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SHORT COMMUNICATION

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# Effect of rhizobial isolates and nitrogen fertilizer on shisham (*Dalbergia sissoo Roxb.*) soil properties

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## ABSTRACT

A treatment combination of isolates and N-based fertilizer was observed for effect on the rhizospheric soil of *Dalbergia sissoo*. Various blends of two plant growth-promoting rhizobacteria (PGPR1 and PGPR2) and nitrogen fertilizers (Treatments T1 to T10) have been applied to analyze their effects on the rhizospheric soil's microbial count, physicochemical properties, and available soil NPK contents. The treatments did not produce any significant changes in the soil's pH, electrical conductivity, or organic carbon content. However, a significant increase was observed in the nitrogen, phosphorus, and potassium contents of the rhizospheric soil, with the highest increase observed in soil treated with treatment T4, composed of the two rhizobacterial isolates utilized in this study.

## KEYWORDS

Bacillus; Compatibility; PGPR; Consortium

## ARTICLE HISTORY

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## Introduction

According to the United Nations, the amount of nitrogen in the hydrosphere, lithosphere, and atmosphere has almost doubled in the last century due to anthropogenic activity. The excessive use of synthetic nitrogen fertilizers to cater to the unprecedented agricultural growth to feed the fast-expanding global population resulted in a flood of excess nitrogen, adversely affecting land and aquatic ecosystems. Algal blooms and dead zones devoid of aquatic life have increasingly materialized as run-off of synthetic fertilizers from adjoining farmlands enter and disrupt freshwater and marine ecosystems. Nitrogenous emissions from agricultural activities, like nitrous oxide, are potent greenhouse gases contributing to global warming. An estimated 40% to 60% of nitrogen inputs in cropping are irretrievably lost to the environment. This lost nitrogen content not only wreaks havoc on the environment but also compromises the farmer's profits, who must buy more fertilizers each time he plants a crop. Optimal use of nitrogen fertilizers is thus paramount to both sustainable economics and sustainable agriculture. Capturing nitrogen into the products is the most efficient way to reduce nitrogen losses through nitrous oxide formation, ammonia volatilization, nitrate leaching, and run-off into groundwater or waterways.

*Dalbergia sissoo* is a versatile native Indian tree with high nitrogen-fixing ability, making it suitable for agroforestry and land reclamation projects. Widely used in their native countries for reforestation programs, these trees offer a multitude of benefits; the robust root system and predisposition to suckers enhance its capacity to stabilize soil and manage erosion along stream and river banks, and the leaves augment the soil with nitrogen upon decomposing [1]. The tree is also economically advantageous, given the numerous products it offers, including high-quality timber, fodder, broad leaves for making disposable utensils, and traditional medicines.

Plant growth-promoting rhizobacteria (PGPRs) are diazotrophic bacteria that can colonize plants' roots or rhizosphere and promote the latter's growth through various regulatory molecules. These microorganisms can directly or indirectly enhance their hosts' fitness. PGPRs play an essential role in sustainable agricultural and forestry practices as biofertilizers. Prior studies have demonstrated the growth-enhancing effects and increased stress tolerance in plants grown using biofertilizers made of PGPRs and compost [2]. Using PGPRs as biofertilizers has been shown to enhance nitrogen fixation, increase the growth and grain yield of maize crops from 24 to 34%, and promote seed germination rate and early development [3,4]. Inoculating PGPR species during sugarcane cultivation has also been reported to enhance crop production and yield while considerably lowering the costs of fertilizers [5].

Nitrogen-based fertilizers are largely used to cultivate manifold nurseries of economically important plants and trees [6]. The effect of chemical-based fertilizers is affecting the soil properties in such practices. The use of biofertilizers to accelerate plant growth and minimize the effect of such fertilizers is essential. *D. sissoo* is an economically important tree, and nursery practices of such tree species also affect the nitrogen around the soil [7]. Therefore, using PGPRs to improve the quality and growth during developmental stages and sustain the development of such nitrogen-fixing tree species is of tremendous importance.

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## Materials and Methods

The physicochemical properties of *D. sissoo* soils at the beginning and end of the experiment were studied from the second nursery trial study by Dhiman et al. [8]. The compatible rhizobial isolates were used in treatment combination with N fertilizer as represented in 10 treatments, namely T1 to T10 in replicates. Analysis of the potting mixture soil for different physicochemical properties was done. A viable microbiological count was also enumerated for the potting mixture treatments. The gathered soil samples were air-dried in the shade, ground into a powder with a wooden mallet, and then run through a 2mm sieve for initial sample preparation. The physicochemical properties of the samples were then analyzed using the standardized methods represented in Table 1. The recorded soil and microbiological properties were statistically analyzed as described by Sheoran et al. [9].

**Table 1.** Methodology for soil samples analysis.

| Properties | Method | References |
|---|---|---|
| pH (1:2.5) (soil: water suspension) | The soil suspension was settled to determine pH potentiometrically. | [10] |
| EC (dS $m^{-1}$) | The conductivity meter was used to measure the supernatant liquid's conductivity. | [10] |
| Organic Carbon (%) | Organic carbon was determined by the Chromic acid titration method. | [11] |
| Available N (kg $ha^{-1}$) | Available nitrogen was determined by the alkaline permanganate method. | [12] |
| Available P (kg $ha^{-1}$) | Sodium Bicarbonate ($NaHCO_3$) at alkaline pH was used to extract available phosphorus and determined spectrophotometrically. | [13] |
| Available K (kg $ha^{-1}$) | Available potassium was extracted by normal neutral ammonium acetate and determined on a flame photometer | [14] |

## Results

The data on to the initial physicochemical status of the soil are presented in Table 2. A perusal of the data showed that the soil was nearly neutral (pH 6.9), EC (0.36dS$m^{-1}$), and organic carbon was 1.5%. The available N (290.4kg/ha) and P (52.5kg/ha) contents were in medium range. However, available K (263.8kg/ha) was in the high range.

Compared to an uninoculated control, applying rhizobial isolates, both individually and in combination with nitrogen fertilizer, dramatically increased the rhizospheric microbial population. The highest count (2.0 × $10^8$cfu/g soil) was recorded in the rhizosphere treated with T4 (PGPR1+PGPR2), whereas the treatment T1 (uninoculated control) yielded the lowest count (0.4 × $10^8$cfu/g soil).

The physicochemical properties of treatments T1 to T10 were also analyzed for the microbial count and available soil NPK contents. The data revealed that none of the treatments influenced soil pH, electrical conductivity (EC), or organic carbon (OC) significantly over control. The corresponding values recorded were 6.9-7.1 for pH, 0.36-0.39 for electrical conductivity, and 1.5-1.8 percent organic carbon. All the treatments registered a significant increase in available NPK content. The highest available N content (349.7kg/ha) and available P content (66.8kg/ha) of soil were recorded with treatment T4 (PGPR1+PGPR2), which was statistically at par with treatment T6 (PGPR1+40%N). However, the minimum (289.5kg/ha) available N content and available P content (52.7kg/ha) of soil were recorded with treatment T1 (uninoculated control was treated). The maximum available K content (308.4kg/ha) of soil was recorded with treatment T4 (PGPR1+PGPR2), which was statistically at par with the T6 treatment. The minimum (269.6kg/ha) available K content of soil was recorded with T1 (uninoculated) control.

Figure 1 shows the percent increase of NPK after the completion of the experiment over control. The treatment T4 (PGPR1+PGPR2), i.e., a consortium of two selected isolates, registered a 27.34 percent increase for N, 22.00 percent for P, and a 14.35 percent increase for K over treatment T1 (non-inoculated control).

**Table 2.** Physicochemical of soil before and after the experiment.

| Treatment | pH | Organic Carbon (%) | EC ($dSm^{-1}$) | Available N (kg/ha) | Available P (kg/ha) | Available K (kg/ha) | Microbial count (×$10^8$ cfu/g) |
|---|---|---|---|---|---|---|---|
| Before experiment | | | | | | | |
| Untreated potting soil | 6.9 | 1.5 | 0.36 | 290.4 | 52.5 | 263.8 | 9.2 × $10^6$ cfu/g |
| After experiment | | | | | | | |
| T1 (Control) | 6.9 | 1.7 | 0.36 | 289.5 | 52.7 | 269.6 | 0.9 |
| T2 (PGPR1) | 7.1 | 1.5 | 0.36 | 296.8 | 55.4 | 273.1 | 1.4 |
| T3 (PGPR2) | 7 | 1.6 | 0.38 | 292.7 | 54.2 | 271.8 | 1.5 |
| T4 (PGPR1+PGPR2) | 7.2 | 1.5 | 0.39 | 349.7 | 66.8 | 308.4 | 2 |
| T5 (PGPR1+20%N) | 7 | 1.7 | 0.38 | 308.2 | 57.5 | 279.4 | 1.6 |





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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| T6 (PGPR1+40%N) | 7.1 | 1.6 | 0.37 | 335.8 | 64.2 | 303.2 | 2 |
| T7 (PGPR1+60%N) | 6.9 | 1.7 | 0.37 | 322.3 | 60.1 | 294.5 | 1.8 |
| T8 (PGPR2+20%N) | 7 | 1.6 | 0.37 | 301.4 | 56.3 | 278.3 | 1.5 |
| T9 (PGPR2+40%N) | 7 | 1.8 | 0.38 | 328.5 | 62.7 | 302.7 | 1.8 |
| T10 (PGPR2+60%N) | 6.9 | 1.8 | 0.39 | 315.9 | 59.4 | 285.6 | 1.7 |
| CD (0.05) | NS | NS | NS | 18.129 | 2.33 | 17.152 | 4.61 |

Source of isolates: PGPR1= Burkholderia caribensis, PGPR2= Paraburkholderia sp.

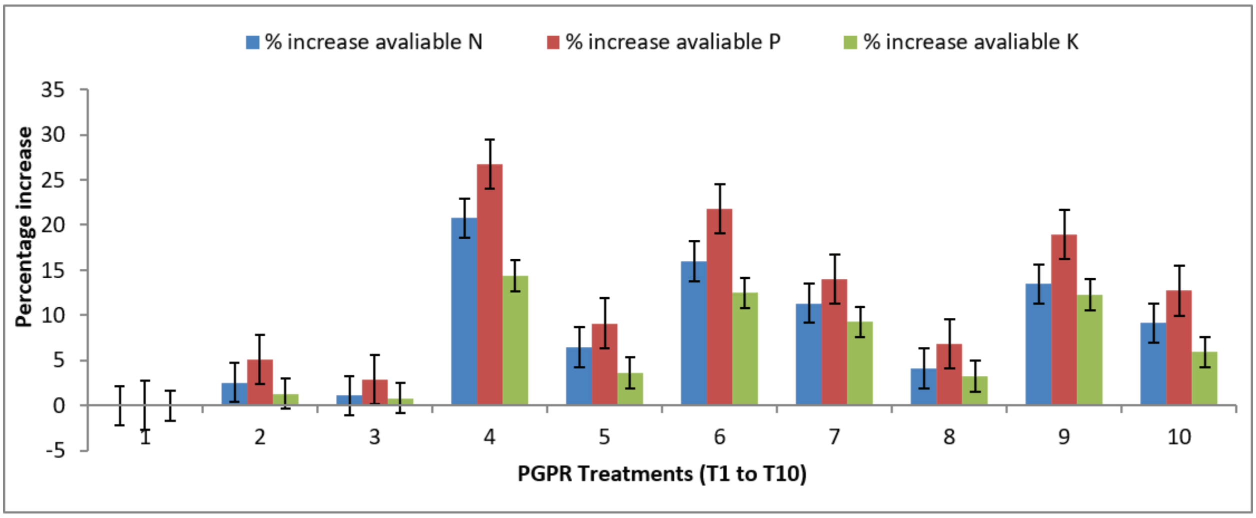


**Figure 1.** Percent increase in NPK content of soil over control at the end of the experiment.

## Discussion

The multitude of beneficial microorganisms residing in a plant's rhizosphere can significantly enhance the plant's nutrient assimilating capacity, promote its growth, increase its robustness against disease and abiotic stress, and even remove toxic compounds. These microorganisms can form symbiotic relationships with various parts of the plant: the roots (rhizosphere), stem (endosphere), and even leaves (phyllosphere). The soil-residing rhizobacteria and plant-residing endophytes both promote the growth of their host plant through various mechanisms.

The PGPR has a variety of roles in plant nutrition, protection, and hormone behavior with environmental changes [15-18], their part in influencing the rhizosphere is critically important. The increase in available N contents in soil may be due to the mineralization of organic matter and fixation of atmospheric nitrogen by the symbiotic rhizobial isolates. The most established and important direct mechanisms comprise nitrogen fixation, increased production of growth-regulating hormones like auxins, cytokinins, and gibberellins, and inorganic phosphate solubilization. These processes lead to rhizoremediation and biofertilization, stimulating root growth and increasing the plant's tolerance against abiotic stresses like droughts and increased salinity. PGPRs also indirectly mediate plant growth and fitness by inhibiting the growth and activity of pathogenic organisms on the plant. The PGPRs counter plant pathogens' adverse effects through various mechanisms, including antibiotic production, pathogen quorum inhibition, induction of systemic resistance, cell wall-degrading enzymes, and antioxidants [19].

Both *D. sissoo* and PGPRs have become consequential factors in sustainable agriculture owing to their ability to replenish the soil with essential nutrients. This study demonstrates the positive increments in the NPK content of the rhizospheric soil around the roots of *D. sissoo* upon treatment with PGPRs. Our results conform with those reported by Dhiman et al. and Korir et al. in terms of the nitrogen-fixing capacity of the bacterial isolates used [7,20]. The increase in the P availability might be due to the activities of phosphate-solubilizing microorganisms, which might have brought some P from the unavailable pool to the available pool. The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increases nutrient availability to host plants [21]. Similar results were reported by Totey et al. in *D. sissoo* and *Acacia nilotica*; they studied the combined effect of Rhizobium inoculation along with application of different levels of N dosages of fertilizer and found Rhizobium with fertilizer was superior over control [22]. Several workers have reported increased soil nutrient status with rhizobial inoculation [23].

## Conclusions

The inoculation of rhizospheric bacteria into the potting mixture for growing *D. sissoo* effectively increased the nutrient-retaining property of the soil. The consortium of the two rhizobial isolates used in this study showed the highest increment in the NPK content of the soil without much alterations in other physicochemical properties like pH, electrical conductivity, and organic carbon content. Thus, these two isolates can be used along with *D.sissoo* plantations to rejuvenate the soil and sustain soil health without using chemical fertilizers. Further studies can be conducted to increase the scalability of using this method to reclaim barren



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agricultural lands or replenish the soil between crops in a sustainable and eco-friendly method.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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