

Impact of Pendimethalin Herbicide on Plant Growth-Promoting Rhizobacteria and Crop Growth

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Abstract: Pendimethalin is extensively used as a pre-emergence herbicide for weed management in legume cultivation. Its excessive application could adversely affect beneficial rhizospheric microorganisms and crop productivity. The present study was conducted to evaluate effect of pendimethalin on plant growth-promoting rhizobacteria (PGPR) and growth performance of chickpea (*Cicer arietinum*). Present study was focused on three important PGPR strains namely *Rhizobium* spp., *Bacillus* spp., and *Pseudomonas* spp. under different pendimethalin concentrations. A pot culture experiment was designed with four treatments viz., control (0 mL/L), below recommended dose (5.0 mL/L), recommended dose (10 mL/L) and above recommended dose (20 mL/L) of pendimethalin. Sterilized soil was inoculated with PGPR consortium and chickpea seeds were bioprimes before sowing. Various biochemical parameters viz., microbial population, phosphate solubilization, indole acetic acid (IAA), siderophore production and Hydrogen Cyanide (HCN) production were analyzed along with plant growth indicators such as germination, shoot and root length, nodulation and fresh biomass. The results disclosed concentration-dependent inhibitory effect of pendimethalin on both PGPR activity and chickpea growth. *Pseudomonas* spp. exhibited the highest PGP activity in control conditions whereas *Rhizobium* spp. exhibited maximum sensitivity to herbicide stress. Higher herbicide concentrations significantly reduced microbial viability, nodulation, IAA production, and plant biomass, leading to chlorosis and stunted growth in chickpea. The present study was limited to pot conditions and short-term observations. Future studies should focus on field-scale validation, molecular mechanisms of herbicide toxicity and development of herbicide-tolerant PGPR formulations for sustainable agriculture.

Keywords: Pendimethalin, PGPR, Chickpea, *Rhizobium* spp., *Bacillus* spp., *Pseudomonas* spp. Herbicide Toxicity and Sustainable Agriculture

1. Introduction

Soil health is essential for agricultural productivity, relying on complex biological, chemical and physical interactions within soil (Xing et al., 2025). It is mainly influenced by organic matter, microbial diversity, monoculture and synthetic biocides (Ṫopa et al., 2025). Presently, herbicide market is growing in India with 427.56 Million (USD) (Mordor Intelligence, 2026). This was started since Green Revolution. Mordor Intelligence (2026) also reported that the herbicide market in India would reach USD 647.53 million by 2031 to meet growing food demand across the country. The excessive and prolonged use of these herbicides has adversely affected soil health, biodiversity, and beneficial microorganisms such as bacteria, fungi, and earthworms (Ahmad et al., 2024). Bioaugmentation entails introduction of specific microorganisms or enzymes capable of degrading pollutants into contaminated environments (such as soil or wastewater) to accelerate degradation of hazardous substances (Nzila et al., 2016). It is a significant bioremediation technique commonly utilised to improve the efficacy of microbial communities (e.g., *Rhizobium*, *Azospirillum*, *Bacillus* spp., *Aspergillus*, *Trichoderma* and Mycorrhizal fungi) in agricultural fields to enhance crop yields and to control unwanted weeds, pests and pathogens (Aljabri, 2025).

PGPR are increasingly recognized as sustainable agents that enhance soil health through mechanisms like nitrogen fixation, phosphate solubilization and phytohormone production e.g., IAA and so on (Barbosa Santos et al., 2023). Herbicides (or weed killers) are chemicals or natural substances that control unwanted plants and boost crop yields by removing weeds that compete for resources in crop fields (Kraehmer et al., 2014). Common herbicides

include Glyphosate, Pendimethalin, 2,4-D, Paraquat, and Bispyribac-sodium have been used in India to control weeds (Maity et al., 2026). Pendimethalin has been reported as a harmful pre-emergence herbicide for soil microbiota due to its broad-spectrum, moderately persistent nature in soil which inhibits microbial cell division. Besides targeting to weeds, it also harms beneficial microorganisms by acting as mitotic inhibitor, reduce soil microbial biomass and hamper essential nutrient cycling (Bhat et al., 2021). Therefore, we have conducted a primary evaluation of indigenous rhizobacterial strains and examined the impact of Pendimethalin on plant growth and PGPR bacteria in an *in vitro* experimental model.

2. Materials and Methods

The experimental setup was prepared to assess effect of Pendimethalin on legume crops namely chickpea (*Cicer arietinum*) and PGPR bacteria. The *Rhizobium* spp., *Bacillus* spp. and *Pseudomonas* spp. were selected for study under PGPR bacteria. Pendimethalin 30 % EC were purchased with brand name TATA PANIDA from local market.

Isolation of PGPR from Rhizosphere soil

One gram of rhizosphere soil was suspended in 10 ml of sterile distilled water to make a 1/10 (w/v) soil suspension. Serial dilutions were subsequently prepared using sterile water. A 0.1 mL aliquot of suspension was spread onto 20 mL of solidified Yeast Extract Mannitol Agar (YEMA), Nutrient Agar Media (NAM) and Cetrinide Agar Media (CAM) and incubated at 37°C for 48 hours. After then colonies were counted and expressed as Colony Forming Unit (CFU) per mL (Sultana et al., 2020).

Experimental Design and Site Preparation

The experiment was conducted using pot culture design to evaluate impact of Pendimethalin on PGPR (viz., *Rhizobium* spp., *Bacillus* spp. and *Pseudomonas* spp.) and legume plant growth as mentioned by Hammok & Al-mandeel (2020) and Shubham et al. (2023) with slight modifications. Four treatment groups were established based on concentration of Pendimethalin applied to the soil:

- POT 1 (Control): 0 mg (No herbicide applied).
- POT 2 (Below Recommended): 5.0 mL/Liter Pendimethalin.
- POT 3 (Recommended Level): 10 mL/Liter of Pendimethalin (as prescribed by manufacturer).
- POT 4 (Above Recommended): 10 mL/Liter mg of Pendimethalin.

Plastic pots were filled with 5.0 kg of sterilized soil to ensure that any observed microbial activity was strictly derived from the introduced PGPR strains.

Inoculation of PGPR and Sowing

Pure cultures of *Rhizobium* spp., *Bacillus* spp., and *Pseudomonas* spp. were grown in their respective broth media viz., Yeast Extract Mannitol Broth (YEMB) for *Rhizobium* spp. and Nutrient Broth for *Bacillus* spp. and *Pseudomonas* spp. (Sultana & Pindi, 2025) at approximately 10^8 to 10^9 CFU/mL concentration. Chickpea seeds were surface-sterilized using 0.1% $HgCl_2$ and ethanol, then coated with the bacterial consortium using a 1% carboxymethyl cellulose (CMC) adhesive for bioprimering of seeds (Singh et al., 2020). Six seeds were sown per pot and later thinned to three healthy seedlings after germination.

Herbicide Application in Sites

Pendimethalin was prepared in aqueous solutions to achieve the target concentrations as per experimental design. It was then applied to all prepared sites (POT-1 to POT-4) as a pre-emergence soil drench 24 hours after sowing. The control pots received an equal volume of distilled water to maintain consistent moisture levels across all sites.

Assessment of PGPR Biochemical Activities

Total nitrogen content was estimated by using Kjeldahl method (Saha & Sen, 2024). Phosphate solubilization efficiency was measured by the diameter of the clearance zone on Pikovskaya's agar (Pikovskaya, 1948). IAA production was quantified using Salkowski's reagent and spectrophotometric analysis (Guardado-Fierros et al., 2024). Siderophore production was detected by clear zone diameter on Chrome Azurol S (CAS) agar (Nivetha et al., 2021). HCN synthesis was monitored via the filter paper (picric acid) method (Bakker & Schippers, 1986).

Plant Growth and Health Analysis

The chickpea plants were carefully harvested to maintain their root structure. Shoot height and root length were measured with a graduated scale. The number of functional nodules in the root system was recorded manually. Fresh weight was noted immediately after harvest. Plant health was visually evaluated, paying attention to leaf color (chlorosis), stem turgidity and any herbicide-induced deformities like club-root swelling.

Statistical Analysis

All data were collected in triplicate. The mean values and standard deviations were calculated to determine significance of inhibitory effect of herbicide across four treatment levels.

3. Results and Discussion

Three bacterial strains viz., *Rhizobium* spp., *Bacillus* spp. and *Pseudomonas* spp. were identified using colony, morphological and biochemical characterization shown in Table 1. The *Rhizobium* spp. was also screened using YEMA media supplemented with Congo Red. *Rhizobium* colonies was remained cream colour. Similarly, *Pseudomonas* spp. was cultivated on cetrimide agar for screening purposes. As it secretes the yellow-green pigment pyoverdine, *Pseudomonas* colonies were identified accordingly.

Table 1: Colony, Morphological and Biochemical characterization of PGPR Bacterial isolates

Feature	<i>Rhizobium</i> spp.	<i>Bacillus</i> spp.	<i>Pseudomonas</i> spp.
Gram Stain	Negative	Positive	Negative
Cell Shape	Rod-shaped	Rod-shaped bacilli	Straight or slightly curved rods
Endospore	Negative	Positive	Negative
Colony Morphology	Circular, convex, mucoid (glistening) and semi-translucent colony	Large, irregular, flat and matte-waxy appearance of colony	Circular, moist, slightly raised and mucilaginous surfaces
Pigmentation	Cream	Off-white	Yellow-green
Catalase Test	Positive	Positive	Positive
Oxidase Test	Positive	Negative	Positive
Starch Hydrolysis	Negative	Positive	Negative
Citrate Utilization	Negative	Positive	Positive

The plant growth-promoting (PGP) traits of three bacterial strains viz., *Rhizobium* spp., *Bacillus* spp. and *Pseudomonas* spp., were evaluated across four treatment sites (POT 1 to POT 4). All strains showed consistent decline in performance as pot number increased. *Pseudomonas* spp. exhibited highest overall microbial concentration and PGP activity in POT 1 reached 3.8×10^9 CFU/g, IAA production of 40.2 μ g/ml, siderophore clear zone of 20.5 mm and strong (+++) HCN production (Table 1). *Bacillus* spp. was recorded with highest phosphate solubilization at 72.6% in POT 1 with a

concentration of 2.5×10^9 CFU/g. In contrast, its IAA and siderophore levels were at 34 μ g/ml and 14.2 mm, respectively. Further, *Rhizobium* spp. generally showed lower values compared to other strains with its maximum performance in POT 1 with concentration of 1.2×10^9 CFU/g, 48.3% of P-solubilization and 26.5 μ g/ml of IAA. The microbial concentrations in all strains significantly decreased from POT1 to POT 4 with *Rhizobium* dropping to its lowest point of 1.1×10^5 CFU/g and failed to produce detectable HCN (Table 2).

Table 2: Biochemical Activities of PGPR Bacteria

Bacterial Strain	Treatment Site	Microbial Conc. (CFU/g)	P-Solubilization (%)	IAA Production ($\mu\text{g/ml}$)	Siderophore (Clear zone in mm)	HCN Production
Rhizobium spp.	POT 1	1.2×10^9	48.3	26.5	8.5	+
	POT 2	8.5×10^7	40.8	20.1	6.2	+
	POT 3	4.2×10^6	28	12.4	3.0	-
	POT 4	1.1×10^5	15.2	6.8	1.2	-
Bacillus spp.	POT 1	2.5×10^9	72.6	34	14.2	++
	POT 2	1.8×10^8	65.2	28.5	11.5	++
	POT 3	9.5×10^7	45.3	19.2	7.8	+
	POT 4	3.2×10^6	30.8	11	4.5	+
Pseudomonas spp.	POT 1	3.8×10^9	60.4	40.2	20.5	+++
	POT 2	2.4×10^9	55	33.8	18.2	+++
	POT 3	1.2×10^8	42.2	26.4	12.0	++
	POT 4	5.5×10^7	25.7	15.6	8.1	+

POT 1: Control (0 mg/L Pendimethalin); POT 2: Below Recommended Level (5.0 mL/L Pendimethalin); POT 3: Recommended Level (10 mL/L Pendimethalin); POT 4: Above Recommended Level (20 mL/L)

The chickpea growth and health indicators across four treatment sites (POT 1 to POT 4) exhibited progressive decline in all measured parameters. POT 1 showed most robust chickpea growth with 98% of germination rate, 24.5 cm of shoot height, and 14.2 cm of root length. These plants also produced highest biomass and nodulation with a fresh weight of 5.2 g and 22 nodules with appearing lush and dark green shown in Table 3. In contrast, POT 4 had least performance with germination rate of 58%, shoot height to 11.4 cm and root length to 4.2 cm. Likewise, nodulation was severely inhibited in POT 4 with only 2.0 nodules of chickpea

was recorded and it reached to a lowest fresh weight of only 1.8 g. It was showed visible chlorosis and brittle club-roots also. Hammok et al. (2020) disclosed notable decrease in pods, pod weight (g), and seed weight after pendimethalin treatment. POT 2 and POT 3 showed intermediate values with POT 3 specifically characterized by pale green coloration and stunted growth during present study. Notably, Shabana et al. (2001) reported declines in photosynthetic efficacy, cell counts, chlorophyll a levels, growth, and dry weight in green algae as pendimethalin concentration increased.

Table 3: Chickpea Growth and Health Indicators

Treatment Site	Germination (%)	Shoot Height (cm)	Root Length (cm)	Nodule Count	Fresh Weight (g)	Health and Appearance
POT 1	98	24.5	14.2	22	5.2	Lush, dark green and strong stems.
POT 2	92	21.8	11.5	16	4.5	Healthy and slightly smaller leaves.
POT 3	75	16.2	6.8	7.0	3.1	Pale green and stunted growth.
POT 4	58	11.4	4.2	2.0	1.8	Chlorosis and brittle club-roots.

POT 1: Control (0 mg/L Pendimethalin); POT 2: Below Recommended Level (5.0 mL/L Pendimethalin); POT 3: Recommended Level (10 mL/L Pendimethalin); POT 4: Above Recommended Level (20 mL/L)

The results showed direct and negative correlation. The concentration of pendimethalin increased from control (POT 1) to above-recommended dose (POT 4). The primary impact of pendimethalin was displayed drastic reduction in microbial viability. Table 1 Showed that *Rhizobium* spp. was most sensitive isolate with its population was declined from 1.2×10^9 CFU/g (control) to mere 1.1×10^5 CFU/g in POT 4 (pendimethalin above-recommended dose). This decline was possibly due to chemical nature of pendimethalin which interferes with microtubule assembly and cell division (Licitra et al., 2026). A high concentration of pendimethalin could disrupt metabolic pathways of sensitive bacteria (Ni et al., 2016). This microbial suppression results in deprivation of essential secondary metabolites produced by them and delivered to the plants for their growth (Narayanan & Glick, 2022). For instance, HCN production was completely inhibited in *Rhizobium* at recommended and above-recommended levels (POT 3 and 4).

The correlation between suppressed bacterial biochemical activity and reduced plant growth was notable. In the control (POT 1), *Pseudomonas* spp. produced high levels of IAA ($40.2 \mu\text{g/ml}$) and siderophores (20.5 mm). These metabolites are essential for root elongation and iron absorption. However, at the highest dose of pendimethalin in POT 4, IAA

production across all strains decreased by more than 60%. This hormonal deficiency coupled with direct inhibition of plant mitosis caused by pendimethalin presents significant concern in plant growth (Kim et al., 2023). As a result, it accounts for reduced root length (4.2 cm) and shoot height (11.4 cm). Plants entered a state of nutritional starvation and exhibited a pale green, chlorotic appearance without active PGPR to solubilize phosphorus (Ehinmitan et al., 2024). As we found, these were dropped from 72.6% to 30.8% in *Bacillus* spp. The herbicide was also seen in nodule counts. Chickpeas rely on *Rhizobium* for symbiotic nitrogen fixation (Mahto et al., 2025). The data showed that nodulation dropped by nearly 70% compared to the control at recommended dose of pendimethalin (POT 3). Remarkably, we observed only 2.0 nodules in POT 4 during the present study. Moreover, nitrogen deficiency caused chlorosis (Paponov et al., 2021) and massive loss in fresh weight (Zhao et al., 2005) which fell from 5.2 g to 1.8 g in succeeding POT 1 to POT 4. Wagner and Nádasy (2006) stated that elevated levels of pendimethalin diminish the fresh and dry weights of the entire vegetative parts and roots of peas. Therefore, present experimental observations exhibited that pendimethalin exerted a significant inhibitory effect on both PGPR and physiological development of chickpea plants.

4. Conclusion

The present study was successfully assessed impact of pendimethalin herbicide on PGPR activity and growth performance of chickpea under controlled pot culture conditions. The investigation revealed that the increase in concentrations of pendimethalin was significantly suppressed microbial population, biochemical activities and plant growth parameters. Among tested bacteria, *Pseudomonas* spp. was showed comparatively higher tolerance and better PGPR traits whereas *Rhizobium* spp. was found to be highly sensitive to herbicide stress. We have also noted that reductions in phosphate solubilization, IAA production, siderophore synthesis and nodulation were directly affected by germination, root-shoot development, biomass accumulation and overall plant health. Severe chlorosis and stunted growth were observed at herbicide doses above the recommended rate. These findings divulged that indiscriminate use of pendimethalin can negatively affect beneficial soil microbiota and chickpea productivity. Therefore, balanced herbicide application and integration of compatible PGPR inoculants are essential for sustainable crop management. Future research could be extended towards long-term field studies, molecular-level interaction analysis and formulation of herbicide-resistant biofertilizers to minimize ecological damage with sustainable agricultural productivity.

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This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from Other Sources

Not Applicable

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