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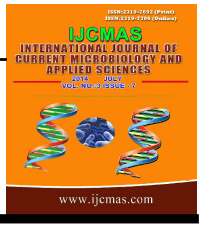

Original Research Article

Effect of Plant growth-promoting Microorganisms on Quality Seedling Production of *Feronia elephantum* (Corr.) in Semi-Arid Region of Southern India

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Keywords

Feronia elephantum, *Azospirillum*, AM fungi, *Pseudomonas* Seedling quality.

A B S T R A C T

Feronia elephantum (Corr.) belongs to the family Rutaceae and a native species of Indian subcontinent. 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 *Feronia elephantum* in tropical nursery conditions. The bioinoculants such as *Azospirillum* (*Azospirillum brasilense*), AM fungi (*Glomus fasciculatum*) and *Pseudomonas* (*Pseudomonas flurorescens*) were isolated, mass multiplied and inoculated individually and in combinations. Seedlings were kept at 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.

Introduction

Feronia elephantum(Corr.) 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 has been reported in traditional medicine as a curative for various 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 advantages, non-government organizations and medicinal plant cultivators have planted ally cropping and avenue trees in the public land in urban area and highways. Since the trees are planted in barren land it is necessary to apply fertilizer for nutrient management of *Feronia*. Long term application of synthetic chemical

fertilizers will damage the physico-chemical and biological properties, 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 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 & P by 25%. Stimulate plant growth, activate soil biologically, restore natural fertility and provide protection against drought and some soil borne diseases.

Nitrogen fixing bacteria of genus *Azospirillum* is an important non-symbiotic associative microorganisms, it fixes atmospheric nitrogen in 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* spps 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. 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 biofertilizers to improve the growth and nutrient uptake of *Feronia elephantum* seedlings. Hence, the present study was undertaken to find out the suitable bio-inoculants and it combinations for the quality seedling production.

Materials and Methods

Seeds

Feronia elephantum fruits were collected from a single plus tree, located at the semi-arid region of Alagar hills in Madurai district of Tamil Nadu, India. 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 in room temperature.

Pseudomonas and *Azospirillum*

Isolation of *Pseudomonas*

Pseudomonas fluorescens was isolated by the serial dilutions and pour plate method using King's B medium (King et al., 1954). 1mL of soil 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+ 2^oC 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+2^{o}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^{o}C and mass multiplied for further use.

Isolation of *Azospirillum*

N-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. The cultures were incubated at 32^{0}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^{0}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

AM fungus (*Glomus fasciculatum*) was isolated and recorded as dominant species in the rhizosphere soil of *F.elephantum.* It was multiplied in pot culture in the sterilized mixture of sand: soil (1: 1 v/v) and maintained in the roots of *Sorghum vulgare* as the host plant. The inoculum contained extrametrical hyphae, chlamydospores and infected root segments were added in the root zones of each seedling.

Treatment

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 AM fungus were inoculated by making holes in the rhizosphere of seedlings.

T_1- *Azospirillum* (*Azospirillum brasilense*)
T_2-ArbuscularMycorrhizal Fungi (AMF) (*Glomus fasciculatum)*
T_3-*Pseudomonas* (*Pseudomonas fluorescens*)
T_4- *Azospirillum* + AMF
T_5- *Azospirillum* + *Pseudomonas*
T_6- *Pseudomonas* + AMF
T_7- *Azospirillum*+ *Pseudomonas* + AMF
T_8- 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, India. 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 identical nursery condition up to 180 days.

Seedling survival percentage was calculated using the following formula:

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

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^{o} C in a paper bags placed in hot air oven 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 ^{0}C. The colonies were counted on third day for *Pseudomonas* and *Azospirillum* using colony counter and expressed the population (cfu) colony-forming unit per gram of soil.

Assessment of Mycorrhizal infection

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 KOH (w/v) and boiled in a water bath at 90 ^{o} C for 15 minutes. The roots were rinsed with repeated washing with water and lightened in H_2O_2 (3 ml of 20 % NH_4OH in 30 ml H_2O_2) 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 H_2O, 50 ml 1 % HCl) containing 0.05% trypan blue. The trypan blue solution was poured off and the roots were de-stained in acidic glycerol at room temperature. The stained roots were mounted in a glass slide and percentage of infection was calculated.

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

Seedlings Quality Index

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

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

Root collar diameter (mm)
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

The data were statistically analyzed by analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test ($P< 0.05$) (1955).

Results and Discussion

Microbial population of potting media

Analysis of nursery soil used for growing *Feronia* seedlings shows that the population of *Pseudomonas* and *Azospirillum* reported as 1.3 x 10^8 and 1.2 x 10^6 cfu/g of soil respectively, assessment of AM spore density was recorded as 8 spores/100g soil.

Growth enhancement estimation
Shoot length

Significant increase in shoot length was recorded in *F.elephantum* 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* (T_7) was found to be the most effective combination in increasing the growth of seedlings at all stages. Among the individual inoculation, *Azospirillum* (T_1) showed higher shoot length and statistically on par with AM fungi (T2) inoculated seedlings. Within double inoculations, AM fungi + *Azospirillum* (T4) is superior when compared with other dual inoculations. It was registered 40.90% and18.18% increase over control in single and dual inoculations.

Basal diameter

The result revealed that the difference between, treatments and their interaction were found to be highly significant (Table 1). Combined inoculation of *Azospirillum* + AM fungi+ *Pseudomonas* (T_7) 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* (T_4) registered higher basal diameter but it was on par with AM fungi+ *Pseudomonas* (T_5).

Root length

Significant increase in Root length was recorded in *F. elephantum* 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* (T_7) was found to be the most effective combination in increasing the growth of seedlings at all stages. Among the individual inoculation, *Azospirillum* (T_1) showed higher shoot length and statistically on par with AM (T_2) inoculated seedlings. Within double inoculations, AM fungi + *Azospirillum* (T_4) 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 - 2. 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* (T_7). It was recorded 77.47 % increase over control. It was followed by inoculation with *Azospirillum* + AM fungi (T_4). Among single and double inoculations, *Azospirillum* (T_1) and *Azospirillum* + AM fungi (T_4) 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* (T_1) 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* (T_7) followed by *Azospirillum* + AM fungi (T_4). It was recorded 100.18% increase over control. It was also statistically on par with *Azospirillum* + AM fungi (T_4) and *Azospirillum* + *Pseudomonas* (T_5). Among single and double inoculations (T_1) *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 2).

Total biomass of seedling

Seedling biomass maximum in the treatment comprising of *Azospirillum* + AM fungi + *Pseudomonas* (T_7) 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 (T_1) 75.25% more than the control (Table 2).

Seedling quality index

Maximum good quality seedlings were obtained in treatment (T_7) *Azospirillum* + *Pseudomonas* + AM Fungi recorded highest seedling quality index. Among the single inoculation, *Azospirillum* (T_1) and AM Fungi (T_2), double inoculation, AM Fungi +*Azospirillum* (T_4) had higher seedling quality index than other treatments (Fig. 1).

Nutrient content estimation

Nitrogen

Total Nitrogen content of *F. elephantum* seedlings inoculated with biofertilizers had significantly increased over control (Table 3). The highest Nitrogen content was observed in seedlings inoculated with *Azospirillum* + AM + *Pseudomonas* (T_7) followed by double inoculation of Azospirillum + AM (T_4). The above treatment accounted 319%, 251% and 203% more than that of control respectively. Statistically there is no significant difference between *Pseudomonas*+ AM + (T_6), *Azospirillum* + *Pseudomonas* (T_5). Among single and double inoculations, *Azospirillum* (T_1) and AM + *Azospirillum* (T_4) recorded higher nitrogen content when compared to other treatments.

Phosphorus

The phosphorus content was the highest in the seedlings treated with *Azospirillum*+ AM + *Pseudomonas* (T_7) followed by *Azospirillum*+ AM (T_4) and AM + *Azospirillum* (T_5) registering and 280% more than that of control plants respectively. Among single inoculations, *Azospirillum* (T_1) 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 (T_7) followed by *Azospirillum* + AM (T_4). Among single inoculations, plants inoculated with *Azospirillum* (T_1) inoculated seedlings contained more K, Ca and Mg than other single treatments (Table 3).

Microorganisms are commonly found organized in micro-colonies in the rhizosphere soil. At the same time these micro-colonies benefit from the nutrients secreted by plant root systems they can also directly or indirectly stimulate plant growth. These 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).

In the present study, the shoot and root height, collar diameter, dry matter and quality seedlings were higher in the *Feronia* 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*. Similarly, previous report also supports the plant growth of agronomically important field crops by 10 to 30% in the field experiment (Okon, 1985; Sumner, 1990). 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 equisetifoli*a (Rajendran et al., 2003), *Moringa* o*leifera* (KasthuriRengamani et al., 2006), *Acacia* n*ilotica* (Rajendran and Jayashree, 2007), *Delonix* r*egia* (Meenakshisundaram et al., 2011), *Erythrina* i*ndica* (Rajendran, 2012), *Azadiracta* i*ndica* (Alagesaboopathi and Rajendran, 2009) and *Tectona* g*randis* (Subramanian et al., 2000; Rajan et al., 2000).

Phosphate plays a major role in the root development (Kuccy, 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 mycorrhizal infection in roots of seedlings were found to be higher in the inoculated seedlings. It is also recorded that increased nutrient content especially P uptake. The present result corroborate with earlier report of Abbott and Robson (1982) and quality seedling production of

Azadirechta i*ndica* (Alagesaboopathi and Rajendran, 2009), *Casuarina* e*quisetifolia* (Rajendran et al., 2003), *Acacia* n*ilotica* (Rajendran and Jayashree, 2007), *Delonix* r*egia* (Meenakshisundaram et al., 2011) and *Tectona* g*randis* (Rajan et al., 2000) This can be attributed to the increased absorbing surface area due to extensive external network of mycelium produced by the AM fungi in association with the host root system (Howeler et al., 1981).

In the present study AM fungi inoculation enhanced the plant growth and biomass of *F. elephentum* seedlings this can be attributed to the increased absorbing surface area due to extensive external network of mycelium produced by the AM fungi in association with the host root system[40]. In the present study, triple inoculantion with AM fungi + *Azospirillum* +*Pseudomonas* influence the growth and biomass of *F. elephentum* 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., 1086; 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* (T_7) were found exert the maximum influence on various growth parameters of *F. elephantum* 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* influence the growth and biomass of *F. elephantum* seedlings. It is relevant to mention here that *Pseudomonas* by virtue of its capacity to multiply certain growth promoting substances like IAA and GA might induce the growth of *F. elephantum* 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 *F. elephantum* 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.

Table.1 Effect of bio-inoculants on the growth of *Feronia elephantum* seedlings (180 days after inoculation)

S.No	Treatments	No of leaves	Collar diameter (mm)	Shoot length (cm)	Root length (cm)	Total length (cm)
1	T_1	39^{c}	$0.66^{c} \pm 0.013$	$31.72^{e} \pm 0.442$	$69.32^{d} \pm 1.511$	$101.04^{d} \pm 1.953$
2	T_2	37^{c}	$0.64^{c} \pm 0.017$	$27.42^{e} \pm 0.190$	$66.14^{c} \pm 1.599$	$93.56^{c} \pm 1.789$
3	T_3	33^{b}	$0.52^{b} \pm 0.014$	$24.26^{ab} \pm 1.001$	$44.90^{a} \pm 1.307$	$69.16^{a} \pm 2.308$
4	T_4	42^{d}	$0.56^{b} \pm 0.015$	$26.60^{cd} \pm 0.620$	$54.76^{b} \pm 1.192$	$81.36^{b} \pm 1.812$
5	T_5	35^{b}	$0.54^{b} \pm 0.016$	25.42^{bcd} 0.815	$54.60^{b} \pm 1.756$	$80.02^{b} \pm 2.571$
6	T_6	34^{b}	$0.54^{b} \pm 0.012$	$24.80^{abc} \pm 1.215$	$45.84^{a} \pm 0.761$	$70.64^{a} \pm 1.976$
7	T_7	48^{de}	$0.73^{d} \pm 0.013$	$33.42^{e} \pm 1.215$	$75.44^{d} \pm 1.132$	$108.86^{d} \pm 2.347$
8	T_8	26^{a}	$0.35^{a} \pm 0.013$	$22.74^{e} \pm 1.215$	$44.40^{a} \pm 1.307$	$67.14^{a} \pm 2.522$

Means followed by a common letter(s) in the same column are not significantly different at the 5 % level by DMRT

T_1- *Azospirillum*;T_2-ArbuscularMycorrhizal Fungi (AMF) ;T_3-*Pseudomonas* ;T_4- *Azospirillum* + AMF; T_5- *Azospirillum* + *Pseudomonas*; T_6- *Pseudomonas* + AMF;T_7- *Azospirillum*+ *Pseudomonas* + AMF T_8- Control (Sand: Red soil: Farm Yard Manure 2:1:1

Table.2 Effect of bio-inoculants on the biomass (g/dry wt.) of *Feronia elephantum* seedlings (180 days after inoculation)

S. No	Treatments	Leaf	Shoot	Root	Total Biomass
1	T_1	9.22^{c}	30.24^{c}	46.01^{c}	85.47^{c}
2	T_2	8.20^{b}	24.01^{b}	43.83^{c}	76.04^{c}
3	T_3	8.44^{b}	22.87^{b}	30.89^{b}	62.20^{b}
4	T_4	8.76^{b}	24.08^{b}	41.43^{c}	74.27^{c}
5	T_5	8.65^{b}	23.04^{b}	38.14^{b}	69.83^{b}
6	T_6	8.55^{b}	22.87^{b}	33.27^{b}	64.69^{b}
7	T_7	10.48^{d}	33.01^{d}	54.43^{d}	97.92^{d}
8	T_8	7.88^{a}	18.60^{a}	27.19^{a}	53.67^{a}

Means followed by a common letter(s) in the same column are not significantly different at the 5 % level by DMRT

T_1- *Azospirillum*;T_2-ArbuscularMycorrhizal Fungi (AMF) ;T_3-*Pseudomonas* ;T_4- *Azospirillum* + AMF; T_5- *Azospirillum* + *Pseudomonas*; T_6- *Pseudomonas* + AMF;T_7- *Azospirillum*+ *Pseudomonas* + AMF T_8- Control (Sand: Red soil: Farm Yard Manure 2:1:1)

Table.3 Total Biomass, Nutrient concentration and nutrient content (g/seedling) of *F.elephantum* seedlings inoculated with different bio-inoculants (180 days after inoculation)

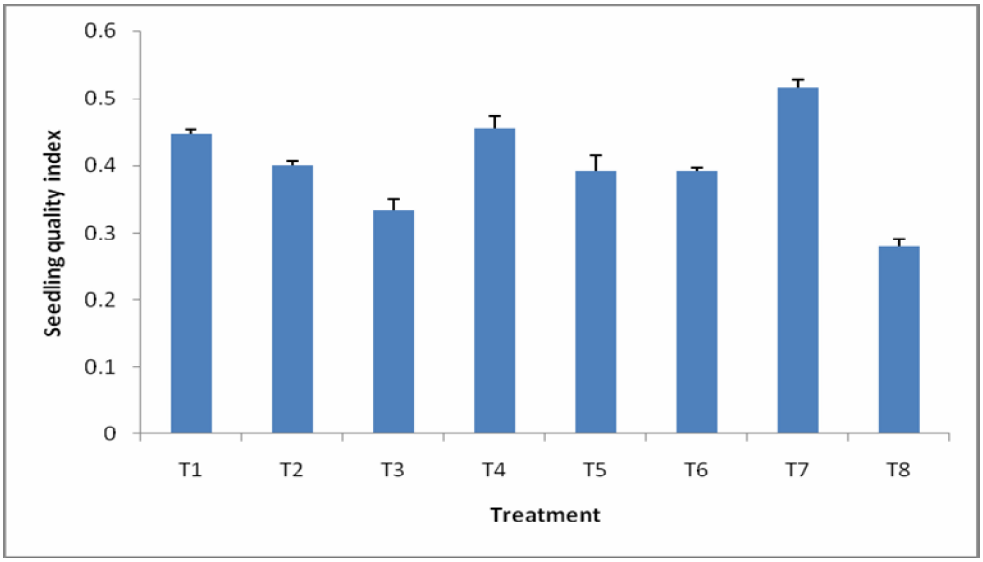
Treatments	Total Biomass	N (%)	P (%)	K (%)	Ca(%)	Mg (%)
T_1	85.47[c]	2.02 (1.371)	0.100 (0.067)	1.321 (0.897)	1.410 (0.957)	0.29 (0.196)
T_2	76.04[c]	2.22 (1.504)	0.102 (0.069)	1.320 (0.820)	1.405 (0.873)	0.28 (0.174)
T_3	62.20[b]	2.42 (1.851)	0.103 (0.078)	1.326 (1.011)	1.426 (1.087)	0.32 (0.244)
T_4	74.27[c]	2.04 (1.096)	0.100 (0.053)	1.321 (0.710)	1.405 (0.755)	0.30 (0.161)
T_5	69.83[b]	2.04 (1.315)	0.101 (0.065)	1.322 (0.852)	1.406 (0.906)	0.30 (0.193)
T_6	64.69[b]	2.00 (1.122)	0.098 (0.055)	1.300 (0.729)	1.406 (0.789)	0.31 (0.174)
T_7	97.92[d]	2.44 (2.133)	0.107 (0.093)	1.389 (1.214)	1.486 (1.299)	0.36 (0.314)
T_8	53.67[a]	1.94 (0.893)	0.101 (0.044)	1.300 (0.569)	1.400 (0.613)	0.31 (0.135)

Means followed by a common letter(s) in the same column are not significantly different at the 5 % level by DMRT

Figures are parenthesis is nutrient uptake (g/plant)

T_1- *Azospirillum*;T_2-ArbuscularMycorrhizal Fungi (AMF) ;T_3-*Pseudomonas* ;T_4-*Azospirillum* + AMF; T_5- *Azospirillum* + *Pseudomonas*; T_6- *Pseudomonas* + AMF;T_7-*Azospirillum*+ *Pseudomonas* + AMF T_8- Control (Sand: Red soil: Farm Yard Manure 2:1:1)

Fig.1 Seedling quality index of *Feronia elephantum* inoculated with bio-inoculants



T_1- *Azospirillum*;T_2-ArbuscularMycorrhizal Fungi (AMF) ;T_3-*Pseudomonas* ;T_4-*Azospirillum* + AMF; T_5- *Azospirillum* + *Pseudomonas*; T_6- *Pseudomonas* + AMF;T_7-*Azospirillum*+ *Pseudomonas* + AMF T_8- Control (Sand: Red soil: Farm Yard Manure 2:1:1)

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 *Feronia elephentum.* Bio fertilizers 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 *F. elephentum* seedlings in a stipulated period, thereby producing good quality planting stock.

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