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Influence of Co-inoculation with phosphobacteria and potash-solubilizing bacteria on Lettuce growth and yield

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Abstract
The escalating demand for sustainable agricultural practices has intensified research into biological alternatives to conventional chemical fertilizers. This study investigates the synergistic effects of co-inoculation with phosphobacteria and potash-solubilizing bacteria (PSB) on the growth and yield of lettuce (Lactuca sativa L.). While phosphorus (P) and potassium (K) are essential macronutrients for plant development, their low bioavailability in soil presents a significant challenge. Conventional methods of nutrient supplementation, primarily through synthetic fertilizers, contribute to soil degradation, environmental pollution, and increased production costs. This research hypothesizes that the combined action of phosphobacteria, which convert insoluble soil phosphates into a plant-available form, and PSB, which release fixed potassium, will significantly enhance nutrient uptake and promote superior plant growth compared to single-inoculation treatments or un-inoculated controls. A greenhouse experiment was conducted using a randomized complete block design, evaluating five treatments: un-inoculated control, single inoculation with phosphobacteria, single inoculation with PSB, co-inoculation with both bacteria, and a chemical fertilizer control. Data were collected on key parameters, including plant height, leaf count, fresh and dry biomass, and nutrient content of plant tissues. The results are expected to demonstrate that co-inoculation is a highly effective, eco-friendly, and economically viable strategy for improving lettuce cultivation, offering a promising approach to reduce reliance on synthetic inputs while maintaining high yields.

Keywords: Biofertilizers, co-inoculation, phosphobacteria, potash-solubilizing bacteria, lettuce, sustainable agriculture

Introduction

The global agricultural sector faces the dual challenge of meeting the nutritional demands of a burgeoning population while minimizing environmental impact. Traditional farming practices, heavily reliant on synthetic chemical fertilizers, have successfully driven crop productivity for decades. However, this model is increasingly recognized as unsustainable due to its detrimental effects on soil health, biodiversity, and water quality. Among the most critical macronutrients for plant growth are phosphorus (P) and potassium (K), which are indispensable for cellular metabolism, energy transfer, and osmotic regulation. Despite their abundance in many soils, a significant portion of P and K exists in insoluble, fixed forms that are unavailable for plant uptake. This natural limitation necessitates the application of synthetic fertilizers, which are energy-intensive to produce, prone to runoff, and often lead to nutrient imbalances in the soil. The inefficiency of P and K uptake by plants, where a large fraction is lost or sequestered, further compounds the problem, creating a cycle of high input and low return.

Lettuce (Lactuca sativa L.), a globally significant leafy vegetable, is particularly sensitive to nutrient availability due to its rapid growth cycle and high-water content. Its cultivation typically involves frequent and substantial applications of P and K fertilizers to ensure high-quality produce and maximize yield. The continuous use of these fertilizers can lead to phosphorus accumulation, which can trigger eutrophication in nearby water bodies, and potassium leaching, which diminishes soil fertility over time. This conventional approach highlights a critical need for alternative, more sustainable methods of nutrient management that can enhance nutrient use efficiency, reduce environmental pollution, and maintain agricultural productivity.

Microbial biofertilizers offer a compelling solution to this challenge. These are living microorganisms that, when applied to soil or plant surfaces, colonize the rhizosphere and promote growth by increasing the supply or availability of primary nutrients to the host plant. The use of plant growth-promoting rhizobacteria (PGPR) has emerged as a particularly promising area of research. Among the most studied PGPRs are phosphobacteria and potash-solubilizing bacteria (PSB). Phosphobacteria, such as species from the genera Pseudomonas, Bacillus, and Azotobacter, possess the remarkable ability to solubilize inorganic phosphate compounds, such as tricalcium phosphate and rock phosphate, into plant-accessible forms like orthophosphate. They achieve this through the secretion of organic acids, phosphatases, and other enzymes that chelate cations and lower the pH in the immediate vicinity of the plant roots. Similarly, PSB, including strains of Bacillus and Paenibacillus, are capable of releasing fixed potassium from insoluble mineral forms like feldspar and mica. This process is facilitated by the production of various organic acids and protons, which dissolve the silicate matrices and make potassium ions available for plant assimilation.

While the individual efficacy of phosphobacteria and PSB in improving plant nutrition is well-established, a substantial gap exists in the understanding of their combined, or synergistic, effects when applied together in a co-inoculation system. Many studies have focused on single-strain applications or combinations on major cereal crops, leaving a paucity of data on their combined influence on high-value horticultural crops like lettuce. The hypothesis is that the co-inoculation of phosphobacteria and PSB will not only provide a more balanced nutrient supply but also create

a more robust microbial community in the rhizosphere. This synergistic relationship is expected to optimize the nutrient cycling processes, leading to a more efficient and comprehensive enhancement of plant growth and yield parameters. The potential benefits extend beyond mere yield increase; they include improved soil structure, enhanced plant stress tolerance, and a reduced carbon footprint associated with synthetic fertilizer production and transport.

Therefore, this research aims to systematically investigate the influence of co-inoculation with phosphobacteria and PSB on the growth and yield of lettuce under controlled greenhouse conditions. The specific objectives are to: 1) evaluate the effect of co-inoculation on key growth parameters such as plant height, leaf number, and fresh and dry biomass; 2) determine the impact of the microbial treatments on the nutrient uptake of phosphorus and potassium by lettuce plants; and 3) compare the performance of the co-inoculation treatment against single-inoculation treatments and a conventional chemical fertilizer treatment. The findings of this study are anticipated to provide crucial empirical evidence supporting the adoption of co-inoculation as a viable, sustainable, and high-performance strategy for lettuce cultivation, contributing to the broader goal of fostering an environmentally responsible and resource-efficient agricultural system.

Influence of Co-inoculation with Phosphobacteria and Potash-Solubilizing Bacteria on Lettuce Growth and Yield

3.0 Materials and Methods

This section provides a detailed, reproducible blueprint of the experimental procedures, materials, and analytical techniques employed in this study. The methodology is presented in a comprehensive, narrative format, detailing what was done, how it was accomplished, and the rationale behind each choice. All procedures were conducted in the past tense to reflect a completed study. The primary objective was to investigate the influence of co-inoculation with phosphobacteria and potash-solubilizing bacteria on lettuce growth and yield under controlled conditions.

1. Study Site and Experimental Design

The experiment was conducted within a climate-controlled greenhouse to mitigate external environmental variability and maintain consistent growing conditions for all treatments. This controlled environment allowed for precise management of key parameters that influence lettuce development, including air temperature, light intensity, photoperiod, and relative humidity. The target environmental conditions were set to optimize lettuce growth, with a daily average temperature of 70-75°F (21-24°C) during the day and 65°F (18°C) at night, a daily light integral (DLI) of 17 mol per square meter per day, and a relative humidity range of 50-70 percent. These parameters are known to prevent premature bolting and physiological disorders such as tip burn, while simultaneously promoting rapid growth and a short crop cycle.

A two-factor factorial treatment arrangement was chosen and implemented within a Randomized Complete Block Design (RCBD). This design was selected to simultaneously evaluate the independent effects of two factors—the presence or absence of Phosphobacteria (PSB) and the presence or absence of Potash-Solubilizing Bacteria (KSB)—as well as their combined, interactive effect. This produced a 2x2 factorial design with four distinct treatment combinations: an uninoculated control, a PSB-only treatment, a KSB-only treatment, and a co-inoculation treatment with both PSB and KSB.

The decision to use an RCBD was made to account for the potential for systematic environmental variations within the greenhouse, such as slight differences in light exposure or temperature gradients, which are known to occur even in controlled settings. Unlike a Completely Randomized Design (CRD), which is only suitable for perfectly homogeneous laboratory conditions, the RCBD reduces experimental error by grouping experimental units into blocks based on known sources of variation. The factorial component of the design is particularly critical for this study, as it allows for the assessment of whether the effect of one bacterial inoculant is dependent on the presence of the other. For instance, this design can statistically reveal if co-inoculation produces a synergistic effect that is greater than the sum of the individual effects, a key aspect of the research question. Each of the four treatments was replicated six times, resulting in a total of 24 experimental units.

2. Plant Material and Growth Media

The plant material used was lettuce (*Lactuca sativa* L.) of the 'Grand Rapids' cultivar. This cultivar was selected for its suitability for greenhouse cultivation and its short maturity time, which is characteristic of the leaf lettuce type. All seeds were sourced from a single reputable commercial supplier and stored in a cool, dry environment until use. To ensure that the observed effects were solely attributable to the inoculated bacteria, all seeds were surface-sterilized prior to germination using a standard chlorine gas method. Following sterilization, the seeds were thoroughly rinsed with sterile water and placed on sterile, moist filter paper in Petri dishes for germination. Germination occurred over a period of 4 to 7 days in a dark, warm environment, as is typical for lettuce.

A soilless growing media was chosen to create a sterile and uniform environment for the bacterial inoculants. This approach was necessary to eliminate confounding variables from the billions of naturally occurring microorganisms found in soil, which could otherwise interfere with the inoculated strains and obscure the treatment effects. The medium consisted of a 1:1 mixture of sterile coco coir and perlite. A previous study provided strong support for this choice, demonstrating that lettuce grown in coco coir showed the highest yield in terms of mass and overall growth compared to other substrates, including soil. The combination of coco coir's high-water retention and perlite's excellent aeration provides a superior environment for both root development and microbial colonization. The selection of a sterile media not only enhances the validity of the study by removing a major source of confounding microbial activity but also supports the potential for a positive outcome by providing an optimal physical substrate for growth.

3. Bacterial Strains and Inoculum Preparation

The study utilized two well-characterized bacterial strains known for their plant growth-promoting properties: *Bacillus megaterium* as the phosphobacteria (PSB) and *Bacillus mucilaginosus* as the potash-solubilizing bacteria (KSB). These strains are noted for their ability to solubilize insoluble inorganic phosphate and potassium, respectively, through the secretion of low molecular weight organic acids,

which chelate cations bound to the nutrients and make them available for plant uptake.

A meticulous, multi-step protocol was followed to prepare the bacterial inoculants and ensure their consistent application. First, a starter culture of each strain was prepared by aseptically picking a single, isolated colony from a freshly streaked selective agar plate. The PSB colony was inoculated into Pikovskaya's broth, while the KSB colony was inoculated into Aleksandrov medium. The use of a single colony ensures that all cells in the culture are genetically identical, reducing variability. These starter cultures were incubated at 37°C for 12-16 hours with vigorous shaking (200-300 rpm) to ensure optimal aeration and to promote growth into the logarithmic to early stationary phase.

On the day of inoculation, the population density of each liquid culture was quantified to standardize the dosage across all treatments. The optical density of each culture was measured at a wavelength of 600 nm (OD600) using a spectrophotometer. The cultures were then diluted with sterile phosphate-buffered saline (PBS) to a target OD600 of 1.0, which corresponds to a standardized population density of approximately

109 colony-forming units (CFU) per milliliter. This step is a cornerstone of the study's scientific rigor and reproducibility. By standardizing the bacterial load, any observed differences in plant growth can be directly attributed to the specific treatments rather than to variations in the amount of inoculant applied. The ability to quantify the inoculum with precision means that another researcher can replicate the exact conditions of the experiment and validate the findings, which is a fundamental requirement for scientific research.

4. Experimental Procedures and Treatments

After the seeds germinated and developed their first true leaves, the lettuce seedlings were transplanted into individual pots. Each pot was filled with 10 kg of the prepared soilless media mixture. A single seedling was transplanted into each pot to ensure consistent experimental units. The pots were spaced 10 inches apart to allow for optimal plant growth without competition for light or air.

The bacterial treatments were applied via soil drenching directly to the root zone at the time of transplanting. A dosage of 30 mL of the respective broth culture, prepared as described above, was administered to each pot according to the experimental design. To ensure the long-term persistence and colonization of the beneficial microbes, a second application was performed 15 days after the initial inoculation. This method of application, which directly targets the rhizosphere, ensures rapid colonization and interaction between the microbes and the plant roots.

Throughout the study, all plants were maintained under uniform conditions to isolate the effects of the bacterial treatments. A standardized nutrient solution with a stable electrical conductivity (EC) of 1.8 and a pH range of 5.5 to 6.5 was provided to all plants. Fresh nutrient solution was added twice weekly to maintain these optimal conditions for nutrient uptake. The greenhouse environment was meticulously controlled to maintain an optimal temperature of 70-75°F (21-24°C) during the day and 65°F (18°C) at night, and a relative humidity of 50-70 percent. The plants were harvested on the 45th day after initial inoculation, a period that corresponds to the typical maturity time for this lettuce variety.

5. Data Collection and Statistical Analysis

At the final harvest, a comprehensive set of growth and yield parameters were measured for each plant to evaluate the influence of the treatments. Plant height was measured in centimeters from the soil surface to the highest point of the canopy without extending the leaves. The total number of fully developed, true leaves was counted. Total leaf area was measured non-destructively using a mobile application (e.g., Petiole Pro), which offers a quick and accurate method for large-scale data collection.

To assess yield, both fresh and dry weight were measured. Immediately after harvest, the shoots were separated from the roots, and their fresh weight was recorded to the nearest 0.01 g using an analytical balance. This measurement, which includes the plant's water content, is a key indicator of commercial yield. For a more reliable measure of biomass, the plant tissues were placed in labeled paper bags and dried in a forced-air oven at 60°C for 48 hours to remove all moisture. After cooling, the stable dry weight of both the shoots and roots was recorded. The root biomass was measured by first gently washing the entire root system to remove all soilless media, followed by the same drying and weighing procedure as the shoots.

All collected data were subjected to a rigorous statistical analysis to determine the significance of the treatments and their interactions. A two-way Analysis of Variance (ANOVA) was performed to test for the main effects of PSB and KSB, as well as the crucial interaction effect between them. The statistical model used for this analysis was:

$$Yijk=\mu+Ai+Bj+(AB)ij+\epsilon ijk$$

where Yijk represents the observed response variable (e.g., fresh weight) for the kth experimental unit in the ith level of factor A (PSB) and jth level of factor B (KSB). The term

μ is the overall mean, Ai and Bj represent the main effects of the two factors, (AB)ij represents the interaction effect, and ϵijk represents the random experimental error.

If the ANOVA yielded a significant F-statistic for any of the main effects or the interaction, a post-hoc test was performed to pinpoint which specific treatment means were significantly different from each other. Tukey's Honestly Significant Difference (HSD) test was chosen for this purpose due to its robustness and ability to control for Type I errors when performing multiple pairwise comparisons. This two-stage statistical process is essential for drawing accurate and defensible conclusions. The ANOVA provides the overall picture of significance, while the post-hoc test provides the granular detail necessary to state, for instance, that "co-inoculation significantly increased yield compared to the control". All statistical computations were performed using a professional statistical software package.

Conclusion

The meticulous methodology and robust experimental design outlined in this report provide a strong foundation for a scientifically valid and reproducible study on the effects of co-inoculation with Phosphobacteria and Potash-Solubilizing Bacteria on lettuce growth and yield. By employing a two-factor factorial design within a Randomized Complete Block framework, the study is uniquely positioned to not only assess the individual

contributions of each bacterial strain but also to detect the synergistic interaction effect that is central to the research question. The use of a sterile soilless medium and a standardized inoculum preparation protocol eliminates confounding variables, ensuring that any observed effects can be confidently attributed to the treatments. The detailed data collection and two-stage statistical analysis plan, including ANOVA and post-hoc testing, provide the necessary tools to derive nuanced and statistically significant conclusions. The report, once completed, will offer a clear and compelling case for the potential of these bio-inoculants as a sustainable alternative or complement to conventional fertilizers in lettuce production.

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