Chapter

Sustainable Development of Horticulture and Forestry through Bio-Inoculants

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Abstract

The role of microorganism is very critical in nutrient management of horticulture and plantation forestry. They are conductors of the nutrient management orchestra as they provide by inputs in terms of micro and macronutrients besides organic matter and can be called as bio-inoculants (biofertilizers). Biofertilizers play a vital role in fixing the atmospheric nitrogen and mobilization of phosphorous, sulfur, manganese, copper, and iron in the soil. Symbiotic (Rhizobium and Frankia) and nonsymbiotic microorganisms (*Azospirillum*) are known to improve the soil fertility by fixing the atmospheric nitrogen. Arbuscular mycorrhizae fungi (AM fungi) and phosphobacterium have ability to transfer insoluble phosphate into soluble form. Moving in this direction it becomes imperative to understand as forest microbiologist and pathologist, the roles played by microorganism in diverse plants-soil-microbe interaction to analyze their effectiveness in improving their efficiency. Biofertilizers are economy and environmentally safe, and there is a growing awakening among the tree growers and farmers. In agriculture, advantages of biofertilizer application are better known, but in tree crops, the utility of biofertilizers is still in an experimental stage. The review paper is collective evident for the compatibility of different biofertilizers and their augmentation effect on the production of quality seedling and nutrient management of tropical horticulture and plantation forestry.

Keywords: arbuscular mycorrhizae fungi (AM fungi), bio-inoculants (biofertilizers), horticulture, plantation forestry, sustainable nutrient management, Rhizobium, Frankia

1. Introduction

Plants are being an important component of socio-economic condition of human life and culture. Ever since the beginning, tree crops have furnished use with three of life's essentials, wood, food and oxygen. Besides these, they provide additional necessities to human being such as shelter, fuel wood, fodder for livestock, ethno medicine, architectural, agriculture implements, building construction tools, sound and wind barriers, soil improvement through litter production and nitrogen fixation in association with Rhizobium and Frankia. Many drugs which derived from plants generally have been replaced by more potent synthetic ones and trees remain a source for some drug ingredients for pharmaceutical industry. They play an important role in ecosystem services through carbon sequestration, improving air quality, climate amelioration, and conservation of water and supporting wildlife. They also reduce the atmospheric temperature and the impact of greenhouse gases by maintaining low levels of carbon dioxide.

Plant growth and productivity is generally regulated by the availability of soil nutrients. One of the major efforts to increase the plant productivity is through management of nutrients, which can be achieved by application of fertilizers. However, application of chemical fertilizers is not eco-friendly and economically viable in current scenario. Other alternative method is supplement of bio-inoculants (bio-fertilizers) for sustainable development of horticulture and forestry crops. Bio-inoculants are plant growth promoting beneficial microorganisms such as the species of *Azospirillum, Azotobacter, Bacillus, Ecto and Endo-mycorrhizal* fungi*, Frankia, Pseudomonas, Rhizobium*, *Trichoderma,* etc. Such microorganisms accelerate certain microbial process to augment the extent of availability of nutrients in the form, which can be assimilated by plants and also maintain the plant health by controlling diseases.

2. Rhizosphere and microbial interaction

Various types of microorganisms inhabit air, water and soil. They play an important role in restoring the physical, chemical and biological property of soil. Rhizosphere ecology and microbial interactions are responsible for key environmental processes, such as the bio-geo chemical cycling of nutrients, organic matter and maintenance of plant health and soil quality [1]. Among the microbial population, both beneficial and harmful bacteria as well as fungal species were found, but the microbial population was low when compared to rhizosphere soil [2].

Rhizosphere is the physical location in soil where plants and microorganisms interact. The interest in the rhizosphere microbiology derives from the ability of the soil microbiota to influence plant growth and vice versa. The presence of microorganisms in the rhizosphere will increase root exudation and it was found that 5–10% of the fixed carbon was exudates from the root under sterile condition, on the introduction of beneficial microorganisms, root exudation rate increases by 12–18%. The interaction between bacteria and fungi associated with plant roots may be beneficial, harmful or sometimes neutral for the plant, and effect of a particular bacterial species may very as a consequence of soil environmental conditions [3]. The beneficial microbes can be divided into two major types based on the living nature; free living (that live in soil) and symbiotic relationship with the plant root nodule of legume and actinomycete plants [4].

3. Bio-inoculants

Bio-inoculants are beneficial microorganisms for nutrient management, plant growth and are eco-friendly and natural inputs providing alternate source of plant nutrients, thus increasing farm income by providing extra yields and reducing input cost also. Bio-inoculants increase crop yield by 20–30%, replace chemical N and P by 25%, stimulate plant growth, enhance soil biodiversity, restore natural fertility and provide protection against drought and some soil borne plant pathogens. The role of bio-inoculants has already been proved extensively in enhancing the mineralization processes of organic matter and helping the release of nutrients, utility of soil organic matter contents and cations exchange capacity [5] and therefore, bio-inoculants are gaining importance in agriculture for the past few decades. However, the scientific exploitation of bio-inoculants in horticulture and forestry is scanty in developing countries like India.

4. Classification of bio-inoculants for tree crops

1. Nitrogen fixing symbiotic microorganisms (*Rhizobium* and *Frankia*)

2. Nitrogen fixing non-symbiotic microorganisms (*Azospirillum, Azotobacter* and blue-green algae)

3. Phosphate solubilizing microorganisms (*Arthrobacter, Pseudomonas, Bacillus, Aspergillus*)

4. Phosphate mobilizing microorganisms (Ecto and Endo—Mycorrhizal fungi)

5. Potash mobilizer (*Bacillus* sp., *Pseudomonas* sp.)

6. Sulfur uptake (*Pseudomonas, Klebsiella, Salmonella, Enterobacter, Serratia* and *Thiobacillus*)

7. Zinc solubilizer (*Bacillus subtilis, Thiobacillus thiooxidans* and *Saccharomyces* sp.)

8. Iron uptake (*Pseudomonas fluorescens*)

9. Plant growth promoters (*Pseudomonas* sp., *Bacillus* sp., *Serratia* sp.)

5. Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria are group of bacteria that actively colonize roots and increase plant growth and yield [6]. It enhances plant growth and productivity by synthesizing phytohormones, increasing the availability and facilitating the uptake of nutrients by decreasing heavy metal toxicity in the plants, antagonizing the plant pathogens [7]. The mechanisms by which PGPR promote growth are not fully understood [8], against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and fungicidal compounds [9] and also solubilization of mineral phosphates and other nutrients [10].

5.1 Azospirillum

Azospirillum species are free-living N_2-fixing bacteria commonly found in soils and in association with roots of agriculture, horticulture and forestry species [11]. *Azospirillum* are known to act as plant growth promoting rhizobacteria (PGPR) and stimulate plant growth directly either by synthesizing phytohormones or by promoting improved N nutrition through biological nitrogen fixation (BNF). PGPR also produce several the growth promoting substances including IAA, GA3, Zeatin and ABA [12]. Presently there are seven species have been identified in this genus, *A. amazonense* [13], *A. brasilense, A. lipoferum,* [14], *A. doebereinerae* [15], *A. halopraeferens* [16], *A. irakense* [17] *and A. largimobile* [18].

Applications of plants with Azospirillum have promoted plant growth of agronomically important field crops by 10–30% in the field experiment [19, 20]. Nursery experiments proved that the inoculation of tree cops with *Azospirillum* could result in significant changes in various growth parameters, particularly shoot and root growth, biomass, nutrient uptake, tissue nitrogen content, leaf size of several shola tree species [21] and *Casuarina equisetifolia* [22, 23], *C. cunninghamiana* Mig. [24], *Moringa oleifera* [25], *Acacia nilotica* [26], *Azadirachta indica* [37], *Delonix regia* [28], *Erythrina indica* [29], *Feronia elephantum* [30], *Jatropha curcas* [31]. Two years old *Casuarina equisetifolia* plants treated with bio-inoculants in field condition improve the growth of plants by 90% over uninoculated control [32]. *Azospirillum lipoferum* treated with *Jatropha curcas* under field conditions has increased the shoot length by 44.85% and primary and secondary root length by 39.3 and 37.5% respectively. Similarly, the root and shoot biomass also increased by 24.01 and 15.04% leaf area by 28.57% increase over control and the other *Azospirillum* species such as *A. brasilense*, *A. haloference* and *A. amazonense* [33]. The stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms including secretion of phytohormones (auxins and gibberellins), biological nitrogen fixation, and enhancement of mineral uptake of plants [8] due to the ability of synthesis of in vitro phyto-hormones such as IAA, gibberellins, cytokinin [34, 35] and produced by ethylene [36].

5.2 Effect of bio-inoculants and biochemical changes of tree crops

Plants inoculated with *A. brasilense* were always characterized by a higher chlorophyll concentration. Inoculation of crops caused a statistically significant increase of chlorophyll content in the case of oats in 1996 (15%) and wheat in 1997 (15%). Chlorophyll appeared to be a sensitive indicator of inoculation effect, which was also supported by Bashan et al. [37]. *A. lipoferum* inoculated *Jatropha curcas* seedlings has increase in level of chlorophyll a, b and carotene and such increase was maximum by 31.98, 14.5 and 18.9% and protein content (37.35%) amino acid (26.33), lipids (8.9) and carbohydrates (9.37) when compare to control plant under field conditions [31]. The total chlorophyll and soluble protein content was found to be higher in the *Moringa oleifera* seedlings inoculated with *A. brasilense* [25].

5.3 Pseudomonas

The genus *Pseudomonas* is one of the most diverse gram-negative non-spore forming, motile, rod shaped bacteria with an important metabolic versatility and pathogenicity [38]. Morphologically this genus is straight or slightly curved rods and produced yellowish green pigment in King's B. Medium. Plant growth promoting rhizobacteria consisting of primarily *Pseudomonas fluorescens* and *P. putida* were identified as important organisms with ability for plant growth promotion and effective disease management properties. The population density of fluorescent pseudomonas in the rhizosphere in usually reduced by AM fungi colonization [34, 39, 40]. Many strains of genus *Pseudomonas* possess the capability to promote plant growth [41], due to their 1-aminocyclopropane-I carboxylate deaminase activity, indole acetic acid (IAA) and siderophore production [42], PGPR can exert a beneficial effects on plant growth by suppressing soil borne pathogens [43], improving mineral nutrition [44] and phytohormone synthesis [45].

6. Arbuscular mycorrhizal (AM) fungi

The symbiotic association between fungus and root systems of higher plants is called mycorrhiza, which literally means root fungus. Ectomycorrhizae and entomycorrhizae or arbuscular mycorrhizae (AM) are playing important role in phosphorus and micronutrients uptake by tree species. The AM fungi association is endotrophic, and has previously been referred to as vesicular-arbuscular mycorrhiza (VAM), this name has been dropped since 1997 in favor of AM fungi, because all fungi are not produced vesicles [46]. Arbuscular mycorrhizal fungi belong to the division Zygomycetes and order Glomales. There are six genera of AM fungi have been identified and are *Glomus, Gigaspora, Aculospora, Scutellospora, Entrophosphora,* and *Sclerocystis. Acaulospora* and *Scutellospora* belong to Gigasporaceae; *Glomus* and *Sclerocystis* belong to Glomaceae [47]. Arbuscular mycorrhizal fungi (AMF), belonging to the phylum *Glomeromycota,* are obligate symbiotic fungi forming mutualistic associations with the roots of most of the tropical plants. Increased access to low-mobility soil mineral nutrients has been considered to be main beneficial effect of AMF on their host plants [48]. In addition, they have been shown to improve the uptake of Zn, Cu, S, Mg, Ca, K and other nutrients [49]. The AM fungal mycelia have been reported to stabilize soil through the formation of soil aggregated [50].

Arbuscular mycorrhizal (AM) fungi are the most widespread type and ecologically important root fungal that form symbiosis with 80% of land plant species which depend upon them for growth [51]. AM fungal symbiosis is characterized by fungal penetration of root cortical cells forming microscopic branched structures called arbuscules that increase that increase efficiency of plant-fungus metabolite exchange [48]. These microsymbionts occur widely under various environmental conditions with beneficial effects on soil structure improvement [52, 53] and have great importance due to their higher capacity to increase growth and yield through efficient nutrient uptake in infertile soils, water uptake and drought resistance in plants [54].

6.1 A combined effects AM fungi and *Pseudomonas* in tree species

The interaction between *Pseudomonas* and the arbuscular mycorrhizal fungus, *Glomus clarum* NT4 on spring wheat grown under gnotobiotic condition was investigated [55]. Although plant growth responses varied, positive response to Pseudomonad inoculants was obtained. Shoot biomass enhancement ranged from 16 to 48%, whereas enhancement ranged from 82 to 137% for roots. Typically, dual inoculation positively influenced the magnitude of response associated with any organism applied alone.

The highest mycorrhizal root colonization and number of AM fungal spores, and pseudomonas population were observed when *G. fasciculatum* and *P. monteilii* were coinoculated on to *Coleus forskohlii* plants [56] under organic field condition. Negative effects of *Glomus intraradices* on population of PGPR, *P. fluorescens* DF57 were shown by Ravnskov et al. [57] and suggested that competition for inorganic nutrients might explain the effect, since the mechanism did not require cell-to-cell contact. Marschner et al. [58, 59] suggested that similar negative effects of *Glomus intraradices* on *P. fluorescens* 2-79RL might be due to mycorrhizal induced decreases in root exudation, affecting the composition of the rhizosphere soil solution. *P. fluorescens* 92rk and P190r, and *G. mosseae* BEG12, inoculated alone, promoted tomato plant growth. Plant growth promotion by florescent pseudomonads has been ascribed to the suppression of phytopathogenic soil-borne microorganisms [43, 60]. Moreover, co-inoculation of three microorganisms showed synergistic effects compared with single inoculated plants and reports demonstrate additive effects on plants on plant growth of AMF and rhizobacteria [61, 62].

6.2 Effect of AM spores in rhizosphere of three species

The occurrence of AM spores depends upon the environmental conditions, plant species and soil type. There are two different types of AM spores such as *Acaulospora* and *Glomus* were observed in non-rhizosphere soil. Among the two different AM spore, Glomus was the dominant one. Spore density was very low 8 spore/100 g of soil [63, 64]. Analysis of root colonization was higher in mycorrhizal than non- mycorrhizal plants. Santhaguru et al. [65] reported that VAM infection was 100% in *Albizia amara, Peltophorum pterocarpum* and *Pongamia glabra, 80% in Derris scandens* 78% in *Erythrina variegata*, 18% in *Pterlobium* and16% in *Prosopis chilensis*. However, there is no VAM fungi infection in five plant species viz. *Albizia lebbeck, Bauhinia tomentosa, Cassia, Prosopis juliflora* and *Tamarindus indica* at Alagar Hills of Tamil Nadu, India. Similarly, AM Fungi colonized with several tree species semi-arid zone of South India, 1, 2 and 3 years old *Casuarina equisetifolia* [2], *Leucaena leucocephala* [66], *Feronia elephantum* with AM fungi (*Glomus fasciculatum*), *Samanea saman* [67]. Similarly, 16 different species of Arbuscular mycorrhizal fungi were isolated from rhizosphere of teak (*Tectona grandis*) among these *Glomus* and *Aculospora* found in dominant species and seedlings inoculated with combination of Arbuscular fungi had good quality seedlings and increased shoot height compared to with individual AM fungus in *Tectona grandis* [68].

6.3 Role of bio-inoculants on plant growth and metabolites

Leucaena leucocephala seedlings were inoculated with different types of vesicular-arbuscular mycorrhizal fungi found that the collar diameter increment of between 18 and 123% [66]. Similarly, *Pterocarpus indicus* inoculated with vesicular-arbuscular mycorrhizal fungi improve the shoot diameter [69], root collar diameter in sweet gum seedlings by 268% [70]. *Feronia elephantum* with AM fungi (*Glomus fasciculatum*) increase the plant growth especially root length and was recorded the root length increment was up to 84% [30]. Similarly shoot length was higher in *Samanea saman* [67] Mycorrhiza colonization also protect the roots from the soil pathogens [71]. AM fungi significantly increase the net photosynthesis by increasing total chlorophyll and carotenoid contents ultimately increasing carbohydrate accumulation. The chlorophyll content, fresh weight and leaf area are higher in mycorrhizal plants than in non-mycorrhizal plants but differences are significant only under draught stress conditions [72]. In mycorrhizal infected groundnut roots, high concentrations of ortho-hydroxy phenols were present. This type of phenols has been known to play an important role in plant disease resistance [73]. Inoculation of AM fungi is enhancing the plant quality by stimulating the synthesis of secondary metabolites which can be important for plant tolerance to abiotic and biotic stresses [74]. According to Morandi et al. [75] the Phenolic substances, such as phytotoxins are synthesized when the root is infected by a pathogen. They are non-specific toxic substances, which can be considered to play a role in disease resistance. Kapoor et al. [76] observed a significant increase in the density of glandular trichomes in the medicinal plant *Artemisia annua* following inoculation with the AM fungi *G. macrocarpum* and *G. fasciculatum* contributing to enhance artemisinin content in the plants.

The chlorophyll a, chlorophyll b, total chlorophyll and Carotenoid contents increased in mycorrhizal seedlings compared with non-mycorrhizal tree seedlings of *Cassia siamea*, *Delonix regia*, *Erythrina variegata*, *Samanea saman* and *Sterculia foetida* [77]. A significant enhancement in biochemical parameters like total chlorophyll content, soluble protein and NRase activity in *Pongamia pinnata* seedling 10.7, 48.5 and 43.6% increase over control with the combined inoculation of Rhizobium, Phosphobacteria and AM fungi [78]. Similarly, an increase in chlorophyll content and soluble protein was observed in *Ziziphus mauritiana* when inoculated with AM fungi [79] and *Dalbergia sissoo* inoculated with Rhizobium and mycorrhizae [80] and in Shola species inoculated with Azospirillum + Phosphobacteria and AM fungi [22]. Eucalyptus seedlings inoculated with mixed *Glomus mosseae, Trichoderma viride* and *Glomus fasciculatum* increases the phosphorous content of shoot and root over control. Then increased rate of P uptake and inflow in roots is regarded as the major contribution of AM infection [81]. The AM colonization increased initially up to 45 days but decreased thereafter [82].

6.4 Effect of AM fungi on growth and nutrient content

The fundamental importance of the mycorrhizal associations in restoration and to improve the revegetation is well recognized [83]. Arbuscular mycorrhiza colonized plants showed significant increment in height, biomass production and girth as compared to non mycorrhizal plats. Growth, biomass and P uptake were higher were higher on dual inoculation of *G. fasciculatum* and *G. macrocarpum* as compared to uninoculated tree species under both nursery and field condition. Tropical trees inoculated with AM fungi have shown increased nutrient uptake and growth, withstanding the transplant stock, hostile conditions like drought resistance and survival of *Acacia holosericea* [84]. *Casuarina equisetifolia* seedlings inoculated with AM (*Glomus fasciculatum*) increased shoot and root biomass [23, 24], *Eucalyptus tereticornis* [85] *Tectona grandis* [68] *Santalum album*, *Acacia auriculiformis*, *Grevillea robusta*, *Eucalyptus camaldulensis*, *Bombax ceiba* [86, 87] and *Albizia lebbeck* [88].

Inoculation with *Glomus mosseae* and *G. fasciculatum* along with other nitrogen fixing and phosphate solubilizing organism improved the quality and growth of neem seedlings, owing to greater absorption of nutrients, under nursery conditions in unsterilized soil [89], AM fungus (*G. fasciculatum*) and Rhizobium treated *Acacia nilotica* seedlings recorded an increase in shoot and root biomass [90]. Beneficial effects of AMF, such as growth promotion, increased root branching, lengths of lateral roots, specific root length and root diameter [91], protection against pathogens [92] and tolerance to abiotic stresses [93], could be due to positive interactions between mycorrhizae and associated microorganisms such as Pseudomonas, Arthrobacter and Burkholderia in a particular environment [94].

Combined inoculation of *Glomus fasciculatum* and Rhizobium on the growth of *Prosopis juliflora* seedlings showed better growth on shoot length and biomass. It was found that *G. fasciculatum,* Scutellospora sp., *G. leptotichum* and *G. mossease* were most efficient for *Dalbergia sissoo*, *Acacia auriculiformis*, *A. nilotica* and *Dalbergia latifolia,* respectively, and increase in plant biomass and height was to the extent of 34 and 24%, respectively, in *Dalbergia sissoo*, 126 and 50% in *A. auriculiformis*, 48 and 24% in *Dalbergia latifolia* and 100 and 112% in *Acacia nilotica* [95].

7. Trichoderma

The genus Trichoderma is the most common fungi found in all climatic condition. It can be isolated in all type of soil. It is also found in plant root, rotting wood, plant litter and seed. Fungi of the genus Trichoderma are important biocontrol agents (BCAs) of several soil borne phytopathogens. Trichoderma use different mechanisms for the control of phytopathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes. In addition, *Trichoderma* could have a stimulatory effect on plant growth 48 as a result of modification of soil conditions.

Shoot length and fresh weight were more in *Eucalyptus saligna* seedlings inoculated with *Trichoderma viride*. The greater height and fresh weight of *Acacia nilotica* inoculated with Trichoderma due to the Trichoderma species produce growth hormones which result in better growth of shoots. *Trichoderma* sp. co-inoculated with *Azotobacter* sp. and *Bacillus megaterium* showed a significant increase on the growth of Teak and Indian red wood under nursery condition [96]. The growth promoting substances are known to cause enhanced cell division and root development [97]. Similarly, many strains of Bacillus pseudomonas and Trichoderma have been implicated in improvement of overall growth of many crop plants [98].

8. Rhizobium

Rhizobium belongs to family Rhizobiaceae and the bacteria have the ability to reduce N_2 and thereby "fix" atmospheric nitrogen using the enzyme nitrogenase. It colonizes the roots of species legumes to form tumor like growths called root nodules, which act as biofactories of ammonia production (**Figure 1**). The process of biological nitrogen fixation was discovered the Dutch microbiologist Martinus Beijerinck. Rhizobia (e.g., Rhizobium, Mesorhizobium, Sinorhizobium) fix atmospheric nitrogen or dinitrogen, N_2 into inorganic nitrogen compounds such as ammonium, NH_4, Which is then incorporated into amino acids, which can be utilized by the plant. Plants cannot fix nitrogen on their own, but need it in one form or another to make amino acids and protein. Because legumes form nodules with rhizobia, they have high levels of nitrogen available to them. Rhizobium is a soil habitat bacterium, which is able to colonize the legume roots and fixes the atmospheric

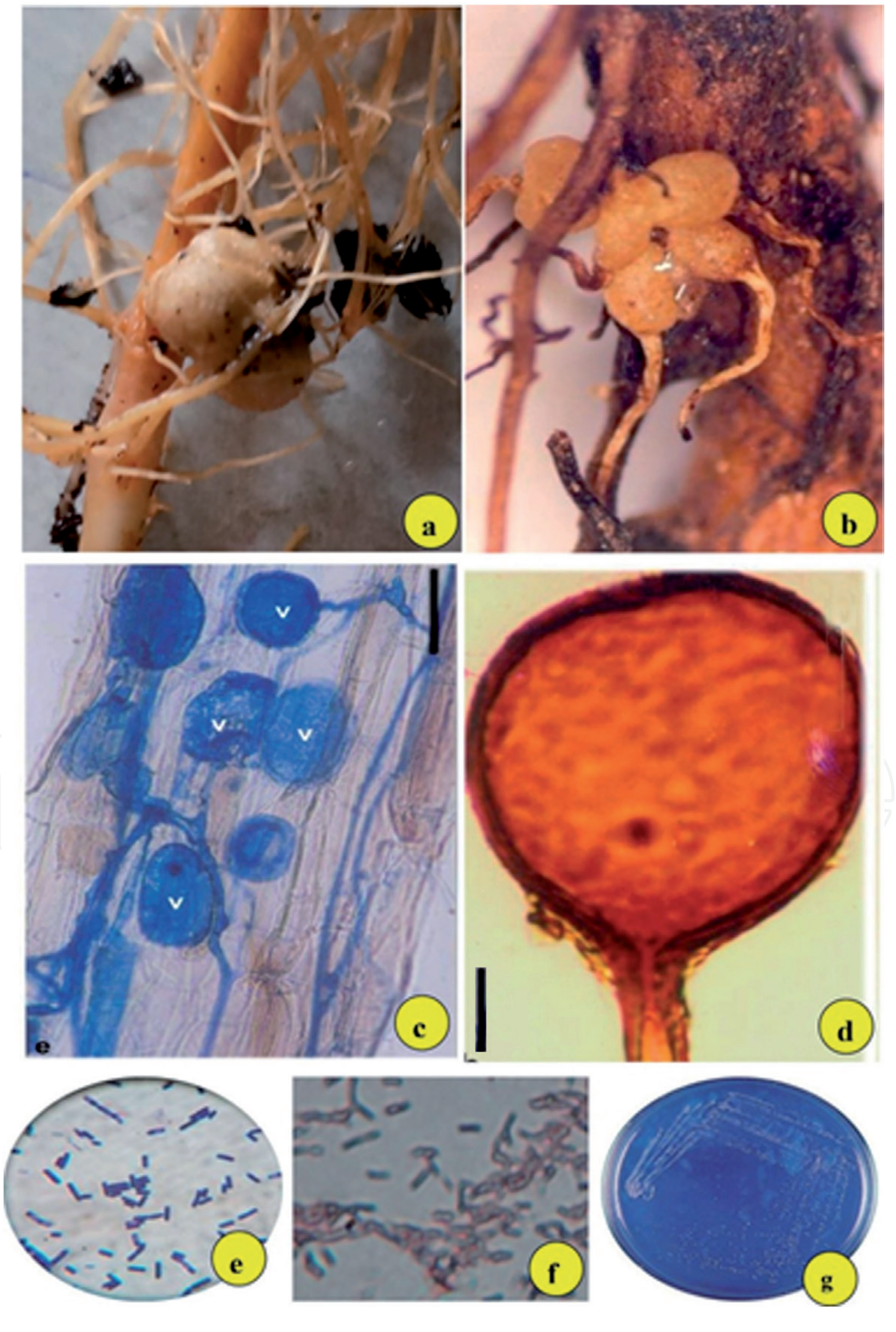

Figure 1.
(a) Rhizobium root nodule, (b) Frankia *root nodule, (c) AM fungi infection, (d) AM fungi spore, (e) phosphate solobilizing bacteria* Bacillus *sp., (f)* Paenibacillus polymyxa, *(g)* Azospirillum brasilense.

nitrogen. Rhizobium associated with nodulated legume trees have an outstanding potential for fixing atmospheric nitrogen (*Sesbania cannabina* and *Leucaena leucocephala*) can fix up to 75–584 kg N ha^{-1} yr^{-1} [99]. In recent year use of Rhizobium culture has been routinely recommended as an input in legume tree species cultivation. Rhizobium helps to boost up the tree growth by insoluble nutrients available for plant. Seedling treated with Rhizobium biofertilizer found to remarkable increase in growth and nodulation of *D. sissoo* [100], *A. nilotica* [26] *Albizzia* sp. [101].

8.1 Rhizobium with helper microbes

High nitrogen yield was estimated in the *Pongamia pinnata* seedling inoculated with Rhizobium + Phosphobacteria + VAM fungi [78]. Increased N content in the plant sample of various tree seedling, co-inoculated with different biofertilizers [102]. Similarly increase in biomass production due to VAM fungi inoculation with *Acacia* sp. [103] and in *Albizzia* sp. [104]. Rhizobium inoculation + PSB with 25% N significantly increase the average 53 nodule no./seedling was followed by only Rhizobium with 25% N (38 nodule/seedling) inoculation in *Acacia nilotica* shoot length increased from 58.50 to 78.75 cm, collar diameter from 5.05 to 6.15 mm and nodulation increased 0.071 to 0.342 g/seedling [105].

8.2 *Azospirillum* and *Rhizobium* interaction

Dual inoculation of *Azospirillum* and *Rhizobium* with legume plant has been found to increase plant-growth when compared with single inoculations. *Azospirillum* is considered a helper bacteria to *Rhizobium* by stimulating nodulation, nodule function, and possibly plant metabolism. Similarly, phytohormones produced by *Azospirillum* promoted epidermal-cell differentiation in root hairs that increased the number of potential sites for rhizobial infection and more nodule development [106]. Dual inoculation of AM fungi with *Rhizobium* improved nodulation, plant dry weight, N and P contents of *Leucaena leucocephala* in a P deficient soil compared to single inoculation with either organism [107] .

8.3 *Azotobacter* plant interaction

Azotobacter is a free living (non-symbiotic), aerobic, nitrogen fixing organism and these gram negative bacteria belongs to family Azotobacteriaceae. There are seven species of *Azotobacter* viz. *A. beijerinckii, A. chroococcum, A. vinelandii, A. paspali, A. agilis, A. insignis* and *A. macrocytogenes. A. chroococcum* appeared more in acidic soils and arable soils while *A. beijerinckii* in neutral and alkali soils. Apart from nitrogen, this organism is capable of producing antibacterial and antifungal compounds, hormones and siderophore [108]. Individual or combined inoculations stimulated the plant growth and significantly increased the concentrations of indole 3-acetic acid (IAA), P, Mg, N, and total soluble sugars in agri crop. Bioinoculants co-inoculation of nitrogen fixing organism *Azotobacter* and phosphate solubilizing microorganisms *Bacillus megaterium* showed a significant increase on the growth of teak and India red wood under nursery condition [96].

Azotobacter inoculated strawberry plants attained maximum height (24.92 cm) more number of leaves per plant (26.29), more leaf area (96.12 cm^2), number of runners per plant (18.70), heavier fruit (10.02gm), more fruit length (35.9 mm), and more fruit breadth (22.91 mm) as compared to all other treatment [109]. Similarly, combined application of manure + Azotobacter + wood ash + phosphorous solubilizing bacteria + oil cake improved significantly fruit diameter (3.11 cm), length (3.95 cm), volume (20.397 cm^3), weight (11.11 g), total sugars (7.95%), total soluble solids (9.01'B), acidity (0.857), TSS:acidity ratio (11:12) and yield (238.95 g/plant) [110].

Name of the species	Azospirillum/Azotobater			Frankia/Rhizobium			Phosphate solubilizing bacteria			AM fungi			Combination of bio-fertilizers			Reference
	CD	SL	BM	CD	SL	BM	CD	SL	BM	CD	SL	BM	CD	SL	BM	
Casuarina equisetifolia L.	14.2	55	19.6	17.6	23	23	6.8	406	11	18	19.5	23	63.5	62.2	115.0	[22]
Acacia nilotica L.	61.0	60	26	125	57	56	NA	NA	NA	96	60	48	236.3	131.2	156.8	[26]
Azadirachta indica (A) Juss	1.1	2.34	3.2	NA	NA	NA	NA	5.5	3.74	0.7	7.08	5.4	0.67	16.0	7.49	[27]
Moringa oleifera L.	75	22	276	NA	NA	NA	5.04	6	17	NA	NA	NA	5.6	11.7	176.5	[25]
Mangifera indica (L.) Delile	12.0/13.3	12.7/ 13.7	—	NA	NA	NA	NA	NA	NA	14.73	14.5	NA	11.8	12.9	NA	[117]
Delonix regia (Hook.) Raf.	5.8/4.2	5.2/ 4.3	17.8/5.8	NA	NA	NA	NA	NA	NA	3.5	2.42	0.9	13.0	7.9	21.2	[28]
Tectona grandis L.f.	––/69.8	––/ 0.65	––/28.2	NA	NA	NA	31.7	0.65	106.8	6.3	7.2	37.4	114.3	26.8	258.9	[116]
Samanea saman (Jacq.) Merr.	60.0	20.6	35.8	NA	NA	NA	54.4	6.68	19.5	78.9	16.9	30.9	108.6	46.9	74.9	[67]
Feronia elephantum L.	71.4	39.5	55.5	NA	NA	NA	48.8	6.68	15.9	82.9	20.7	41.7	122.8	47.0	92.4	[30]
Gmelina arborea (Roxb.)	11.6	13.9	38.8	NA	NA	NA	11.9	8.8	27.5	11.9	9.43	63.4	25.6	21.9	166.4	[118]

CD, collar diameter; SL, shoot length; BM, biomass; NA, not applicable.

Table 1.
Growth and biomass increases (percentage increased over control) of horticulture and forestry crops treated with bio-inoculants.

9. Frankia with actinorrhizal plants

Frankia is a genus of Actinomycetes, belongs to family Frankiaceae and an ability to fix the atmospheric nitrogen in symbiotic association with *Casuarina* species in tropical and temperate environmental condition. These microorganisms usually invade root hairs of Casuarina and developing within cortical cells in lobes of the resultant nodules. Frankia are able to convert the nitrogen gas in the atmosphere into amino acids, which are the building blocks of proteins. Frankia exchange nitrogen for carbohydrates from the plant. As the plant drop organic matter, or when the plants die, the nitrogen from their tissues is made available to other plants and organisms. This process of accumulating atmospheric nitrogen in plants and recycling it through organic matter is the major source of nitrogen in tropical ecosystems. Various agro-forestry practices such as alley cropping, improved fallow, and green manure/cover cropping exploit this natural fertility process by using nitrogen fixing plants.

Casuarina equisetifolia seedling inoculated with Frankia strains showed improved growth, biomass and tissue N content over control seedlings [24, 111, 112]. Nitrogenase activity of Frankia strains were significantly ($p < 0.05$) and negatively correlated with a tissue N content [111]. Similarly, under nursery experiments, the growth and biomass of *C. equisetifolia* rooted stem cuttings inoculated with Frankia showed three times higher growth and biomass than uninoculated control and improved growth in height (8.8 m), stem girth (9.6 cm) and tissue nitrogen content (3.3 mg/g) than uninoculated controls in field condition [112]. Frankia inoculated Casuarina seedlings planted in farm forestry improve the tree growth and biomass in the field condition [2, 112] and improve the nutrient cycling of actinorrhizal plants through high amount of litter production and decomposition [113]. Combined inoculation of Azospirillum, Phosphobacteria, AM fungi and *Frankia* produced excellent growth and biomass of *C. equisetifolia* seedlings due to co-inoculation with *Frankia* through improved nitrogen fixation [22, 114] (**Table 1**).

10. Methods of inoculation

10.1 Inoculation methods of *Azospirillum, Rhizobium* and phosphobacteria

Seed or nursery stage is best for application of bio-fertilizers. Suitable methods for forestry species is seed coating and inoculation in polythene bag. Two grams of carrier culture (10^{-8} cfu/g) can be applied in rhizosphere of seedlings in the polythene bags in the nursery.

10.2 Inoculation with seeds

Inoculation requirement varies from the size of the seeds. Normally 200 g of lignite/peat soil based culture (10^8 cfu/g) is need for every 8–10 kg of seeds of the tree species. A slurry is formed by mixing the inoculant with cooled rice gruel (250 ml). The required quantity of seeds is added in the slurry and mixed thoroughly so that each seed is coated with the black colored inoculant. The treated seeds are then shade dried for 30 min and sown.

10.3 Inoculation in the nursery mother bed

Two hundred grams of lignite based carrier culture of *Rhizobium* or *Azospirillum* (10^8 cfu/g) is required for 4 m × 1 m mother bed. It has to be spread uniformly and mixed thoroughly before sowing of seeds.

10.4 Seed treatment

Ten percent sugar or gum arabic solution or rice porridge is to be prepared to serve as a sticker for culture cells applied to seeds. This solution is to be sprinkled on required seeds and then the seeds spread on a polythene sheet and mixed uniformly. The peat based culture is sprinkled uniformly over the sticker-coated seeds and mixed simultaneously. After treatment the seeds are air dried in 1 h then the seed can be dipped in nursery mother bed.

10.5 Dipping seedlings

In case of transplanted seedlings, the seedlings from the nursery beds are uprooted and tipped in a suspension of biofertilizers before planting.

10.6 Inoculation in the nursery seedlings

Two gram of lignite based culture (10^8 cfu/g) is added to rhizosphere of the seedlings a week after transplanting. In the case of AM 5 g of vermiculate based culture can be used. The cultures may be mixed together and applied near the root zone. If necessary, the inoculant may be made bulk by mixing with the finely powered farm yard manure or sand for easy application.

10.7 Inoculation of out plantings

Ten grams of lignite based culture (10^8 cfu/g) is required per seedling which are to be planted in the field directly from the mother bed, in the form of naked root seedlings. Otherwise, 200 g of lignite based culture can be mixed with 10 l of water, and roots of seedlings can be dipped in it before planting.

11. Advantages of biofertilizers

- Biofertilizers have number of advantages than synthetic fertilizers. Bio fertilizers can facilitate not only supply of nutrients, but also produces vitamins and plant growth hormones. They prevent soil erosion by producing capsular polysaccharides and also control plant pathogens.

- Biofertilizers, will be isolated from the rhizosphere soil of host plant hence huge amount need not be spent for mother culture. It can be cultivated under normal laboratory condition using conventional media and fermentors within short span of time. Production method is very simple and production cost is cheaper than chemical fertilizers.

- Chemical fertilizers are required in huge quantity for land application. The physical optimum levels for getting the maximum grain yield for the medium duration rice hybrid $CORH_2$ was found to be 151:66:57 kg N, P_2O_5 and K_2O ha^{-1} [115]. But in case of biofertilizers, 1 g of carrier based inoculum of Azospirillum and phosphobacterium contain with a population load of 10^{-9} and 10^{-8} and approximately 12,500 infective propagule/10 g of soil [22]. Hence, very less quantity is sufficient and it may get multiplied into many fold as the optimum environmental conditions in the nursery and field. As the propagules multiply in the field they need not be applied repeatedly.

- It helps to improve the seed germination and induces the healthy seed emergence due to production of growth promoting hormones, gibberellins and cytokinin-like biologically active substances. Biofertilizers promote better root formation in trees for efficient absorption and assimilation of water and nutrients.

- Biofertilizers are involved in the litter decomposition and the breakdown of minerals into available form to plants. It directly facilitates the function of rhizoids in terms of absorption and translocation of minerals and water.

- Biofertilizers do not pollute the soil, whereas excess application of chemical fertilizers creates soil pollution. Biofertilizers are effective in promoting and maintaining the soil fertility which helps a better balance in the plantation forest ecosystem in terms of nutrient availability and cycling of nutrients.

- Due to the strong colonization of biocontrol microorganism and their secretory substances, the tree plants cultivated under this pattern will exhibit a strong resistance against an array of infectious disease caused by plant pathogens.

11.1 Limitations of bio-fertilizer utilization in forestry and horticulture

Apart from the advantages, biofertilizers have certain limitations. Lack of awareness on benefits of bio inoculants among the farmers and tree growers. Adequate availability and quality assurance of bioinoculants are being the limiting factors. Competition between native and introduced microbial population in the cultivated field also identified as a limiting factor. Hence, a preliminary analysis on the cultivable land about the native microflora, physico-chemical parameters is essential to overcome such limitations.

12. Conclusion

Bio-inoculants are renewable, cost effective, eco-friendly and economically viable population of beneficial microorganisms providing an alternate source of plant nutrients, thus increasing farm income by providing extra yields and reducing input cost. Bio-inoculants increase crop yield by 20–30%, replace synthetic fertilizers of N & P by 25%. Stimulate plant growth, activate soil biologically, restore natural fertility and provide protection against drought and some soil borne plant pathogens. Application of Bio-fertilizers in combined form in Horticulture and Forestry will play an important role in improving the soil fertility by supply of macro and micronutrients, organic carbon, accumulation of soil enzymes, suppression of plant pathogen by bioactive substances. This will have direct impact on socio-economy of tree growing farmers, maintain sustainability in natural soil ecosystem, wood and food crops availability in future. Therefore, the development of more efficient and sustainable agriculture strategies, guarantied food supply for an expanding world population and minimizing damage to the environment is one of the greatest challenges for humankind today. It is inferred that under appropriate management, the use of more efficient bioinoculants, co-inoculation with other bioinoculants lead to an increased growth and biomass of tree species in nutrient impoverished soil.

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