

**EFFECT OF BENEFICIAL MICROORGANISMS ON QUALITY SEEDLING
PRODUCTION OF *AEGLE MARMELLOS* UNDER NURSERY CONDITIONS**

E. Mohan and K. Rajendran

Centre for Research and P.G. Department of Botany,
Thiagarajar College, Madurai-9, Tamil Nadu, India
Email: easmohan@yahoo.co.in

Abstract

Aegle marmelos L. is a native species of Indian subcontinent, which belongs to the family Rutaceae.. The fruits of the plant are edible and considered to be traditional medicine in southern part of India. To improve the seedling quality, nursery experiments were conducted to select the suitable bio-inoculants for *Aegle marmelos* in tropical nursery conditions. The bioinoculants such as *Azospirillum* (*Azospirillum brasilense*), Arbuscular mycorrhizal (AM) fungi (*Glomus fasciculatum* syn *Rhizophagus fasciculatus*) and *Pseudomonas* (*Pseudomonas fluorescens*) were isolated, mass multiplied and inoculated individually and in combinations. Seedlings were grown for 180 days under nursery conditions. Shoot length, root length, collar diameter and biomass were recorded at 180 days after inoculation. The experimental result showed that the shoot length, root length, collar diameter and biomass were increased above 77.47 % in combined inoculation of *Azospirillum* + AM fungi + *Pseudomonas* when compare to control. The combined inoculation of bioinoculants is beneficial for increasing growth, biomass and good quality seedling production.

Key words: Wood Apple, Bioinoculants, Seedling quality, Nursery condition.

INTRODUCTION

Aegle marmelos L. belongs to the family Rutaceae is native to the Indian sub-continent. It is widely distributed plant throughout India and occupies an important role in folk medicine. The fruit pulp of the plant is used in traditional medicine to cure ailments such as diarrhea, purities', impotence, dysentery, heart disease, vomiting, and anorexia, and has also been used for the treatment of asthma and tumors, as a liver tonic and peptic ulcer (Anurag Mishra et al., 2009).

Due to these health benefits, non-government organizations and medicinal plant cultivators have planting *A. marmelos* in all cropping and as avenue trees in the public land in urban area and highways. Since the trees are planted on barren land, fertilizer application is essential for nutrient management of wood apple. Long term application of synthetic chemical fertilizers will damage the physico-chemical and biological properties of the soil. At present the pinch of fertilizer consumption are being felt more in India, since the country cannot afford to either import the required fertilizer of high cost and subsidize the sale to the farmers or build new fertilizer plants at formidable cost. Hence, farmers and medicinal plant cultivators are prepared to take to an organic farming by using bio-inoculants. Bio-inoculants are cost effective and eco-friendly in providing an 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 fertilizers by 25%. They stimulate

plant growth, activate soil biologically, restore natural fertility and provide protection against drought and some soil borne diseases (Zimmer, 1988).

Growth promoting rhizobacteria are classified as biofertilizers, plant stimulators or biological control agents, according to the degree to which they can fix nitrogen, directly promote growth or protect plants against plant pathogens (Bloemberg and Lugtenberg, 2001). Several methods have been suggested to explain the phenomenon of plant growth-promotion when agronomic crops are inoculated with rhizobacteria. These include increases in the nitrogen fixation, the production of auxin, gibberellin, cytokinin, ethylene, the solubilization of phosphorus and oxidation of sulfur, increases in nitrate availability, the extra-cellular production of antibiotics, lytic enzymes, hydrocyanic acid, and increases in root permeability, strict competition for the available nutrients and root sites, as well as the induction of plant systemic resistance (Chanway, 1997; Kloepper, 1993).

Nitrogen fixing bacteria of genus *Azospirillum* is an important non-symbiotic associative microorganism that fixes atmospheric nitrogen in the soil (Krishnamoorthy, 2002) and augments nitrogen fixation (Vijayakumari and Janardhanan, 2003). *Azospirillum* promotes seedling growth, biomass and nutrient uptake (Sekar, 1995; Rajendran et al., 2003; Kasthuri Rengamani et al., 2006). It also increases root biomass, root surface area, root diameter, density and length of root hairs (Okon and Kapulnik, 1986). In agriculture, advantages of bioinoculants application are better known, but in tree species the utility of biofertilizer is still in experimental stage (Kabi et al., 1982; Basu and Kabi, 1987). Many root colonizing bacteria including the nitrogen fixing *Azospirillum* and phosphorus solubilizing *Pseudomonas fluorescens* are known to produce growth hormones which often lead to the increased root and shoot growth (Govindarajan and Thangaraju, 2001; Wong and Sternberg, 1979).

Soil used for the production of planting stock in nurseries of semiarid zone of Tamil Nadu is very poor in nutrient content and beneficial microbial population (Mohan *et al.*, 2012). Even though the soil is mixed with farm yard manure (FYM) the quality of seedlings is very poor due to insufficient of effective microbes, the rate of mineralization and nitrogen fixation is very low. This problem can be overcome by providing suitable bioinoculants to improve the growth and nutrient uptake of *Aegle marmelos* seedlings. Hence, the present study was undertaken to find out the suitable bio-inoculants and its combinations for the quality seedling production.

MATERIALS AND METHODS

Seeds

Aegle marmelos fruits were collected from a single plus tree, located at the semi-arid region of Alagar hills in Madurai district of Tamil Nadu located at 10° 05' N latitude and 78° 16' E longitude. Seeds were separated by removing the pulp and seeds were dried in shade, dried seeds were graded and uniform size was used for raising seedlings. Seedlings were raised in a mixture of unsterilized sand in germination tray at room temperature (32°C).

Isolation of *Pseudomonas*:

Pseudomonas fluorescens was isolated by the serial dilutions and pour plate method using King's B medium (King et al., 1954). One mL of soil (obtained from Thiagarajar College campus) suspension from aliquot dilutions (10^5 to 10^8) was aseptically added. Twenty ml of sterilized, melted and cooled medium was added and poured in each petri-plate and incubated at $28 \pm 2^\circ\text{C}$ for 24 h. Well separated individual colonies with yellow-green and

blue white pigments were marked and detected by viewing under UV light. The individual colonies were picked up with sterilized loop and transferred on to fresh King's B medium. The plates were incubated at $28\pm 2^{\circ}\text{C}$ for 24 h. The single colonies developed were transferred to King's B medium slants and the pure cultures so obtained were stored in refrigerator at 4°C and mass multiplied for further use.

Isolation of *Azospirillum*:

Nitrogen-free semisolid Malate medium (NFB) was used to isolate *Azospirillum* (Dobereiner et al., 1976). *Casuarina* roots were washed in sterile distilled water and in 25 mM phosphate buffer, pH 6.8, followed by three more washings in sterile distilled water (Baldani and Dobereiner, 1980). The root samples were cut into pieces (5 to 8mm long) and placed in 10 ml serum vials containing 5 ml of NFB medium. Other vials containing NFB medium were inoculated with rhizosphere soil of *Casuarina* plantation. The cultures were incubated at 32°C for 24-72 h. White; dense, undulating pellicle formed just 1-3 mm below the surface of the medium was streaked on to Congo red plates and incubated at 32°C for 72 h. After the incubation period, small scarlet colonies were observed, indicating the presence of *Azospirillum* sp. The isolated *Azospirillum* colonies were mass multiplied in nutrient broth (Rodriguez Caceres, 1982).

Isolation of AM fungi

Arbuscular Mycorrhizae fungus (*G. fasciculatum*) was isolated and recorded as more number in the rhizosphere soil of *A. marmelos*. It was multiplied in pot culture in the sterilized mixture of sand: soil (1: 1 v/v) and maintained in green house in the roots of *Sorghum vulgare* as the host plant. The inoculum contained extrametrical hyphae, spores and mycorrhizal roots bits were added in the root zones of each seedling.

Treatment

Fifteen days after germination the seedlings were transplanted in 13×26 cm. size polyethylene bags with a potting mixture of unsterilized sand: red soil: farm yard manure (2: 1: 1). Ten days after transplantation in polyethylene bags 10 grams of peat soil based culture of *Azospirillum*, *Pseudomonas* and *Glomus* were inoculated by making holes in the rhizosphere of seedlings.

T₁- *Azospirillum* (*Azospirillum brasilense*)

T₂-ArbuscularMycorrhizal Fungi (AMF) (*Glomus fasciculatum*)

T₃-*Pseudomonas* (*Pseudomonas fluorescens*)

T₄- *Azospirillum* + AMF

T₅- *Azospirillum* + *Pseudomonas*

T₆- *Pseudomonas* + AMF

T₇- *Azospirillum*+ *Pseudomonas* + AMF

T₈- Control (Sand: Red soil: Farm Yard Manure 2:1:1)

Experimental design

Nursery experiments were conducted at the Tamilnadu Forest Department nursery located at Madurai, Tamil Nadu at $10^{\circ} 05'$ N latitude and $78^{\circ} 16'$ E longitude. The experiment was set up in a completely randomized block design (RCBD) with 8 treatments and twenty four replicates. All the plants were kept under nursery condition up to 180 days.

$$\text{Seedling survival percentage} = \frac{\text{Number of seedlings present in each treatment}}{\text{Total number of seedlings transplanted in each treatment}} \times 100$$

Harvesting and measurement

180 days after transplanting from each treatment, a total of 12 seedlings were randomly selected height and basal diameter were recorded. Seedlings were carefully uprooted without disturbing the root system and washed in running tap water. Excess of water was wiped out by placing them between folds of blotting paper. The seedlings were cut at collar region, dried separately at 70° C in a paper bags placed in hot air oven for 48 hrs and estimation of biomass (root and shoot dry weight) was carried out using top pan electronic balance.

Quantitative estimation of the microorganisms

Dilution plate counting method was employed for the enumeration of microbial population in the soil samples. An appropriate dilution with King’s B medium for *Pseudomonas* (King et al., 1954) N-free semi solid malate medium for *Azospirillum* (Dobereiner et al., 1976) were used for respective organisms. An aliquot of 1 ml of the respective dilution was spread in sterile petriplates of 90-mm diameter and dispensed with respective media. Plates were rotated gently thrice in clockwise and anticlockwise direction to ensure uniform distribution of the soil suspension. The plates were incubated at 28 °C. The colonies were counted on third day for *Pseudomonas* and *Azospirillum* using colony counter and expressed the population (cfu/g of soil).

Assessment of Mycorrhizal colonization

Mycorrhizal root infection was assessed following the procedure of Phillips and Hayman (1970). The root segments were placed in a 2.5 % aqueous solution of 10% KOH (w/v) and boiled in a water bath at 90 ° C for 2hrs. The roots were rinsed with repeated washing with water and lightened in H₂O₂ (3 ml of 20 % NH₄OH in 30 ml H₂O₂) for 10-45 minutes. They were again thoroughly rinsed with water several times and acidified by soaking in 40- 50 ml of 1 % HCl for 3 min. Acidified roots were stained in an acidic glycerol solution (500 ml glycerol, 450 ml H₂O, 50 ml 1 % HCl) containing 0.05% trypan blue for one day. The trypan blue solution was poured off and the roots were de-stained in acidic glycerol at room temperature.

$$\text{Percentage of Root colonization} = \frac{\text{Number of root bits showing AM infection}}{\text{Total number of root bits examined}} \times 100$$

Seedlings Quality Index

Seedlings Quality Index was calculated using the formula of Dickson (1960).

$$\text{Seedlings Quality Index (SQI)} = \frac{\text{Total weight (g/ plant)}}{\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Shoot weight (g/plant)}}{\text{Root weight (g/plant)}}}$$

Nutrient Analysis

The oven-dried plant samples were ground to pass through a 0.5 millimeter plastic sieve before digestion and samples were taken for the bio-chemical analysis.

Nitrogen and Phosphorous

The dried plant material was ground in a mortar and pestle and the total nitrogen content was estimated by the conventional micro-Kjeldahl method. Total phosphorus was estimated by the method of Fiske-Subbaro as modified by Bartlett (1925).

Estimation of total potassium, calcium and magnesium

One gram of plant sample was digested with tri-acid mixture with HNO_3 : H_2SO_4 : HClO_4 in the ratio of 9:2:1 until it became colorless. After digestion it was filtered and the volume was made up to 100 ml. Potassium in the extract was determined using a flame photometer. Calcium and Magnesium were determined by the Versenate method as described by Jackson (1973).

Statistical analysis

Statistical tests were performed with SPSS for window version 11.5. The data obtained from the various biometrical observations were subjected to the statistical analysis by analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test ($P < 0.05$) (Duncan, 1955).

3. RESULTS AND DISCUSSION

Microbial population of potting media

Analysis of nursery soil used for growing *A.marmelos* seedlings shows that the population of *Pseudomonas* and *Azospirillum* reported as 1.3×10^8 and 1.2×10^6 cfu/g of soil respectively, assessment of AM spore density was recorded as 8 spores and arbuscular mycorrhizal propagules /100g soil in addition to.

Growth enhancement

Shoot length

Significant increase in shoot length was recorded in *A. marmelos* seedlings inoculated with different biofertilizers compared to un-inoculated control at 180 days after inoculation (Table 1). Analysis of growth data revealed that the combined inoculation of *Azospirillum* +AM fungi+ *Pseudomonas* (T₇) was found to be the most effective combination in increasing the growth of seedlings at all stages. Among the individual inoculation, *Azospirillum* (T₁) showed higher shoot length and statistically on par with AM fungi (T₂) inoculated seedlings. Within double inoculations, AM fungi + *Azospirillum* (T₄) is 18.18% – 40.90% superior when compared with other dual inoculations. **Basal diameter**

The result revealed that the difference between, treatments were found to be highly significant (Table 1). Combined inoculation of *Azospirillum* + AM fungi+ *Pseudomonas* (T₇) showed significantly superior growth than other treatments on 180 days after inoculation. Thus registered 108.57% increase over control respectively. Among double inoculation, *Azospirillum*+ AM fungi (T₄) registered higher basal diameter but it was on par with AM fungi+ *Pseudomonas* (T₅).

Root length

Significant increase in total root length was recorded in *Aegle marmelos* seedlings inoculated with different biofertilizers compared to un-inoculated control (Table 1). Analysis of

growth data revealed that the combined inoculation of *Azospirillum* + AM fungi+ *Pseudomonas* (T₇) was found to be the most effective combination in increasing the growth of seedlings at all stages. Among the individual inoculation, *Azospirillum* (T₁) showed higher shoot length and statistically on par with AM (T₂) inoculated seedlings. Within double inoculations, AM fungi + *Azospirillum* (T₄) is superior when compared with other dual inoculations. It was recorded that 56.81% and 22.72% increase over control in single and dual inoculations (Table.1).

Shoot biomass

The data pertaining that the shoot biomass increase in the treated seedlings, presented in Table - 1. The result indicated that significant responses were observed among the treatments evaluated at 180 days after biofertilizer inoculation. The largest significant biomass in the shoot was recorded in seedlings inoculated with *Azospirillum* + AM + *Pseudomonas* (T₇). It was recorded 77.47 % increase over control. It was followed by inoculation with *Azospirillum* + AM fungi (T₄). Among single and double inoculations, *Azospirillum* (T₁) and *Azospirillum* + AM fungi (T₄) were the most effective in producing shoot biomass. It was recorded 62.58 % and 29.46% increase over control.

Root Biomass

The data of root biomass showed significant difference between the treatments. Inoculation of *Azospirillum* (T₁) alone and in combination with other biofertilizers was found to increase significantly root biomass when compared to other treatments. Root biomass was the highest in *Azospirillum* +AM fungi+ *Pseudomonas* (T₇) followed by *Azospirillum* + AM fungi (T₄). It was recorded 100.18% increase over control. It was also statistically on par with *Azospirillum* + AM fungi (T₄) and *Azospirillum* + *Pseudomonas* (T₅). Among single and double inoculations (T₁) *Azospirillum* treated seedlings and combination with *Azospirillum* + AM fungi showed better root biomass than other treatments. . It was recorded 69.21 % and 52.37% increase over control (Table 1).

Total biomass

Seedling biomass maximum in the treatment comprising of *Azospirillum* + AM fungi + *Pseudomonas* (T₇) and it was 90.95% more than that of control. Seedlings inoculated with *Azospirillum* + AM Fungi(T₄) recorded 64.90% highest biomass followed by *Azospirillum* alone (T₁) 75.25% more than the control (Table 1).

Seedling quality index

Maximum good quality seedlings were obtained in treatment (T₇) *Azospirillum* + *Pseudomonas* + AM Fungi recorded highest seedling quality index. Among the single inoculation, *Azospirillum* (T₁) and AM Fungi (T₂), double inoculation,AM Fungi +*Azospirillum* (T₄) had higher seedling quality index than other treatments (Fig. 1).

Nutrient content

Nitrogen

Total Nitrogen content of *A. marmelos* seedlings inoculated with biofertilizers had significantly increased over control (Table 1). The highest Nitrogen content was observed in

seedlings inoculated with *Azospirillum* + AM + *Pseudomonas* (T₇) followed by double inoculation of *Azospirillum* + AM (T₄). The above treatment accounted 319%, 251% and 203% more than that of control respectively. Statistically there is no significant difference between *Pseudomonas*+ AM + (T₆), *Azospirillum* + *Pseudomonas* (T₅). Among single and double inoculations, *Azospirillum* (T₁) and AM + *Azospirillum* (T₄) recorded higher nitrogen content when compared to other treatments.

Phosphorus

The phosphorus content was the highest in the seedlings treated with *Azospirillum*+ AM + *Pseudomonas* (T₇) followed by *Azospirillum*+ AM (T₄) and AM + *Azospirillum* (T₅) registering and 280% more than that of control plants respectively. Among single inoculations, *Azospirillum* (T₁) had more Phosphorous content than the rest (Table 3).

Potassium, Calcium and Magnesium

K, Ca, and Mg content in the seedlings showed the highest amount in combination of *Azospirillum* + *Pseudomonas* + AM (T₇) followed by *Azospirillum* + AM (T₄). Among single inoculations, plants inoculated with *Azospirillum* (T₁) inoculated seedlings contained more K, Ca and Mg than other single treatments (Table 3).

Discussion

Microorganisms are commonly found organized in micro-colonies in the rhizosphere soil. At the same time these micro-colonies benefit from the substances secreted by plant root systems they can also directly or indirectly stimulate plant growth. In the present study, the shoot and root height, collar diameter, dry matter and quality seedlings were higher in the *Aegle* seedlings inoculated with bioinoculants. The increase of growth may be attributed to high accumulation of chlorophyll and protein in the plant tissue by the application of nitrogen fixing bacteria of the genus *Azospirillum*. It increase in germination rate, plant height, leaf size, enhanced minerals and water uptake, increased dry matter accumulation, root surface area, root diameter density and root hair (Tien et al., 1979). To support the earlier reports, the present study, *Azospirillum* inoculated seedlings showed better growth and root biomass when compared to the control. Growth may be attributed due to increased root biomass and accumulation of nitrogen (Wong and Sternberg, 1979) and the production of gibberellins and cytokinin like substances (Tien et al., 1979) which promote the growth of the seedlings. The above results corroborate with earlier studies conducted on tree species of *Casuarina equisetifolia* (Rajendran et al., 2003), *Moringa oleifera* (Kasthuri Rengamani et al., 2006), *Acacia nilotica* (Rajendran and Jayashree, 2007), *Delonix regia* (Meenakshisundaram et al., 2011), *Erythrina indica* (Rajendran, 2012), *Azadiracta indica* (Alagesaboopathi and Rajendran, 2009) and *Tectona grandis* (Subramanian et al., 2000; Rajan et al., 2000).

Phosphate plays a major role in the root development (Kucy, 1987). Stribley (1987) reported that P seems to be the most important nutrient involved for the plant growth and other nutrients such as N, K, Ca, and Mg are translocated along with AM hyphae. Inoculation with AM fungi is known to enhance plant growth by improving the mineral nutrient of the host plant (Abbott and Robson, 1982).

In the present study AM fungi inoculation enhanced the plant growth and biomass of *A. marmelos* seedlings this can be attributed to the increased root absorbing surface area due to extensive external network of mycelium produced by the AM fungi in association with the host root system. In the present study, triple inoculation with AM fungi + *Azospirillum*

+*Pseudomonas* have promote the growth and biomass of *A. marmelos* seedlings and it was also corroborate with earlier report of Priya Rani et al. (1991).

Variability in plant growth promotion and disease suppression by strains of *Pseudomonas* spp. has been reported in field trials conducted with sugar beet (Suslow and Schroth, 1982), wheat (Weller and Cook, 1983) and potato (Bakker et al., 1986; Burr et al., 1975; Schippers et al., 1987). Similarly, in the present study *Pseudomonas* inoculated seedlings produced better plant height, stem girth and total biomass. It may be due to inoculation of phosphate solubilizing microorganism *Pseudomonas* which has shown stable and consistent capacity to solubilize insoluble phosphorus and thus making it available to plants and suppression of pathogenic microorganism. The combined treatment *Pseudomonas* + AM fungi + *Azospirillum* (T₇) were found exert the maximum on various growth parameters of *A. marmelos* recorded. Similarly, *P. fluorescens* inoculation significantly increased plant growth, dry matter production and yield of tomato crop (Yan et al., 2003) and inoculation with *Pseudomonas fluorescens* + *Azotobacter chroococcum* + *Azospirillum brasilense* in combination recorded higher plant growth and yield as well as control of the nematode in tomato crop (Yan et al., 2003).

In the present study dual inoculation of AM Fungi with *Pseudomonas* influenced the growth and biomass of *A. marmelos* seedlings. It is relevant to mention here that *Pseudomonas* by virtue of its capacity to produce certain growth promoting substances like IAA and GA might induce the growth of *A. marmelos* seedlings (Siddiqui, 2003; Ramamoorthy, 1982). Among all the treatments are combined inoculations of *Azospirillum* + *Pseudomonas* +AMF produced excellent growth, biomass and tissue nutrient concentration. The greater height, diameter and dry matter of the *A. marmelos* seedlings due to co-inoculation of all the biofertilizers might strongly improve accumulation of nitrogen due to *Azospirillum* (Gaur, 1990), more phosphorus uptake by *Pseudomonas* (Gunjal and Patil, 1992) and AM fungi (Young et al., 1988).

The total chlorophyll and soluble protein content was found maximum in the seedlings inoculated with *Azospirillum*. This increase is in agreement with other findings (Singh et al., 1983) and was attributed to the greater supply of nitrogen to growing tissues (Arther and Kawlis, 1994). Similarly increase in chlorophyll and soluble protein content was also recorded in shola species (Sekar et al., 1995) with inoculation of *Azospirillum* + Phosphobacterium.

4. CONCLUSION

Long-term sustainability in agriculture and forestry is possible only through the use of low cost farm grown inputs with minimum use of synthetic fertilizers, which work in harmony with nature. This study is a holistic approach of sustainable utilization of bio-resources and management with locally available resources in cultivation of *A. marmelos*. Bioinoculants act as perpetually renewable inputs helping in better tree crop nutrient management and maintenance of soil health, better soil and water management leading to improved forestry practices. It is inferred that under appropriate technology, the use of efficient microbial inoculants helps to produce the quality seedlings which may perform well in impoverished soil. The present study clearly shows that the application of bio inoculants such as nitrogen fixing bacteria *Azospirillum*, phosphate solubilizing bacteria and bio control agent of *Pseudomonas* and nutrient mobilizing microorganisms of AM Fungi plays a

significant role in increasing the growth response of *A. marmelos* seedlings in a stipulated period, thereby producing good quality planting stock.

5. REFERENCES

- Abbott, L.K. and Robson, A.D. (1982) The role of vesicular arbuscular mycorrhizal fungi in agriculture and selection of fungi for inoculation Aus, J Agric, Res. 33, 389-408.
- Alagesaboopathi, C. and Rajendran, K. (2009) Effect of bioinoculants on quality seedling production of *Azadirachta indica* (A.) Juss, J Phytol Res. 22 (1), 125 – 130.
- Anurag, M., Sandeep, A., Rajiv Gupta, M., Rajesh Kumar, P. and Ashish Kumar, S. (2009) Effect of *Feronia elephantum* (Corr) Fruit Pulp Extract on Indomethacin induced Gastric Ulcer in Albino Rats, Trop J Pharm Resear. 8(6), 509-514.
- Arther, D.A.J. and Kawlis, N.R. (1993) Influence of Vesicular arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency, Plant Physiol. 101, 147-160.
- Bakker, P.A., Lamers, J.G., Bakker, A.W., Marugg, J.D., Weisbeek, P.J. and Schippers, B. (1986) The role of siderophores in potato tuber yield increase by *Pseudomonas putida* in a short rotation of potato. Neth. J Plant Pathol. 92, 249-256.
- Baldani, V. L. D. and Dobereiner, J. (1980) Host-plant specificity in the infection of cereals with *Azospirillum* spp. Soil Biol. Biochem. 12, 433-439.
- Basu, P.K. and Kabi, M.C. (1987) Effect of application of biofertilizers on the growth and nodulation of seven forest legumes, Ind For. 113 (4), 249-257.
- Bloemberg, G. V. and Lugtenberg, B.J.J. (2001) Molecular basis of plant growth promotion and bio-control by rhizobacteria, current Opinion Plant Bio. 4, 343-350.
- Burr, T.J., Schoth, M.N. and Suslow, T. (1978) Increased potato yield by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*, Phytopathol. 68, 1377-1383.
- Chanway, C.P. (1997) Inoculation of tree roots with plant growth-promoting soil bacteria, An emerging technology for reforestation. For Sci. 43, 99-112.
- Dickson, A., Aa, L., Leaf and Fosner, J. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *For Chron.* 36: 10-13.
- Dobereiner, J., Marriell, I.E. and Nery, M. (1976) Ecological distribution of *Spirillum lipoferum* Beijerinck. Can. J Microbiol. 22, 1464-1473.
- Duncan, D.B. (1955) Multiple range and multiple f-tests. Biometrics. 11, 1-42.

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- Fiske, Cyrus. H. and Subbaro, Y. (1925) The colorimetric determination of phosphorous, J of Biol, chemi 66 (2) 375-400. Jackson, M.L, Soil Chemical Analysis.(1973) Printice Hall of India (Pvt) Ltd., New Delhi.
- Gaur, A.C. 1990 Phosphate solubilising microorganisms as biofertilizers, Omega scientific publishers, 175.
- Govindarajan, K. and Thangaraju, M. (2001) Use of biofertilizers in quality seed production. Recent techniques and participatory approaches on quality seed production, Department of seed science and Technology, Coimbatore, 127-130.
- Gunjal, S.S. and Patil, P.L. (1992) Mycorrhizal control of wilt in Casuarinas. Agroforestry Today. 4, 14-15.
- Howeler, R.H., Edwards, D.G. and Asher, C.J. (1981) Application of the flowering solution culture techniques to studies involving mycorrhizae, Plant and Soil. 59, 179 – 183.
- Jackson, M. L. (1973) Soil chemical analysis. Printice hall of India (Pvt) Ltd., New Delhi.
- Kabi, M.C., Poni, S.C. and Bhaduru, P.N. (1982) Potential of nitrogen nutrition of leguminous crops through rhizobial inoculation in West Bengal soils, Trans 12th International Congress of Soil Sci. 6, 1-52.
- KasthuriRengamani, S., Jothibas, M. and Rajendran, K. (2006) Effect of bioinoculants on quality seedlings production of Drumstick (*Moringa oleifera* L.) J Non-Timber For Pro. 13 (1), 41-46.
- King, E.O., Ward, M.K. and Raney, D.E. (1954) Two simple media for the demonstration of Pyocyanin and fluorescein, J, Lab, Clin, Medi. 44, 301-307.
- Kloepper, J.W. (1993) Plant growth-promoting rhizobacteria as biological control agents, P, 255-274 in Soil microbial ecology-applications in agricultural and environmental management, Meeting, F.B, Jr, (ed.), Marcel Dekker, New York.
- Krishnamoorthy, G. (2002) Agrobook, Editor, Usha printers, New Delhi, Apr-June, 22-24.
- Kucy, R.M.N. (1987) Increased phosphorus uptake by wheat and field beans inoculated with a phosphorus solubilizing *Penicillium bilaji* strain and with vesicular arbuscular mycorrhizal fungi, Appl Environ Micro. 52, 2699-2703.
- Meenakshisundaram, M., Santhaguru, K. and Rajendran, K. (2011) Effects of Bioinoculants on quality seedling production of *Delonix regia* in tropical nursery conditions. Asia J of Biocheml and Pharm Rese. 1(1), 99-107.
- Okon, Y. and Kapulnik, Y. (1986) Development and Function of *Azospirillum* inoculated roots. Plant Soil. 90, 3-16.
- Philips, J.M. and Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasite and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 55, 158-161.

- Priya Rani, S., Aggarwal, A. and Mehrotra, R.S. (1998) Growth responses in *Acacia nilotica* inoculated with VAM fungus (*Glomus fasciculatum*), *Rhizobium* sp. And *Trichoderma harzianum*. J of Mycopath resear, 36(1), 13-16.
- Rajan, S.K. Reddy, B.J.D and Bagyaraj, D.J. 2000. Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. *For Ecol. Manage.* **126**: 91-95.
- Rajendran, K. (2012) Effects of bioinoculants on seedling growth, biochemical changes and nutrient uptake of *Erythrina indica* L., In Semi-Arid Region of Southern India, J Biomet Biostat. 3(2), 1-6.
- Rajendran, K. and Devaraj, P. (2004) Biomass and nutrient distribution and their return of *Casuarina equisetifolia* inoculated with biofertilizers in the farm land. Biomass and Bioen. 26 (3), 235-249.
- Rajendran, K., Sugavanam, V. and Devaraj, P. (2003) Effect of biofertilizers on quality seedling production of *Casuarina equisetifolia*, J Trop For Sci. 15 (1), 82 – 96.
- Rajendran, K. and Jayashree, S. (2007) Effect of biofertilizers on quality seedling production of *Acacia nilotica*, J of Non-timber for pro. 14(1), 1-5.
- Ramamoorthy, A. (1982) Studies on interaction between phosphobacteria and nitrogen fixing microorganisms in relation to production of pearl millet (*Pennisetum americanum*), M.Sc.(Ag), Thesis, Tamilnadu Agricultural University, Coimbatore.
- Rodriguez Caceres, E.A. (1982) Improved medium for isolation of *Azospirillum* spp, Appl, Environ, Microbiol. 44, 990-991.
- Schippers, B., Bakker, A.W. and Bakker, P.A.H.M. (1987) Interactions of deleterious and beneficial rhizosphere organisms and the effect of cropping practices, Annu, Rev, Phytopathol. 25, 339-358.
- Sekar, I., Vanangamudi, K., Suresh, K. and Suresh, K.K. (1995) Effects of biofertilizers on the seedling biomass VAM colonization, enzyme activity and phosphorous uptake in the shoal tree species, My forest. 31 (4), 21-26.
- Siddiqui, Z.A. (2003) Effect of plant growth promoting bacteria and composed organic fertilizers on the reproduction of *Meloidogyne incognita* and tomato growth. Bioresource Technol. 95, 223-227.
- Singh, M., Jagadish Singh., Kalyan Singh, (1983) Effect of phosphorous and bio fertilizers on chlorophyll content of leaves and laghaemoglobin content of fresh nodules in kharif grain legumes. Indian J of Agro. 28(3), 229-234.
- Stribley, D.P. (1987) Mineral nutrition In :Ecophysiology of VAMycorrhizal plants (G.R. Safir ed.), CRC Press, Boca Raton, Florida, 193-211.

- Subramanian, K., Madal, A.K. and Ram Babu, N. (2000) Chundamannil M,&Nagarajan B, Site, Technology and Productivity of Teak plantations in India. In: Enters T., Nair C.T.S. (eds): Site, Technology and Productivity of Teak Plantations, FAO, FORSPA Publication No 24, 51–68.
- Sumner, M.E. (1990) Crop responses to *Azospirillum*, In: Stewart, B.A,(ed) Ad in soil sci, Springer,New York, 53-123.
- Suslow, T.V. and Schroth, M.N. (1982) Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield, Phytopathol. 72 (9), 199-206.
- Swaminath, M.H. and Vadiraj, B.A. (1988) Nursery studies on the influence of *Azospirillum* biofertilizers on the growth and dry matter of forestry species, Myforest. 24, 289-294.
- Tien, T.M., Gaskin, M.H. and Hubbell, D.H. (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of Pearl Millet (*Pennisetum americanum* L.) Appl, Environ, Microbiol. 33, 1016-1024.
- Verma, R.K. and Jamaluddin, R. (1995) Association and activity of arbuscular mycorrhizae of teak (*Tectona grandis*) in central India. In fores. 121, 553-539.
- Vijayakumari, B. and Janardhanan, K. (2003) Effect of biofertilizers on seed germination, seedling growth and biochemical changes in silk cotton [*Ceiba pentanndra* (Linn.) Gaertn.] Crop Res. 25(2), 328-332.
- Weller, D.M. and Cook, R.J. (1983) Suppression of take-all of wheat by seed treatments with fluorescens Pseudomonas, Phytopathol. 73, 463-469.
- Wong, P.P. and Sternberg, N.E. (1979) Characterization of *Azospirillum* isolates from nitrogen fixing roots of harvested Sorghum plants. Appl, Environ Microbiol. 38, 1189-1191.
- Yan, Z., Reddy, M.S. and Kloepper, J.W. (2003) Survival and Colonization of rhizobacteria in a tomato transplant system, Can J Micro. 49, 383-389.
- Young, C.C., Juang, T.C. and Chao, C.C. (1988) Effect of *Rhizobium* and VAM inoculation on nodulation and soybean yield in sub-tropical fields. Biol Fertil Soils. 6, 165 – 169.