the production site (about 102-103 CFU g1), however it is often lower than that of organic carriers. Vermiculite, perlite, and bentonite are also available in various nations, although their use is often limited because to the difficulties in formulating a formulation using them. Once sprayed, the oil retains water around the organism, slowing evaporation. This is especially advantageous for organisms that are susceptible to dehydration or for horticultural crops that have irrigation systems. Water-in-oil emulsions allow chemicals to be added to the oil and/or aqueous phases, potentially improving cell viability as well as release kinetics. Bacteria have been included in alginate beads for a variety of species, both sporulating and non-sporulating. Other AMF structures have also been entrapped in alginate matrixes or beads made from various polymers. Mycorrhizal fungi spores were trapped in an alginate film formed in a PVC-coated fibreglass screen [95], and roots of leek seedlings inoculated with this alginate film containing G. mosseae spores were heavily colonised after only a few weeks of growth in greenhouse conditions. Spores obtained from monoxenic cultures implanted in beads produced similar results. It has also been demonstrated that filamentous fungus such as *Aspergillus* and *Actinomycetes* can be included.

VI. Liquid biofertilizer

Among the several approaches for producing biofertilizers, Dr.Teruo Higa of japan established the notion of effective microorganisms, which is available in liquid form, in 1991 (Setboonsarng *et al.*,1999; Ngampimol & Kunathigun *et al.*, 2008). For the creation of nitrogen fixing biofertilizers, bacteria are inoculated into growing media and allow to generation (Bhattacharjee *et al.*,2008; Datta *et al.*, 2015). The survival of microorganisms during storage is one of the most difficult problems in inoculate technology and several factors such as the culture medium, the physiological state of the microorganisms. When harvested, the dehydration process, the rate of drying, storage temperature and water activity of the inoculum all affect their shelf life. As a result, research into extending the shelf life of inoculants or developing new carrier forms of liquid biofertilizer. (O'Callaghan et al.,2016; Chaube *et al.*, 2018). The bacteria in these liquid biofertilizer have two year shelf life. They can endure tempreture of upto 55 degree Celsius, as well as ultraviolet rays. The count can reach 109 c.f.u./ml, which is quite high is kept steady for up to two years. As a result the 1 ml of liquid biofertilizer is applied equivalent to applying 1 kg of 5 month old meat biofertilizers with carriers (1000 times). Because of this , Formulation that are liquid in the field, the application is also very simple and straightforward. (Chaudhari *et al.*,2021; Tamilkodi & Victoria *et al.*, 2018). The manufacturing method is quick and inexpensive (Samal *et al.*,2020; Choube *et al.*, 2018).

VII. Types of liquid biofertilizers

7.1 Bacterial biofertilizer

7.1.1 *Rhizobium spp.*

Rhizobium spp is a soil habitat bacteria that can grow legume roots and symbiotically fix atmospheric nitrogen. *Rhizobium* has a wide range of shape and physiology, from free-living to nodular bacteria. They are the most effective biofertilizer in terms of nitrogen fixation. They are classified as cross inoculation group because they contain seven genera and are extremely selective for forming nodules in legumes. The first Rhizobium inoculant was made in the United States and commercialised by private company in the 1930s, and Fred has described the odd scenario that existed at the time (Tamilkodi *et al.*, 2018). For diverse legume crops, microbial activities of Rhizobium have been calculated to fix 40-250 kg N/ha/year. When the plants have finished growing, The corrected N2 is released, allowing it to be used by anyone. This fertilises the soil by attracting other plants.

7.1.2 Azotobacter spp.

chrococcum is the most common *Azotobacter* species found in arable soils and is capable of fixing N2 (2-15 mg N2 fixed /g of carbon source) in culture media. The bacterium creates a lot of slime, which helps the soil clump together. Due to a lack of organic matter and the presence of antagonistic microorganisms in Indian soils, the number of A. chrococcum rarely exceeds 105/g soil (Al Abboud *et al.*,2014).

7 1.3 *Azospirillum* spp

Azospirillum is a bacterium that has been found to fix a significant amount of nitrogen in the rhizosphere of nonleguminous plants like as grains, millets, oilseeds, cotton, and other plants in the range of 20-40 kg N/ha. Because of its ability to induce copious roots in a variety of plants such as rice, millets, and oilseeds even in upland circumstances, *Azospirillum* is regarded as an excellent biofertilizer (Tamilkodi et al.,2018). *Azospirillum lipoferum* and *A. brasilense* (*Spirillum lipoferum* in previous literature) are graminaceous plant soil, rhizosphere, and intercellular spaces inhabitants. They form a symbiotic association with graminaceous plants through associative symbiosis. Aside with nitrogen fixation, *Azospirillum* inoculation also provides growth promoting substance (IAA), disease resistance, and drought tolerance(Al Abboud *et al.*,2014).

7.2 Fungal biofertilizers

Fungal biofertilizers are made up of fungal inoculum that works directly or indirectly on plant development and yield through several methods. Phosphate solubilizing microorganisms are fungal biofertilizers that have been utilised to increase plant development by increasing phosphorus uptake in plants (Tamilkodi *et al.*,2018).

7.2.1 *Aspergillus spp*

Aspergillus niger is a fungus found in nature that is filamentous, global, and produces black spores. It is frequently segregated from soil, plant debris, and the interior environment. Soilborne fungus, such as *Aspergillus sp.*, play a significant role in phosphate solubilization (Arcand and Schneider *et al.*,2006). Because both fungi can solubilize both organic and rock phosphates, co-inoculation of these two bacteria will increase phosphate availability to plants, lowering the need for synthetic fertilisers. The fungus *Aspergillus niger* has been researched for its ability to solubilize inorganic phosphates by producing acids (Tamilkodi *et al.*,2018).

7.2.2 *Trichoderma spp.*

Trichoderma species are widespread saprobes found in soil and root environments, and they may be easily isolated from soil, rotting wood, and other organic matter. *Trichoderma* species have been used as mycofungicides with great success because they are fast growing, have a high reproductive capacity, inhibit a broad spectrum of fungal diseases, have a variety of control mechanisms, are excellent competitors in the rhizosphere, have the ability to modify the rhizosphere, are tolerant or resistant to soil fungicides, have the ability to survive in unfavourable conditions, are efficient in utilising soil nutrients, and have strong abiotic resistance (Tamilkodi *et al.*,2018).

7.2.3 *Penicillium spp*

Penicillium is a big fungus found in the air, on bread, and in soil. Different species of these fungus create a variety of secondary metabolites, including antibacterial drugs like penicillin and antifungal drugs like griseofulvin, as well as a variety of poisonous chemicals for people and animals. Plant diseases cause fruit to decay, while one variety causes a human sickness in Asia.

VIII. Methods of application biofertilizers:-

8.1 Seed treatment

Using an adhesive such as gum acacia, 10 kg of seeds are treated with 20 ml of biofertilizer mixed (Debnath *et al.*,2020; Mahajan *et al.*, 2003).

8.2 Seedling roots dip

The seedling roots of transplanted crops are treated with a biofertilizer solution for half an hour before transplantation in the field. The seedling necessary for one acre are inoculated using 2-2.5 kg biofertilizers in this approach. The biofertilizer is a appropriately combined with a glass of water. The seedling roots dip into the mixture of inoculum (Kumawat *et al.*,2020; Maliwal *et al.*, 2020).

8.3 Soil /field application

Fruit, crops, sugarcane and other crops that require localised application are the most common uses for this technology. 20 g of biofertilizer mixed with compost should be placed to the ring of one sapling when it is time to plant fruit trees (Das *et al.*,2018; Kuila *et al.*, 2022).

8.4 Foliar application

Plants are sprayed with micro bacterial culture in liquid form, which are absorbed through the stomatal hole. Alternatively, spray epidermal cells on the leaf surface 1% methylobacterium biofertilizer concentration in liquid early in the morning or late night (Reddy *et al.*,2020; Poornimmal *et al.*, 2020).

IX. Advantages of biofertilizers

They are both environmental friendly and financial effective.

1. Their use results in soil enrichment and the soil quality increase over time. Though they may not produce immediate results, the long term results are amazing (Young M.D. *et al.*,2020; Kumar *et al.*, 2017).
2. These fertilizer capture nitrogen from the atmosphere and make it available to plant immediately. (Gruber *et al.*,2008; Lehghari *et al.*, 2016).
3. The production of growth promoting hormones by fertilizers promotes root multiplication.
4. Biofertilizers containing microorganisms that help the root plants out enough nutrients (Bhardwaj *et al.*,2014; Kumar *et al.*, 2017).
5. Special cell protectants or chemicals that promote the production of resting spores or cysts are present.
6. Possibilities for combat with the indigenous population are increased.
7. Doses are ten times lower than those used in carrier based biofertilizers.
8. Excessive enzymatic activity due to the lack of contaminants (Barman *et al.*,2017; Poornimmal *et al.*,2020).
9. The release of growth-promoting hormones by biofertilizers improves root proliferation.
10. Microorganisms break down complicated nutrients into basic nutrients so that plants can use them.
11. Biofertilizer is made up of microorganisms that help the host plants get enough nutrients.
    and guarantee that their physiology is properly developed and regulated.
12. They can help increase agricultural yields by 10% to 25%.
13. To some extent, biofertilizers can protect plants from soil-borne illnesses.

X. Limitations of liquid biofertilizer

- Biofertilizers are never used in conjunction with chemical fertilizers.
- Biofertilizers are never used in combine with fungicides.
- There can't be any direct sunlight.
- Kept between 0 and 35 °C at room temperature (Kumar *et al.*,2013; Tamilkodi *et al.*, 2018).

Conclusion : They concluded that liquid biofertilizer is inexpensive, environmental friendly, easy carrier and effective for plants in agriculture.

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