IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, Sep 2022

A Review on an alternative source of Nutrition: Bio-fertilizers

Archana Negi, Assistant Professor

College of Agriculture Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India

Email id- archie.negi@gmail.com

ABSTRACT: ***By forming a symbiotic connection, some bacteria fix atmospheric nitrogen in the root nodule of plants. Rhizobium infects legume trees for nodulation, while Frankia is an actinomycete recognized for forming actinorhizal symbioses with nonlegumes. Azospirillium, on the other hand, is a nitrogen-fixing symbiotic organism. The fungus known as vesicular arbuscular mycorrhizal (VAM) aid in the absorption of phosphorus by plants. The current research looked at these four bacteria. Frankia, Rhizobium, and Azospirillum were inoculated to Casuarina equisetifolia, Acacia nilotica, and Eucalyptus tereticornis, either individually or in combination with VAM. In comparison to the control, the inoculation resulted in a significant increase in biomass. In comparison to the control, Casuarina equisetifolia and Acacia nilotica produced more nodules with greater nodule dry mass and nodule nitrogenase activity. It is essential to note that in all three tree species, dual inoculation with Frankia VAM, Rhizobium VAM, and Azosprillum VAM produced greater output than single inoculation.***

KEYWORDS: ***Azospirillum, Biofertilizer, Nitrogen Fixation, Rhizobium, VAM.***

1. INTRODUCTION

The yield of tree crops in a sustainable production system is mainly dependent on the activity of suitable microbes through nutrient exchange among trees. Despite the fact that the rhizosphere appears to be too complex to manipulate, specific microorganisms such as mycorrhizal fungi, actinorhizae, associative nitrogen fixers, and Rhizobium spp. increase tree productivity by increasing nitrogen input and availability of other minerals, particularly phosphorous, in the tree ecosystem. Rhizobacteria that stimulate plant development are known as plant growth boosting rhizobacteria (PGPR). Many scientists highlight the importance of studying the synergistic impact of many kinds of microorganisms on tree production, thus there is a need to focus on plant microbe-microbe interactions rather than plant microbe interactions. Frankia, Rhizobium, Azospirillum, and vesicular arbuscular mycorrhiza (VAM) fungi were used to enhance production in Casuarina equisetifolia, Acacia nilotica, and Eucalyptus tereticornis. Surprisingly, these microorganisms are environmentally benign and have a cost advantage over inorganic fertilizers in terms of tree production.

With the adoption of suitable production methods, many fast-growing tree species may offer a high output of biomass. The leguminosae family of trees is one of the most widely planted on the Indian subcontinent. Leguminosea is often grown on infertile or marginal terrain. It grows quickly, has many purposes, and is simple to cultivate and maintain. It may establish a symbiotic connection with Rhizobium species since it is a leguminous plant. Biological nitrogen fixation is the process by which bacteria transform atmospheric nitrogen into a form that may be used by plants (BNF). BNF reduces the requirement for nitrogen fertilizer application. In many soils, nodule bacteria are either missing or insufficient in quantity or quality to satisfy legume nitrogen needs. In order to enhance biomass productivity or growth potentials, highly efficient rhizobia cultures must be inoculated into the seed or seedling under these circumstances. Chemical fertilizers are too costly

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, Sep 2022

and harmful to the environment to be used indefinitely. Rhizobium species, on the other hand, seem to be a socially, economically, and ecologically feasible option for increased biomass production [1]–[3].

Biological Nitrogen Fixation

There are over 640 tree species that fix atmospheric nitrogen, the bulk of which are members of the Leguminosae family. Because they can grow and flourish on nitrogen-deficient soils, nitrogen-fixing trees (NFTs) are an excellent choice for reforesting damaged areas. This ability comes from a one-of-a-kind relationship with symbiotic bacteria from the Rhizobium genus or actinomycetes from the Frankia genus. Rhizobium nodulates legumes in general, whereas Frankia nodulates a variety of nonleguminous tree crops. Symbionts that fix nitrogen provide between 50 and 200 kg N hm-2. NFTs grow rapidly and withstand a wide range of soil conditions, in addition to their ability to fix nitrogen [4]–[7].

VAM rely on plant roots to establish themselves and/or carbon to contribute to their development. Though many kinds of mycorrhizal connections have been documented, the most prevalent are ectomycorrhizae and VAM, also known as endomycorrhizae. VAM, which are obligatory symbionts, cannot be grown in artificial media like the majority of ectomycorrhizae.

Selecting the Most Appropriate Strain for Inoculation:

The development of an appropriate procedure to discover and isolate the most efficient mycorrhizae–nitrogen-fixing tree strains is a major focus of mycorrhizae–nitrogen-fixing tree research. Because the effectiveness of host inoculation is dependent on the capacity of organisms to survive local circumstances (e.g., soil pH) and perform their roles, identification is critical. The researchers utilized a variety of techniques to determine which strain was best for inoculation. Preliminary screening of a large number of isolates was utilized, which eliminated the requirement for time-consuming in vivo screening for specific characteristics such acid aluminum stress resistance and reduced the number of candidate isolates. This method was used to find Rhizobium strains that were appropriate for acid soil. Symbionts must be screened in the field under the same soil and environmental conditions as the region to be revegetated. It's also a good idea to test symbionts for tolerance to various soil and environmental conditions. When the same isolation does not have the highest score for each criterion, selecting a single isolate becomes difficult (i.e., plant dry mass, percentage of root infection, phosphorous content of plant tissue, number of nodules and total nitrogen content, or alternatively, nitrogenase activity). Cluster analysis is used in one study to pick the two best isolates from a pool of many that were evaluated. Several important characteristics are given equal weight in this technique of examination. The organism must be separated from big, healthy nodules in the case of root nodule symbionts. The isolate should be verified by observing nodule formation after inoculation of aseptically produced host seedlings. Frankia, on the other hand, is difficult to grow in the lab and must be given to its host seedlings (Casuarina equisetifolia) as crushed nodule suspensions. Inoculating the plant with the symbiont to test for the development of an efficient symbiotic relationship and then re-isolating the same organism from the plant is the best way of authenticating a particular isolate. Serological methods may be used to validate the strain's identification. Several months or years after inoculation, strain identification is performed to check for the existence of an injected symbiont.

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, Sep 2022

Repeated testing is particularly essential at degraded sites, where adverse soil conditions have a high probability of eradicating the organism.

Mycorrhizae and Rhizobium/Frankia Succession:

On temperate tree species, several research on ectomycorrhizal succession have been conducted. However, there hasn't been a comprehensive investigation of this phenomena in tropical species. The significance of researching tree species' mycorrhizal status and mycorrhizal succession at all phases of tree development. If succession is not carefully observed, the potential of neglecting the main species and an opportunistic organism as the symbiont cannot be discounted. Because trees are perennials with variable metabolite needs at various phases of their life cycle, knowing the succession sequence of Mycorrhizae and Rhizobium/Frankia species/strains is very essential in tree crop production. Some experts questioned the practice of isolating VAM, Rhizobium, and Frankia from mature trees (4 to 6 years old) of Acacia auriculi, Casuarina equisetifolia, and Pterocarpus marsupium for inoculation of seedlings at the establishment phase because different symbionts form associations with the species at different stages to fit growth. In deteriorated soil, the primary issue is seedling establishment and survival; thus, isolating symbionts from seedlings or younger plants seems suitable.

Microbial Symbiont Contribution to Host Plant (Symbiotic Efficiency):

The nitrogen and phosphorous absorbed by the root system independently of symbionts are often confused with the nitrogen and phosphorous fixed by nodule symbionts and the soil phosphorous really supplied to the plant by Mycorrhizae. However, tagged isotopes of N and P may be used to quantify symbiont-supplied N and P. (15N and 32P). The 15N dilution technique revealed that approximately 10% of total plant nitrogen came from the atmosphere in Rhizobium-inoculated Gliricidia sepium. Atmospheric nitrogen made up 34 to 61 percent of total nitrogen in Rhizobium-inoculated Leucaena leucocephala, whereas N from supplementation made up 5-6 percent. Using the 15N isotope dilution method, the impact of dual inoculation with VAM (Gigasporamargarita) and Rhizobium in acid soils with low P and N levels was investigated in Trifolium subterraneum and Lotus pendunculatus. At 10 kg mh12, the N obtained from the atmosphere increased by 350 percent (on a per plant basis) and plant biomass increased by 68 percent in Trifolium subterraneum compared to plants infected with Rhizobium alone. When N was increased to 100 kg mh-2, the quantity of nitrogen obtained from the atmosphere decreased while the amount of nitrogen derived from the soil increased. However, increasing the quantity of nitrogen fertilizer had no impact on the proportion of plant nitrogen obtained from the atmosphere in similar treatments of Lotus pedunculatus infected with VAM and Rhizobium. Because of the wide range of reactions to fertilizer treatments, it's important to establish a baseline amount of fertilizer for each species before prescribing experimental dosages. Dose-response curves depicting seedlings against increasing fertilizer dosages may be used to determine appropriate base line doses.

Biofertilizer Inoculation

Inoculants containing one or more beneficial microorganism strains (or species) prepared with an easy-to-use and cost-effective carrier material are known as plant growth-promoting microorganisms (PGPM). 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

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, Sep 2022

selection of an acceptable carrier, and the creation of proper delivery systems are all important elements of PGPM inoculation technology [8].

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 [9].

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 [10].

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

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, Sep 2022

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 critical elements of this manufacturing method:

1. I the utilization of AMF species that are well-known;
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. Altering the mineral nutrient levels in the soil
4. The right mix of AMF species and host plant.

2. DISCUSSION

There are just a few options for delivering PGPM to crops in the field. Farmers are hesitant to invest in specialist equipment for the production of microbial-based goods. As a result, prepared inocula should be simple to apply using conventional agricultural equipment and techniques. Inoculation may be done on plant material or in the soil. Because the latter technique requires less time, it may be more convenient for the farmer, but it also need a larger quantity of inoculant. Solid or liquid formulations may be used for soil inoculation. The inert substance is usually combined with the inoculum in the factory, but it may also be mixed by the farmer before application, particularly when liquid formulations are employed. The use of fertilizers made by combining organic matrixes and insoluble phosphates with the addition of selected P-solubilizing microorganisms can also be considered a method of applying PGPM to crops because it increases the availability of nutrients (especially P) to plants and, as a result, affects the plant's tolerance to soil pathogens.

Annual crops may be inoculated by spreading the inoculum over the soil surface, alone or with seeds, or by in-furrow application, seed dressing, or coating; tree crops can be infected by root dipping or seedling inoculation. When inocula must be dispersed to the soil, application to previously established orchards or plantations may provide certain technical challenges. Liquid formulations applied via a fertigation system may meet the requirement to provide PGPM as near to the root system as feasible. The feasibility of using alginate beads and polyurethane foam as carriers to deliver PGPM into a water solution has been demonstrated (Malusá, Trzcinski, and Treder, unpublished observations); however, the foam must be soaked in the water tank for some time or the beads must be dissolved in a citrate solution. Powder ingredients may also be buried near the roots with the use of a harrow-like instrument and a distributor. Irrigation thereafter would boost inocula transfer to the roots and perhaps improve inoculation effectiveness by providing better moisture conditions that encourage bacterial mobility in the soil.

Microbial communities in the soil may be able to attenuate or neutralize the effects of PGPM. The injected strains in the soil or on the root rhizosphere were only recovered 30–40 days after inoculation in the case of PGPR. As a result, applying PGPM three to four times throughout the growth season, with a two- to four-week gap between each treatment, increases the efficacy of PGPM applications.

3. CONCLUSION

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, Sep 2022

The growth of infected seedlings is clearly greater than that of control seedlings, as shown by the preceding discussion. As a result, inoculating seedlings with Rhizobium, Rhizobium Frankia, or Rhizobium VAM may boost seedling production in a plantation site. The ability of nitrogen-fixing bacteria in the soil should be investigated, and if the soil lacks such bacteria, appropriate strains of these bacteria, fungus, and VAM may be added individually or in combination to restore the trees' vigor and development. Thus, inoculation with an appropriate strain of these cultures substantially improves crop development in acidic soils, low-phosphorus soils, and soils lacking in nitrogen-fixing bacteria.

REFERENCES

[1] G. Cvijanovic, O. Dozet, V. Ukic, and N. Uric, "The importance of biofertilizers in sustainable production of corn, wheat and soybean," *Int. Conf. Compet. Agro-food Environ. Econ. Proc.*, 2015.

[2] W. S. Wong, S. N. Tan, L. Ge, X. Chen, and J. W. H. Yong, "The Importance of Phytohormones and Microbes in Biofertilizers," 2015.

[3] B. Ritika and D. Utpal, "Biofertilizer, a way towards organic agriculture: A review," *African J. Microbiol. Res.*, 2014, doi: 10.5897/ajmr2013.6374.

[4] P. M. Vitousek, D. N. L. Menge, S. C. Reed, and C. C. Cleveland, "Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems," *Philos. Trans. R. Soc. B Biol. Sci.*, 2013, doi: 10.1098/rstb.2013.0119.

[5] C. Santi, D. Bogusz, and C. Franche, "Biological nitrogen fixation in non-legume plants," *Annals of Botany*. 2013, doi: 10.1093/aob/mct048.

[6] R. Darnajoux *et al.*, "Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: importance of molybdenum availability and implications for current biological nitrogen fixation estimates," *New Phytol.*, 2017, doi: 10.1111/nph.14166.

[7] F. Mus, A. B. Alleman, N. Pence, L. C. Seefeldt, and J. W. Peters, "Exploring the alternatives of biological nitrogen fixation," *Metallomics*. 2018, doi: 10.1039/c8mt00038g.

[8] M. Abd Alla, D. Farrag, and R. Knany, "Interaction Effects Between Nitrogen Levels And Biofertilizer Inoculation Methods On Okra Growth, Yield And Pods QualitY.," *J. Plant Prod.*, 2015, doi: 10.21608/jpp.2015.52434.

[9] M. M. Tawfik, A. Bahr, and A. K. M. Salem, "Response of Kaller Grass (*Leptochloa fusca* L.) to biofertilizer inoculation under different levels of seawater irrigation," *J. Appl. Sci. Res.*, 2006.

[10] A. Namvar and T. Khandan, "Response of wheat to mineral nitrogen fertilizer and biofertilizer (Azotobacter sp. and Azospirillum sp.) inoculation under different levels of weed interference," *Ekologija*, 2013, doi: 10.6001/ekologija.v59i2.2711.