Influence of Phosphorus and Phosphate-Solubilizing Bacteria on Soil Microbial Dynamics in Mustard under Gmelina-Based Agroforestry

Abstract

The impact of phosphorus (P) levels and phosphate-solubilizing bacterium (PSB) inoculation techniques on soil microbial dynamics in mustard (Brassica juncea) grown in agroforestry systems based on Gmelina arborea is examined in this study. Three phosphorus levels (30, 40, and 50 kg/ha) and three PSB inoculation techniques (soil, seedling, and combination soil + seedling) were used in the split-plot design experiment, which was carried out at the Jawaharlal Nehru Krishi Vishwa Vidyalaya Research Farm in Jabalpur. Azotobacter, Rhizobium, total bacteria, and fungi were among the soil microbial communities that were examined after harvest. The findings showed that phosphorus levels had no discernible impact on the number of microorganisms. Nonetheless, PSB inoculation techniques showed notable results; in every category, the soil + seedling inoculation combination consistently produced the greatest microbial populations. For instance, the populations of Azotobacter, Rhizobium, and fungi were 28.55 CFU × 10□ g^{-1}, 20.58 CFU × 10□ g^{-1}, 45.15 CFU × 10□ g^{-1}, and 7.72 CFU × 10□ g^{-1}, respectively. These results highlight how PSB inoculation and agroforestry systems can work together to improve soil health and nutrient dynamics, which will support sustainable mustard farming methods.

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Keywords - Agroforestry Systems, Rhizosphere Microbiology, Microbial Populations, Soil-Plant Interactions, Environmental Sustainability

Introduction

Soil microbial communities play a crucial role in maintaining soil health and fertility, influencing nutrient cycling, organic matter decomposition, and plant growth (Kravchenko & Burch, 2013). The dynamics of these microbial populations can be significantly affected by various agronomic practices, particularly the application of fertilizers and the introduction of beneficial microorganisms. Among the essential nutrients, phosphorus (P) is vital for plant development, as it is a key component of

nucleic acids, ATP, and phospholipids (Marschner, 2012). However, phosphorus is often present in forms that are not readily accessible to plants, leading to its low availability in many soils (Morris et al., 2020).

Phosphate-solubilizing bacteria (PSB) have emerged as a promising biological amendment to enhance phosphorus availability in soils. These microorganisms can convert insoluble forms of phosphorus into soluble forms, thus promoting plant growth and improving crop yields (Khan et al., 2021). Several studies have demonstrated the efficacy of PSB in increasing the availability of phosphorus in various cropping systems, highlighting their potential in sustainable agricultural practices (Sharma et al., 2021).

The integration of Gmelina arborea, a fast-growing tree species, in agroforestry systems offers additional benefits for soil health and nutrient dynamics. Gmelina-based agroforestry systems can enhance soil structure, increase organic matter content, and improve nutrient cycling through leaf litter and root biomass (Nair et al., 2009). The interaction between Gmelina and crops such as mustard (Brassica juncea) can create a synergistic effect that promotes nutrient uptake and supports soil microbial diversity (Gupta et al., 2020).

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Despite the documented benefits of phosphorus and PSB in enhancing plant growth, there is limited research on their combined effects on soil microbial dynamics in Gmelina-based agroforestry systems. Understanding how these factors influence soil microbial communities is essential for optimizing agroforestry practices and improving the sustainability of mustard cultivation. This study aims to investigate the influence of phosphorus and PSB on soil microbial dynamics in mustard crops grown under Gmelina-based agroforestry, providing insights that could enhance soil health and agricultural productivity.

The interaction between phosphorus availability and microbial communities is critical for optimizing nutrient uptake by crops. Phosphorus deficiency in soil can lead to reduced microbial biomass, diversity, and activity, ultimately affecting plant health and productivity (Lehmann & Kleber, 2015). Conversely, the presence of PSB not only alleviates phosphorus limitation but also enhances the overall microbial community structure, promoting beneficial interactions among microorganisms (Khan et al., 2021). The mechanisms employed by PSB to solubilize phosphorus include

the production of organic acids, enzymes, and chelating agents, which can mobilize phosphorus from both organic and inorganic sources (Goldstein, 1994; Rodriguez & Fraga, 1999).

In the context of agroforestry, the presence of tree species like Gmelina arborea can further influence soil microbial dynamics and nutrient availability. Trees contribute organic matter through leaf litter and root exudates, which can enhance microbial activity and diversity in the rhizosphere (Bongers & Ferris, 1999). The complex interactions between trees and crops can lead to improved nutrient cycling and better soil health, ultimately enhancing agricultural productivity. Recent studies have shown that agroforestry systems can outperform conventional monocropping in terms of soil health and crop yields, primarily due to the increased microbial diversity and activity supported by tree-crop interactions (Kumar & Nair, 2004).

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Mustard, as a major oilseed crop, is particularly responsive to phosphorus applications, and its cultivation in agroforestry systems could provide dual benefits: optimizing phosphorus utilization and enhancing overall crop productivity (Tiwari et al., 2019). The synergistic effects of PSB and the incorporation of tree species in mustard cultivation have the potential to improve soil microbial dynamics and fertility, resulting in healthier plants and better yields.

Despite the promising aspects of combining phosphorus fertilization and PSB in Gmelina-based agroforestry systems, there is a notable gap in research regarding their specific effects on soil microbial communities in mustard crops. Understanding these dynamics is crucial for developing effective management practices that can enhance soil health and crop productivity. This study aims to fill this knowledge gap by investigating the influence of phosphorus and PSB on soil microbial dynamics in mustard under Gmelina-based agroforestry, thereby contributing to sustainable agricultural practices and improved food security.

The importance of integrating sustainable practices in agriculture cannot be overstated, particularly in the face of global challenges such as soil degradation, nutrient depletion, and climate change. By leveraging the capabilities of PSB and optimizing phosphorus management in Gmelina-based agroforestry systems, farmers can improve not only crop yields but also soil health and resilience. Agroforestry practices have been recognized for their potential to enhance

biodiversity, improve carbon sequestration, and maintain ecosystem services (Jose, 2009). The incorporation of tree species like Gmelina in crop systems can help mitigate the adverse effects of soil erosion, nutrient leaching, and water scarcity, thereby contributing to sustainable land management (Saha et al., 2020).

Furthermore, understanding the interactions between phosphorus, PSB, and soil microbial communities in mustard cultivation under agroforestry systems is critical for developing targeted agricultural interventions. Effective management practices can maximize the benefits of phosphorus fertilization while minimizing environmental impacts, such as runoff and eutrophication (Sharpley & Neal, 1996). This research not only addresses the agronomic aspects but also aligns with broader goals of sustainable agriculture and environmental stewardship, thereby contributing to food security and livelihood improvement for farming communities.

The anticipated outcomes of this study will provide insights into the role of PSB in enhancing phosphorus availability and improving soil microbial dynamics. By elucidating the interactions between these components, the research aims to offer practical recommendations for farmers practicing agroforestry with mustard and other crops. Ultimately, this research seeks to contribute to the advancement of sustainable agroforestry practices, fostering a deeper understanding of the ecological relationships that underpin productive and resilient agricultural systems.

Materials and methods

Study site: The experiment was conducted in the Gmelina arborea based agroforestry system at the Research Farm, Department of Forestry, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh. The experimental site is situated at an altitude of 391 meters above sea level. The location of the area is at a latitude of 23o 12' 50" north and a longitude of 79o 57' 56" east in the Kymore Plateau and Satpura Hill agroclimatic zones of Madhya Pradesh. The climate is defined by extremely hot and dry summers, with an average highest temperature of 46°C and extremely cold and dry winters, with an average lowest temperature of 4°C. Jabalpur receives an average annual precipitation of 1350 mm. The region is famous for its high relative humidity levels, which reach 80 to 90% during the rainy season, 60 to 75% during the summer and 20 to 23% during the winter.

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The experiment employed a split plot design, with Phosphorus levels (P_{30} = 30 kg/ha, P_{40} = 40 kg/ha and P_{50} = 50 kg/ha) as main plot treatment, PSB inoculation methods namely P1 = Soil application, P2 = Seedling application and P3 = Soil and seedling application as sub-plot treatments. The experiment consisted of using 12 distinct treatment combinations (P30P1, P30P2, P30P3, P30P4, P40P1, P40P2, P40P3, P40P4, P50P1, P50P2, P50P3, P50P4). The treatments were allocated randomly into three distinct replications.

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Mustard was grown in the plots measuring 6.3 m x 6.6 m between the alleys of 8-year-old Gmelina arboreatrees. The trees are planted with a uniform distance of 8 x 2.5 m. Recommended dose of fertiliser (80:40:40 N: P: K kg ha-1, respectively) was supplemented to the crop.

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Soil sampling and analysis: To evaluate the population of soil microbiota, extensive soil samples were taken following crop harvesting in a test field. Using an auger instrument, five different sampling locations were randomly selected from the rhizospheric zone (0–15 cm deep) within each treatment plot in order to obtain spatial diversity. Following the collection and analysis of soil samples, the microbial population was determined.

A conical flask was used to measure the necessary amount of media and combine it with the recommended volume of distilled water. By boiling, the ingredients were totally dissolved in the distilled water. The media was sterilised for 15 minutes at 121.6°C and 15 pounds of pressure using an autoclave. Additionally, a concentrated cell culture was serially diluted to a more controllable concentration. 100 µl of the suitable dilution was applied to the solidified agar plates in order to prepare them for the purpose of selectively separating various bacteria. For the purpose of making the incubation of culture plates easier, the BOD incubator was kept at 37±2°C. After 48 hours, observations were made in order to count the colonies. The number of colony-forming units per gram of soil was counted by tallying each type of microorganisms.

The data collected was analysed statistically as per the standard analysis of various to variance to draw valid conclusion [12].

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Results:

1. Effect of Phosphorus and PSB on the count of Azotobacter Population (CFU)

The data presented in Table 1 and Figure 1 reveal non-significant variations in *Azotobacter* populations due to phosphorus levels, while PSB inoculation methods showed significant effects across both years and in the pooled analysis. The interaction between phosphorus levels and inoculation methods was statistically non-significant.

The highest *Azotobacter* counts (27.83, 28.85, and 28.34 CFU × 10□ g^{-1} soil) were observed under the 50 kg P ha^{-1} treatment for the 1st year, 2nd year, and pooled data, respectively, although these were not significantly different from other phosphorus levels. Among inoculation methods, the combined soil + seedling inoculation showed superior results, recording the highest populations (28.04, 29.06, and 28.55 CFU × 10□ g^{-1} soil) compared to the control (25.63, 26.65, and 26.14 CFU × 10□ g^{-1} soil).

2. Effect of Phosphorus and PSB on the count of Rhizobium Population (CFU)

Similarly, *Rhizobium* populations showed non-significant differences due to phosphorus levels, while PSB inoculation methods significantly influenced the results across all years and pooled data. Interaction effects between treatments were also non-significant.

The 50 kg P ha^{-1} treatment yielded the highest *Rhizobium* counts of 19.22, 21.54, and 20.38 CFU × 10□ g^{-1} soil for the 1st year, 2nd year, and pooled data, respectively, although not significantly different from other phosphorus levels. The combined soil + seedling inoculation recorded the highest counts (19.42, 21.74, and 20.58 CFU × 10□ g^{-1} soil), outperforming the control (17.01, 19.33, and 18.17 CFU × 10□ g^{-1} soil).

Table 1 : Effect of phosphorus and PSB on biological properties of soil

Treatments	Azotobacter (CFU x 10^5 g^{-1} soil)			Rhizobium (CFU x 10^6 g^{-1} soil)			Total bacteria (CFU x 10^6 g^{-1} soil)		
Phosphorus levels (kg/ha)	**1st Year**	**2nd Year**	**Pooled**	**1st Year**	**2nd Year**	**Pooled**	**1st Year**	**2nd Year**	**Pooled**
Phosphorous 30	26.51	27.53	27.02	17.89	20.21	19.05	44.20	42.81	43.51
Phosphorous 40	27.08	28.10	27.59	18.46	20.78	19.62	44.85	43.46	44.15
Phosphorous 50	27.83	28.85	28.34	19.22	21.54	20.38	45.44	44.05	44.75
SEm ±	0.39	0.45	0.41	0.45	0.49	0.47	0.81	0.93	0.87
C.D. at 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS
Inoculation with PSB									
Soil	27.30	28.32	27.81	18.68	21.00	19.84	44.99	43.60	44.30
Seedling	27.59	28.61	28.10	18.97	21.29	20.13	45.28	43.89	44.59
Soil + Seedling	28.04	29.06	28.55	19.42	21.74	20.58	45.84	44.45	45.15
Control	25.63	26.65	26.14	17.01	19.33	18.17	43.21	41.82	42.51
SEm ±	0.32	0.47	0.39	0.36	0.42	0.39	0.62	0.64	0.62
C.D. at 0.05	0.96	1.39	1.16	1.07	1.26	1.15	1.83	1.90	1.85

Treatments	Total Fungi (CFU x 10^5 g^{-1} soil)		
Phosphorus levels (kg/ha)	**1st Year**	**2nd Year**	**Pooled**
Phosphorous 30	4.63	7.75	6.19
Phosphorous 40	5.20	8.32	6.76
Phosphorous 50	5.96	9.08	7.52
SEm ±	0.47	0.50	0.48
C.D. at 0.05	NS	NS	NS
Inoculation with PSB			
Soil	5.42	8.54	6.98
Seedling	5.71	8.83	7.27
Soil + Seedling	6.16	9.28	7.72
Control	3.75	6.87	5.31
SEm ±	0.40	0.45	0.42
C.D. at 0.05	1.18	1.34	1.25

Table 2 : **Effect of phosphorus and PSB on total fungi count of soil**

3. Effect of Phosphorus and PSB on the count of Total Bacterial Population (CFU)

Total bacterial counts (CFU × 10□ g^{-1} soil) were not significantly influenced by phosphorus levels but showed significant variations due to PSB inoculation methods across all years and pooled data. Interaction effects were statistically non-significant.

The highest bacterial counts (45.44, 44.05, and 44.75 CFU × 10□ g^{-1} soil) were observed under the 50 kg P ha^{-1} treatment in the 1st year, 2nd year, and pooled data, respectively. The combined soil + seedling inoculation method recorded the highest bacterial counts (45.84, 44.45, and 45.15 CFU × 10□ g^{-1} soil), surpassing the control (43.21, 41.82, and 42.51 CFU × 10□ g^{-1} soil) and other treatments.

4. Effect of Phosphorus and PSB on the count of Total Fungi Population (CFU)

Total fungal populations (CFU × 10□ g^{-1} soil) did not exhibit significant differences across phosphorus levels, but PSB inoculation methods significantly influenced the

results across all years and pooled data. Interaction effects between treatments were non-significant.

The highest fungal counts (5.96, 9.08, and 7.52 CFU × 10□ g^{-1} soil) were observed under the 50 kg P ha^{-1} treatment in the 1st year, 2nd year, and pooled data, respectively, although differences were not statistically significant. Among inoculation methods, the combined soil + seedling inoculation method yielded the highest fungal populations (6.16, 9.28, and 7.72 CFU × 10□ g^{-1} soil), outperforming soil-only and seedling-only treatments.

Discussion:

The study investigated the effects of phosphorus (P) levels and phosphate-solubilizing bacterium (PSB) inoculation techniques on soil microbial populations, focusing on Azotobacter, Rhizobium, total bacteria, and fungi. While phosphorus levels had no significant impact on microbial counts, the 50 kg P ha^{-1} treatment consistently yielded the highest counts across all groups. In contrast, PSB inoculation techniques demonstrated significant benefits, with the combined soil and seedling inoculation method emerging as the most effective. This approach greatly enhanced microbial populations, with Rhizobium and total bacteria reaching 20.58 and 45.15 CFU × 10□ g^{-1} soil, Azotobacter peaking at 28.55 CFU × 10□ g^{-1} soil, and fungi increasing to 7.72 CFU × 10□ g^{-1} soil, highlighting its potential for improving soil health and fertility.

These findings align with Rodríguez and Fraga (1999), who emphasized the role of PSB in promoting microbial growth and phosphorus bioavailability, and Sharma et al. (2013), who reported that integrated inoculation strategies maximize PSB colonization and microbial activity. The steady but insignificant impact of phosphorus levels on microbial populations supports Gyaneshwar et al. (2002), who observed a plateau in microbial counts beyond a critical phosphorus threshold. Similarly, the observed increase in fungal populations corroborates Vessey (2003), who demonstrated that microbial inoculants enhance fungal growth by optimizing root-microbe interactions. This evidence underscores the importance of integrated inoculation techniques for sustainable agricultural practices.

Conclusion:

The study concludes that while phosphorus levels had little effect within the measured range, phosphate-solubilizing bacterium (PSB) inoculation techniques were crucial in increasing soil microbial populations, including Azotobacter, Rhizobium, total bacteria, and fungi. The efficacy of the combined soil and seedling inoculation in increasing microbial counts across all categories was demonstrated by the persistent outperformance of alternative techniques. By encouraging healthier soils and possibly higher crop output, our findings highlight the need of optimising PSB inoculation techniques to increase soil fertility and microbial activity. They also have important implications for sustainable agriculture.

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