IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876

A Review on Inoculation Technology of Bio-Fertilizers

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ABSTRACT: The usage of fertilizers based on helpful microorganisms is becoming more popular as the need for ecologically acceptable farming methods grows. These organisms, which range from bacteria to yeasts and fungus, belong to a broad range of genera, classes, and phyla, and may promote plant nutrition via a variety of methods. Furthermore, research into the interactions between plants, soil, and other microbes is revealing insight on their interrelationships, potentially opening up new avenues for agricultural use. Despite the fact that inoculating plants with these microbes is a well-known technique, developing inocula that have a dependable and consistent impact in the field remains a barrier for their widespread usage. The technique used to make inoculas and the carrier used in the formulation are both critical to their success. This article focuses on how to address inoculation problems in order to enhance the effectiveness of beneficial microorganisms used to boost plant growth and production.

KEYWORDS: Biofertilizers, Inocula, Microorganisms, PGPM, Plant.

1. INTRODUCTION

Environmental problems such as freshwater contamination, energy conservation, and soil erosion are pushing farmers to adopt environmentally friendly farming techniques. Voluntary certification programs (e.g., GlobalGAP or organic farming schemes) as well as legally binding laws (e.g., the EU Directive 2009/128 aimed at the adoption of sustainable pest management techniques) encourage the use of environmentally friendly practices. In this perspective, reducing the use of chemical fertilizers while increasing the use of organic fertilizers is seen as a necessary step in reducing the environmental impact of agricultural operations.

In recent years, a number of organic fertilizers have been developed that serve as natural stimulators of plant growth and development. Products based on plant growth-promoting microorganisms are a subset of this kind of fertilizer. Arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and nitrogen-fixing rhizobia, which are not generally called PGPR, are three main families of microorganisms that are helpful to plant nutrition. Even if the precise definition of these groups is still uncertain, microbial inoculants based on these microorganisms may be categorized into various categories depending on their application. Nonetheless, the term "biofertilizer" is most frequently used to describe products that include soil microorganisms that increase plant availability and absorption of mineral nutrients (like rhizobia and mycorrhizal fungi). Biofertilizers, according to Vessey's definition, are substances that contain living microorganisms that colonize the rhizosphere or the interior of the plant when applied to seed, plant surfaces, or soil, and promote growth by increasing the supply or availability of primary nutrients to the host plant. Phytostimulators, which typically include auxin-producing microorganisms and induce root elongation, are another type of PGPM-containing products [1], [2].

The use of these products is becoming more popular as nutrient absorption efficiency improves and society expects more green technology in production, resulting in higher agrochemical prices. In addition, biofertilizers and phytostimulants have secondary positive effects that make them more effective as bioinoculants. Microorganisms including Rhizobium and Glomus spp. have been proven to aid in the prevention of plant diseases.

Plants were first inoculated with PGPM in the early twentieth century, when a product containing Rhizobium sp. was patented. Since the late 1950s, mycorrhizal fungi have been shown to enhance plant development via P absorption, despite the fact that they have been used as biofertilizers for a few decades. Since then, research activities in these areas have gradually grown, resulting in the selection of many strains with a variety of advantageous characteristics in recent years.

The registration of several strains for both biocontrol and biofertilization has resulted from policies supporting sustainable agricultural production and extensive research that has improved the effectiveness and consistency of microbial inocula, with mycorrhizal and PGPR preparations being marketed in several countries. However, owing to the unpredictability and inconsistency of findings across laboratory, greenhouse, and field research, a broader use of microbial inoculants, particularly those functioning as phytostimulators and biofertilizers, has been hindered. The cause of these inconsistencies is a lack of knowledge of the intricate connections that exist between the system's components: the plant, microorganisms, and environmental circumstances, especially soil conditions. In addition, the lack of proper formulations and the costly and time-consuming registration processes are among the obstacles preventing the widespread use of PGPM [3], [4].

The current article focuses on a variety of problems connected to inoculant formulation in order to enhance the efficacy of PGPM usage in agriculture, especially for plant nutrition.

2. INOCULATION TECHNOLOGY

Formulations comprising one or more beneficial microorganism strains (or species) prepared with an easy-to-use and cost-effective carrier material are known as PGPM inoculants. The major problem to be addressed in order to enable widespread use of biofertilizers is the development of methods for producing vast amounts of pure inocula with high infectivity potential. The utilization of a suitable formulation of inocula preparations, the selection of an acceptable carrier, and the creation of proper delivery systems are all important elements of PGPM inoculation technology.

2.1. Preparation of the inoculum:

The use of fermenters to produce chosen bacteria and yeasts in pure cultures is a widespread technique. As a result, after the strain(s) for the inoculum have been chosen, an industrially standardized manufacturing method may be established. Unlike biopesticides, however, in the case of biofertilizers, the cost of manufacturing is a significant restriction, since the fertilizer's price must not surpass that of conventional fertilizers in order to ensure market viability. As a result, a variety of low-cost organic matrixes (e.g., whey, water sludges, composts, etc.) have been investigated as PGPM growth medium. Using agroindustrial wastes enhanced with rock phosphate is another way to save manufacturing costs. Free or immobilized microorganisms that generate organic acids are introduced to the matrix during composting or fermentation, increasing phosphate solubilization and making it more accessible to plants.

Biofilms have recently been suggested as a potential method for producing efficient plant inocula. A biofilm is made up of microbial cells immersed in a self-produced polymeric matrix (EPS) and adhered to an inert or living surface, which gives structure and protection to the microbial population. In the soil, there are three kinds of biofilms: bacterial (including Actinomycetes), fungal, and fungal-bacterial biofilms. Biofilms are produced on both abiotic and biotic surfaces, with fungus serving as the biotic surface in the development of fungal-bacterial biofilms. Biofilms are formed by the majority of plant-associated bacteria found on roots and in soil. As a result, utilizing PGPM strains that form biofilms may be a method for making inocula formulation and manufacturing easier. Furthermore, using the biofilm as a carrier, biofilm-based inocula may help in the creation of biofertilizers [5]–[7].

While ectomycorrhizal fungi may be generated under fermentation conditions, AMF inocula synthesis is more challenging owing to the requirement for a plant host for mycorrhizal fungus growth. Pot cultures using soil mixes or other methods were employed in the initial efforts to produce AMF inocula (such as aeroponics). However, in the late 1980s, the discovery of monoxenic cultures allowed for the synthesis of AMF under tight control. To generate spores, a technique using split-plate cultures and Ri T-DNA altered carrot roots was devised. Despite the fact that the technique has a greater efficiency, producing 15.000 spores per Petri dish on average 4-5 months after starting the production cycle, it has mostly been utilized for physiological and laboratory research. Douds' technique is improved by changing the medium in the distal compartment every two months and refilling the carbon supply in the proximal compartment with glucose at the same time. In 7 months, about 65.000 spores were produced. However, since the yearly cost of generating one spore was projected to be up to 30–50 USD, depending on the technique employed, such approaches are mostly used for the manufacture of batches of spores for trials or the maintenance of genebanks. A large-scale in vitro generation of mycorrhizal fungus has recently been suggested, which may be implemented on a commercial basis. It stresses the selection of Ri T-DNA converted host roots for various AMF species, as well as the selection and management of the growth media and the use of quality assurance methods.

Commercial inoculants containing AMF species, on the other hand, are still primarily produced by growing host plants under controlled conditions, with the inclusion of various fungal structures (spores, mycelium hyphae) and mycorrhizal root residues from the plants used as propagating material in the inoculant (i.e., sorghum, maize, onion, or Plantago lanceolata). This might be considered a traditional technique, in which sand/soil substrates and/or other materials (e.g., zeolite, perlite) are utilized to mass-produce AM fungal inoculum for large-scale applications in pots, bags, or beds. The following are crucial elements of this manufacturing method:

1. The application of well-known AMF species,
2. The selection of a host species with a short life cycle, sufficient root growth, a high degree of colonization by a wide variety of AM fungus, and tolerance to relatively low phosphorus levels,
3. Manipulation of soil mineral nutrient levels
4. The right mix of AMF species and host plant

It is feasible to get inoculum densities of 80–100 thousand propagules per litre with this method. This necessitates diluting the inoculum with a carrier in order to prepare a commercial product.

Enhanced formulations may contain two or more species of distinct PGPM, given that microbial interactions between bacteria and mycorrhizal fungus have been found to develop naturally in the soil, increasing mycorrhizal symbiosis [36, 37]. Plant development may be stimulated by microbial consortia through a variety of methods that enhance nutrient uptake and suppress fungal plant diseases. The many processes suggested to explain such growth stimulation relate to an increased rate of nutrients cycling as a result of increased soil microbial content and microorganism biodiversity in soil where mycorrhizal plants are cultivated.

When compared to single inoculation, simultaneous inoculation with various PGPR and/or AMF frequently resulted in enhanced growth and yield due to better nutrient absorption. Interactions between bacteria and AM fungus do have positive effects on nutrient absorption, especially when PGPR and N2-fixing bacteria are involved.

Maize and ryegrass inoculated with A. brasilense with AMF produced N and P levels similar to fertilizer-grown plants. Due to the general non-specificity of AMF fungus colonization of specific plant species/cultivars, coinoculation with various AMF species is more likely to be successful. Plant growth has also been found to benefit from a synergistic interaction between AM fungus and various PGPR species, including Azospirillum, Azotobacter, Bacillus, and Pseudomonas species. When mycorrhizal fungi were coinoculated with PGPR, root colonization by AMF increased. Plants infected with a combination of Glomus deserticola and Rhizobium trifoli produced four times more nodules than plants treated with a single R. trifoli, while coencapsulated R. trifoli and Yarrowia lipolytica produced increased mycorrhization and nodulation. The use of nodule-inducing rhizobia and AM fungi increased the efficiency of both P and N absorption. Synergistic effects of mycorrhizal and nodule symbioses on infection rate, mineral nutrition, and plant development are common. When PGPM were used as commercial biofertilizers comprising consortia of various microorganisms, coinoculation led in improved mineral nutrient absorption and growth.

All of these instances demonstrate the use and greater effectiveness of biofertilizers made up of several species with various growth-promoting mechanisms. The availability of numerous PGPR and AMF strains that have been evaluated in a variety of crops and under a variety of field circumstances should enable for the creation of commercially viable consortia.

2.2. Carriers:

The carrier is the bulk of the inoculant (in terms of volume or weight) that aids in the delivery of a sufficient quantity of PGPM in a physiologically acceptable form. Organic, inorganic, or synthetic materials may be used to make the carrier. The primary variables influencing a carrier's decision are availability and cost.

The container should be constructed to give the PGPM with an appropriate microenvironment and ensure that the product has a long shelf life (at least 2-3 months for commercial purposes, possibly at room temperature). The formulation should be simple to disperse or dissolve in the soil volume near the root system. A good carrier should have the following characteristics: good moisture absorption capacity, ease of processing and absence of lump-forming materials, near-sterility or ease of sterilization by autoclaving or other methods (e.g., gamma irradiation), low cost and availability in sufficient quantities, and good pH buffering capacity. A strong adherence to seeds is also essential for carriers that will be utilized to coat seeds. A standardized composition that ensures chemical and physical stability, suitability for as many PGPM species and strains as possible, the ability to mix with other compounds (e.g., nutrients or adjuvants), and being composed of biodegradable and nonpolluting compounds are all factors that influence the carrier's appropriateness. If the inoculant is used as a seed coating, the carrier must ensure that the PGPM on the seed survives since seeds are not usually planted right away after being coated. The PGPM's survival is critical both throughout the bio product's storage period and after it is planted. Because the inoculant must compete with natural soil microorganisms for nutrients and livable niches, as well as survive against grazing protozoa, carrier materials that make nutrients and/or habitable micropores accessible to the PGPM, especially in the case of bacteria, are more appropriate [8], [9].

The bio-physical fertilizer's shape is determined by the kind of carrier used. Dry inoculants may be made from a variety of soil materials (peat, coal, clays, inorganic soil), organic materials (composts, soybean meal, wheat bran, sawdust, and so on), or inert materials (sand, sand, sand, sand, sand, sand, sand, sand, sand, sand, sand, sand, sand, sand (e.g., vermiculite, perlite, kaolin, bentonite, silicates). Broth cultures, mineral or organic oils, and oil-in-water suspensions may all be used to make liquid inoculants.

Solid carriers are often in the shape of powder, granules, or beads. Powder material sizes may range from 75 m to 0.25 mm in standard sizes. Granules and beads vary in diameter from 100–200 m to 3–4 mm. Powdered inoculants may be used to coat seeds, or they can be suspended in a liquid and applied straight to the furrow, or the seeds/plants can be dipped in it just before sowing/planting.

Bacteria may also be preserved via lyophilization, which allows for excellent survival rates without the need for a carrier. However, a cryo-protectant must be introduced throughout the procedure, since this is required to protect the bacterial cell membrane and cytoplasm from dehydration. Mannitol is an effective protectant, however microcrystalline cellulose has lately emerged as a viable alternative owing to its slower breakdown kinetics in soil and long-term inoculum stability at ambient temperature. Microbial cultures that have been lyophilized may be used directly or integrated into a solid carrier [10].

3. DISCUSSION

To enhance the effectiveness of PGPM inocula applications in the field, a deeper knowledge of the various circumstances and characteristics of the interrelationships in the soil-plant-microorganism system is required. Many variables, especially for bacteria, have a role in determining their rhizocompetence. Several environmental factors, in addition to the genotype and physiological state of the inoculated strain, influence the size and composition of the populations sustained by the rhizosphere: soil pH, mineral nutrients, and water content; plant species, genotype, and physiological state; and the presence of other microbial species.

Several isolates with plant growth promotion or biocontrol characteristics have been discovered in recent decades. However, our understanding of PGPM behavior at the root level and their role in the field is currently restricted.

Studies employing inoculant combinations are offering a new approach to the issue, given the positive benefits of PGPR and AMF. These findings may aid in the development of huge consortia of inocula that promote plant growth in a synergistic manner or have multitasking capabilities. However, such combinations are more technically challenging since they make it more difficult to create an inoculant that fits the various strains and types of microorganisms. The development of biofilm-based carriers or encapsulation methods that enable the creation of macro-capsules with a core and an envelope may aid the development of microbial consortia-based bio-fertilizers.

4. CONCLUSION

The majority of inoculants chosen so far have been for annual crops (mainly legumes, cereals, and some vegetables). Other agricultural areas, such as fruit and vegetable production, and especially organic farming and integrated production systems, are seeing increased demand for synthetic inputs, which are either prohibited or restricted by law. Because of the use of inert substrates and controlled growing circumstances, commercial inoculants may find success in soilless and protected crops, where the predictability of PGPM treatments should be greater than in open fields. Specific strains for all of these crops may help to increase the inocula market and assist the transition in agriculture toward more sustainable production methods. However, the development of technology aimed at delivering the inocula efficiently, most likely by modifying sprayers and sprinklers often used for plant protection or irrigation, may help to expand the usage and dependability of PGPM treatments in agricultural settings. The efforts to alleviate abiotic stress conditions in crops (i.e., drought, salt, inorganic, and organic pollutants) and improve food quality will be the future difficulties in choosing PGPM. Improvements in the production process for microbial inocula consortiums, the development of new nanoparticle-based carriers, and the optimization of application devices and application times for polyannual crops are all issues that require more research in order to expand the use and efficiency of PGPM in agriculture. Such a requirement is also driven by legislative choices that promote sustainable practices, as well as the evaluation of the safety of plant protection agents, which is now ongoing in both the EU and the US, and which has the potential to expand the market for PGPM.

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