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COMPARATIVE EFFICACY OF CHARCOAL AND PITMOSS BASED PSEUDOMONAS SPP. INOCULANTS ON PLANT GROWTH AND PHOSPHATE SOLUBILIZATION

Manoj D. Jadhav*	Asst. Professor, Department of Microbiology, Siddharth Arts, Commerce & Science College, Jafrabad, Dist. Jalna, Maharashtra *Corresponding Author
Ajaykumar G. Jadhav	Professor, Department of Microbiology, Government Institute of Science, Chh. Sambhajinagar, Maharashtra

ABSTRACT Phosphorus (P) is a vital macronutrient often limited by soil immobilization, necessitating sustainable solutions like Phosphate Solubilizing Bacteria (PSB). In this study, potent Pseudomonas spp. were isolated from diverse rhizospheric soils and characterized through morphological and biochemical assays. A pot culture experiment was conducted using a Completely Randomized Design (CRD) to evaluate the bio-efficacy of the isolate when integrated with two distinct carrier materials: Charcoal and Pitmoss. Growth parameters, including germination (P), leaf count (Q), plant height (R), and leaf size (S), were systematically monitored over a 15-day period. Findings revealed that while both carriers enhanced vegetative growth, the Pitmoss-based PSB formulation (Treatment D) demonstrated superior efficiency. By Day 15, Treatment D achieved a maximum plant height of 10.1 cm and a leaf count of 44, significantly outperforming charcoal-based treatments and uninoculated controls. Statistical analysis confirmed that carrier selection is a decisive factor in maintaining microbial viability and maximizing nutrient bioavailability. This study underscores the potential of Pseudomonas-enriched Pitmoss as a high-performance biofertilizer for sustainable chickpea (Cicer arietinum L.) cultivation, offering an eco-friendly alternative to synthetic phosphatic inputs.

KEYWORDS : Pseudomonas Spp., Phosphate Solubilization, Pitmoss, Charcoal, Biofertilizer, CRD.

INTRODUCTION

Phosphorus (P) is an indispensable macronutrient, serving as a structural component of nucleic acids and phospholipids, and playing a central role in energy transfer through ATP (Adenosine Triphosphate). Despite its abundance in the earth's crust, P is often the primary limiting factor for agricultural productivity due to its high reactivity. When applied to the soil as chemical phosphatic fertilizers, a staggering 70% to 90% of the soluble phosphorus is rapidly immobilized through precipitation with metals like Aluminum (Al^{3+}) and Iron (Fe^{3+}) in acidic soils, or Calcium (Ca^{2+}) in alkaline soils, making it unavailable for plant uptake [5]. Consequently, farmers often over-apply these fertilizers to compensate for this loss, leading to a massive accumulation of "legacy phosphorus" that frequently leaches into aquatic ecosystems, causing eutrophication and the degradation of surface water bodies.

The biochemical liberation of this sequestered phosphorus is primarily driven by soil microorganisms. These microbes utilize specialized mechanisms to transition P from insoluble inorganic and organic pools into bioavailable forms [1]. However, the modern agricultural paradigm's heavy reliance on synthetic inputs has disrupted these natural microbial cycles. The excessive and prolonged use of chemical fertilizers has been clinically linked to deteriorating human health, including increased risks of stomach cancer, goiter, and the life-threatening condition methemoglobinemia, or "Blue Baby Syndrome," in infants, caused by nitrate-heavy groundwater contamination [4]. As global food demand intensified, the agricultural sector became increasingly tethered to these agrochemicals for reliable crop yields [2]. Yet, this dependency has triggered a cascade of negative environmental impacts, most notably the development of chemical resistance in soil pathogens and the destruction of non-target beneficial microbial populations [7].

In response to these ecological challenges, Plant Growth-Promoting Rhizobacteria (PGPR) have emerged as a cornerstone of sustainable biotechnology. These rhizospheric bacteria facilitate plant development through diverse direct and indirect mechanisms, including biological nitrogen fixation, the production of iron-chelating siderophores, and the solubilization of mineral phosphates. Within this group, Phosphate Solubilizing Bacteria (PSB) are uniquely capable of hydrolyzing complex phosphorus compounds. Research has identified the genera Bacillus and Pseudomonas as the most potent and consistent solubilizers in varied soil profiles [3].

The application of these Phosphate Solubilizing Microorganisms (PSMs) has shown the potential to increase total crop yields by up to 70%. Specifically, Pseudomonas spp. have been documented to enhance the symbiotic efficiency of plants by increasing the number and dry weight of nodules, ultimately leading to superior grain quality and total biomass [6].

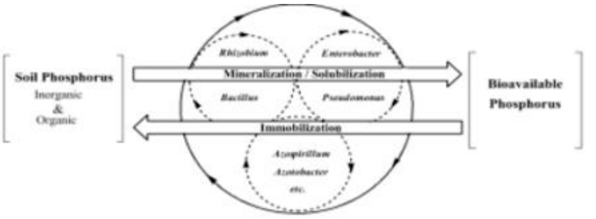

Figure 1. Schematic Representation of Phosphorus Transformation In Soil Mediated by Various Microbial Genera (Adapted from Gaur, 2002 [3])

MATERIALS AND METHODS

Sample Collection and Microbial Isolation :

Rhizospheric soil samples were collected aseptically from five different plants and stored at 4°C. Phosphate Solubilizing Bacteria (PSB) were isolated using the serial dilution-agar plating technique. 10 g of soil was suspended in 90 mL sterile water, and dilutions up to 10^{-7} were prepared. Aliquots (100 µl) were spread-plated on Pikovskaya's (PVK) Agar and incubated at 30°C for 2–7 days. Colonies exhibiting distinct halo zones were purified by re-streaking on PVK agar and maintained on nutrient agar slants.

Characterization of PSB Isolate:

The screened isolate underwent morphological and biochemical evaluation for identification: Morphology: Gram staining was performed to determine Gram reaction and cellular shape under oil immersion. IMViC Tests: Standard assays including Indole production (Kovac's reagent), Methyl Red (MR), Voges-Proskauer (VP), and Citrate utilization (Koser's Citrate medium) were conducted at 37°C for 24 hours. Carbohydrate Fermentation: The ability to ferment glucose, sucrose, and lactose was tested using fermentation broth equipped with Durham's tubes to detect acid and gas production.

Carrier Preparation and Pot Assay: The growth-promoting potential of the isolate was evaluated on Chickpea (Cicer arietinum L.) using a Completely Randomized Design (CRD) with three replicates. Carrier materials, specifically Charcoal and Pitmoss, were ground, sieved through a 200-mesh screen, and sterilized at 121°C for 15 minutes to ensure the removal of any indigenous microflora. For the inoculation process, surface-sterilized seeds were soaked in a 48-hour-old active PSB broth for two hours before being integrated with their respective carriers.

To ensure statistical accuracy and comparative analysis, four distinct treatment groups were established: Treatment A consisted of Charcoal without PSB, Treatment B utilized Charcoal with PSB, Treatment C served as the Pitmoss control without PSB, and Treatment D combined Pitmoss with the PSB inoculant. These treated seeds were sown in pots containing sterilized soil and maintained under greenhouse conditions, with data on germination percentage, plant height, and leaf count and size recorded systematically from day 3 to day 15.

RESULTS

Primary screening was based on the formation of distinct halo zones (zones of clearance) around the colonies, which indicates the solubilization of tricalcium phosphate. As shown in Table 1 and Figure 1, isolate 1b demonstrated the highest solubilizing activity with a clearance zone of 11 mm, followed by isolate 2a with 7 mm. Isolate 3d exhibited the minimum clearance zone of 5 mm. Based on these results, isolate 1b was identified as the most efficient phosphate solubilizer and was prioritized for further biochemical characterization and pot assays.

Table 1. In-vitro Phosphate Solubilizing Activity of Bacterial Isolates

Sr no.	Isolate Code	Zone of clearance (in mm)	Solubilization Rating
1	2a	7	Moderate
2	1b	11	High
3	3d	5	Low

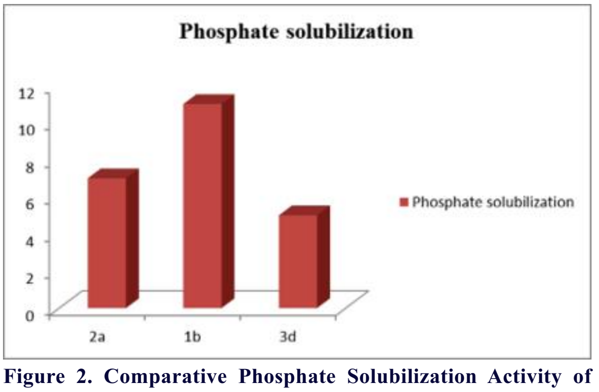

Figure 2. Comparative Phosphate Solubilization Activity of Isolates 2a, 1b, and 3d

The comparative performance of the bio-inoculants was evaluated using four primary growth indices: Germinated seeds (P), No. of leaves (Q), Height of plant (R), and Size of leaves (S) These parameters were monitored across four distinct experimental groups, categorized as Treatment A (Charcoal without PSB), Treatment B (Charcoal with PSB), Treatment C (Pitmoss without PSB), and Treatment D (Pitmoss with PSB). The data, recorded periodically over a 15-day growth cycle, are presented in Table 2.

Table 2: Effect of Carrier Materials and PSB on Growth Parameters (Day 3 to Day 15)

Treatment	Day	P	Q	R	S
A	3	2	1	1	0.1
	7	3	8	3.5	0.4
	15	4	28	7	0.9
B	3	4	4	1.5	0.2
	7	4	17	5	0.6
	15	4	42	9.2	1.1
C	3	3	2	1	0.1
	7	4	11	3	0.4
	15	4	30	8.1	0.9
D	3	4	4	2	0.3
	7	4	18	6	0.7
	15	4	44	10.1	1.2

Note: Only representative data for initial, middle, and final growth stages are shown for brevity.

Data presented in Table 2 illustrates the progressive growth of Cicer arietinum L. over a 15-day period. Treatment D (Pitmoss + PSB) exhibited the most significant growth enhancement, achieving a maximum plant height of 10.1 cm and a leaf count of 44 by the final observation day, outperforming both the charcoal-based carrier and uninoculated controls.

Statistical analysis using ANOVA confirmed that the growth variations between treatments were highly significant ($P < 0.05$). Specifically, the superior vegetative development in Treatment D —reaching a maximum height of 10.1 cm and 44 leaves—compared to the charcoal-based Treatment B (9.2 cm height, 42 leaves) underscores that the selection of a high-efficiency carrier matrix like Pitmoss is as critical as the microbial strain itself for achieving maximum bio-efficacy (P approx. < 0.001).

DISCUSSION

The superior performance of the Pitmoss-based PSB inoculant (Treatment D) is attributed to the carrier's high moisture retention and surface area, which facilitated prolonged microbial survival and enhanced phosphorus solubilization for plant uptake.

CONCLUSIONS

The present study successfully demonstrates the high efficacy of isolated Pseudomonas spp. in solubilizing insoluble inorganic phosphorus, thereby promoting the vegetative growth of Cicer arietinum L. (Chickpea). Experimental results reveal that the selection of an appropriate carrier material is a critical determinant of bio-inoculant performance; specifically, the Pitmoss-based formulation (Treatment D) consistently outperformed Charcoal-based and control treatments, achieving a maximum plant height of 10.1 cm and a leaf count of 44. These findings suggest that the integration of potent Phosphate Solubilizing Bacteria (PSB) with high-retention organic carriers can significantly enhance nutrient bioavailability and crop productivity.

Consequently, this biotechnological approach offers a sustainable, eco-friendly, and cost-effective alternative to synthetic phosphatic fertilizers, facilitating the transition toward "Green Agriculture" and reducing the environmental risks associated with agrochemical over-reliance.

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