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Bioinoculation Strategies for Enhanced Growth and Development of Economically Important *Dalbergia latifolia* Roxb

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Abstract

The present experiment was conducted to evaluate the plant growth properties of the native rhizospheric microflora of *Dalbergia latifolia*, an important medicinal, timber and biofuel plant. A systemic isolation, characterization and identification of rhizospheric soil, collected indigenously, initially produced 45 numbers of fungi and 3 numbers of bacteria. Both groups of organisms were characterized for their extracellular useful activity, especially phosphate solubilisation potential, and presented in terms of solubilisation index and solubilisation efficiency. Two fungi and two bacteria with good phosphate solubilization activity, along with NPK and organic manure, were selected and used in a pot culture experiment to promote the growth and development of *Dalbergia latifolia* under nursery conditions. Data recorded on 90 Days of plant growth exhibited the growth promotion of inoculated plants over control uninoculated experimental set. Results obtained on morphological and physiological growth parameters exhibited that the application of *Aspergillus flavus* followed by *Burkholderia territorii* + org. manure showed maximum shoot height and inoculation of *Burkholderia anthina* + *Aspergillus sp*. + NPK was significantly higher leaf area. Significantly longer root has been observed in *Burkholderia territorii*. However, a difference was observed in the number of leaves in *Aspergillus flavus*. + Org. manure and higher numbers of branches were observed in *Aspergillus flavus* only as compared to uninoculated control. Coinoculation of *Aspergillus flavus* + *Penicillium simplicissimum* showed better performance in fresh and dry shoot biomass as compared to other treatments. *Burkholderia territorii* + Org.manure produced higher fresh biomass of roots and coinoculation of bacteria *Burkholderia territorii* + *Burkholderia anthina* helped in enhancing root dry biomass. Dual inoculation of *Aspergillus flavus* + *Penicillium simplicissimum* gives better result in both fresh biomass of leaves and dry biomass of leaves. Application of *Aspergillus flavus* + *Penicillium simplicissimum* enhanced the total fresh biomass of the seedlings whereas supplementation of *Burkholderia anthina* + *Penicillium simplicissimum* enhanced the total dry biomass. Coinoculation of *Aspergillus flavus* + *Penicillium simplicissimum* showed higher RGR and coinoculation of *Burkholderia anthina* + *Aspergillus flavus* + NPK showed quite higher Leaf area ratio (LAR cm^2gm^{-1}). Co inoculation of *Burkholderia territorii* + *Burkholderia anthina* + Organic Manure showed highest root-shoot ratio and Coinoculation of *Penicillium simplicissimum* + Org. manure showed higher Net assimilation rate (NAR). However, Bacterial and fungal inoculants were also used as potent bioinoculants for the growth and establishment of crops of forest tree species.**Keywords:** Dalbergia latifolia; Aspergillus flavus; Penicillium simplicissimum; Burkholderia territorii; Burkholderia anthina.

1. Introduction

Dalbergia latifolia Roxb. a member of the Fabaceae family and subfamily Papipionoideae, is a highly valued timber tree that is commonly found in Bihar, Bundelkhand and Central India [1, 2]. The tree has thin compound leaves, gray bark that peels in long fibres, thin, irregular, short cracked compound leaves, and branches of small flowers. It possesses a long cylindrical bole with high density and strength and is in high demand internationally due to its superior wood properties [3, 4]. However, the increasing demand for this wood due to its high quality properties has led to a continuous reduction in forest land. The regeneration status of these plants in forest areas is unknown [5]. For this purpose, monitoring *D. latifolia's* natural habitat is crucial. Regeneration of unknown forest areas and preservation of valuable tree species are required in natural forests. Apart from its woody properties, it has a lot of pharmacological properties. Tannins, one type of phenolic compound produced from the bark are used for the treatment of diarrhea, worms, indigestion and leprosy [6, 7]. The bark extracts of *D. latifolia* showed a potent reduction in glycemia, which indicates an ability to reduce blood sugar levels [8].

Research over the years has demonstrated the positive effects of various microorganisms on plant growth. Some reports have emphasized the importance of bio inoculants in improving plant health. Specific studies have highlighted the benefits of nitrogen-fixing bacteria such as Azospirillum and Rhizobium, and phosphate- solubilizing bacteria, including Trichoderma species and arbuscular mycorrhizal (AM) fungi [9, 10]. When these microorganisms are used either individually or in combination, they have been shown to significantly increase chlorophyll and haemoglobin content in plants. As discussed by Gupta et al. in 2020, the development of bio inoculants from microorganisms used an alternative approach to decrease the reliance on chemical fertilizers [11]. Bio inoculants, which contain beneficial microorganisms, play a significant role in promoting plant growth, especially under adverse conditions. This promotion is primarily due to the bioactive compounds produced by these microorganisms. A study by Rajesh et al. in 2013 found that combining Rhizobium, Azospirillum, phosphate-solubilizing bacteria, *Trichoderma harzianum*, and different species of Glomus (such as *Glomus intraradices* and *Glomus fasciculatum*) led to improved seedling growth. This was evident from the enhanced chlorophyll and haemoglobin content in the seedlings [12].

Microorganisms are crucial in the solubilization of minerals through biomineralization, making essential nutrients more available to plant roots. This is particularly important in tropical soils where phosphorus often becomes fixed and unavailable in its free form. Researchers demonstrated that phosphate-solubilizing bacterial strains like *Serratia marcescens* and *Pseudomonas sp.* significantly increased dry biomass in nursery conditions, by up to 99% and 94% respectively, compared to controls [13]. Another study by Tilak et al. (2005) showed that for the plant Eragrostis tef, *Pseudomonas fluorescens* and *Bacillus subtilis* increased root dry weight and root-shoot ratio, whereas *Burkholderia cepacia* and *Bacillus coagulans* did not show similar effects [14]. To enhance the nutritional needs of *Casuarina equisetifolia* seedlings, combining *G. geosporum*, *Paenibacillus polymyxa*, and Frankia as inoculants has proven effective, as noted [15]. Additionally, a study on *Eugenia dysenterica* seedlings by highlighted the benefits of applying phosphate-solubilizing microorganisms (PSM) and arbuscular mycorrhizal fungi (AMF) through various inoculation methods. This co-inoculation led to higher levels of essential nutrients such as iron, magnesium, and potassium in the leaves [16].

Furthermore, the use of organic manure, derived from animal by-products, has been shown to enhance nutrient availability in the soil. Organic manure not only stimulates microbial activity but also decomposes harmful elements, improves soil texture, and increases water-holding capacity. The excessive use of chemical fertilizers like NPK can lead to reduced plant growth and weaker root systems. Organic manure can mitigate these issues and also reduce environmental pollution. The novelty of the study lies in the comprehensive exploration of native microflora and their extracellular activities on growth and development of *Dalbergia latifolia* plant. It provides valuable insights that can be used to cultivate plants and forestry practices in a sustainable way.

The present study investigates the plant growth-promoting properties of native rhizospheric microflora associated with *Dalbergia latifolia*. Initial isolation and characterization efforts led to the identification of microorganisms from the rhizospheric soil, which were then evaluated for their extracellular phosphate solubilization potential. The combination of this microflora, along with NPK and organic manure, can serve as effective bioinoculants, enhancing the growth and establishment of forest tree species. Looking ahead, this approach holds promising prospects for sustainable agriculture and forestry, potentially reducing dependency on chemical fertilizers and promoting healthier, more resilient ecosystems. Future research could further optimize these bioinoculant combinations and explore their applications across diverse plant species and environmental conditions, contributing to more sustainable and environmentally friendly agricultural practices.

2. Materials and Methods

2.1. Isolation, Identification and Characterization of Native Micro Flora

Soil samples from the native plantation of *Dalbergia latifolia* were collected by digging 10 inches from soil or root interface of both of the plants, were pooled and brought to polythene bags in the laboratory for analysis. Serial dilution and direct inoculation method was followed for the isolation of bacteria and fungi from the collected soil sample on Sabouraud Dextrose agar medium and Nutrient agar medium. Mixed scattered colonies were obtained. Then bacterial and fungal colonies were separated, purified and maintained for further analysis [17].

2.2. Analysis of Phosphate Solubilising Efficiency

The phosphate solubilization ability of all the bacterial and fungal isolates was tested by using Pikovskaya's agar medium. Detection of clear zone around the colony indicated estimation of the phosphate solublization ability of microorganisms. The halozone diameter and colony diameters were measured at 7 days after inoculation. Solubilisation efficiency (SE) and solubilisation index (SI) was calculated as per formula referenced by (Elias et al., 2016; Qureshi et al., 2012) [18, 19]. The following formulae were used to calculate SE and SI:

Solubilization Efficiency (SE%) = Halozone diameter/colony diameter ×100

Solubilization Index (SI) = Halozone diameter (mm) / colony diameter (mm)

2.3. Inoculation Studies and Pot Experiments

The experiment was done in polybags containing red laterite soil on the nursery grown plant *Dalbergia latifolia* of Regional Plant Resource Centre, Bhubaneswar. The pH of the soil was 6.2. Deep brown colour seeds of *Dalbergia latifolia* were decapsulated and soaked for 12 - 24 hours in warm water at room temperature and sown in

pre-saturated polybags containing 3 – 4 seeds each. Seedlings were developed after 6 days of sowing. Germination rate of seeds were about 74%. Plants of 30 days old were used for inoculation studies.

The experiment was set by using phosphate solubilizing microbes along with recommended dose of fertilizer and organic manure. Two types of fungal and bacterial culture (liquid submerged culture) prepared in Sabouraud dextrose agar medium and Nutrient agar medium of pH 4.5 and 7.2 respectively and added to each pot containing single seedlings of *Dalbergia latifolia* of 30 days old plants. There were 33 nos. of treatments including control sets i.e. un-inoculated plants, un-inoculated plants with NPK (70:50:30 kg ha^{-1}) and un-inoculated plants with organic manure (25gms plant $^{-1}$) were carried out in combination with individual inoculation, individual inoculation with NPK, individual inoculation with organic manure, co-inoculation, co-inoculation with NPK and co-inoculation with organic manure. This supplementation of fungal and bacterial culture was done thrice (25 ml $plant^{-1}$) with monthly intervals and data was recorded for morphological analysis.

Initial observations was taken up to 90 days like root length, shoot height, leaf number, Data on NAR (Net Assimilation rate), LAR (Leaf area ratio), RGR(Relative growth rate), Root shoot ratio were also calculated as per the standard protocol [20-22]. The following formulae were used to calculate the above parameters:

RGR ($gm^{-1}d^{-1}$) = (W2-W1)/ (T2-T1), where, W2 is final biomass, W1 is initial biomass, T1 is initial day (90 days), T2 is final day (180 days).

LAR (cm^2 gm^{-1}) = (L1+L2)/(W1+W2), where, L1 is initial leaf area in cm^2, L2 is final leaf area in cm^2, W1 is leaf dry weight initial in grams and W2 is leaf dry weight final in gram.

NAR (gm^{-1} d^{-1}) = (W2-W1)/t × (L2-L1), where, L1 is initial leaf area in cm^2, L2 is final leaf area in cm^2, W1 is initial leaf dry weight in gram and W2 is final leaf dry weight in gram, t is interval between initial and final reading i.e. 90 days.

Root shoot ratio = root length in cm / shoot height in cm.

3. Results and Discussion

3.1. Characterization of rhizospheric Bacterial and Fungal isolates

Total forty-eight isolates from rhizosphere region of *Dalbergia latifolia* were obtained. Among them 45 fungal isolates and three bacteria were found. All the organisms were screened for phosphate solubilisation activity. 2 bacterial isolates and 2 fungal isolates were phosphate solubililizer which was used further for the experimental studies.

3.2. Analysis of Phosphate Solubilisation Activity

Phosphate solubilisation was measured through plate culture for all selected bacteria and fungi. Interestingly, 3 bacteria and 4 fungi were able to produce clear zones in Pikovskya's medium contains 0.5% TCP in solid agar media. Comparatively larger zone and the highest solubilisation index was observed in *Penicillium simplicissimum* i.e. (60mm and 40mm and 2.86) followed by *Aspergillus flavus* (1.46). Both the fungi had shown highest phosphate solubilisation index also has been presented in (Table-1). Two bacterial isolates have shown halo zone clearly in the Pikovskya's plates and exhibited solubilisation efficiency. Similarly, the profile of solubilisation efficiency was also calculated in bacterial species and *Burkholderia anthina* exhibited highest solubilisation efficiency 1.38 followed by *Burkholderia territorii* i.e. 1.03 (Table-1).

3.3. Evaluation of Phosphate Solubilising Microbial Isolates Under Pot Experiment

Microbial inoculants are recommended in the form of liquid culture along with NPK and organic manure resulted in different pattern of plant growth and raised healthy seedlings of forest trees under nursery conditions as compared to uninoculated control [23-25] . Figure 4 represents the data of shoot height measurements of 90 days old seedlings in the nursery. It is evident that there are prominent and significant differences in the seedling height of control and those of inoculated seedlings. The application of *Aspergillus flavus* followed by *Burkholderia territorii* + org.manure showed maximum shoot height measured 32.70 ±7.21cm and 32.65±8.84cm as compared to uninoculated control. The effect of inoculation of *Burkholderia anthina* + *Aspergillus flavus* + NPK were significantly higher leaf area followed by *Aspergillus flavus* + *Penicillium simplicissimum* i.e. 4.7±1.62cm^2 and 4.45±1.65 cm^2 than the uninoculated control respectively (Figure 3). The inoculation of phosphate solubilising organisms also yielded good root growth in supplemented seedlings as compared to non-supplemented plants. Variable but significantly longer root has been observed in *Burkholderia territorii* followed by *Aspergillus flavus. Burkholderia territorii* yielded 66.98% longer roots as compared to uninoculated control (Figure 5). However, significant difference was observed for the number of leaves in *Aspergillus flavus* + Org. manure i.e. 33.50±7.78 which is 5.49% higher than the control (Fig-1). In case of branches, higher numbers of branches were observed in *Aspergillus flavus* i.e. 8.50±0.71 as compared to uninoculated control (Figure 2).

Nutrient management practices of all the growth parameters and nutrient uptake of *Dalbergia sisso* were found to be effective in the treatment conatining 125% of STV + VAM+ Azospirillum+ Phosphobacteria+ FYM. Dual inoculation of Rhizobium and AM exhibited the increase in growth and biomass of the plant [26]. Supplementation of *Aspergillus ustus* and *Aspergillus tamarii* in case of *P. pinnata* enhancing the plant height and biomass under treated conditions [27]. On this contest, coinoculation of fungal bioinoculants showed higher fresh and dry biomass as compared to control presented. Most of the fungal strains along with organic manure exhibited higher fresh and dry biomass of shoot and root and also leaf as compared to non-supplemented control. Coinoculation of *Aspergillus*

flavus + *Penicillium simplicissimum* showed better performance in fresh and dry shoot biomass i.e. 1.88±0.77g 0.75±0.23g respectively as compared to other treatments. Dual inoculation of both the fungal species enhanced the fresh shoot biomass up to 68.08% and dry shoot biomass up to 57.33% higher than control (Figure 8, 9). *Burkholderia territorii* + Org.manure produced 85.54% higher fresh biomass of roots whereas coinoculation of bacteria *Burkholderia territorii* + *Burkholderia anthina* helped in enhancing root dry biomass by 68.42% over control. They fresh and dry biomass rate of roots was measured up to 0.83±0.07 and 0.38±0.46 over control (Figure 10, 11). Fresh biomass of leaves was having more physiological functions like photosynthesis and respiration as compared to dry biomass of leaves. Dual inoculation of *Aspergillus flavus*+ *Penicillium simplicissimum in* 90 days old plant gave 1.59±0.66g of fresh biomass of leaves as compared to dry biomass of leaves i.e. 0.58±0.21g of which was lower than fresh biomass (Figure 6, 7). Total fresh biomass of the seedlings were measured up to 2.5±0.25g by the applications of *Aspergillus flavus*+ *Penicillium simplicissimum* which was higher than the unsupplemented plants whereas supplementation of *Burkholderia anthina* + *Penicillium simplicissimum* enhanced the total dry biomass up to 0.90±0.46g in plants (Figure 12, 13).

Relative growth rate (RGR $gm^{-1}d^{-1}$) was also changed due to the enhancement of shoot height, leaf area in supplemented seedlings. However coinoculation of *Aspergillus flavus* + *Penicillium simplicissimum* showed higher RGR i.e. 35.40 d^{-1} followed by inoculation of *Burkholderia territorii* +org. manure was also having higher RGR than control. Coinoculation of *Burkholderia anthina* + *Aspergillus flavus* + NPK and *Burkholderia anthina* + *Penicillium simplicissimum* + NPK showed quite higher Leaf area ratio (LAR cm^2gm^{-1}) i.e 27.11 cm^2gm^{-1} as compared to uninoculated control (Figure 15). Co inoculation of *Burkholderia territorii* + *Burkholderia anthina* + Organic Manure showed highest root-shoot ratio i.e. 1.36 as compared to other treatments and non-supplemented control (Figure 14). Coinoculation of *Penicillium simplicissimum* + Org.manure showed higher Net assimilation rate (NAR $gm^{-2}d^{-1}$) followed by *Penicillium simplicissimum* measured 6434.04 and 3736.081 $gm^{-2}d^{-1}$, respectively (Figure 16).The findings of this study suggest that the use of bacterial and fungal inoculants could be a promising approach for enhancing the growth and establishment of forest tree crops.

Table-1. Phosphate solubilisation Efficiency and Index of fungal and bacterial strains

Sl.no.	Organisms	Solubilisation Efficiency (SE%)	Solubilization Index (SI)
1	*Aspergillus flavus*	146.85	1.46
2	*Penicillium simplicissimum*	285.71	2.86
3	*Burkholderia anthina*	138.46	1.38
4	*Burkholderia territorii*	103.22	1.03

Fig-1-16. Effect of different treatments of bioinoculants

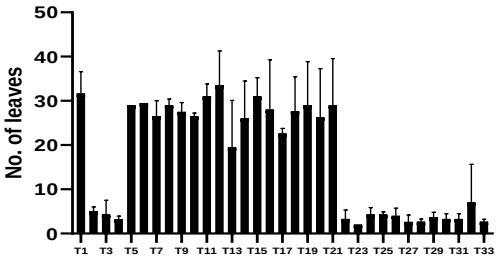

Fig. 1 treatments

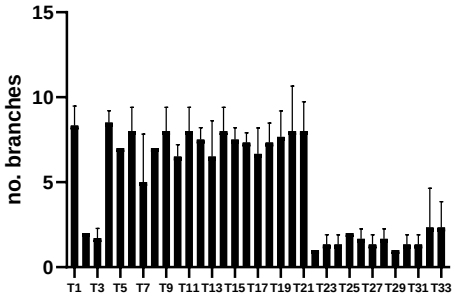

Fig. 2 treatments

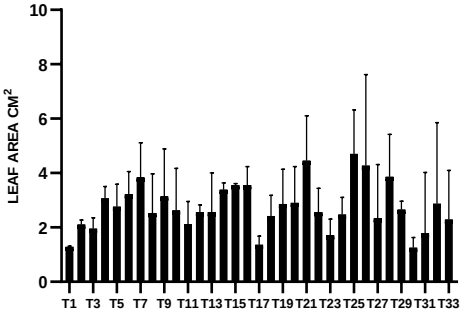

Fig. 3 treatments

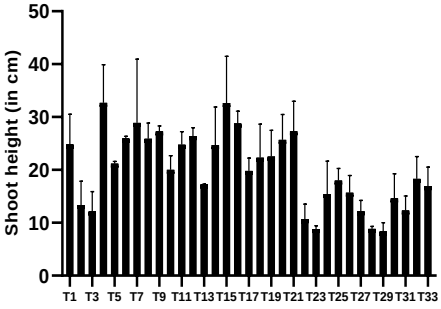

Fig. 4 treatments

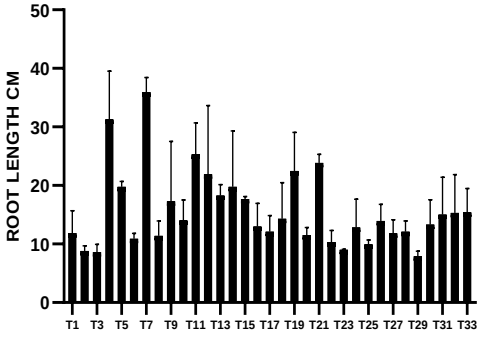

Fig. 5 treatments

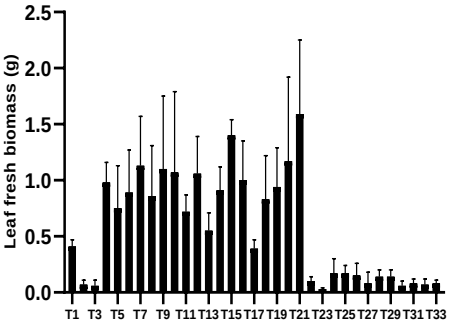

Fig.6 treatments

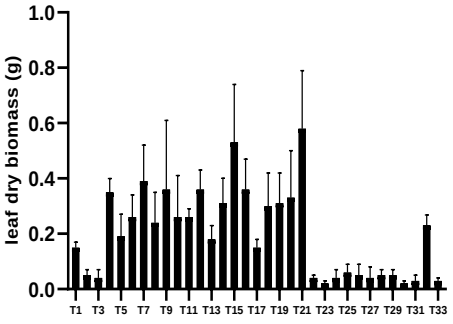

Fig. 7 treatments

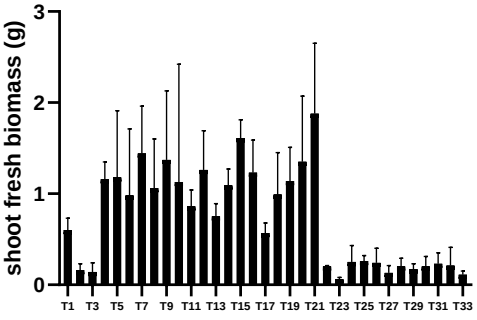

Fig. 8 treatments

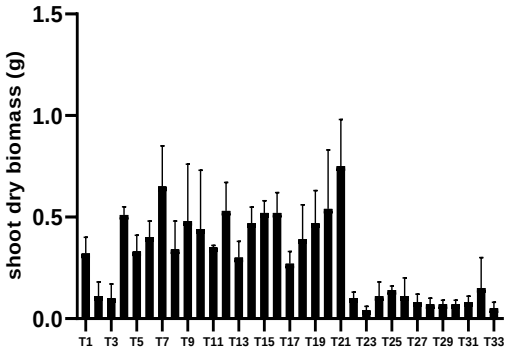

Fig. 9 treatments

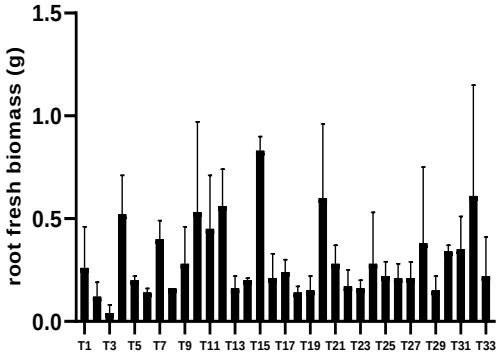

Fig.10 treatments

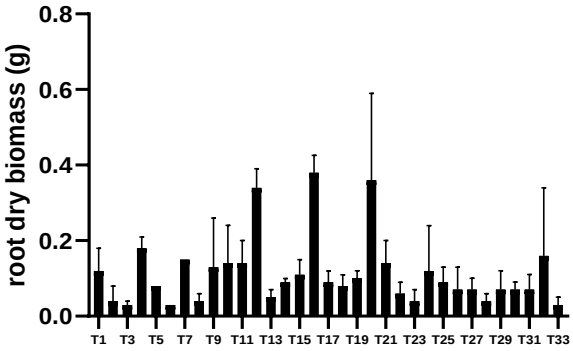

Fig. 11 treatments

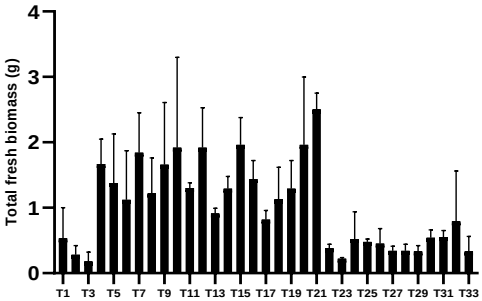

Fig. 12 treatments

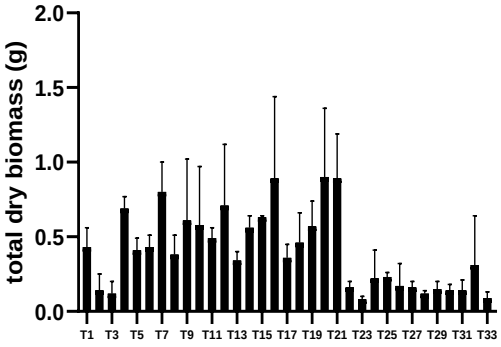

Fig. 13 treatments

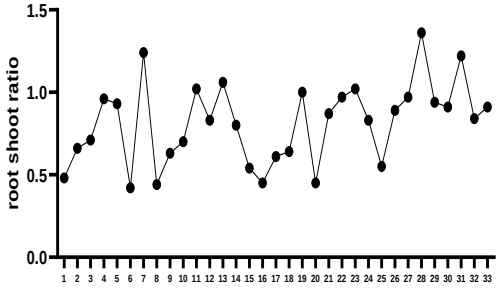

Fig. 14 treatments

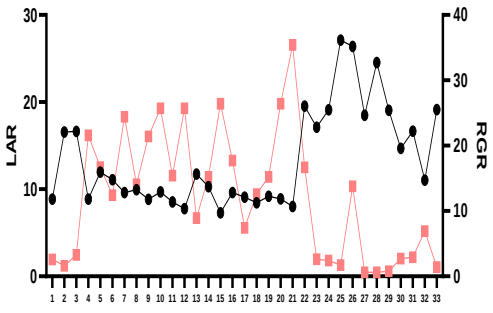

Fig. 15 treatments

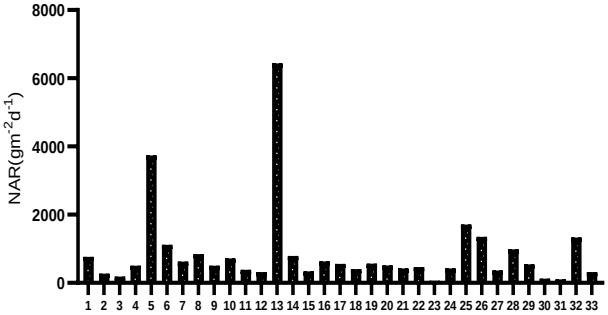

Fig. 16 treatments

Figure-1-16: Effect of different treatments of bioinoculants, NPK and organic manure on the growth of *Dalbergia latifolia*. T1- Control Un-inoculated , T2- Un-inoculated + NPK ,T3- Un-inoculated + Organic manure, T4- *Aspergillus flavus*,T5- *Penicillium simplicissimum*, T6- *Burkholderia anthina*,T7- *Burkholderia territorii*, T8- *Aspergillus flavus* + NPK, T9- *Penicillium simplicissimum* + NPK, T10- *Burkholderia anthina* + NPK, T11- *Burkholderia territorii* + NPK, T12- *Aspergillus flavus* + Organic manure,T13- *Penicillium simplicissimum* + Organic manure, T14- *Burkholderia anthina* + Organic manure, T15- *Burkholderia territorii* + Organic manure, T16- *Burkholderia territorii* + *Burkholderia anthina*, T17- *Burkholderia territorii* + *Penicillium*

*simplicissimum,*T18- *Burkholderia territorii + Aspergillus flavus,* T19- *Burkholderia anthina + Aspergillus flavus,* T20- *Burkholderia anthina + Penicillium simplicissimum,* T21- *Aspergillus flavus + Penicillium simplicissimum,* T22- *Burkholderia territorii + Burkholderia anthina* + NPK, T23- *Burkholderia territorii + Penicillium simplicissimum* + NPK, T24- *Burkholderia territorii + Aspergillus flavus* + NPK, T25- *Burkholderia anthina + Aspergillus flavus* + NPK, T26- *Burkholderia anthina + Penicillium simplicissimum* +NPK, T27- *Aspergillus flavus + Penicillium simplicissimum* + NPK, T28- *Burkholderia territorii + Burkholderia anthina* + Organic manure, T29- *Burkholderia territorii + Penicillium simplicissimum* + Organic manure, T30- *Burkholderia territorii + Aspergillus flavus* + Organic manure,T31- *Burkholderia anthina + Aspergillus flavus* + Organic manure, T32- *Burkholderia anthina + Penicillium simplicissimum* + Organic manure, T33- *Aspergillus flavus + Penicillium simplicissimum* + Organic manure.

4. Conclusions

In conclusion, the study focused to evaluate the efficacy of these phosphate solubilizing microbial isolates in pot experiments with *Dalbergia latifolia* seedlings. The results showed significant differences in various growth parameters between the inoculated seedlings and the uninoculated control. The application of *Aspergillus flavus* and *Burkholderia territorii* along with organic manure resulted in the highest shoot height, while *Burkholderia anthina* and *Aspergillus flavus* with NPK showed the highest leaf area. The inoculation of phosphate solubilizing organisms also enhanced root growth compared to the control. In terms of biomass production, coinoculation of *Aspergillus flavus* and *Penicillium simplicissimum* showed the best performance in fresh and dry shoot biomass, while *Burkholderia territorii* along with organic manure promoted higher fresh biomass of roots. The coinoculation of *Penicillium simplicissimum* with *Burkholderia anthina* resulted in higher total dry biomass of the seedlings. Relative growth rate, leaf area ratio, root-shoot ratio, and net assimilation rate were also influenced by the inoculation of these microbial isolates, indicating their positive effects on plant growth and development. Overall, the study suggests that the use of these bacterial and fungal inoculants could be a promising approach for enhancing the growth and establishment of *Dalbergia latifolia* and other forest tree crops.

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