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Bioinoculation Effect of *Frankia sp*. and Microbial Bioagents on Growth and Development of *Elaeagnus latifolia* L. seedlings: An Endangered Plant Species of North East India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Analyses were done on the role of rhizospheric symbiotic Arbuscular Mycorrhizal (AM) fungus, Non-AM fungus and a bacterium in growth and development of *E. latifolia* L. seedlings.
Study Design and Methodology: The seedlings were inoculated with recognized AM fungal strain (*Glomus mosseae*, Gm 1), non-AM fungus (*Trichoderma harzianum,* Th-13) alone and nitrogen fixing bacteria *Frankia* sp. in combined form (both, dual and triple/ tripartite consortium) and analysed for their effect on growth parameters *i.e.* increase in length, diameter, circumference, Sturdiness quotient (S_q), Biovolume index (B_i) and Plastochron interval index (P_i) of the target plant species.

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Results: Qualitative analysis have revealed that the consortium treatment containing *Glomus mosseae* and *Frankia* sp. (FS-03) resulted in more increase in the length (7.0 ±0.25 cm), diameter (1.3 ±0.25 cm), circumference while the highest biovolume index was observed in the consortium treatment of *Frankia* sp. along with *Glomus mosseae*, *Trichoderma harzianum* viz. Fs-04 (280.96 ± 20.0) and sturdiness quotient of 0.25 including reduced phyto-mortality and initiating rapid phyllogenesis in inoculated seedlings as compared to control seedlings. In contrast to control seedlings, which are covered in depth in this work, a similar pattern with negligible variance in all analyzed parameters as indicated above were also seen in other alone and combined treatments.
Conclusion: As a result, it was shown that both AM alone and consortium inoculations with *Frankia* sp. increased this plant species' adaptation and resilience.

Keywords: Arbuscular mycorrhizal fungi; biovolume index; bio-inoculation; Elaeagnus latifolia; growth parameters.

1. INTRODUCTION

"The application of beneficial microorganisms as bio inoculants appears as an environmental friendly biotechnological tool for sustainable agricultural practices" [1,2]. "Some of the beneficial microbes such as arbuscular mycorrhizal, nitrogen fixing bacteria such as *Frankia* sp., PGPFs (Plant growth promoting fungi) and PGPRs (Plant growth promoting rhizobacteria). Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are considered highly-efficient agents for stress tolerance in host plants and improving soil fertility in rhizosphere" [3]. "The AMF establishes symbiotic relationships with the roots of most of the terrestrial plants, including 80−90% of the vascular plants, and 90% of the agricultural plants, such as cereals, vegetables, and horticultural plants. The application of AMF has been found to increase plant growth and regulation by enhancing nutrient uptake and stress tolerance. AMF plays an important role in laying establishment with a wide range of soil microorganisms. Interactions between the associations can be either positive, negative, or neutral depending on the type of AMF strain and microbes in the rhizosphere. In addition, it helps to acquire nutrients, control root pathogens, and improve plant resilience to stress. Growth promoting bacteria in general and rhizobacteria in particular exhibit extensive interaction in rhizospheric zone. Giving an extraordinary environment inside the rhizosphere, plant releases many compounds as root exudates that is high in sugars, amino acids, organic acids, flavonoids, proteins, and fatty acids. Such root exudates are usually low molecular weight compounds, non-metabolically released" [4].

Likewise, "actinorhizal plants performance is improved by *Frankia* in the different environments. In *Casuarina equisetifolia* and *Casuarina cunninghamiana,* dual inoculation with *Frankia* and mycorrhizal fungi, enhance the trees and seedlings height, depending on the availability of phosphorous contents. Dual inoculation of *Rhizophagus intraradices* and *Frankia* spp. with plants of Black alder, in highly anthropogenic alkaline sediment, increases the shoot length, P and N contents of leaf, overall biomass, and leaf area when compared with another control which was uninoculated the *Rhizophagus intraradices* and *Frankia* spp. treatments alone. In addition to this, dual inoculation increased the symbiosis of AMF, indicating a synergistic effect demonstrated by a high number of nodules in dry weight and a high degree of root development. Although a lot of study and research says that not always a relationship between AMF and Frankia yields good results, sometimes it may be negative" [3]. " In an addition to it, PGPFs (Plant growth promoting fungus) are not only beautiful but play a massive role in the daily life of human beings besides their utilization industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as bio fertilizer and many other ways. Fungal biotechnology has become a complete part of the human welfare. Fungus benefits most plants by suppressing plant root diseases and fungi promote healthier plants by attacking plants pathogens. Fungi also use antagonism to reduce competition by producing antibodies, which inhibit other microorganisms from growing. They produce numerous vitamins which promote plant growth. Beneficial fungi also form protective webs and nets around roots as well as leaves to save the host plants. Fungus also save plants by supplying a protective health to supply both water and phosphorous to the plant roots during droughts" [5].

Thus, the present study was undertaken to enumerate the beneficial effects of the bioinoculants such as PGPRs (Plant growth promoting rhizobacteria), PGPFs (Plant growth promoting fungus) and AMF (Arbuscular mycorrhizal Fungi) in single, dual and tripartite inoculation in the seedlings of *Elaeagnus latifolia* L. *Elaeagnus latifolia* L. belongs to family *Elaeagnaceae,* locally known as *Soh-shang* in Khasi hills of Meghalaya and *MirikaTenga* in Assam, is quite uncommon in Sibsagar (Dikho valley of Assam), Naga hills (Nagaland), Khasi and Jaintia hills of Meghalaya up to an elevation of 1500 above mean sea level (msl) of North-East India [6]. "It is a large evergreen spreading type woody shrub with rusty-shiny scales that are often thorny. The flowers are hermaphrodite and are pollinated by bees. The fruit is oblong in shape with dark pink color at the time of ripening. It flowers during September-December and the light pink coloured fruits are harvested during March-April in 3-4 picking. The people of Meghalaya have found many uses of *Soh-shang* fruit besides enjoying it as fresh fruit" [7,8]. "Fruits are eaten raw and could be utilized for making jam, jelly and refreshing drink.The fruit is considered to be a very rich source of vitamins, minerals and other bioactive compounds. It is also a fairly good source of essential fatty acids, which is fairly unusual for a fruit. The fruits are quite perishable and can be stored only for 3-5 days at room temperature. It is reported that it is capable of reducing the incidence of cancer and also as a means of halting or reversing the growth of cancers" [8,9].

Since, the species, *Elaeagnus latifolia* L. is of multiple usage and has economical importance and is under threat and nearing to its threshold due to extensive exploitation, the present investigation has been undertaken to explore the beneficial microflora such as actinorhizal, endomycorrhizal and endophytic associated with *Elaeagnus latifolia* L. rhizosphere and analysed for their effect of bioinoculation of selected microbial bioagents on growth parameters *i.e.* increase in length, diameter, circumference, Sturdiness quotient (S_q), Biovolume index (B_i) and Plastochron interval index (P_i) of the target plant species.

2. MATERIALS AND METHODS

2.1 Collection of Plant, Soil and Root Samples

Survey was made at selected areas for the collection of plant specimen, *Elaeagnus latifolia* L., (Fig 1) which is known for its symbiotic relationship with several beneficial microbiotas. The rhizospheric soil samples of the plant were also collected to investigate and enumerate important microbial associations. The collected plant specimens were preserved in herbarium sheet for further identification. Rhizospheric soil samples (at least three samples at each location) were taken by digging out a small amount of soil (500 g) adjacent to plant roots up to the depth of 15-30 cm. The collected soil samples were kept in pre-sterilized polythene at 4 ^{0}C to estimate the physico-chemical parameters of soil, mycorrhizal colonization and quantification of AM fungi as well as actinorhizal and endophytic associations.

2.2 Isolation and Characterization of Rhizospheric Microorganisms

In the present investigation, a culture-based approach was primarily used to isolate and characterize the rhizospheric microbes of selected study points. For this, randomly collected soil samples were mixed properly and passed through a 2.0 mm sieve to remove the debris. 1.0 g of soil was then suspended in 10 ml of SDW and incubated in an orbital shaking incubator at 28 °C with periodic shaking at 200 rpm for 30 minutes. 10-fold series dilutions were prepared serially by taking 1 ml of the soil suspension and dispensing it into 9 ml of SDW. Soil particles were allowed to settle and serial dilutions were prepared using SDW. The soil suspension of required dilution was then inoculated into culture media and incubated at optimum temperature for maximum growth of rhizosphere microbes. The colony forming units (cfu) were counted after proper incubation period was over. Three replicates were maintained in each case. Bacterial and fungal colonies were identified and characterized based on their morphological, cultural and reproductive characteristics on the growth media.

2.3 Isolation, Quantification and Root Colonization of Vam Spores

"Wet sieving and decanting technique [10,11] was followed to isolate VAM spores. For this, 50 g of soil was suspended in 500 ml water and decanted by using a series of sieves. Spores retained on the mesh were recovered by repeated washing and transferred to whatman no. 01 filter paper in a petridish and observed under stereo-binocular microscope. The

isolated spores were identified using the keys of various mycologists" [12-16] Websites, www.mycorrhiza.com,www.ffp.csiro.aug.utk/research/mycorrhiza/intro,http://zor.zut.e .pl/Glomeromycota/index.html are also used for identification.

2.4 Mycorrhizal Quantification

A modified method was used for quantitative estimation of AM spores by Gaur and Adholeya [17]. The whatman filter paper was divided into many small sectors and total numbers of spores were counted by adding the number of spores present in each sector under stereo-binocular microscope.

2.5 Growth Media, Isolation and Culture Conditions for Rhizospheric Fungi

Potato dextrose agar (PDA) was used for the isolation of soil fungi using dilution plate technique [18] and 10^5 dilutions. The media were supplemented with 50 µg/ml of streptomycin sulphate to prevent bacterial growth. Fungi were grown at 25±1 °C for 5 days. Three replicates were maintained in each case. Pure colonies were transferred to PDA slants overlaid with mineral oil and stored at 4 °C for further identification. Fungi were characterized based on their cultural, morphological and spore characteristics and identified by consulting various taxonomic monographs [19-22]. The fungi that did not produce spores were characterized as mycelia sterile and those showing no diagnostic morphological characters were included under unidentified strains.

2.6 Isolation and Characterization of Soil Bacteria

Isolation of soil bacteria was done on Nutrient agar (NA) using dilution plate technique of Johnson and Curl, [18] and 10^6 dilutions. The bacterial population was estimated by growing them at 30±1 °C for 48 h. Three replicates were maintained in each case. Pure cultures of bacteria were preserved at 4 ºC in Nutrient agar slants after observing the abundance of bacterial growth, pigmentation and optical characteristics. For long-term storage, isolates were kept in 15% (v/v) glycerol in Nutrient Broth at –20 ºC. Bacterial morphological characters like shape, size, texture, surface, growth, elevation, margin type, consistency, pigmentation, rate of growth etc., as well as physiological and biochemical characteristics were examined in accordance with Cappuccino and Sherman, [23] and Bergey's Manual of Systematic Bacteriology [24]. A modified gram staining method [25] was followed to differentiate the Gram-positive bacterial isolates from Gram-negative strains.

2.7 Production of *In-vitro* Cultures and Mass Multiplication of Selected Indigenous and Putative Bioagents/ Microsymbionts for Bio-Inoculation of Target Plant Species

2.7.1 Mycorrhizal mass multiplication

The mycorrhizal inoculums productions were carried out using soil funnel technique [26]. "Dominant single and efficient AM spore can be used for mass production here. Sorghum, maize, gram and wheat were selected as the best host for starter culture of inoculum production. In the technique, earthen funnels were taken for the germination of seeds. Observation was continued until the root of the seedlings touched the inoculum of AM fungi. The seedlings were raised up to 30 days in the earthen funnels containing sterilized sand and soil at the ratio of 1:3. In the present investigation, 40 g sand was taken against 120 g of soil. The experiment was repeated up to 45-90 days and AM spores were collected by wet sieving and decanting technique" [10,11]. The spores of *Glomus* sp. and *Acaulospora* sp. were utilized in the present investigation for mass multiplication using hosts like *Zea mays* L. (maize) and *Cicer arietinum* L. (chickpea) in bigger earthen pots.

2.7.2 Pot culture

Mass multiplication of dominant AM spores like *Glomus* sp. and *Acaulospora* sp. were carried out using different hosts and substrates in pots. Maize (*Zea mays* L.) and chick pea (*Cicer arietinum* L.) was observed as best hosts for pot cultures of mycorrhizal spores. Sand and soil (1:3) were used as substrates for pot cultures. The pot cultures were maintained for several days. The pots were supplemented with Hoagland solution once in a fortnight. However, KH_2PO_4 was removed from the original solution to observe the effect. The soil containing mycorrhizal spores, mycelium and colonized roots was used, further, to inoculate seedlings and to prepare other pot cultures.

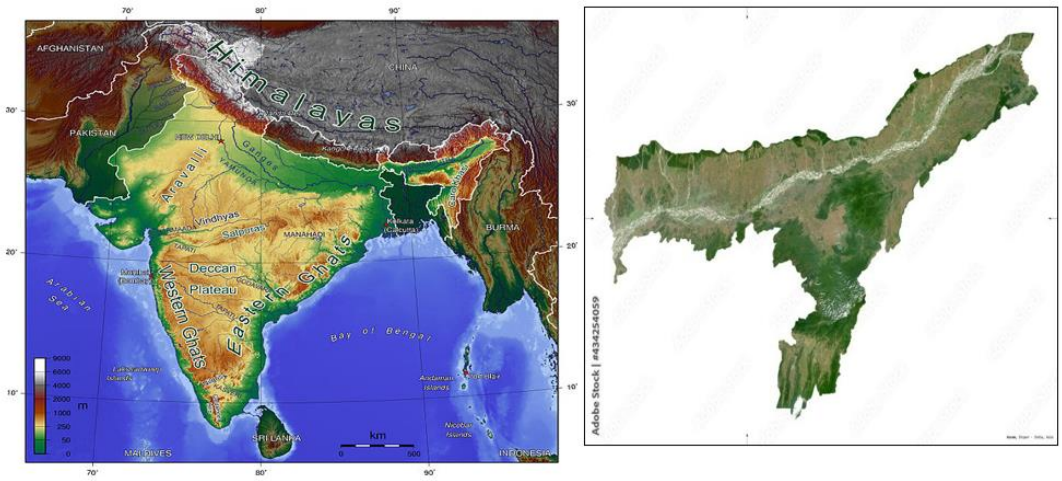

Fig. 1. Study site

2.8 Field Culture

The test inoculants were mass multiplied in field conditions by preparing standard size beds on thin polyethylene sheet (0.5 mm). Care was taken so that no contamination occurred to the inoculants. The experiment was repeated for maintaining the inoculants cultures more viable for further experimentation.

2.9 Mass Multiplication of Selected Fungal Isolate

Trichoderma, being a potent fungal biocontrol agent is known for its antagonistic action against a range of plant pathogens [27,28]. Different organic media like neem cake, coir pith, farmyard manure, decomposed coffee pulp are being used for *Trichoderma* multiplication [29]. However, the method of Parkash and Saikia, [30] was employed in the present investigation for the mass multiplication of *Trichoderma* spp.

2.10 Collection of Soil Samples and Isolation of Compost Fungal Activator

The soil samples were collected from different locations of Assam and Meghalaya of N. E. India. *Trichoderma harzianum,* the fungal stain was isolated from the collected soil samples by using serial dilution plate method on PDA medium. The inoculated plates were incubated at 30^{o}C for 4 days. Fungal colonies were purified by streak plate method on agar slants and incubated at 30^{o}C for 7-8 days. Green conidia forming fungal bodies were selected and microscopic observation was made for fungal identification. In the present investigation, the fungus was identified as *Trichoderma harzianum* (Isolate no. TH-13). The identified fungal isolate was maintained on PDA slants is retained with Mycology and Soil Microbiology Laboratory, (RFRI), Jorhat, Assam, India, for further study and analysis.

2.11 Preparation of Solid Substrate

Saw dust of *Shorea robusta* Gaertn. was used in the present investigation for solid substrate preparation. For this, saw dusts were shade dried and mixed well with wheat bran by adding SDW in the ratio of 3:1:4 w/w where, 03 parts of wheat bran is mixed with 01 parts of saw-dust and 04 parts of water. The moisture of the mixture was maintained up to 50–60%. Autoclaving was done to sterilize the substrate properly.

2.12 Mass Multiplication of *Trichoderma harzianum*

Trichoderma harzianum was grown on synthetic PDA medium (SRL, India) for 7-8 days and incubated at 27–30^{o} ± 1^{o}C. The inoculum was kept in BOD incubator (Labotech, BDI-55 make, India) for 10–12 days for maximum growth and sporulation. The inoculum containing medium was cut into small discs and put in flasks containing wheat bran and saw-dust medium in the ratio of 3: 1: 4 w/w for mass multiplication of *Trichoderma harzianum*. Approximately 50 g substrate was put in 500 ml conical flasks followed by inoculation with 5 mm mycelial mat. It was then incubated at 28 ˚C for 7–10 days. The target bioagent in the form of substrate inoculum

was applied at the time of sowing of target seedlings in the nursery of RFRI, Jorhat, Assam N. E. India.

2.13 Mass Multiplication of Selected Bacterial Isolate/s Mass Multiplication of *Frankia SP.*

Frankia sp. (Isolate FS-I) was mass multiplied on three selective medium such as Nutrient Broth (NB; Murry, Fontaine & Torrey's BAP Medium and Frankial defined minimal (FDM) medium and cultivated for 12 hours in orbital shaking BOD Incubator to record the growth characteristics of the isolated bacteria as well as to observe the effect of inoculums volume on the growth of bacteria.

2.14 Investigation on the Efficacy of Selected Indigenous Putative Bioagents/Microsymbionts on the Target Plant Species In Nursery and Their Role in its Establishment and Conservation

Randomized Block Design (RBD) was made in the nursery of RFRI, Jorhat Assam, India. Selected bioagent was inoculated on the seedling stock of target plant species, *Elaeagnus lalifolia* L. Three different inoculation procedures such as single, double and synergistic were adopted in the present investigation. Three replicates were maintained for each treatment. A treatment without any inoculum served as control. Root trainer (30cm×50m) containing 500 g of substrates like sand and soil at the ratio of 1:2 was used for each treatment along with its replication. Inoculum was applied close to the rhizosphere of the seedlings (at the depth of 5-10 cm).

In our experiment, four treatments were undertaken consisting of mono, dual and tri inoculums of the selected indigenous bioagents consisting of *Glomus mosseae*, *Frankia* sp*.* and *Trichoderma harzianum*. For convenience, the various synergistic treatments were labelled as follows:

C= Control (Non inoculated)

Fs-01 =only *Frankia* sp.

Fs-02 =Treatment/Inoculation of *Frankia* sp. +*Trichoderma* sp.

Fs-03 =Treatment/Inoculation of *Glomus* sp.+ *Frankia* sp.,

Fs-04 = Treatment/Inoculation of *Frankia* sp. + *Glomus* sp. + *Trichoderma* sp.

Different plant growth parameters like shoot height (%), root and shoot dry weight were recorded regularly to evaluate the efficacy of bioinoculants and nodular micro-endosymbiont/s on the growth and development of target plant seedlings.

2.14.1 Bio volume index

Biovolume index of the seedlings were calculated in accordance with Parkash et al., (2011) and Hatchell [31,32].

$B_i = H \times D$

Where B_i = Biovolume index

H= Height of seedlings in cm

D= Diameter of stem in (mm/cm)

Diameter was calculated with the following formula:

$C = \pi r^2$ where C is the circumference or girth of stem of seedlings

$r^2 = \frac{c}{\pi}$, So, D = 2r

2.14.2 Sturdiness quotient (Sq)

The sturdiness quotient reflects the stocky or spindly nature of the seedlings. It is of particular importance in container-grown seedlings where the Sq can get very high on undesirable spindly stock [32]. Sq was calculated with the following formula.

$$Sturdiness\ Quotient\ (Sq) = \frac{Height\ of\ the\ plant}{Diameter\ of\ the\ plant}$$

2.15 Plastochron Interval Index

The plastochron interval index of the seedlings was evaluated after Ist inoculation, using the standard protocol [33].

2.16 Data Analysis

Standard error of means and co-efficient of variance were determined for all the parameters like physico-chemical, mycorrhizal quantification,

growth and yield. Species richness and diversity, similarity and dissimilarity indices of AM fungi were computed using MS Excel software 2007 and SPSS software version 16.0.

3. RESULTS AND DISCUSSION

The increase in height after 90 days of inoculation showed maximum height increase in FS-03 (Treatment/ Inoculation of *Glomus mosseae* + *Frankia* sp.), whereas, minimum increase in height was recorded in FS-04 treatment (Treatment/ Inoculation of *Frankia* sp. + *Glomus mosseae* + *Trichoderma harzianum*) (Fig 2). This results are in accordance with the results obtained by Oliveira et. al., [34] where after a 6-month growth period, alder plants inoculated with both symbionts had significantly greater leaf area, shoot height and total biomass when compared with the uninoculated control, the *Frankia* spp. and the *G. intraradices* treatments alone. However, contradicted to the above mentioned results, in a study [35], it was observed that seedling height were significantly higher in *Casuarina equisetifolia* inoculated with *Frankia* alone. This is due to the host specific symbiosis between *Frankia* and *Casuarina equisetifolia*. The increase in diameter after 90 days of inoculation showed maximum diameter increase (1.3 ±0.25) in FS-03, whereas, minimum increase in diameter (0.5 ± 0.70) was recorded in FS-01 and FS-02 treatment. The coefficient of variance was found to be 0.45 after analysis **(**Fig 3**).** The Quality index was maximum (1.87 ±0.20) in case of FS-02 treatment while it was minimum (1.31 ±0.025) in case of C (Fig 4.). The biovolume index was also maximum (280.96 ±20.0) in FS-04 treatment, while it was minimum (167.53 ±30.0) in FS-01 treatment (Fig 5). The sturdiness quotient (SQ) were also analyzed for the inoculated seedlings and it was found that FS-04 had the highest SQ of 0.25 while the lowest was observed in C (0.20) after 90 days of inoculation (Fig 6).

Plastochrone interval index of *E. latifolia* L. was studied and tabulated in Table 1. It is revealed that the first to second leaf primordium was initiated in control treatment after long interval of 5 days and it ceased after 45 days (7^{th} leaf primordium). However, FS-02 took the longest time interval of 6 days for the initiation of the first leaf primordia. FS-04 treated seedlings continued initiation of leaf primordium even till the 8^{th}-9^{th} leaf primordium.

In our experiment, after 90 days of inoculation, it was observed that the shoot biomass was maximum (40.71±0.15) in Control and it was minimum (24.98 ±0.15) in Fs-02 treatment (Fig. 7). The root biomass was maximum (25.84 ±1.0) in Fs-02 treatment, whereas it was minimum (15.25 ±0.25) in Control (Fig 8). The total biomass yield was maximum (56.88 ±5.5) in Fs-03 treatment and minimum (42.87 ± 5.05) in Fs-01 treatment (Fig 9).

It was observed that, treatment Fs-02 exhibited the maximum increase in leaf length (10.5 ±0.25) and least increase in leaf length was observed in Control (4.6 ±0.20) (Fig 10). The maximum increase in leaf breadth was recorded in Fs-02 (4.5 ±0.25) and the minimum increase in leaf breadth was observed in Control (2.3 ± 0.70) (Fig 11). Regarding the increase in leaf area, the maximum increment was recorded in Fs-02 (30.75±0.05) and the minimum was found in Control (8.38 ±0.15) in sq. cm. (Fig 12). The number of stomata was maximum (40.67 ±1.0) in Fs-01 treatment, whereas it was minimum (25.33±0.25) in Fs-03 treatment (Fig 13). Regarding the number of nodules, the maximum number of nodules were formed in treatment Fs-01 (44) and the least number of nodules were formed in treatment control (13) (Fig 4).

Prior research has indicated that co-inoculating AM fungi with other plant rhizosphere microorganisms increases their effect [36,37]. They noticed that the inoculation of seedlings with the consortium, followed by *G. mosseae, A. laevis, and G. gigantea*, resulted in a significantly higher biomass of shoot and root. Again, the control seedlings showed minimal biomass in their shoots and roots. All inoculation treatments had higher Biovolume indexes (Bi) than the non-inoculated control group; however, consortium treatments had Bi that was comparable to the other inoculation treatments. In comparison to *G. mosseae* and *G. gigantea* treatments, the quality index (Qi) value was likewise higher in the mixed consortium and *A. laevis* treatments, respectively. The Qi value of the control seedlings was low. Despite the fact that the *G. mosseae* treatment made the seedlings taller, biomass was not boosted by more leaves or lateral branches, which is why the Qi value was low. Both *A. laevis* by itself and the consortium treatment (*G. mosseae* + *A. laevis* + *G. gigantea*) showed promise as bioinoculants for raising Garden Rue biomass. Additionally, higher Qi and Bi values were seen in these therapies, indicating that they are of the highest calibre.

Table 1. Plastochron interval index: Time interval (in days) for the initiation of 1st to 2nd to 3rd to 4th& so on leaf primordial

Treatment	Plastochron interval index: Time interval (in days) for the initiation of 1st to 2nd to 3rd to 4th& so on leaf primordia													
	1st - 2nd	2nd- 3rd	3rd- 4th	4th - 5th	5th - 6th	6th - 7th	7th- 8th	8th- 9th	9th- 10th	10th- 11th	11th- 12th	12th- 13th	13th- 14th	14th- 15th
C	5	4	7	9	6	14	0	0	0	0	0	0	0	0
Fs-01	5	3	4	12	7	2	7	5	0	0	0	0	0	0
Fs-02	6	3	4	12	8	2	7	0	0	0	0	0	0	0
Fs-03	3	3	8	6	12	4	0	0	0	0	0	0	0	0
Fs-04	2	3	4	4	8	4	6	7	0	0	0	0	0	0

C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum

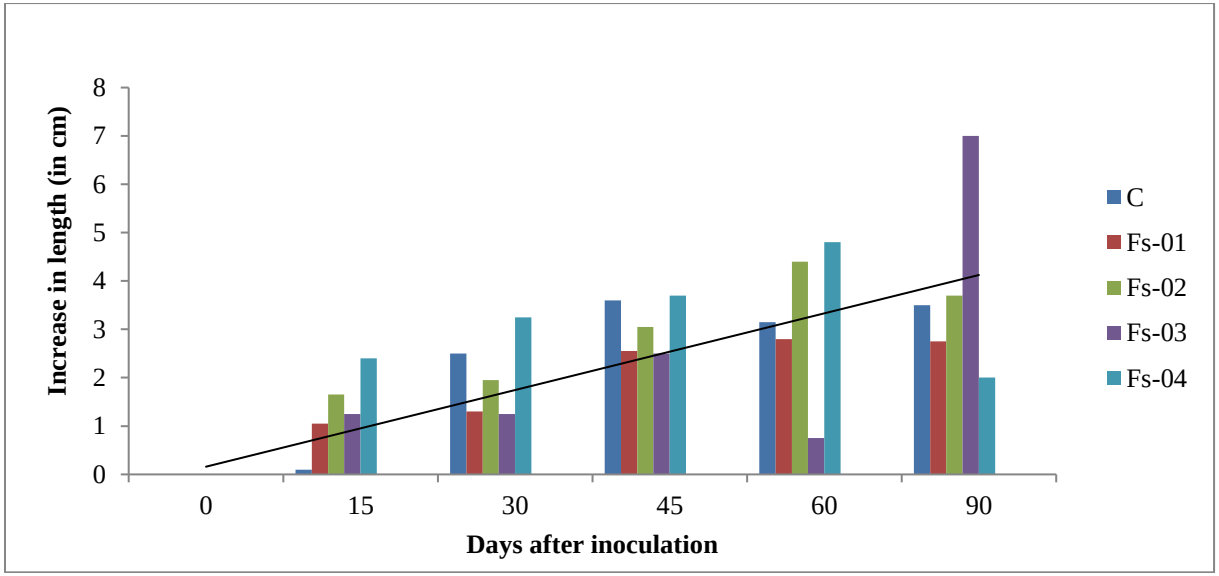

Fig. 2. Histogram showing increase in height of *E. latifolia* L. inoculated seedlings after 90 days of second stage inoculation

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum ; * Data of three replications*

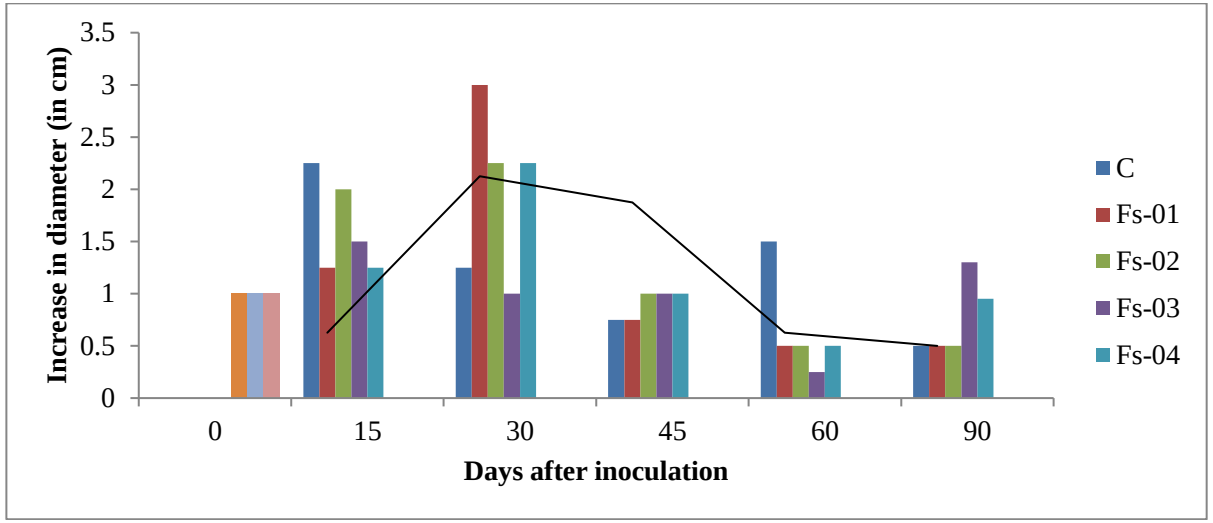

Fig. 3. Histogram showing increase in diameter of *E. latifolia* L. inoculated seedlings after 90 days of second stage inoculation

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

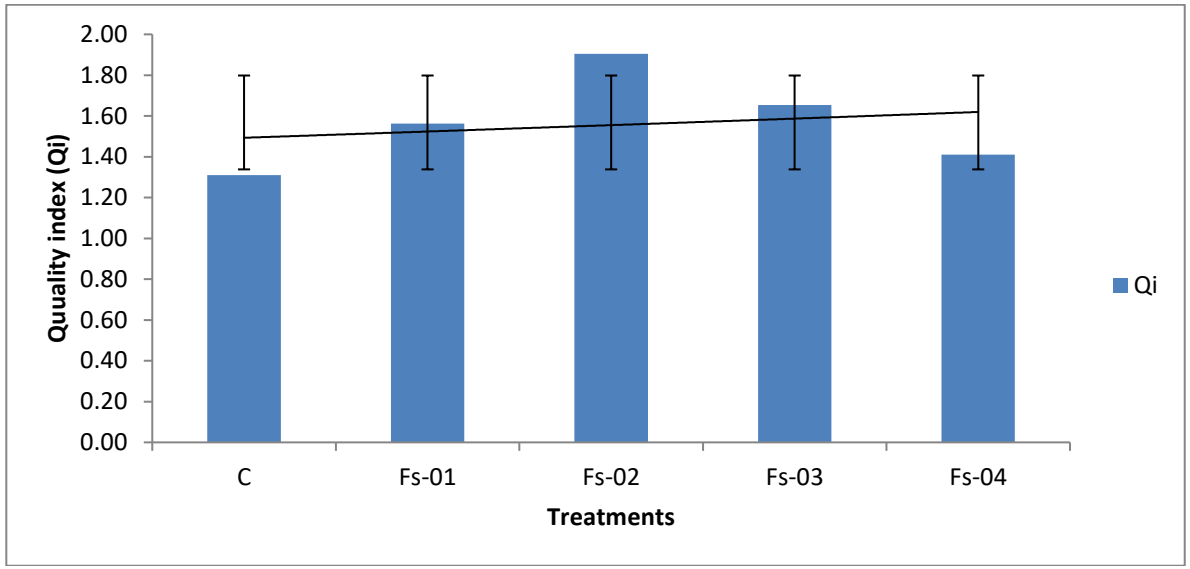

Fig. 4. Quality index of the inoculated seedlings (90 DAI) of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

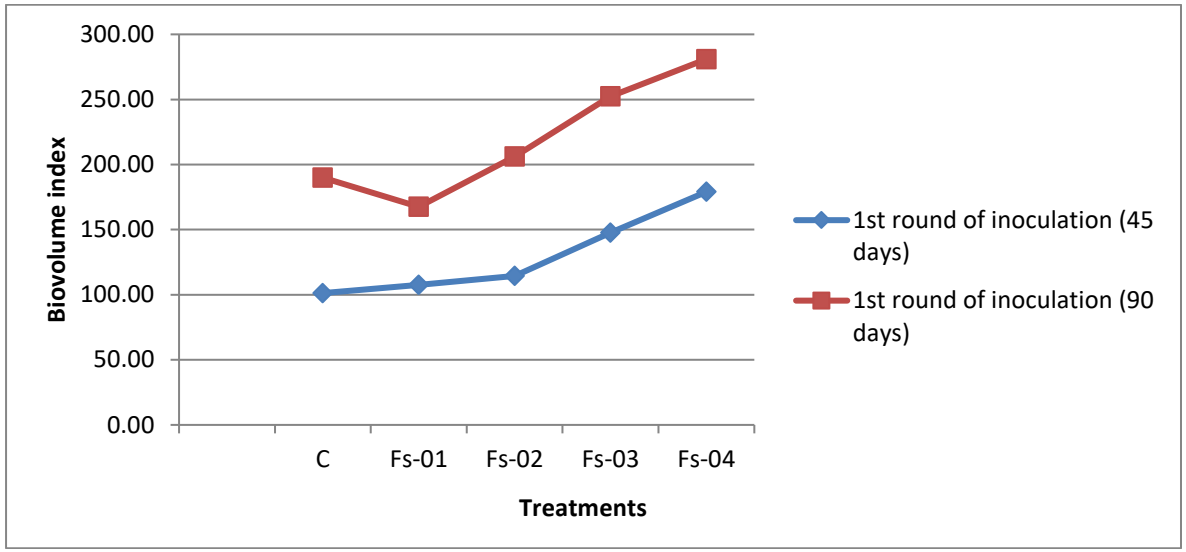

Fig. 5. Line diagram depicting the Biovolume index of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

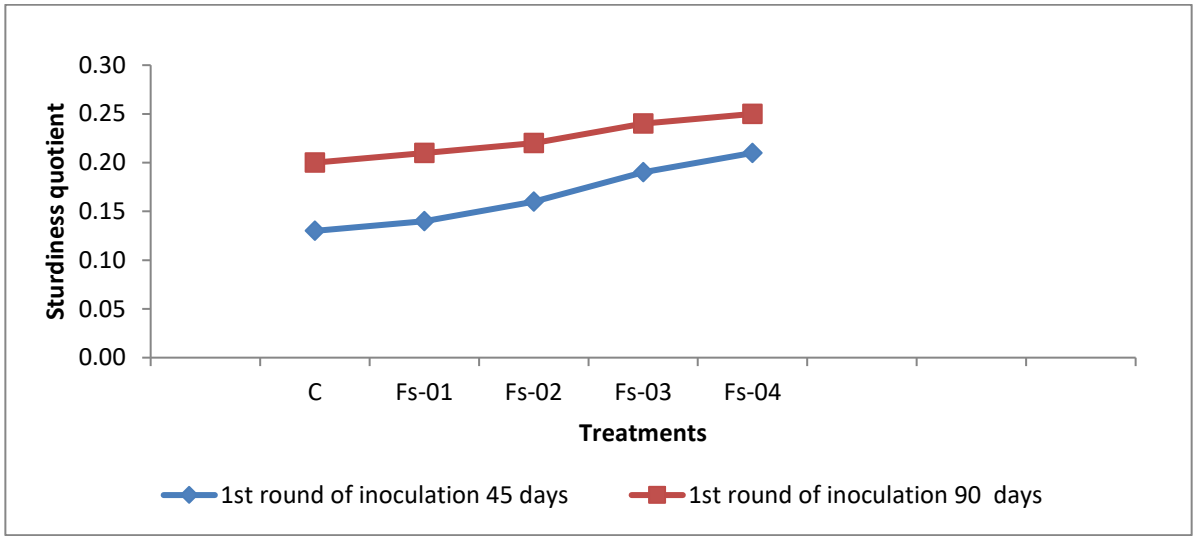

Fig. 6. Line diagram depicting sturdiness quotient of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

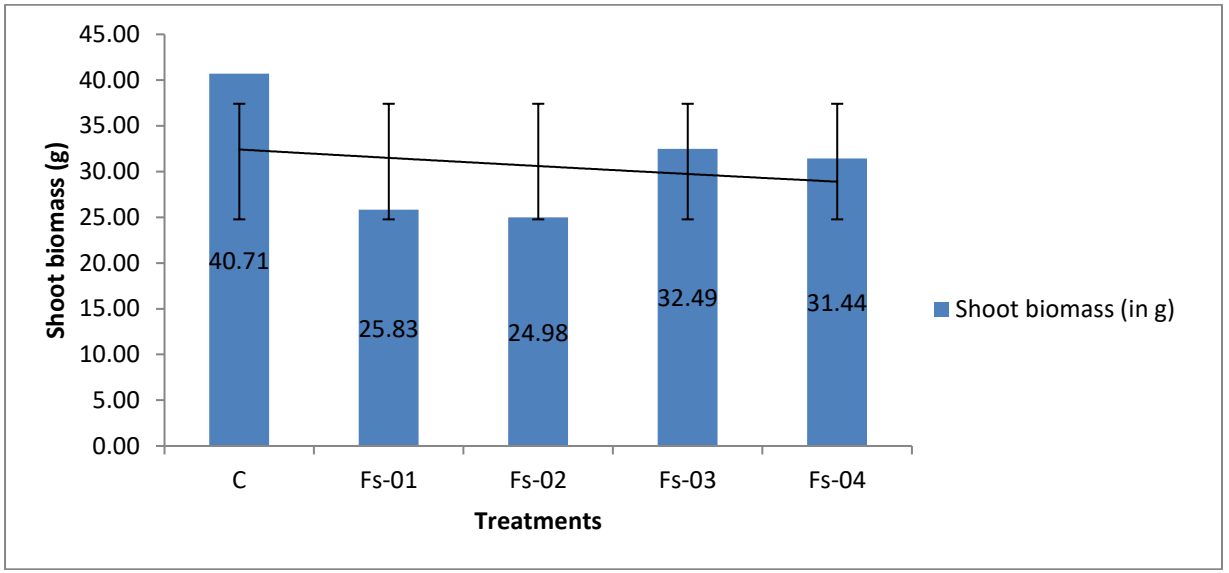

Fig. 7. Shoot biomass (in g) of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

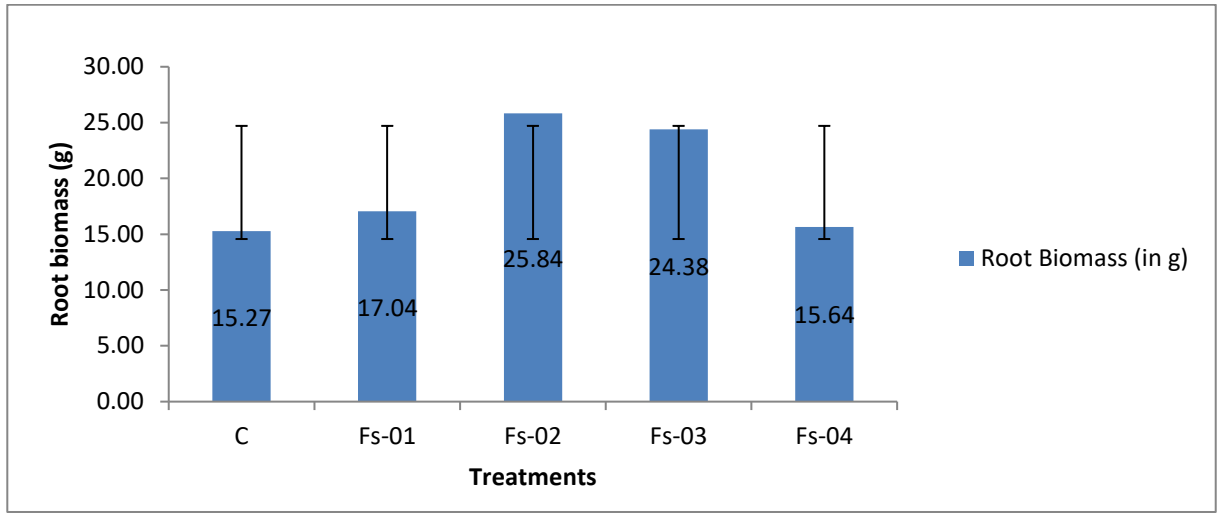

Fig. 8. Root biomass (in g) of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

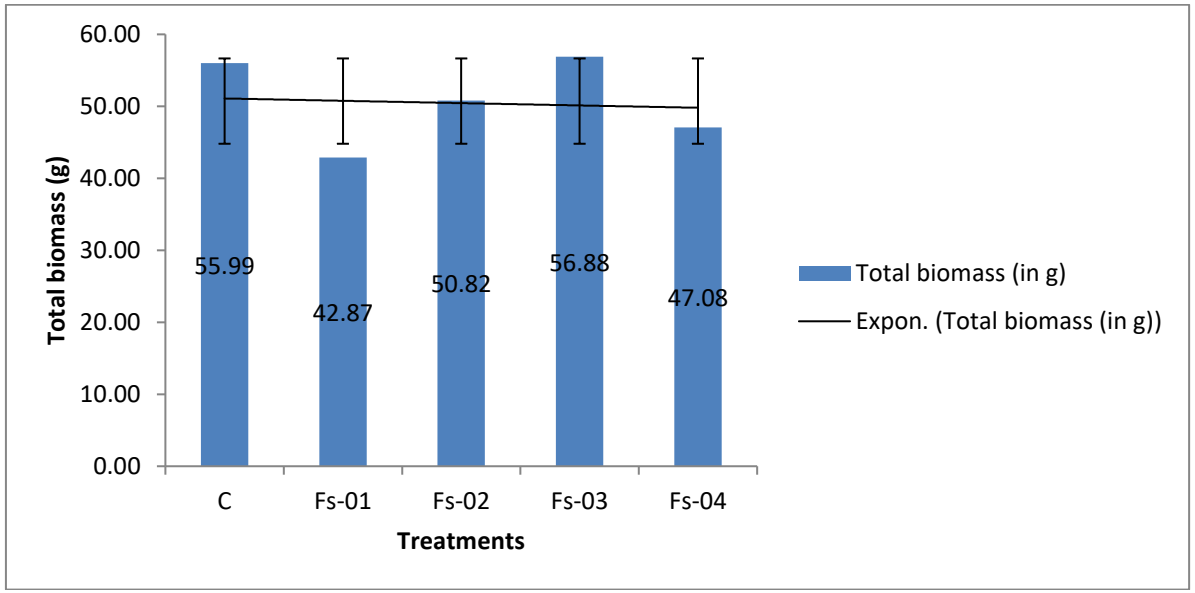

Fig. 9. Total biomass (in g) of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum ; * Data of three replications*

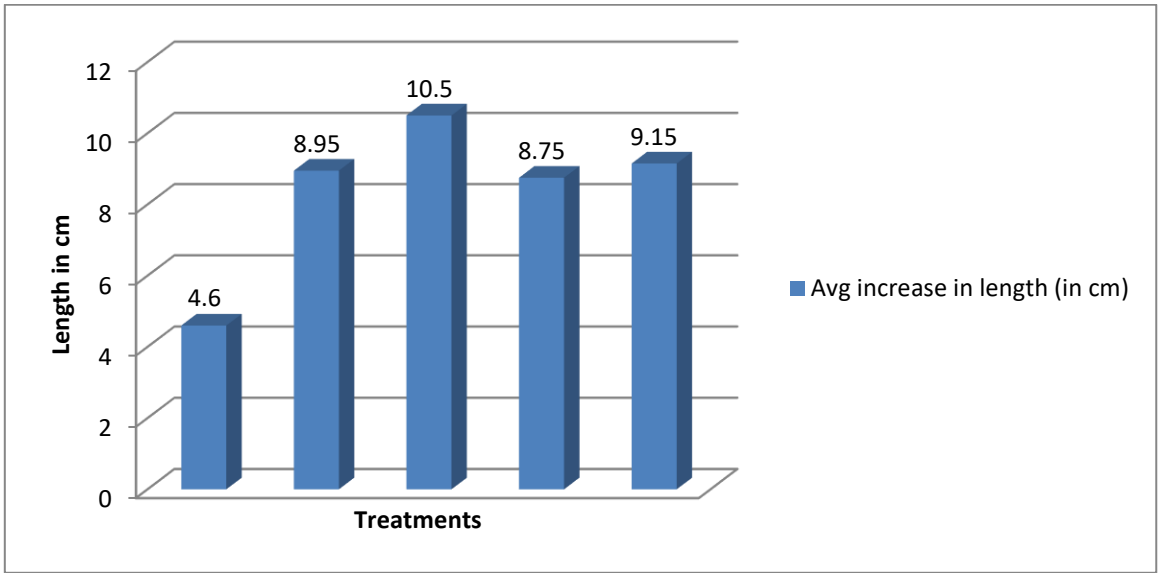

Fig. 10. Average increase in leaf length (in cm) of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum ; * Data of three replications*

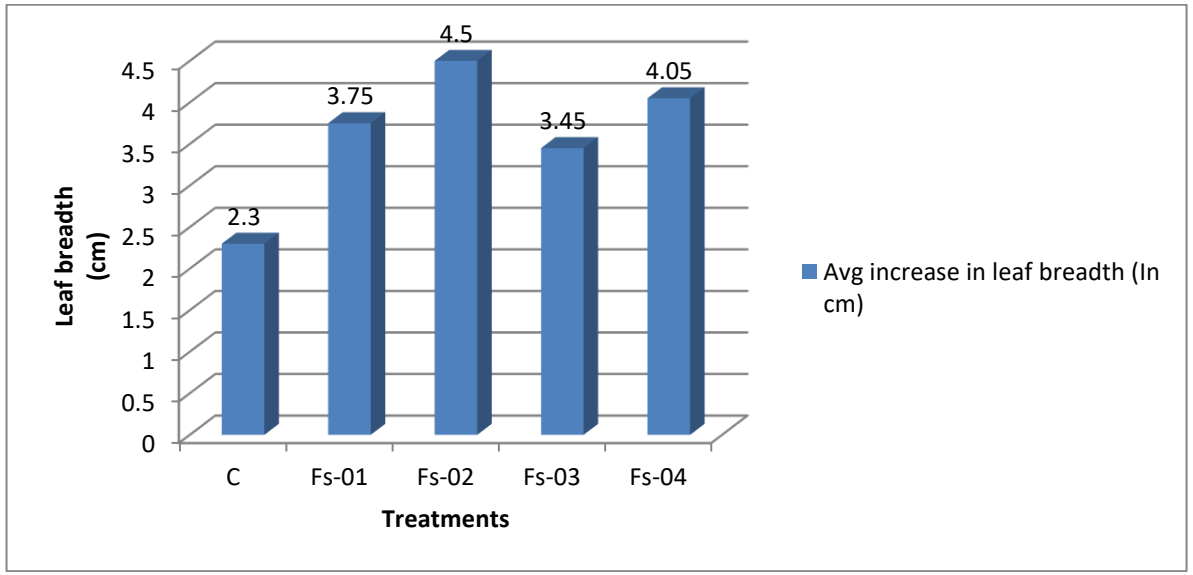

Fig. 11. Histogram showing the average increase in leaf breadth (in cm) of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

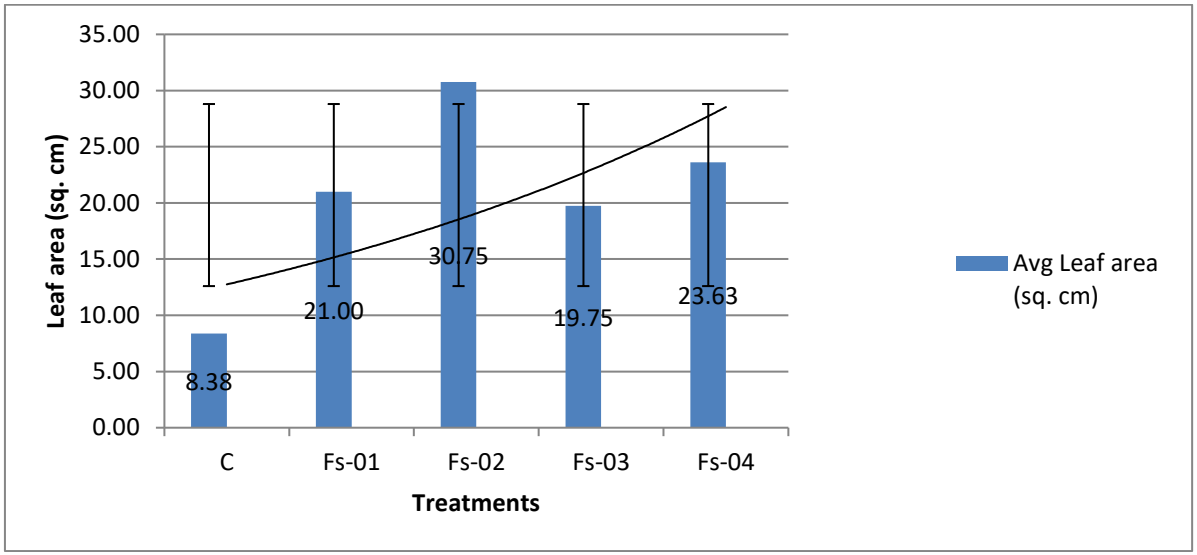

Fig. 12. Histogram showing average increase in leaf area (square cm of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

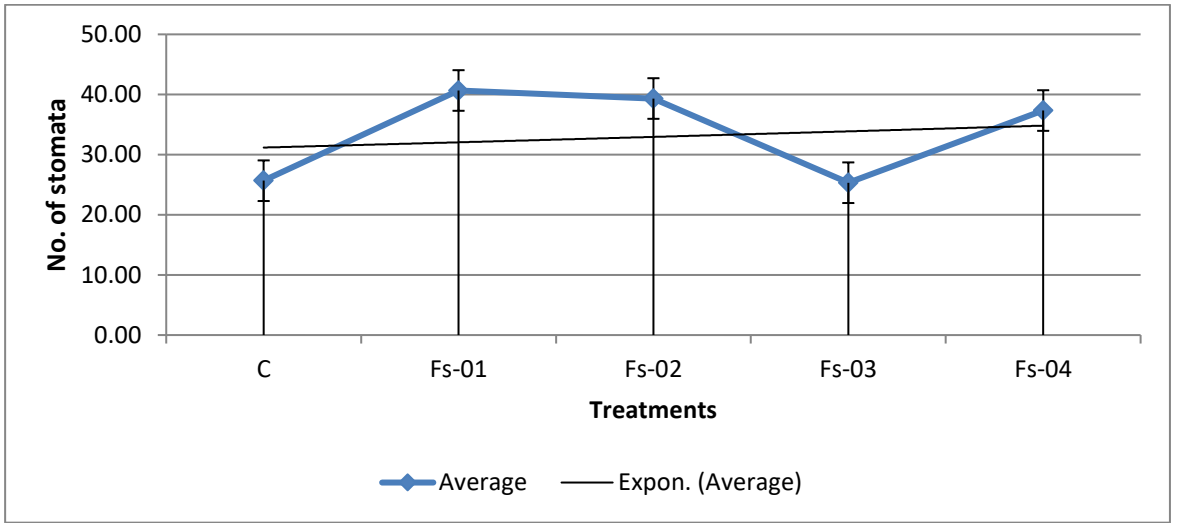

Fig. 13. Effect of inoculation in anatomy of leaf of *E. latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum; * Data of three replications*

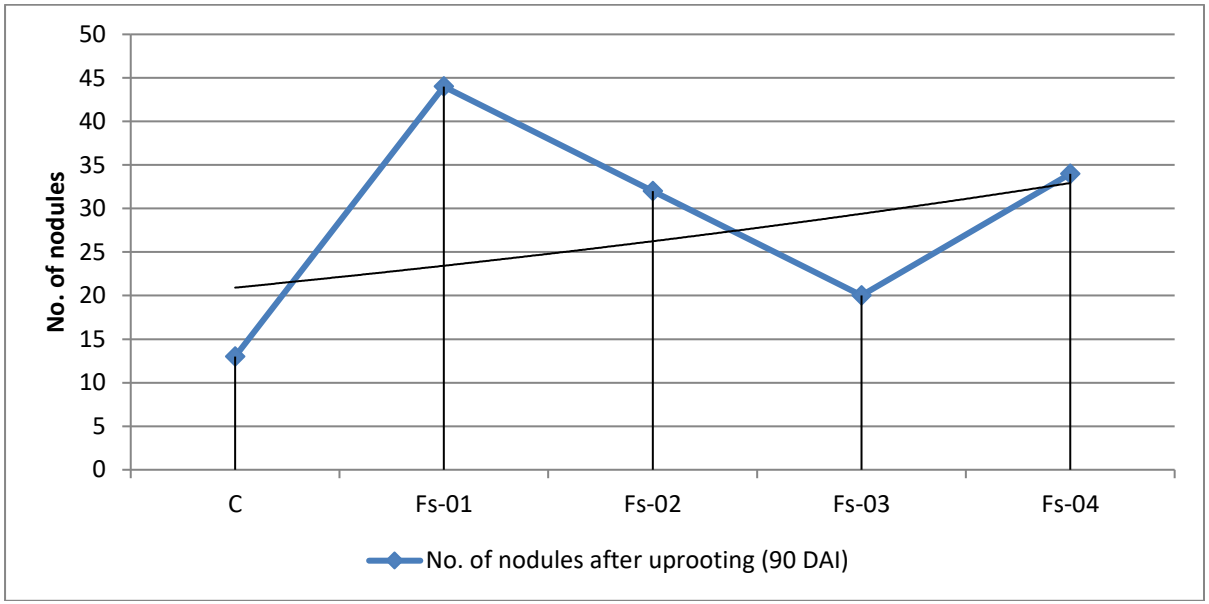

Fig. 14. Number of nodules after uprooting (90 DAI) of *E.latifolia* inoculated seedlings

*C= Control, Fs-01= Only Frankia sp., Fs-02= Inoculation of Frankia sp. + Trichoderma harzianum, Fs-03= Inoculation of Glomus mosseae + Frankia sp., Fs-04= Inoculation of Frankia sp. + Glomus mosseae + Trichoderma harzianum ; * Data of three replications*

4. DISCUSSION

Pan et al., [38] studied “the effect of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Bacteria (PGPR) inoculations on *Elaeagnus aangustifolia* L. in saline soil. The results indicated that, for one-year-old seedlings of *Elaeagnus angustifolia* L., AMF significantly promoted biomass accumulation in aboveground organs, increased the numbers of leaves and branches, and improved the leaf areas, stem diameters and plant height. AMF-mediated morphological characteristics of aboveground organs favoured light interception and absorption and maximized the capacities for photosynthesis, transpiration, carbon dioxide assimilation and gas exchange of *Elaeagnus angustifolia* L. seedlings in saline soil. AMF also promoted root growth, modified root architecture, and enhanced soil enzyme activities”. “*Elaeagnus angustifolia* L. was more responsive to specific inoculation by AMF than by a combination of AMF and PGPR or by solely PGPR in saline soils. Plants cultivated in soil infected with *Glomus fasciculatum* have been found to exhibit higher levels of phytochemical components, fresh and dry shoot and root weight, chlorophyll content, and mycorrhizal colonization” [39]. “The function of the tetra partite interaction of *Alnus sieboldiana, Pseudomonas putida*, a rhizobacterium, *Frankia*, and *Gigaspora margarita*, an arbuscular mycorrhizal fungus, in the growth, nitrogen fixation, and mineral acquisition of *A. sieboldiana* was studied” [40]. The findings demonstrated a synergistic interaction between the rhizobacterium, *Frankia*, and mycorrhizal fungus on *A. sieboldiana* growth. Growth and biomass of *Casuarina* were enhanced by association with *Frankia*. Furthermore, bacteria give plants a high level of tolerance to biotic and abiotic challenges in this symbiotic interaction. Through the use of *C. equisetifolia* infected with Frankia Ceq1, the significance of actinorhizal symbiosis in the rehabilitation of degraded soils was shown [41]. The growth and biomass of Alnus were increased by *Frankia* inoculation. Moreover, more *Frankia* strains were used to inoculate plants, which promote alder growth. *Glomus mossae* inoculation resulted in increased shoot and root dry weights in white clover plants [42]. Greater shoot and dry weight were observed in chick pea plants treated with *Glomus fasiculatum* [43].

A lot of benefits have been known about tripartite symbiosis (nitrogen fixers, plant growth-promoting rhizobacteria or mycorrhizal fungi, and AMF) by a lot of researchers. It was reported that inoculating *C. equisetifolia* plants with AMF, *Azospirillum*, *Frankia*, and phosphobacterium increased the overall height and biomass [3,44].

Price plants that were not inoculated had a much lower dry weight than plants that were colonized by local fungi [45]. It was discovered that the greatest increase in leaf area was observed in Fs-02 (Treatment/Inoculation with *Frankia* sp. + *Trichoderma* sp.). Comparable outcomes were also observed [46]. According to their findings, the inoculated plants' leaf area was significantly larger than that of the control. The plants that were inoculated with *G. mosseae* + *A. laevis* + *T. viride* + *B. japonicum* showed the highest increment in leaf area activity, whereas the control group showed the lowest. The single *G. mosseae* inoculation produced the second-best results, and the triple combination of *G. mosseae* + *A. laevis* + *T. viride* produced the third-best results. The findings show that mycorrhizal fungus, either applied alone or in conjunction with other bioinoculants, considerably increases the leaf area of soybeans. It was observed that in our experiment, treatment Fs-01 (Only *Frankia* sp.) produced the greatest number of nodules, whereas treatment control produced the fewest nodules. Higher shoot and nodule dry weight of mungbean were observed when *Rhizobium* strains were injected [47]. It is commonly known that effective Rhizobium strains that are more capable of completing the rhizobia's natural state not only produce more nodules per plant but also increase the dry weight of the shoot and root. In a study it was found that rhizobium inoculation resulted in higher root dry weight than control [48].

It was further observed that inoculating *Vigna radiata* with *Bradyrhizobium* and *Azotobacter* increased nodulation [49]. Soybeans were found to exhibit increased nodulation following either a single or combined inoculation with *Bradyrhizobium* and AMF [50]. Increased nitrogenase activity, more nodules, and dry weight were the outcomes of the mycorrhizal interaction with *Frankia* sp. on *Alnus nepalensis* [51]. They found that although the quantity and size of nodule lobes varied, the overall weight of lobes per plant did not differ significantly across treatments. The number of nodule lobes formed on plants grown on inoculated soil was higher than on plants that had been grown on non-inoculated soil. In contrast, the largest nodules were found on non-inoculated plants and lobes

from plants grown on inoculated soil were significantly smaller. In another study, the biomass and nutrient uptake of Sorghum plants increased after inoculation with PGPMs alone or in combination with mycorrhiza in a soil-based medium [52]. In another report it was found that, *Banana Berangan* seedlings showed an increase in chlorophyll content, biomass, and the growth of shoots and roots following inoculation with *Bacillus sphaericus* and *Azospirillum* sp. [53-55].

5. CONCLUSION

Climate change threatens natural systems, so research on beneficial effects of mycorrhizal plants (AM), PGPRs and PGPFs is crucial for increased plant tolerance to environmental stresses. Future functional genomics will help identify genes in the target plant species that play key roles in nutrient mobilization, abiotic stress alleviation, and disease suppression. Management of AM fungi, PGPFs and PGPRs and retaining microbial diversity in the rhizosphere are also important for sustainable agricultural strategies. Mycorrhizal and other biotechnological technology can reduce fertilizer and energy inputs while promoting healthy plant growth.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dimpka C, Weinand T, Asch F. Plant–rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environment. 2009;32:1682–1694.
2. Dodd I, Perez-Alfocea F. Microbial amelioration of crop salinity stress. Journal of Experimental Botany. 2012;3415–3428.
3. Singh M, Bisht S, Singh S, Sharma JG. Implications of abiotic stress tolerance in arbuscular mycorrhiza colonized plants: Importance in plant growth and regulation. Journal of applied biology and biotechnology. 2022;10(6):1-11.
   DOI: 10.7324/JABB.2022.100601
4. Mohanty P, Singh PK, Chakraborty D, Mishra S and Pattnaik R. Insight Into the Role of PGPR in Sustainable Agriculture and Environment. Frontiers in Sustainable Food System. 2021;5:667150.
   DOI: 10.3389/fsufs.2021.667150
5. Ahmed T, Shahid M, Noman M, Hussain S, Khan MA, Zubair, M. Plant growth-promoting rhizobacteria as biological tools for nutrient management and soil sustainability, in Plant Growth Promoting Rhizobacteria for Agricultural Sustainability, eds A. Kumar and V. S. Meena (Singapore: Springer). 2019;95–110.
   DOI: 10.1007/978-981-13-7553-8_5
6. Handique L and Parkash V. Locational variation in rhizosphericmycobiota associated with Elaeagnus latifolia, an endemic plant species of N.E. India. Nova Geodesia. 2023;3(4):158.
   Available:https://doi.org/10.55779/ng34158
7. Jeeva S. Horticultural potential of wild edible fruits used by the Khasi tribes of Meghalaya. Journal of Horticulture and Forestry. 2009;1(9):182-192.
8. Seal T. Antioxidant Activity of Some Wild Edible Fruits of Meghalaya State in India. Advances in Biological Research. 2011;5 (3): 155-160.
9. Chhetri RB. Trends in ethno-domestication of some wild plants in Meghalaya, North-east India. Indian Journal of Traditional Knowledge. 2006;5(3): 342-347.
10. Gerdemann JW and Nicholson TH. Spores of mycorrhizal *Endogene*extracted from soil by wet sieving and decanting. Transactions of British Mycological Society, 1963; 46: 235–244.
11. Singh SS and Tiwari SC. Modified wet sieving and decanting technique for enhanced recovery of spores of vesicular arbuscular mycorrhizal (VAM) fungi in forest soils. Mycorrhiza News. 2001;12:12-13.
12. Morton JB and Benny GL. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): New order, Glomales two new sub-orders Glomineae and Gigasporineae and two new families, Aculosporaceae and Gigasporaceae with

emendation of Glomaceae. Mycotaxon. 1990;37:471-491.

13. Mukerji KG. Taxonomy of Endomycorrhizal Fungi: In Advances in Botany, Mukerji KG, Mathur B, Chamola BP and Chitralekha P (eds.) APH Pub. Crop. New Delhi. 1996; 211-221.
14. Schenck NC and Perez Y. Manual for the identification of VA mycorrhizal (VAM) fungi.Univ. of Florida, Synergistic Pub., Florida, USA. 1990; 241.
15. Trappe JM. Synoptic key to the genera and species of zygomycetous mycorrhizal fungi. Phytophathology. 1982;72:1107-1108.
16. Walker C. Systematics and taxonomy of arbuscular endomycorrhizal fungi (Glomales) – A possible way forward. Agronomie. 1992;12:887-897
17. Gaur A and Adholeya A. Effects of particle size of soil-less substrates upon AM fungal inoculum production. Mycorrhiza. 2000;10:43–48.
18. Johnson LF and Curl EA. Methods for the research on Ecology of Soil Borne Plant Pathogens. Minneapolis Burges Publishing Company. 1972;247.
19. Gilman JC. A manual of soil fungi. Iowa State College Press, Ames, Iowa. 1957; 450.
20. Subramanian CV. Hypomycetes, an account of Indian species, except *Cercospora.* ICAR Publication, New Delhi; 1971.
21. Watanabe T. Photomicrographs and illustrations of soil fungi. Soft Science Publications, Tokyo (In Japanese.); 1993.
22. Domsch KH, Gams W and Anderson TH. Compendium of soil fungi.; Academic Press. London; 1980.
23. Cappuccino J and Sherman, GN. Biochemical activities of microorganisms. In: Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co. California, USA.
24. Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST. Bergey's manual of determinative bacteriology, Williams and Wikins co, Baltimore, USA. 1994;9:566
25. Cruickshank R. Medical Microbiology,; 11th ed. Livingstone, Edinburgh. 1965;646.
26. Menge JA and Timmer LW. Procedure for inoculation of plants with VAM in the laboratory greenhouse and field, 59. In: Methods and Principle of Mycorrhiza Research. (Ed. Schenck, N. C.),; American Phytopathology Society, St. Pauls; 1982.
27. Tewari AK, Mukhopadhyay AN. Testing ofdifferent formulations of *Gliocladium virens* againstchickpea wilt complex. Indian Phytopathology, 2001; 54: 67–71.
28. Rini CR and Sulochana KK. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. Journal of Tropical Agriclture. 2007;45:21–28
29. Saju KA, Anandraj M and Sarma YR. On farm production of *Trichoderma harzianum*using organic matter. Indian Phytopathology. 2002;55(3):277–281.
30. Parkash V, Saikia AJ. Production and Multiplication of Native Compost Fungal Activator by Using Different Substrates and Its Influence on Growth and Development of *Capsicum chinensis* Jacq. ''Bhut Jolokia''. Biotechnology Research International; 2015.
31. Parkash V, Aggarwal A, Sharma V. Rhizospheric effect of vesicular arbuscular mycorrhizal inoculation on biomass production of *Ruta graveolens* L. A potential medicinal and aromatic herb. Journal of Plant and Nutrition. 2011;34:1-11.
32. Hatchell GE. Biomass research. *Proc*. Third biennial Southern Silvicultural Res. Conf. Atlanta, Georgia, November, 7-8, 1984. General Technical Report, Southern Forest Experiments, Station, USDA Forest Service. 1985;54:395-402.
33. Parkash V. Utilization of Vesicular Arbuscular mycorrhizal diversity for the quality stock production of some useful forest plant/s species of NongkhyllemReserve forest, Nongpoh, Meghalaya, India. Project Completion Report; (RFRI-12/2008-09/SFM). Rain Forest Research Institute, Jorhat, Assam, India; 2012.
34. Oliveira RS, Castro PML, Dodd JC and Vosatka M. Synergistic effect of *Glomus intraradices* and *Frankia* spp. on the growth and stress recovery of *Alnus glutinosa*in an alkaline anthropogenic sediment. Chemosphere. 2005;60:1462–1470
35. Elumalai S and Raaman N. *In vitro* synthesis of *Frankia* and mycorrhiza with *Casuarina equisetifolia* and ultra structure

of root system. Indian Journal of Experimental Biology. 2009; 47:289-297.

36. Rashmi and Roy AK. Effect of VAM fungi and *Azospirilliumbrasilense*on growth performance of finger millet. Journal of Mycology and Plant Pathology. 2003;33 (3):403-406.
37. Parkash V, Aggarwal A, Sharma S and Sharma D. Effect of endophytic mycorrhizae and fungal agent on the growth and development of *Eucalyptus saligna*Sm. Seedlings. BulletinNational Institute of Ecology. 2004;15:127-131.
38. Pan J, Huang C, Peng F, Zhang W, Luo J, Ma S, Xue X. Effect of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Bacteria (PGPR) Inoculations on *Elaeagnus angustifolia* L. in Saline Soil. Applied Sciences. 2020;10:945. Available:https://doi.org/10.3390/app10030945
39. Selvaraj T. Studies on *Vesicular arbuscular mycorrhizae* of some crop and medicinal plants. Ph.D. Thesis, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India.1989;120.
40. Yamanaka T, Akio akama L, Ching-Yan and Hiroaki O. Growth, nitrogen fixation and mineral acquition of *Alnus sieboldiana*after incubation of Frankia together with *Gigaspora margrita*and *Pseudomonas putida*. The Japanese Forest Society and Springer. Journal of Forestry Research. 2005;21 -26.
41. Tani C, Sasakawa H and Takenouchi K. Isolation of endophytic *Frankia* from root nodules of *Casuarinaequisetifolia*and infectivity of the isolate to host plants. Soil Science and Plant Nutrtion, 2003;49:137–142.
42. Li XL, Marschner H and George E. Acquisition of Phosphorus and copper by arbuscular mycorrhizal hyphae and root to shoot transport in white clover, Plant and Soil. 1991;136:49-57.
43. Champawat RS and Pathak VN. Effect of vesicular-arbuscular mycorrhizal fungi on growth and nutrition uptake of pearl millet. Indian Journal of Mycology and Plant Pathology. 1993;23(1):30-34.
44. Rajendran K, Devaraj P. Biomass and nutrient distribution and their return of *Casuarina equisetifolia*inoculated with biofertilizers in farm land. Biomass Bioenergy. 2004;26:235-49.
45. Dhillion SS. Evidence for host-mycorrhizal preference in native grassland species. Mycological Research. 1992;94:359-362.
46. Alpa N, Tanwar A, AggarwalA and Gupta KK. Impact of endomycorrhizal fungi and other bioinoculants on growth enhancement of *Glycine max* (L.) Merrill. Journal of Applied and Natural Science. 2012;4(1) :111-116.
47. Bhuiya ZH, Islam MR, Ddin MJ and Hoque MS. Performance of some *Rhizobium* inoculants on blackgram (*Vigna mungo*). Bangladesh Journal of Agriculture. 1986; 11:55-63.
48. Begum H, Mohammed S, Rao GK, Raj BB. Efficacy of bleaching powder on the incidence of late wilt and grain yield in maize. Indian Journal of Plant Protection. 1989;17:219–222.
49. Yadav AS, Vashisht RK. Associative effect of *Bradyrhizobium*and *Azotobacter* inoculation on nodulation nitrogen fixed and yield of mungbean (*Vigna radiata* (L) wilcrek). Indian Journal of Microbiology. 1991;31(3):297-299.
50. Singh HP. Response to inoculantion with *Bradyrhizobium*, Vesicular arbuscular mycorrhizaand phosphate solublizing microbes on soybean in a Millisol. Indian Journal of Microbiology. 1994;34:27-37.
51. Tiwari SC. *Alnus nepalensis* D. Don biomass production and growth response to inoculation with *Frankia* and vesicular arbuscular mycorrhiza, In: Adholeya, A. and Singh, S. (Eds.) Mycorrhizae: Biofertilizers For The Future. TERI, Lodhi Road, New Delhi, India. 1995;184-188.
52. Ojuederie OB, Babalola OO. Microbial and plant-assisted bioremediation of heavy metal polluted environments: A review. International Journal of Environmental Research. Public Health. 2017;14:1504.
53. Baset MM, Shamsuddin ZH, Wahab Z, Marziah M. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured' musa'plantlets under nitrogen-free hydroponics condition. Australian Journal of Crop Science. 2010;4:85–90.
54. Goswami M, Chakraborty P, Mukherjee K, Mitra G, Bhattacharyya P, Dey S, Tribedi

P. Bioaugmentation and biostimulation: A potential strategy for environmental remediation. Journal of Microbiology and Experimentation. 2018;6:223–231.

55. Dhawi F. The Role of Plant Growth-Promoting Microorganisms (PGPMs) and Their Feasibility in Hydroponics and Vertical Farming. Metabolites. 2023;13:247. Available:https://doi.org/10.3390/metabo13020247

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