**Influence of Phosphatic Fertilizers (PROM and DAP) on Soil Microbial Diversity Across Different Plant Growth Stages of Watermelon**

**Abstract**

Phosphatic fertilizers significantly influence soil microbial communities, playing a vital role in nutrient cycling and plant growth. This study investigates the impact of Phosphate Rich Organic Manure (PROM) and Di-Ammonium Phosphate (DAP) in different combination on soil microflora at different growth stages (vegetative, flowering, fruiting, and harvesting) in watermelon (Citrullus lanatus) cultivation. Soil microbial populations (bacteria and fungi) were analyzed across six treatments (T0–T5) with varying proportions of PROM and DAP. The results indicate that PROM application enhances microbial diversity and abundance due to its organic matter content, supporting beneficial bacterial and fungal communities. In contrast, DAP application leads to an initial surge in microbial activity followed by a decline, likely due to soil acidification and nutrient imbalance. Mixed treatments containing both PROM and DAP exhibited intermediate effects, balancing phosphorus availability and microbial sustainability. The study highlights the critical role of organic phosphorus sources in maintaining soil microbial health, promoting plant growth, and ensuring long-term soil fertility. Findings emphasize the need for integrated phosphorus management strategies to optimize soil microbial ecology and agricultural productivity. The study suggests that a balanced application of PROM and DAP can maximize phosphorus efficiency while sustaining microbial diversity, thereby supporting sustainable agricultural practices.

**Keywords:** PROM, DAP, Soil microflora, Plant growth stages, Soil health

**Introduction**

Soil microflora is an essential component of the soil ecosystem, playing a crucial role in nutrient cycling, organic matter decomposition, and overall plant growth (Richardson et al., 2009). The balance of soil microbial communities determines the efficiency of nutrient transformations and directly impacts soil health and crop productivity. Among the various soil nutrients, phosphorus is a key element required for plant growth, yet its availability in soil is often limited due to fixation in insoluble forms (Bashan & de-Bashan, 2010). Phosphatic fertilizers are widely used to supplement phosphorus levels in soil. Two commonly used sources are Phosphate Rich Organic Manure (PROM) and Di-Ammonium Phosphate (DAP). PROM is an organic amendment that provides phosphorus in a slow-release form, enhancing microbial activity and improving soil health. In contrast, DAP is a synthetic fertilizer that provides an immediate source of phosphorus but may lead to soil acidification and microbial imbalances when used excessively. Studies by Malhotra et al. (2018) indicate that organic phosphorus sources such as PROM significantly enhance microbial diversity, whereas excessive chemical fertilizers lead to long-term soil degradation.

Soil microflora includes a diverse array of microorganisms such as bacteria, fungi, actinomycetes, algae, and protozoa, each contributing uniquely to soil functioning. Bacteria dominate in number and are key players in nutrient solubilization, nitrogen fixation, and organic matter mineralization (Sylvia et al., 2005). Fungi, including mycorrhizal species, form symbiotic relationships with plant roots and aid in phosphorus and water uptake (Smith & Read, 2008). Actinomycetes, a group of filamentous bacteria, decompose complex organic compounds and contribute to humus formation (Goodfellow & Williams, 1983). Though less abundant, protozoa regulate bacterial populations through predation, while algae contribute to soil formation and nitrogen fixation in surface layers (Clarholm, 1985). The functional activity and abundance of these groups are directly influenced by nutrient availability and fertilization practices.

The application of these fertilizers have varying effects on soil microbial communities. Organic amendments such as PROM encourage the proliferation of beneficial microbes, including phosphate-solubilizing bacteria (PSB) and mycorrhizal fungi, which play a central role in phosphorus mobilization (Khan et al., 2019). These microorganisms convert insoluble phosphorus into bioavailable forms, facilitating plant uptake. In contrast, excessive use of DAP can reduce microbial diversity by altering soil pH and nutrient availability, thereby negatively affecting the soil enzymatic activities (Adesemoye et al., 2009; Smith & Read, 2010). Several studies suggest that integrated nutrient management using both organic and inorganic sources can optimize phosphorus availability while sustaining soil microbial health. The presence of organic matter in PROM supports nitrogen-fixing bacteria such as *Rhizobium* and *Azotobacter*, which further contributes to soil fertility (Gyaneshwar et al., 2002).

Furthermore, the influence of phosphatic fertilizers on microbial populations varies across different plant growth stages. During the vegetative stage, microbial activity is primarily driven by root exudates, promoting beneficial bacterial populations (Marschner et al., 2011). At the flowering and fruiting stages, microbial diversity peaks as nutrient demand increases, while the harvesting stage often shows a decline in microbial populations due to reduced root exudation and nutrient depletion (Van der Heijden et al., 2008). The ability of PROM to sustain microbial populations throughout these stages highlights its advantages over DAP for long-term soil fertility.

This study aims to evaluate the effects of PROM and DAP on soil microflora at different plant growth stages in watermelon cultivation. Specifically, it examines bacterial and fungal population dynamics in response to different fertilizer treatments. Understanding the impact of these fertilizers on microbial communities will provide insights into sustainable phosphorus management practices for improved soil fertility and crop productivity.

**Material and methods**

**Description of the study area**

The experimental site, ZS Farm in Sunarian Kalan, Rohtak, Haryana, lies at an altitude of 222 m and features a subtropical steppe climate. The region experiences hot summers and cool winters, with sandy loam soils conducive to watermelon cultivation.

**Climate and soil type**

The city of Rohtak, is located at 222 meters above sea level. It is influenced by a subtropical steppe climate. The average temperature is 30.2ºC (86.36ºF), which is 4.23% higher than the average for India. Typically, Rohtak receives about 10.3 millimeters (0.41 inches) of precipitation annually, with an average of 18.18 rainy days (4.98% of the time). The soils are loamy sand to sandy loam on the surface and sandy loam to clay loam on the sub-surface.

**Treatments and experiment design**

The crop selected for the study was the Sagar King variety of watermelon, scientifically known as *Citrullus lanatus* (Thumb.) Mastum. & Nakai. The pot experiment comprised 5 different combinations of PROM and DAP with 5 replications, as shown in table 1. The doses of nitrogen and potassium are the same for all treatments as recommended by FCO, while phosphorus was applied according to different PROM and DAP combinations. PROM consists of 88.3% bio-compost, 10.4% rock phosphate and the remaining concentration of various fungi (*Trichoderma viridae, Trichoderma herzanium, Aspergillus awamori, Aspergillus niger, Penicillium chrysogenum*), bacteria (*Cellulomonas* spp., *actinomyces, Strepto*myces spp., *Azotobacter chroococcum*), phosphate solubilizing bacteria (*Bacillus megaterium, Bacillus polymyxa, Psudomonas striata*), potash mobilizing bacteria (*Fraturea aurentia*), Sulfur oxidizing microorganism (*Thiobacillus thioxidanes*), iron and zinc solubilizing bacteria (*Thiobacillus ferroxidanes*) and silicon solubilizing bacteria. The fertilizer was applied every 3-4 weeks during the growing season at three growth phases. During the seedling stage, balanced 20-20-20g of N/P/K was applied; at the pre-flowering stage, 10-20-20g of N/P/K and 10-15-20g of N/P/K was applied during the fruit development stage.

**Table 1: Different concentrations of PROM and DAP in various combinations of treatments.**

|  |  |  |  |
| --- | --- | --- | --- |
| S. No. | Treatments | Dose of PROM | Dose of Chemical DAP |
| 1 | T0 (control) | 0% | 0% |
| 2 | T1 | 100% | 0% |
| 3 | T2 | 75% | 25% |
| 4 | T3 | 50% | 50% |
| 5 | T4 | 25% | 75% |
| 6 | T5 | 0% | 100% |

**Microbial Analysis**

Microbial populations in soil samples were assessed using the standard colony-forming unit (CFU) count technique as described by Madigan et al. (2018). Soil samples (10 g each) were collected from the rhizosphere at a depth of 0–15 cm using a sterile auger. The samples were placed in sterile polyethylene bags and stored at 4°C to preserve microbial viability and analyzed within 24 hours of collection, following the guidelines of Torsvik and Øvreås (2002).

For microbial enumeration, each 10 g soil sample was suspended in 90 mL of sterile 0.85% saline solution and homogenized by shaking at 120 rpm for 30 minutes, as per Atlas (2010). Serial dilutions ranging from 10⁻¹ to 10⁻8 were prepared by transferring 1 mL aliquots from the homogenized mixture into successive tubes containing 9 mL of sterile saline solution, in accordance with Cappuccino and Sherman (2014). Aliquots of 100 µL from the appropriate dilutions were spread onto nutrient agar for bacterial enumeration and potato dextrose agar for fungal enumeration using a sterile glass spreader. The plates were incubated at 28±2°C for 24–48 hours for bacterial colonies and 72 hours for fungal colonies, following the protocol by Difco (2009). After incubation, colonies were manually counted using a digital colony counter, and results were expressed as CFU per gram of soil for both bacteria and fungi (Pelczar et al., 2015).

Microbial identification was carried out based on morphological characteristics, including colony shape, pigmentation, and margin. Selective media such as MacConkey agar for Gram-negative bacteria and Mannitol Salt Agar for *Staphylococcus* spp. were used for differentiation, as outlined by Prescott et al. (2020).

**Results**

The microbial population in soil varied significantly across different treatments and different plant growth stages. The highest bacterial and fungal counts were observed in treatments with higher proportions of PROM (T1, T2, and T3), whereas treatments with higher proportions of DAP (T4 and T5) exhibited lower microbial activity. The microbial population generally increased from the vegetative stage to the flowering stage, followed by a gradual decline during the fruiting and harvesting stages.

**Table 2: Effect of different treatments (T0, T1, T2, T3, T4 and T5) on soil microbial count at various plant growth stages (Vegetative, flowering, fruiting and harvesting)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S. No. | Treatments | Vegetative Stage (CFU x 108/g soil) | Flowering Stage (CFU x 108/g soil) | Fruiting Stage (CFU x 108/g soil) | Harvesting Stage (CFU x 108/g soil) |
| 1 | T0 | **B:** 3.9 ± 1.4**F:** 1.5 ± 0.4 | **B:** 7.5 ± 1.2**F:** 4 ± 0.6 | **B:** 5.6 ± 1.0**F:** 3.4 ± 0.5 | **B:** 4.8 ± 1 .5**F:** 2 ± 0.5 |
| 2 | T1 | **B:** 10 ± 1.5**F:** 5.5 ± 1 | **B:** 17 ± 2.5**F:** 10 ± 1.5 | **B:** 14.5 ± 2**F:** 8 ± 1.2 | **B:** 12.8 ± 2**F:** 7 ± 1 |
| 3 | T2 | **B:** 13 ± 2**F:** 6 ± 1.2 | **B:** 21 ± 3**F:**11 ± 2 | **B:** 19 ± 3**F:** 10 ± 2 | **B:** 16 ± 3**F:** 8.5 ± 2 |
| 4 | T3 | **B:** 9.5 ± 2**F:** 5 ± 1 | **B:** 13.5 ± 3**F:** 8 ± 1.8 | **B:** 12.6 ± 2.5**F:** 6.5 ± 1.5 | **B:** 11 ± 2**F:** 5.5 ± 1 |
| 5 | T4 | **B:** 7 ± 1.5**F:** 4.8 ± 1 | **B:** 11 ± 2.5**F:** 6.5 ± 1.3 | **B:** 10 ± 2**F:** 6 ± 1.2 | **B:** 9 ± 2**F:** 5.5 ± 1 |
| 6 | T5 | **B:** 5.5 ± 1**F:** 4 ± 0.8 | **B:** 9.5 ± 2**F:** 6 ± 1.1 | **B:** 8.8 ± 1.5**F:** 5 ± 1 | **B:** 7.5 ± 1**F:** 4.5 ± 1 |

\*T0: control, **T1:** 100% PROM**, T2:** 75% PROM + 25% DAP**, T3:** 50% PROM + 50% DAP, **T4:** 25% PROM + 75% DAP,and **T5:** 100% DAP; **\*B:** Bacterial count, and **F:** Fungal count.

**Vegetative Stage**

During the vegetative stage, the microbial population was at its lowest across all treatments. However, significant differences were observed among the treatments. The control group (T0) recorded the lowest microbial population (B: 3.9 ± 1.4 × 10⁸ CFU/g, F: 1.5 ± 0.4 × 10⁸ CFU/g). The application of 100% PROM (T1) resulted in a notable increase in microbial counts (B: 10 ± 1.5 × 10⁸ CFU/g, F: 5.5 ± 1 × 10⁸ CFU/g), highlighting PROM’s key role in boosting microbial activity. The highest microbial count was observed in T2 (75% PROM + 25% DAP) (B: 13 ± 2 × 10⁸ CFU/g, F: 6 ± 1.2 × 10⁸ CFU/g), suggesting a synergistic effect from PROM combined with a small amount of DAP. Conversely, T5 (100% DAP) showed the lowest microbial counts among the treated groups (B: 5.5 ± 1 × 10⁸ CFU/g, F: 4 ± 0.8 × 10⁸ CFU/g), indicating that high levels of chemical phosphorus could suppress microbial populations.

**Flowering Stage**

The microbial population increased significantly at the flowering stage across all treatments. The highest microbial activity was recorded in T2, with bacterial and fungal counts of 21 ± 3 × 10⁸ CFU/g and 11 ± 2 × 10⁸ CFU/g, respectively. This was followed by T1, which recorded counts of B: 17 ± 2.5 × 10⁸ CFU/g, F: 10 ± 1.5 × 10⁸ CFU/g. Treatments with balanced PROM and DAP application (T3 and T4) exhibited moderate microbial populations, while T5 again recorded the lowest among treated groups (B: 9.5 ± 2 × 10⁸ CFU/g, F: 6 ± 1.1 × 10⁸ CFU/g). These observations underscore the superior influence of PROM-rich treatments in promoting microbial proliferation compared to DAP-alone.

**Fruiting Stage**

Microbial populations declined slightly during the fruiting stage compared to the flowering stage, although PROM-based treatments continued to support higher microbial activity. T2 and T1 sustained the highest bacterial and fungal counts—T2 (B: 19 ± 3 × 10⁸ CFU/g, F: 10 ± 2 × 10⁸ CFU/g) and T1 (B: 14.5 ± 2 × 10⁸ CFU/g, F: 8 ± 1.2 × 10⁸ CFU/g). T3 and T4 showed gradual reductions in microbial loads—T3 (B: 12.6 ± 2.5 × 10⁸ CFU/g, F: 6.5 ± 1.5 × 10⁸ CFU/g) and T4 (B: 10 ± 2 × 10⁸ CFU/g, F: 6 ± 1.2 × 10⁸ CFU/g). The lowest activity was again observed in T5, with microbial counts of B: 8.8 ± 1.5 × 10⁸ CFU/g, F: 5 ± 1 × 10⁸ CFU/g.

**Harvesting Stage**

At the harvesting stage, microbial populations declined further across all treatments. T2 maintained the highest microbial count (B: 16 ± 3 × 10⁸ CFU/g, F: 8.5 ± 2 × 10⁸ CFU/g), followed by T1 (B: 12.8 ± 2 × 10⁸ CFU/g, F: 7 ± 1 × 10⁸ CFU/g). The microbial populations in T3 and T4 continued to decline—T3 (B: 11 ± 2 × 10⁸ CFU/g, F: 5.5 ± 1 × 10⁸ CFU/g) and T4 (B: 9 ± 2 × 10⁸ CFU/g, F: 5.5 ± 1 × 10⁸ CFU/g). T5 remained the least effective, with bacterial and fungal counts of 7.5 ± 1 × 10⁸ CFU/g and 4.5 ± 1 × 10⁸ CFU/g, respectively. These findings reinforce the long-term microbial benefits of PROM-based fertilization compared to sole chemical phosphorus input.

**Figure 1: Effect of different treatments on soil bacterial count at various plant growth stages**

**Figure 2: Effect of different treatments on soil fungal count at various plant growth stages.**

Across all treatments, the highest microbial counts were observed at the flowering stage, followed by a decline in the fruiting and harvesting stages. The peak microbial population at the flowering stage is likely due to enhanced root exudation, which provides an organic carbon source for microbial communities.

 **Statistical Analysis**

The results were calculated and statistically examined using an analysis of variance (ANOVA). The Pearson correlation coefficient of pairwise correlation analysis was performed. Statistical significance was considered as P<0.05.The tests were conducted using SPSS version 26.

**Discussion**

The present study demonstrated that soil microbial populations varied significantly across different fertilization treatments and plant growth stages, reflecting the profound influence of nutrient inputs and plant phenology on soil biological dynamics. Among the treatments, those involving PROM led to markedly higher microbial abundance and activity, especially during the flowering stage, underscoring the potential of organic amendments in promoting soil microbial health. These findings are in agreement with prior research that highlights the role of organic inputs in enriching microbial diversity, stimulating enzymatic activity, and improving overall soil biological quality (Chen et al., 2022; Zhang et al., 2016).

Soil microflora, including bacteria, fungi*, actinomycetes*, and *archaea*, are integral to maintaining ecosystem functionality. These microbial communities facilitate key soil processes, including nutrient mineralization, organic matter decomposition, nitrogen fixation, and the suppression of soil-borne diseases (van der Heijden et al., 2008). Bacteria and *actinomycetes* are especially important for nitrogen and phosphorus mineralization, while fungi are primarily responsible for breaking down complex organic substances like lignin and cellulose. The application of PROM helps create a favorable environment for these microorganisms by providing easily accessible organic carbon, enhancing soil structure, improving aeration and water retention, and stabilizing soil pH factors that collectively support microbial growth and diversity (Bhattacharyya et al., 2018; Huang et al., 2014).

The highest microbial counts were observed in treatment T2 (75% PROM + 25% DAP), especially during the flowering stage, closely followed closely by T1 (100% PROM). This trend strongly suggests that organic phosphorus inputs provide sustained nutrient availability and support rhizosphere microbial proliferation. Interestingly, T2 also showed elevated microbial counts, indicating that the strategic inclusion of a small amount of chemical phosphorus may synergistically enhance microbial activity by rapidly addressing short-term phosphorus demands while PROM fulfills longer-term nutrient and carbon needs. This integrated approach is supported by Singh et al. (2022), who reported improved soil biological activity and nutrient cycling when organic and inorganic phosphorus sources were applied in combination.

In contrast, treatments dominated by chemical phosphorus (T4 and T5) consistently exhibited lower microbial populations at all growth stages. High DAP concentrations may induce soil acidification, alter osmotic balance, and suppress sensitive microbial taxa, thereby destabilizing the soil microbial community (Chen et al., 2019). Manna et al. (2005) also noted that excessive chemical fertilization can lead to nutrient imbalances and long-term soil degradation. Moreover, such conditions may suppress beneficial microbes, including phosphate-solubilizing bacteria (PSB), mycorrhizal fungi, and nitrogen-fixing organisms, which are vital for nutrient transformation and plant-microbe symbiosis.

Microbial populations followed a characteristic temporal pattern: increasing from the vegetative stage to a peak during flowering, and then declining during the fruiting and harvesting stages. This progression reflects the influence of root activity on the soil microbiome. During early vegetative growth, roots begin exuding simple sugars and organic acids that serve as substrates for microbial proliferation. The flowering stage typically corresponds with peak root exudation and nutrient demand, creating an optimal environment for microbial metabolism (Huang et al., 2014). Root exudates also act as signaling molecules, modulating microbial community structure and facilitating beneficial plant-microbe interactions.

The decline in microbial counts during the fruiting and harvesting stages can be attributed to reduced root activity, exhaustion of labile carbon sources, and increased microbial competition for limited nutrients. However, the persistence of relatively higher microbial counts in PROM-treated soils even during the later stages of crop growth underscores the advantage of organic inputs in maintaining soil biological resilience. Organic amendments decompose slowly and continue to release nutrients and carbon over time, thereby supporting microbial life beyond the peak growth phases (Bhattacharyya et al., 2018).

The ecological implications of these findings are significant. PROM-based treatments not only promote microbial abundance but also contribute to a more functionally diverse and resilient soil microbiome. Enhanced microbial activity, in turn, facilitates nutrient solubilization, disease suppression, and improved plant growth—factors critical to sustainable crop production. Furthermore, PROM contributes to the buildup of soil organic matter and improves soil physical properties such as porosity, water-holding capacity, and aggregate stability, creating a favorable microhabitat for microbial communities (Huang et al., 2014). In contrast, the consistent reduction in microbial populations under high-DAP treatments highlights the unintended consequences of excessive chemical fertilizer use. While DAP can provide immediate nutrient availability, its long-term use risks soil acidification, disruption of microbial networks, and reduced soil fertility. These effects may ultimately compromise the productivity and sustainability of agricultural systems (Chen et al., 2019).

**Conclusion**

This study reinforces the value of integrated nutrient management strategies that incorporate both organic and inorganic sources to optimize plant growth while preserving the health and diversity of soil microbial communities. The results confirm that PROM-based fertilization strategies significantly enhance soil microbial activity, particularly during the critical flowering and fruiting stages of watermelon growth. Treatments with a combination of organic and chemical phosphorus (e.g., T2: 75% PROM + 25% DAP) provided the highest microbial counts, suggesting that a judicious mixture of organic and inorganic fertilizers may offer optimal soil health benefits. Future research should explore the long-term impacts of PROM application on soil microbial diversity and its role in sustainable crop production.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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