International Journal of Advanced Biochemistry Research 2024; SP-8(5): 389-397

International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(5): 389-397
www.biochemjournal.com
Received: 06-03-2024
Accepted: 20-04-2024

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An overview of biofertilizers in agriculture with special reference to mulberry

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DOI: https://doi.org/10.33545/26174693.2024.v8.i5Sf.1210

Abstract
Fertilizers play a crucial role in supplying essential nutrients to plants, notably nitrogen (N), potassium (K), and phosphorus (P), thereby enhancing crop yield. However, their usage has been associated with various health risks, soil health, soil infertility and environmental pollution. Consequently, consumers are increasingly favoring organic farming practices, including the use of organic manure and fertilizers. In recent times, biofertilizers have gained prominence as a significant contributor to biological nitrogen fixation. There is a shift toward the use of biofertilizers because they provide nutrition through natural processes like zinc, potassium and phosphorus solubilization, nitrogen fixation, production of hormones and protect the plant from different plant pathogens and stress conditions. They provide the nutrition in is suffcient amount necessary for healthy crop development to fulfill the demand of the increasing population worldwide. They offer an economically viable and environmentally sustainable means of nourishing plants. Biofertilizers serve as a cost-effective renewable nutrient source that complements chemical fertilizers. Particularly among small and marginalized farmers, biofertilizers have garnered attention due to their affordability. This review will focus on biofertilizers, types and their significance in agriculture with special reference to mulberry cultivation.

Keywords: Biofertilizer, VAM. Azotobacter, health hazards, crop yield, affordability

Introduction

Mulberry plant is important economic crop grown extensively for the production of leaf for feeding mulberry silkworm (*Bombyx mori* L.). A sericigenous insect which is cultivated for the production of silk cocoons converted into amazing products of silken fabrics. It is grown throughout the year under tropical conditions. The quality of silk cocoon production is directly dependent on the quality of mulberry leaf fed to silkworms. It is estimated that 38.2% of success rate is directly due to quality leaf (Rahmathulla, 2012) [43]. Continuous production of leaf for a longer time results in depletion of soil nutrients and unless these nutrients *viz.*, NPK are replenished back into the soil system, the quality of leaf is bound to suffer. Farmers do not generally apply nutrients to soil in their right or recommended dosage *viz.*, 300: 120: 120 kg NPK in the case of mulberry under temperate conditions either due to their non-availability, erratic availability or even because of the fact that they cannot afford to do so. (Baqual *et al.*, 2017) [5]. Biofertilizers contribute essential nutrients to plants through natural processes such as nitrogen fixation, phosphorus solubilization, and the synthesis of growth-promoting substances, thereby enhancing plant growth (Mishra and Barolia, 2020) [34]. They offer a promising avenue for reducing reliance on chemical fertilizers and pesticides in agriculture. Biofertilizer comprises of live microorganisms, is a vital component applied to seeds, plant surfaces, or soil in agriculture. It colonizes either the rhizosphere or the interior of the plant, playing a crucial role in promoting growth by augmenting the supply or availability of essential nutrients to the host plant. They represent eco-friendly organic inputs that are more cost-effective than their chemical counterparts. Several microorganisms are commonly used as biofertilizers including nitrogen-fixing soil bacteria and cyanobacteria, phosphate-solubilizing bacteria, used along with the combination of molds and fungi (Umesha *et al.*, 2018) [57].

Similarly, phyto-hormone producing bacteria are also used in biofertilizer formulation. They provide the growth promoting substances like indole acetic acid (IAA), amino acids, and vitamins to the plant and improve the productivity and fertility of the soil while maintaining the crop yield (Parikh and James, 2012) [41]. Utilizing microorganisms such as Bacillus, Pseudomonas, Lactobacillus, photosynthetic bacteria, nitrogen-fixing bacteria, Trichoderma fungi, and yeast, biofertilizers form symbiotic associations with plant roots, converting complex organic matter into easily absorbable compounds. This not only maintains the natural soil habitat but also enhances crop yield by 20-30%, while replacing 25% of chemical nitrogen and phosphorus inputs. Moreover, biofertilizers offer protection against drought and certain soil-borne diseases, further emphasizing their importance in sustainable agriculture (Kapoor *et al.*, 2015) [27]. Biofertilizers, in their ready-to-use form, contain live beneficial microorganisms. When applied to seeds, roots, or soil, they activate the microorganisms, thereby enhancing soil health. By mobilizing nutrients through their biological activity, biofertilizers contribute to rebuilding lost microflora and improving overall soil health (Raghuvanshi, 2012).To simplify application, biofertilizers are packaged in suitable carriers like lignite or peat. These carriers also play a crucial role in ensuring adequate shelf life for the product (Singh *et al.*, 1999) [53]. Rhizobium stands out as the most extensively researched and vital genus among nitrogen-fixing bacteria (Baqual *et al.*, 2017) [5]. Azospirillum species play a significant role in boosting yields of cereal and forage grasses. They achieve this by enhancing root development in well-colonized roots, increasing the uptake of water and minerals from the soil and facilitating biological nitrogen fixation (Nousheen *et al.*, 2021) [64]. Biofertilizers have demonstrated significant promise as supplementary, renewable, and environmentally friendly sources of plant nutrients. They play a crucial role in Integrated Nutrient Management and Integrated Plant Nutrition Systems (Raghuwanshi, 2012) [42]. Biofertilizers grown naturally not only enhance crop yields but also pose no harm to humans. Moreover, they contribute to fostering better sustainable economic development for farmers and their respective countries (Mishra and Dash, 2014) [35]. This review is mainly focused on microbial inoculants that could potentially increase crop productivity. The knowledge gained from this review will help to understand the importance of biofertilizers in the agriculture and sericulture industry and overcome the problems associated with the use of chemical fertilizers.

Significance of biofertilizers in agricultural practices

Biofertilizers play a crucial role in enhancing soil fertility (Kapoor *et al.*, 2015) [27]. Moreover, applying them to the soil enhances soil structure and reduces reliance solely on chemical fertilizers. In lowland environments, employing a combination of BGA (Blue-Green Algae) and *Azospirillum* has shown notable advantages in enhancing LAI (Leaf Area Index). Additionally, the utilization of biofertilizers has resulted in increased grain yield and harvest index. Introducing a combination of *Azotobacter, Rhizobium*, and VAM (*Vesicular Arbuscular Mycorrhiza*) has demonstrated the highest boost in both straw and grain yield for wheat plants, especially when rock phosphate is used as a phosphorus fertilizer. Azolla presents an affordable and eco-friendly solution, contributing to soil enrichment through carbon and nitrogen enhancement (Nousheen *et al.*, 2021) [64]. Certain biofertilizers that are commercially accessible are also utilized for crops. It has been documented that microorganisms such as *Bacillus subtilis, Thiobacillus thioxidans*, and *Saccharomyces sp.* can serve as bio-fertilizers to solubilize bound micronutrients like zinc. Soybean plants, similar to numerous other legumes, have the ability to fix atmospheric nitrogen symbiotically. It's estimated that approximately 80 to 90% of the nitrogen requirement for soybean can be met through symbiosis (Bieranvand *et al.*, 2003) [10]. Bio-control, an advanced method of disease management, holds substantial potential in agriculture (Hoffmann-Hergarten *et al.*, 1998) [23]. The application of antagonist bacteria like *Rhizobium* and *Bradyrhizobium* has shown significant efficacy in managing root knot infections in mungbean plants (Khan *et al.*, 2006) [29]. The growth, yield, and quality of specific plants have been notably enhanced by the application of biofertilizers containing bacterial nitrogen fixers, phosphate, and potassium solubilizing bacteria, as well as microbial strains of certain bacteria (Kapoor *et al.*, 2015) [27].

Types of biofertilizers

Biofertilizers are grouped into different types on the basis of their functions and mode of action. The commonly used biofertilizers are nitrogen fixer (N-fixer), potassium solubilizer (K-solubilizer), phosphorus solubilizer (P-solubilizer), and plant growth promoting rhizobacteria (PGPR) (Mahdi *et al*., 2010) [32]. In one gram of fertile soil, up to 1010 bacteria can be present, with a live weight of 2000 kg/ha (Raynaud and Nunan, 2014) [46]. The presence of bacteria in the soil depends upon the physical and chemical properties of the soil, organic matter, and phosphorus contents, as well as cultural activities. However, nutrient fixation and plant growth enhancement by bacteria are key components for achieving sustainable agriculture goals in the future. Microbes also facilitate various nutrient cycles in the ecosystem.

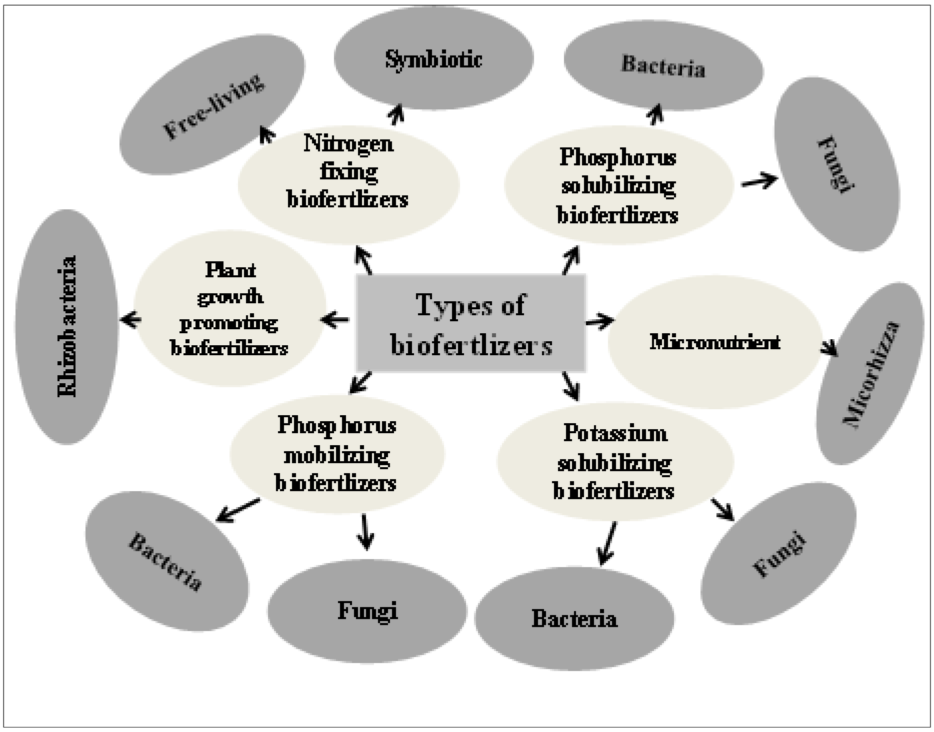


Fig 1: Types of biofertilizers (Nousheen *et al.*, 2021) [64].

Nitrogen fixing bacteria
Nitrogen is the most limiting nutritional factor for plant growth (Gupta *et al.*, 2012) [22]. The atmosphere contains about 80% of the nitrogen in free-state, but most of the plants cannot utilize atmospheric nitrogen. A specialized group of microbes are required to fix this nitrogen and make it available to the plant. These microorganisms are known as biological nitrogen fixers (BNFs). They transform the inert N2 into plant-usable organic form (Reed *et al.*, 2011) [47]. Nitrogen fixation can provide 300–400 kg N/ha/yr and increase the crop yield by 10–50%. In plants, up to 25% of total nitrogen comes from N-fixation (Nosheen *et al.*, 2021) [39]. The roots of plants release substances into the soil, which support colonization and nitrogen fixation by bacteria in the rhizosphere of plants. They can efficiently substitute for chemical fertilizers to a varied extent, thus reducing the chemical load from the environment. They are classified into *Rhizboum, Azotobacter, Azolla, Azospirillium and Frankia* (Brahmaprakash and Sahu, 2012) [11].

Rhizobium
Rhizobium, belonging to the family Rhizobiaceae, operates in a symbiotic manner and is specifically associated with nitrogen fixation in legumes. It has the capability to fix nitrogen ranging from 50-100 kg/ha, but solely with leguminous crops (Kapoor *et al.*, 2015) [27]. Within the family Rhizobiaceae, various genera are encompassed, including *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium,* and *Allorhizobium* (Graham and Vance, 2000) [21]. Rhizobium proves beneficial for various leguminous crops such as chickpeas, red-gram, peas, lentils, black gram, soybeans, groundnuts, as well as forage legumes like berseem and lucerne. This bacterium colonizes the roots of specific legumes, forming tumor-like growths known as root nodules. These nodules act as ammonia production factories, aiding in the enhancement of plant growth and nitrogen fixation (Parul *et al.*, 2022). Rhizobium possesses the capacity to fix atmospheric nitrogen through a symbiotic association primarily with legumes and certain non-legumes such as Parasponia (Mishra and Barolia, 2022). The population of Rhizobium in the soil is contingent upon the presence of legume crops within the field; in their absence, the population tends to decline. Rhizobium is commonly used in agronomic practices to ensure adequate nitrogen (approximately 80% of biologically fixed N comes from symbiosis) and have potential to replace chemical N fertilizers (Rubio-Canalejas *et al.*, 2016) [49]. Rhizobium maintains the soil fertility along with higher crop yields (Arora *et al.*, 2017) [2]. Rhizobium strains were found to increase growth and yield parameters in comparison to the control (Datta *et al.*, 2015) [16].

Technique of rhizobium isolation from root nodules
Isolation techniques for Rhizobium species involves selecting intact root nodules from a healthy Sysbania exaltata plant. Among these nodules, a pink juvenile one was chosen and transferred to a drop of sterile water in a Petri dish. The nodule is crushed between two glass slides, releasing nitrogen-fixing Rhizobium bacteria into the sterile water drop. A smear of the crushed root nodule is streaked onto a yeast extract mannitol agar (YEMA) plate containing 1% Congo red dye. Subsequently, the culture is incubated at 20 to 25 °C for three days (Kapoor *et al.*, 2015) [27].

Azotobacter
Azotobacter is a free-living, nitrogen-fixing diazotrophic bacterium plays an important role in the nitrogen cycle because of its various metabolic functions (Sahoo *et al.*, 2014) [51]. It is present in both alkaline and acidic soil and has the ability to produce vitamins like thiamine and riboflavin (Nosheen *et al.*, 2021) [39]. It belongs to the family Azotobacteriaceae and is used as a biofertilizer for all non-leguminous plants, especially rice, cotton, vegetables, sugarcane, sweet potato, and sweet sorghum. Azotobacter inoculation showed significant increase in seed yield in rapeseed and mustard. It fixes almost 30 kg/N/year; it is

mainly commercialized for sugarcane crop, as it increases the cane yield by 25–50 tons/hectares and sugar content by 10–15%. A. *chroococcum* is the most prevalent species found in the soil, but other species like A. *vinelandii, A. insignis, A. beijerinckii, and A. macrocytogenes* are also found (Kizilkaya, 2009) [31]. Presence **of** A. chroococcum in the rhizosphere of cucumber and tomato was correlated with increased growth and germination of seedlings (Wani *et al.,* 2013) [62]. Another study showed inoculation with *A. chroococcum* caused a significantly increase in plant growth compared to control (Romero-Perdomo *et al.,* 2017) [48]. Azotobacter also produces antifungal compounds and antibiotics that inhibit the growth of several pathogenic fungi in the root zone and help prevent seedling mortality (Wani *et al.,* 2016) [61]. The major limiting factor for the proliferation of Azotobacter is the presence of reduced amount of organic matter in the soils; consequently, the rhizoplane lacks Azotobacter cells (Menendez *et al.,* 2017) [33].

Azospirillium

This bacterium is also essential, as it fixes a considerable amount of nitrogen in the soil. It is associated with the rhizospheric region and fixes up to 20–40 kg N/ha in non-leguminous plants, such as cereals, millets, oilseeds, cotton, and sorghum (Isawa *et al.,* 2009) [25]. It mostly forms a symbiotic association with plants. Several studies have shown the potential of *Azospirillum* for crop improvement (Leite *et al.,* 2019; Galindo *et al.,* 2020) [18]. Azospirillum-inoculated wheat seedlings developed good water status; fresh weight was higher from inoculated seeds than from non-inoculated seeds. A. brasilense were found to synthesize phenyl acetic acid (PAA), an auxin-like molecule with anti-microbial activity. It is demonstrated that the co-inoculation of *A. lipoferum and B. megaterium* provided balanced nitrogen and phosphorus nutrition to the plant and produced a higher yield than inoculation with only Azospirillum (Nosheen *et al.,* 2021) [39].

Phosphorus solubilizing/ Phosphorus mobilizing bacteria

Phosphorus is the second macro-nutrient that is responsible for limiting the growth of plants (Bechtaoui *et al.,* 2021) [7]. It is an important constituent of organic and nucleic acids and is responsible for the synthesis of ATP and several amino acids. P helps in the nodulation process, amino acid synthesis, and proteins in leguminous plants. Soluble form of phosphorus is phosphate anion (orthophosphate), the remaining unavailable form of phosphorus is made into available form via organic acid production by bacteria to makes them available for the plant's nutrition. Examples of phosphate-solubilizing bacteria and fungi (PSB and PSF) are *Bacillus*, *Rhizobium, Aerobacter, Burkholderia, Aspergillus*, and *Penicillium*. Application of Arbuscular fungi can make greater availability of Phosphrus in plants and protects them from stress condition (Nacoon *et al.,* 2020) [37]. Bacillus *subtilis* is also known as PSB which improved safflower growth and protects plants from salinity stress (Zhang *et al.,* 2019). Phosphate-mobilizing microbes can mobilize the immobile forms of phosphorous. They transfer and mobilize the insoluble phosphate from soil layers to the root cortex. Arbuscular *mycorrhiza* is an example of phosphate-mobilizing fungi, in which fungi penetrate the roots and increase the surface area of roots, stimulate metabolic processes, and absorb the nutrients into the roots. Reportedly, phosphorus-solubilizing bacteria (PSB) sometimes act as phosphate mobilizers. Under optimum condition, they have potential to solubilize/mobilize about 30–50 kg P2O5/ha, due to which crop yield could increase by 10–20% (Nosheen *et al.,* 2021) [39].

Mycorrhiza

It is a symbiotic association between the host plant and a certain group of fungi. It is arguably the most important symbiosis on earth (Bucking *et al.,* 2019). This association provides the essential nutrients to the plant, mostly phosphorous and growth hormones, which promote plant growth. They also increase the surface area of roots to increase the absorption of nutrients from the soil and provide resistance to plants against plant pathogens (Dwivedi *et al.,* 2015) [17]. The hyphae of fungi absorb the insoluble phosphorus and convert it into the solubilized form, which is taken up by the plant and, in return, the plant provides shelter and other nutrients to the fungi. These fungi are ubiquitous in geographical distribution and are associated with all crops, except Brasicacea (Nosheen *et al.,* 2021) [39].

Endo mycorrhiza or VAM Fungi

Vesicular arbuscular mycorrhiza (VAM) is the symbiotic association between certain phycomycetous fungi and angiosperm roots. These symbiotic soil fungi colonize the roots of approximately 80% of plant families (Sadhana *et al.,* 2014) [50]. They enhance the transfer of nutrients from the soil into the root system via specialized structures known as vesicles and arbuscules. This association provides many benefits to the plant. The fungal hyphae enhance the uptake of phosphorous and other nutrients as well as increase the root and shoot length. They also help the plant to uptake a large amount of water from the roots. VAM can potentially increase plant tolerance to various biotic and abiotic stresses and could replace the fertilizer requirements of trees and reduce the needs of current levels of chemical fertilizers (Rai and Shukla, 2020) [44]. VAM fungi could contribute to more than twofold increased acquisition of the less mobile nutrients like P, S, Ca, Mg, Zn, and Cu from the rhizosphere. Six genera of fungi have been shown to form mycorrhizal associations *viz.*, *Glomus, Acaulospora, Gigaspora, Sclerocystis, Entrophospora,* and *Scutellospora* (Nousheen *et al.*, 2021) [64]. In addition, VAM fungi enhance the uptake of nutrients by secreting the enzymes and organic acids. An increased concentration of K was found in mycorrhizal plants in comparison to non-mycorrhizal plants (Brahmaprakash *et al.,* 2012) [11].

Plant growth promoting biofertilizer- Rhizobacteria

PGPR is used as biofertilizers; it represents the variation of soil bacteria that live in association with the rhizosphere, rhizoplane associated to root surface, and endophytes present inside the intercellular places (Vandana *et al.,* 2021) [58]. PGPRs are soil bacteria which increase the growth and enhance the tolerance of plants toward stress conditions (Ghosh *et al.,* 2019) [20]. There are diverse mechanisms shown by PGPR which support the plant growth such as N2 fixation, macro- and micronutrient mineralization, secretion of exopolysaccharides, phytohormone production, siderophore, hydrogen cyanide to prevent the growth of phytopathogens, antibiotics, etc. Numan *et al.,* 2018) [40].

Rhizobium *lupini* increased alfalfa growth and enhanced nutrient uptake efficiency. Application of biofertilizers such as *Pseudomonas taiwanensis, Bacillus spp., and Pantoea agglomerans* improved the maize growth, yield, and soil health parameters (Khati *et al.*, 2018) [30]. Application of *Bacillus spp.* improved the plant/soil health parameters and maize productivity. Pseudomonas *taiwanensis* improved maize plant health and soil enzyme activities in the pot experiment. A group of free-living rhizosphere bacteria that colonize plant roots and exert a beneficial effect on plant growth are referred to as PGPR (Beneduzi *et al.*, 2012) [8]. They act as biofertilizers by promoting growth and development of plants, facilitating biotic and abiotic stress tolerance, and helping in the mineralization of the soil by decomposing organic matter. Inoculation of PGPR imparts various beneficial effects to the plant. They increase the tolerance of plant to drought (Ilyas *et al.*, 2020) [24], salinity (Bharti *et al.*, 2013) [9], and biotic stress. They enhance the seed germination and soil fertility (Verma *et al.*, 2016) [59] and promote growth by producing phytohormones including Auxins, IAA, ethylene, gibberellin, etc. (Tahir *et al.*, 2017) [55] They can modulate plant secondary metabolites and bioremediation of heavy metals and pollutants. PGPR includes member of several genera, e.g., *Agrobacterium, Arthrobacter, Alcaligenes, Azotobacter, Acinetobacter, Actinoplanes. Bacillus, Frankia, Pseudomonas, Rhizobium, Micrococcus Streptomyces, Xanthomonas, Enterobacter, Cellulomonas, Serratia, Flavobacterium, Thiobacillus*, etc. (Yadav *et al.*, 2017) [63]. The detailed contribution of PGPR to plant growth promotion and their modes of action has been included in several reviews (Swarnalakshmi *et al.*, 2020) [54].

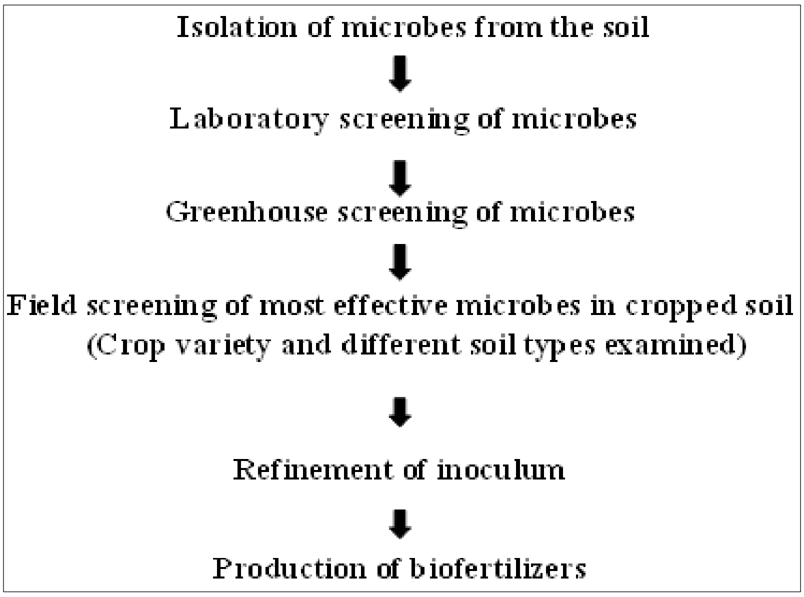
Table 1: Research work conducted on application of biofertilizers in mulberry.

Research conducted	Results	References
Mulberry cuttings treated with *A. chroococum* and *Azospirrillum brasilense* prior to planting.	Increased the sprouting and rooting percentage of cuttings thereby ensuring the establishment of more mulberry cuttings	(Gangawar and Thangavelu, 1992) [19]
Application of *Azotobacter chroococum* in Kanva-2 variety of mulberry.	50% curtailment of nitrogen in Kanva 2 variety of mulberry	(Das *et al.*, 1994) [15].
Application of *Azotobactor. chroococum* in mulberry.	Incidence of major foliar diseases of mulberry caused by *Cercospora moricola*, *Phyllactinea corylea*, *Cerotelium fici*, *Fusarium plallidoroseum* and *Pseudomonas syringae* was found to be effectively reduced	(Sharma *et al.*, 1996) [52].
Integrated organic manures packages of practices which included *Azotobacter*, VAM, seriphos, vermicompost and green manure application to V-1 mulberry.	Improved rearing and grainage parameters of silkworms similar to the standard fertilizers application.	(Jagadeesh *et al.*, 2005) [26].
Co-inoculation with microbial consortium containing phosphate solubilising micro-organisms, nitrogen fixing bacteria (*Azotobacter*) and VA-mycorrhiza to V1 variety of mulberry.	Enhanced growth and yield even with curtailment of nitrogen and phosphorus to the extent of 25-50% of the recommended dose.	Baqual *et al.*, 2005 [3]
The co-inoculation phosphate solubilising micro-organisms, nitrogen fixing bacteria and *arbuscular mycorrhiza* in mulberry.	Enhanced nutrient uptake through leaf i.e., maximum nitrogen (484.12 kg/ha), phosphorus (59.83 kg/ha) and potassium 244.61 kg/ha) uptake through leaf	Baqual and Das, 2006 [6]
Combined effect of biofertilizers (A. chrococum and *Azospirilium*) and in-situ green mauring (Diancha) on leaf yield in mulberry.	The combined effect revealed improved leaf yield along with the 50% curtailment of chemical fertilizers.	Rao *et al.*, 2008 [45]
The dual inoculation of mulberry with phosphate solubilising microorganisms like *Bacillus megaterium*, *Aspergillus awamori* and nitrogen fixing bacteria *Azotobacter chroococum* under varying levels and sources of phosphorus and nitrogen	Beneficial effect on fresh root biomass/sapling, total above ground biomass/sapling, leaf, stem and root nitrogen content of the saplings of V1 and S36 mulberry varieties.	Baqual and Das, 2012 [4]
Effect of three bio-inoculants (*Azotobacter* sp., *Aspergillus awamori* and *Trichoderma harzianum*) application to M5 mulberry plant on silkworm (PM x CSR2) growth, development and cocoon traits.	Larval growth variables and cocoon traits were significantly better and indicated 25 percent reduction of NP application does not adversely affect larval growth and cocoon traits when supplemented with these three microbes.	Waktole and Bhaskar, 2012 [60]
Effect of organic manures specially poultry manure in combination with Azotobactor bio fertilizers	Indicated 50 percent reduction of inorganic nitrogen and 60% reduction of phosphorus application do not adversely affect plant growth, foliar constituents of mulberry when supplemented with the above bio-organic sources of amendments.	Chakraborty and Kundu, 2015 [13]
Effect of biofertilizer Azotobacter chroococcum on the growth of mulberry plant *Morus indica* L. and larval weight, cocoon weight, shell weight, shell ratio and effective rate of rearing (ERR) and length of silk filament of the Bombyx mori	Besides increase in all the parameters, the use of biofertilizer A.c hroococcum has reduced man power and time duration and has also reduced the usage of chemical fertilizer which could curtail the expenses of the farmers	Moorthi *et al.*, 2016 [36]
Combined effect of micronutrient and biofertilizers was studied under existing mulberry garden	Superior growth and yield attributes *viz.*, plant height, number of shoots, number of leaves, leaf area and leaf yield with high leaf moisture retention percentage.	Nazar *et al.*, 2019 [38]
Application of plant growth-promoting fungi (PGPF) in mulberry.	Exhibited increased biomass and length of stems and roots and could be used to promote iron and phosphorus absorption of mulberry as well.	Ting *et al.*, 2023 [56]

Vehicles of biofertilizers: Carriers play a crucial role in enhancing the efficacy of biofertilizers, facilitating easy handling, and extending storage or shelf life. Various materials are utilized as carriers for producing solid biofertilizer products, including clay mineral, diatomaceous soil, and white carbon as mineral carriers, while organic matter carriers include rice and wheat bran, peat, lignite, peat soil, humus, wood charcoal, and discarded feed (Kataria *et al.*, 2022) [28]. Among these, clay mineral and rice bran are commonly employed as carriers. Additionally, to ensure a firm coating of inoculant on seed surfaces, adhesives such as gum arabic, methylethyl cellulose, and vegetable oil are also utilized (Aloo *et al.*, 2022) [1].

Culturing of biofertilizers

The isolated strain is introduced into small flasks containing a suitable medium for inoculum production. Following this, the carrier undergoes autoclaving at 15 psi and 121 °C for 20 minutes. The culture broth is then combined with the carrier at a ratio of 10%, meaning for every 1 kg of carrier, 100 ml of culture broth is utilized. The resulting mixture is spread onto a plastic sheet in a closed environment for air drying. The biofertilizer is subsequently packaged in sterile, airtight plastic bags and stored. For large-scale inoculum production, culture fermenters are employed (Kapoor *et al.*, 2015) [27].



Scheme 1: Mass production of biofertlizers (Kapoor *et al.*, 2015) [27]

General mechanism of Action of Biofertilizers in soil

Biofertilizers play several vital roles in agriculture. They fix nitrogen in the soil and within the root nodules of leguminous crops, thereby making it readily available to plants (Nosheen *et al.*, 2021) [39]. Additionally, they solubilize insoluble forms of phosphate, such as tricalcium, iron, and aluminum phosphate, into forms that plants can easily absorb. Furthermore, biofertilizers produce hormones and anti metabolites that promote root growth and aid in the decomposition of organic matter. When applied to seeds and soil, biofertilizers enhance nutrient availability to plants, leading to yield increases of up to 10-20% without causing any adverse effects on the environment (Kapoor *et al.*, 2015) [27]. Consequently, they significantly improve various plant growth parameters, including plant height, number of branches, root length, shoot length, dry matter accumulation in plant organs, and vigor index.

Application of Azotobacter in mulberry

Azotobacter biofertilizer, a cost effective supplement to chemical nitrogenous fertilizers in mulberry cultivation, is recommended to make sericulture more profitable. Besides, it reduces the deleterious effect of chemical fertilizers on soil health and also reduces the water pollution from nitrate contamination through leaching (Baqual *et al.*, 2017) [5]. It can be applied according to the recommended strategy.

1. Apply 20 kg *Azotobacter* biofertilizer/ha/year (to compensate 150 kg nitrogen) in 5 split doses @ 4 kg each time after every leaf harvest/pruning and intercultural operations.
2. Use phosphorous and potash @ 120 kg/ha/year each in 2 split doses, as per recommendation for irrigated mulberry.
3. Farmyard manure (FYM) should be applied @ 20 tonnes/ha/year as recommended for irrigated mulberry.
4. Apply only 150 kg nitrogen./ha/year instead of 300 kg nitrogen in 5 equal split doses @ 30 kg each time after every leaf harvest/pruning and intercultural operations.
5. The mixed culture of VA-mycorrhiza containing spores of *Glomus fasciculatum* and *Glomus mosseae* is applied to mulberry garden by intercropping technique with maize as mycorrhizal host.

Application of VAM (*Vesicular arbuscular mycorrhiza*) in mulberry

1. Furrows of 10 cm depth are first opened between alternate mulberry rows adjacent to the plant roots and soil based mycorrhizal inoculum is introduced @ 1000 kg /ha having about 10-15 spores /g of inoculum as thin layer by placing in furrows.
2. Maize seeds are sown on the thin layer of inoculum in such a way that a proper contact between maize seeds and the inoculum is established when the seeds are germinated.
3. The furrows are then closed and irrigated.
4. The seeds are allowed to germinate and grown as maize plants between the rows of mulberry.

5. The plants are allowed to grow for a period of one month till the roots of maize develop properly and establish contact with the VAM inoculum placed below.
6. After one month the maize plants are cut at the middle portion with the help of a sickle so as to check the growth and competition of the maize plants with mulberry.
7. In this technique roots of maize plants are colonized quickly by VA-mycorrhiza as soon as the roots come in contact with the VAM inoculum and the population of VA-mycorrhiza increases in the rhizosphere of maize plants.

Quality assurance of biofertilizers
1. Right combinations of biofertilizers needs to be used. It must adhere to specific standards.
2. The inoculants should be carrier-based and contain 108 viable cells per gram of carrier on a dry mass basis within 15 days of manufacture (Kapoor *et al.*, 2015) [27].
3. The pH of the inoculant should fall within the range of 6.0 to 7.5.
4. Cop specific, biofertilisers be used. Other chemicals should not be mixed with the biofertilizers.
5. The inoculums should have a maximum expiry period of 6-8 months from the date of manufacture and should be free from any contaminants, which pose significant challenges to the biofertilizer industry
6. Sufficient gap be given between the application of biofertilisers and chemical fertilizers.
7. Use of expired biofertilisers should be avoided.
8. Both nitrogenous and phosphatic biofertilizers are to be used to get the best results along with organic manures.
9. Each packet of biofertilizer should be labeled with essential information, including the product name, intended leguminous crop, manufacturer's name and address, type of carrier, batch or manufacture number, expiry date, and an ISI mark (Mishra and Barolia, 2020) [34].
10. Biofertilizer packets are stored in cool and dry place away from sun light.

Conclusion
Biofertilizers are gaining popularity across numerous countries and crop varieties. These fertilizers contain living microorganisms that enhance microbial activity in the soil, often supplemented with organic matter to support microbial establishment. In India, soil fertility is gradually declining due to factors such as soil erosion, nutrient loss, toxic element accumulation, water logging, and imbalanced nutrient levels. Organic manure and biofertilizers serve as alternative sources to fulfill crop nutrient requirements. The significance of biofertilizers in agricultural production is considerable. Introducing nitrogen-fixing bacteria through biofertilizers enhances phosphorus levels, thereby impacting sunflower seed oil content and fatty acid proportions. This will not only reduce the burden of stakeholders but will also in long run improve our soil microbial complex for sustainable agriculture and moriculture in particular.

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