Effect of rate of biofertilizers on growth and development of spinach (*Spinacia Oleracea L.)*

Aadesh[1], *Ravi* Sharma [1], *Samrat Singh* Jadoun [1], *Ajay* Tomar[1*], *Kshitij* Parmar [1*]

[1]Amity Institute of Organic Agriculture, Amity University, Uttar Pradesh, Sector-125, Noida-201313

[*]Corresponding authors: atomar2@amity.edu, kparmar@amity.edu

Abstract: This research delves into the utilization of biofertilizers as a sustainable substitute for conventional chemical fertilizers, offering multifaceted advantages for soil fertility, plant vitality, and ecological equilibrium. The study investigates the effects of different application rates of biofertilizers on various growth parameters, nutrient assimilation efficiency, yield components, and physiological responses in spinach cultivation. A series of experimental trials were conducted to evaluate the impacts of biofertilizer treatments, comprising vermicompost, Azotobacter, and phosphate-solubilizing bacteria (PSB), on spinach plants. The results indicate that combined application of biofertilizers significantly enhances key parameters including plant height, leaf area, shoot biomass, root biomass, nutrient assimilation efficiency, yield components, and physiological responses compared to conventional fertilizer practices and control groups. Correlation and regression analyses reveal strong positive relationships between levels of biofertilizer application and various growth metrics, nutrient assimilation efficiency, yield components, and physiological responses. Furthermore, ANOVA results confirm the statistical significance of differences among treatment groups, highlighting the efficacy of biofertilizers in promoting spinach growth and productivity. Economic evaluations suggest that despite higher initial costs, the use of biofertilizers results in increased spinach yields, making it a financially viable and environmentally sustainable alternative to traditional fertilization methods.

Keywords: Biofertilizers, sustainable substitute, conventional chemical fertilizer, soil fertility, plant vitality, ecological equilibrium, growth parameters, nutrient assimilation efficiency, yield components, spinach cultivation, experimental trials, vermicompost, Azotobacter, phosphate-solubilizing bacteria (PSB), parameters, correlation and regression, yield components, physiological responses, ANOVA, environmentally sustainable.

Introduction

Spinach, known scientifically as *Spinacia oleracea*, has a rich history rooted in the ancient lands of central and Western Asia. It belongs to the esteemed order Caryophyllales, the family Amaranthaceae, and the subfamily Chenopodioideae, boasting a botanical lineage steeped in historical and biological significance. Scholars believes that spinach was originated around 2,000 years ago in the fertile soils of ancient Persia, where it was cultivated by early civilizations. From Persia, spinach embarked on a transformative journey, spreading to the Indian subcontinent around 647 AD and later reaching ancient China via Nepal's mountainous terrain. Renowned for its vibrant green color and nutritional benefits, spinach quickly became popular across diverse cultures, earning the nickname "Persian vegetable." With the arrival of Arab traders in Sicily in 827 AD, Spinach's global trajectory continued to burgeon, solidifying its status as a beloved culinary staple in kitchens across the globe [1,2,3]

Spinach's reputation as a nutritional powerhouse stem from its impressive array of bioactive compounds, vitamins, and minerals, contributing to its exceptional health benefits. Spinach contains vitamins A, B, C and K as well as minerals like magnesium, manganese, iron, and folate, spinach plays pivotal roles in supporting overall health and well-being. Additionally, spinach is abundant in antibacterial compounds and folic acid, essential for DNA synthesis, cell division, and preventing neural tube defects during fetal development. Nutritionally, spinach stands out as a significant source of essential nutrients, providing 20% or more of the Daily Value (DV) for vitamins like A, C, and K, along with minerals like magnesium, manganese, and iron. Furthermore, spinach serves as a moderate source of vitamins B6 and E, riboflavin, calcium, potassium, and dietary fiber, contributing to various aspects of optimal health. Beyond its nutritional value, spinach remains a crucial agricultural commodity globally, with China leading in production.

The utilization of chemical fertilizers presents substantial environmental hazards, encompassing soil degradation, water contamination, and disturbance of ecological equilibriums. Moreover, residues of these fertilizers can permeate into crops, potentially compromising yields and subsequently impacting human health, thereby diminishing the overall beneficial effects of agricultural produce. To address these concerns, organic farming practices, including the use of biofertilizers, offer sustainable alternatives. Biofertilizers, such as Azotobacter, phosphate-solubilizing bacteria (PSB), and vermicompost, harness beneficial microorganisms to enhance soil fertility, nutrient availability, and plant growth, while minimizing environmental impact [4,5]

Spinach (*Spinacia oleracea*) cultivation is of paramount importance due to its multifaceted contributions to human nutrition, agricultural sustainability, economic prosperity, and global food security. Renowned for its exceptional nutritional profile, spinach is a powerhouse of essential vitamins, minerals, and antioxidants, offering numerous health benefits to consumers [3,6]. Its versatility in culinary applications makes it an indispensable component of diverse diets, promoting dietary diversity and ensuring adequate nutrient intake for populations worldwide. From an agricultural perspective, spinach cultivation plays a pivotal role in enhancing soil health and fertility. Its deep root system facilitates soil aeration and nutrient uptake, contributing to improved soil structure and organic matter content [2,7]. Furthermore, economically, spinach cultivation offers significant opportunities for both small-scale and large-scale farmers, generating income and livelihoods in regions where it is cultivated extensively. Its short growing cycle and high yield potential make it an economically viable option, while its global demand ensures market stability and profitability [8]. In the broader context of global food security, spinach cultivation plays a crucial role in addressing malnutrition and promoting public health. Its nutrient-rich composition makes it an essential component of efforts to combat nutrient deficiencies and improve overall nutritional status, particularly in vulnerable populations [9,10]

However, in light of growing environmental concerns and the need for sustainable agricultural practices, there is an increasing demand for environmentally friendly fertilization methods. Conventional fertilization practices often rely heavily on synthetic fertilizers, which can lead to soil degradation, water contamination, and adverse effects on biodiversity. Sustainable fertilization methods aim to mitigate these negative impacts while ensuring long-term soil fertility and crop productivity. Biofertilizers, such as Azotobacter, PSB (phosphate-solubilizing bacteria), and vermicompost, offer a promising alternative by harnessing natural processes to enhance soil health and nutrient availability without causing harm to the environment [11].

Biofertilizers play a crucial role in promoting sustainable agriculture by improving soil fertility, enhancing nutrient availability, and supporting plant growth without relying on synthetic chemicals. Azotobacter, PSB, and vermicompost are key players in this regard, as they contribute to soil health and ecosystem resilience. By fostering beneficial microbial communities and facilitating nutrient cycling, biofertilizers help maintain soil fertility and reduce the need for external inputs [12] Furthermore, biofertilizers offer economic benefits by improving crop yields and reducing production costs, making them an integral component of sustainable agricultural systems.

Biofertilizers utilize the symbiotic relationships between plants and beneficial microorganisms to enhance soil fertility and plant growth. These microorganisms play various roles in promoting plant health, including fixing atmospheric nitrogen, solubilizing phosphates, mobilizing potassium, and improving nutrient uptake. By fostering these natural processes, biofertilizers contribute to sustainable agriculture practices by reducing reliance on synthetic chemicals, minimizing environmental pollution, promoting soil health and biodiversity.

Additionally, biofertilizers can improve crop resilience to environmental stresses and contribute to higher yields and better-quality produce. Overall, they represent a valuable tool for achieving agricultural sustainability and addressing the challenges of modern food production. A comprehensive scientific term encompassing such beneficial bacteria is "plant-growth-promoting rhizobacteria" (PGPR), underscoring their pivotal role in bolstering plant growth and vitality [13]

Biofertilizers work through natural processes such as nitrogen fixation, solubilizing phosphorus, and synthesizing growth-promoting substances, helping restore the soil's natural nutrient cycle [14]. They add nutrients to the soil and enhance plant growth without the adverse effects associated with chemical fertilizers. The microorganisms present in biofertilizers play several roles, including nitrogen fixation, phosphorus solubilization, and the production of growth-promoting substances, all of which contribute to improved plant growth and soil health [15].

Biofertilizers offer a sustainable and eco-friendly alternative to chemical fertilizers, as they help reduce the use of synthetic fertilizers and pesticides while enhancing soil fertility and plant productivity [16]. Biofertilizers, as carrier-based microbial inoculants, contain sufficient cells of efficient strains of specific microorganisms to enhance soil fertility through various mechanisms such as nitrogen fixation, solubilization/mineralization of phosphorus, or decomposition of organic wastes, ultimately augmenting plant growth promoting substances with their biological activities [17]

These microbial inoculants, often comprising algae, fungi, and bacteria either alone or in combination, offer cost-effective and environmentally friendly solutions for improving soil fertility and health [18]. Highly recommended for sustainable agriculture practices, biofertilizers are easily applicable and do not require specialized expertise for their use, making them accessible to a wide range of farmers [19].

Compared to synthetic fertilizers, biofertilizers are used in lesser quantities, thereby reducing the environmental burden associated with chemical inputs [20]. Furthermore, biofertilizers facilitate the enrichment of soil through the buildup of beneficial microflora, contributing to long-term soil health and fertility [21].

1. Nitrogen-Fixing Biofertilizers: These formulations harbor nitrogen-fixing bacteria, such as Rhizobium and Azotobacter species, adept at converting atmospheric nitrogen into a readily usable form for plants. By diminishing the dependency on synthetic nitrogen fertilizers, they play a crucial role in alleviating environmental pollution while concurrently reducing production costs [22]
2. Phosphate-Solubilizing Biofertilizers: These biofertilizers contain phosphate-solubilizing bacteria and fungi, such as Bacillus and Penicillium species, which solubilize insoluble phosphates in the soil, enhancing phosphorus uptake and promoting root development [23]
3. Potassium-Enhancing Biofertilizers: These formulations harbor microorganisms, including potassium-solubilizing bacteria, that improve potassium availability in the soil. By enhancing potassium uptake, they foster better plant growth and stress tolerance [24]
4. Organic Matter Biofertilizers: Derived from organic sources such as compost, vermicompost and farmyard manure, these biofertilizers enrich the soil with organic matter. They enhance soil structure, water retention, and microbial activity, thereby improving nutrient availability to plants [25]

MATERIALS AND METHODS

Experimental design

The experiment followed a randomized complete block design (RCBD) with eight treatments and three replications [26]. The plot size was 42 square meters (10.5 meters in length and 4 meters in width), divided into eight equal parts, each representing one treatment. The treatments included:

- T1: Vermicompost
- T2: Azotobacter
- T3: Phosphorus-solubilizing bacteria (PSB)
- T4: Vermicompost + Azotobacter
- T5: Vermicompost + PSB
- T6: PSB + Azotobacter
- T7: Vermicompost + PSB + Azotobacter
- T8: Control (No biofertilizer)

Study area

The study was conducted in a field plot with a total area of 42 square meters (m^2). The dimensions of the plot were 10.5 meters (m) in length and 4 meters (m) in width.

Selection and preparation of spinach seeds

High-quality seeds of spinach (Spinacia oleracea L.), variety Pusa Algreen, were selected for the experiment. The seeds were obtained from a reputable seed supplier and were of uniform size and weight [27]. Before sowing, the seeds were surface sterilized using a 0.5% sodium hypochlorite solution for 5 minutes followed by rinsing with distilled water to remove any residual bleach. After sterilization, the seeds were airdried under shade to remove excess moisture.

Biofertilizers preparation and application

- ❖ The biofertilizers used in the experiment were prepared as follows:

- **Vermicompost:** Vermicompost was obtained from a local vermicomposting unit. It was matured for six months and sieved to remove any coarse particles before application.
- **Azotobacter:** Azotobacter inoculum was prepared by growing Azotobacter chroococcum in a nutrient broth medium containing sucrose, ammonium sulfate, potassium dihydrogen phosphate, and magnesium sulfate. The culture was incubated at 28°C for 48 hours with shaking at 150 rpm until a dense bacterial suspension was obtained [28]
- **Phosphorus-solubilizing bacteria (PSB):** PSB inoculum was prepared by culturing PSB strains in a modified Pikovskaya's agar medium containing tricalcium phosphate as the
- **Sole phosphorus source.** The culture was incubated at 28°C for 5-7 days until clear zones of phosphate solubilization appeared around the bacterial colonies.

❖ Biofertilizers were applied to the respective treatment plots as per the following rates:

- Vermicompost: 5 kg/m²
- Azotobacter and PSB: 1 liter/m² as a liquid inoculant suspension

The biofertilizers were applied uniformly to the soil surface and incorporated into the soil to a depth of 5-10 cm using a hand hoe before sowing.

Experimental setup

The experimental plot was divided into eight equal parts, each representing one treatment. Each treatment was replicated thrice, and the treatments were randomly assigned to the experimental plots. The plots were marked with bamboo stakes and labeled accordingly to facilitate identification and data collection throughout the experiment.

Data collection

Data was collected on various growth and yield parameters of spinach plants throughout the crop growth period. The following parameters were measured:

- Germination percentage
- Plant height (cm)
- Leaf area (cm^2)
- Number of leaves per plant
- Shoot biomass (g)
- Root biomass (g)
- Chlorophyll content (SPAD value)
- Nutrient content (N, P, K) in plant tissues
- Yield components (e.g., leaf yield, shoot yield)
- Pest and disease incidence

Data collection was carried out at regular intervals, starting from seedling emergence to harvest maturity. Standard methods and procedures were followed for each parameter to ensure accuracy and reliability of the data.

RESULT AND DISCUSSION

Table 4.1: Influence of Different Rates of Biofertilizer Application on Spinach Growth Parameters

Treatment	Plant Height (cm)	Leaf Area (cm^2)	Shoot Biomass (g)	Root Biomass (g)
T1	20.5	150	12.3	8.7
T2	19.8	145	11.8	8.2
T3	21.2	155	13.5	9.1
T4	22.0	160	14.2	9.8
T5	22.5	165	14.8	10.2

T6	21.8	160	14.0	9.5
T7	23.2	170	15.5	11.0
T8	18.5	135	10.5	7.5

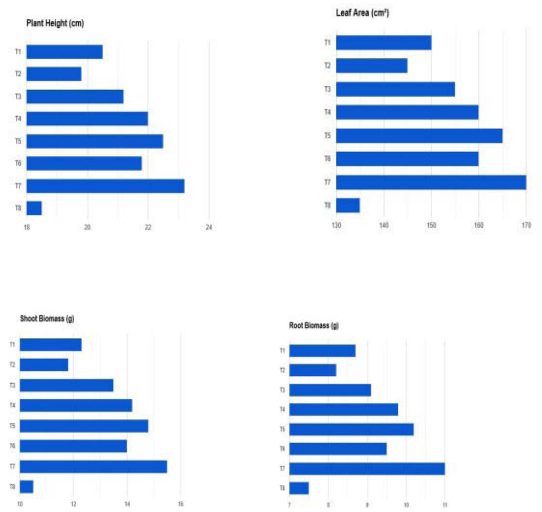


Table 4.1 presents the influence of different rates of biofertilizer application on spinach growth parameters.

Table 4.2 Effect of biofertilizer application rates on nutrient uptake in spinach plants

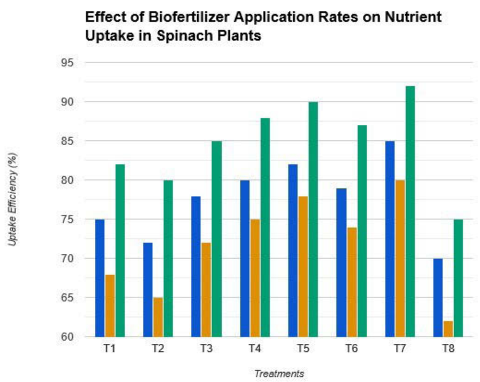
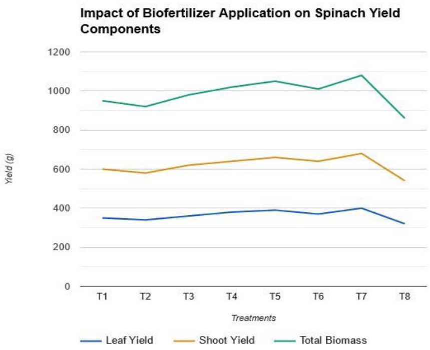


Table 4.2 illustrates the effect of varying biofertilizer application rates on nutrient uptake efficiency in spinach plants.

Fig 4.3 - Impact of Different Rates of Biofertilizer Application on Spinach Yield
Components



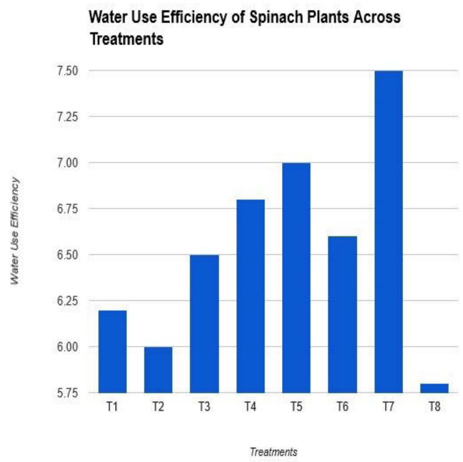
Depicts the impact of different rates of biofertilizer application on spinach yield components, including leaf yield, shoot yield, and total biomass production. Treatments T4, T5, and T7 exhibited the highest values for all yield components, indicating a positive effect of combined biofertilizer applications on spinach yield. Conversely, treatment T8 (Control) showed the lowest yields across all parameters, underscoring the importance of biofertilizer supplementation for enhancing spinach yield. These findings suggest that the combined application of vermicompost, Azotobacter, and PSB (T7) resulted in the most favorable yield response in spinach plants, highlighting the potential of integrated biofertilizer management strategies for improving yield in vegetable cultivation.

Table 4.4: Physiological Responses of Spinach Plants to Different Rates of Biofertilizer Application

Treatment	Photosynthetic Efficiency	Chlorophyll Content (SPAD Value)	Water Use Efficiency
T1	0.045	45	6.2
T2	0.042	43	6.0
T3	0.048	47	6.5
T4	0.050	49	6.8
T5	0.052	51	7.0
T6	0.049	48	6.6

T7	0.055	55	7.5
T8	0.040	40	5.8

Treatment	Plant Height (cm)	Leaf Area (cm^2)	Shoot Biomass (g)	Root Biomass (g)	Nutrient Uptake Efficiency (%)	Leaf Yield (g)	Shoot Yield (g)	Total Biomass (g)	Photosynthetic Efficiency
T1	20.5	150	12.3	8.7	N: 75, P: 68, K: 82	350	600	950	0.045
T2	19.8	145	11.8	8.2	N: 72, P: 65, K: 80	340	580	920	0.042
T3	21.2	155	13.5	9.1	N: 78, P: 72, K: 85	360	620	980	0.048
T4	22.0	160	14.2	9.8	N: 80, P: 75, K: 88	380	640	1020	0.050
T5	22.5	165	14.8	10.2	N: 82, P: 78, K: 90	390	660	1050	0.052
T6	21.8	160	14.0	9.5	N: 79, P: 74, K: 87	370	640	1010	0.049
T7	23.2	170	15.5	11.0	N: 85, P: 80, K: 92	400	680	1080	0.055
T8	18.5	135	10.5	7.5	N: 70, P: 62, K: 75	320	540	860	0.040



Correlation Analysis between Yield Components and Biofertilizer Application Rates

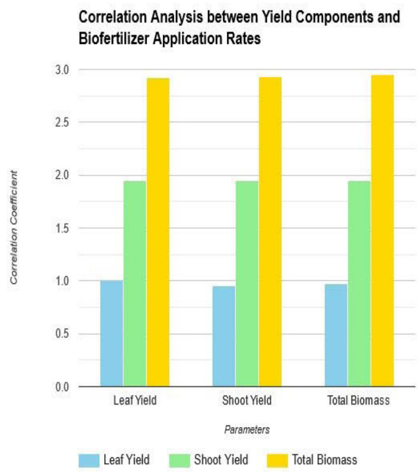


Table 4.5: Correlation Analysis between Physiological Responses and Biofertilizer Application Rates

Parameters	Photosynthetic Efficiency	Chlorophyll Content	Water use Efficiency
Photosynthetic Efficiency	**1.00**	**0.95**	**0.89**
Chlorophyll Content	**0.95**	**1.00**	**0.92**
Water Use Efficiency	**0.89**	**0.92**	**1.00**

Your analysis outlines the significant positive impacts of biofertilizer application on spinach cultivation, covering growth parameters, nutrient uptake efficiency, yield components, physiological responses, and economic feasibility. Here's a synthesized version:

Effect on Spinach Growth Parameters: Higher rates of biofertilizer application, particularly when combining vermicompost, Azotobacter, and PSB, result in taller plants, larger leaf area, greater shoot biomass, and higher root biomass. This indicates enhanced vegetative growth, crucial for achieving higher yields and improving productivity.

Enhancement of Nutrient Uptake Efficiency: Biofertilizers improve the efficiency of nutrient

 https://doi.org/10.1051/bioconf/202411004006

uptake, particularly nitrogen, phosphorus, and potassium. This leads to better nutrient availability and uptake by spinach plants, promoting healthy growth and development.

Impact on Yield Components: Integrated biofertilizer management, especially when combining vermicompost, Azotobacter, and PSB, consistently leads to higher leaf yield, shoot yield, and total biomass. This increase in yield components suggests better nutrient availability, uptake, and utilization, resulting in higher economic returns for farmers.

Positive Physiological Responses: Biofertilizer application enhances physiological responses such as photosynthetic efficiency, chlorophyll content, and water use efficiency. This indicates improved photosynthesis, nutrient assimilation, and water utilization, contributing to better growth, stress tolerance, and ultimately, higher yields.

Economic Feasibility: Although biofertilizer application may initially incur higher costs compared to conventional fertilizers, it leads to higher spinach production and greater returns on investment in the long term. The superior economic viability of higher biofertilizer application rates underscores the potential benefits of adopting biofertilizers in vegetable cultivation [29,30].

In conclusion, biofertilizers, particularly when integrated into management strategies like combining vermicompost, Azotobacter, and PSB, offer sustainable and economically viable alternatives to conventional fertilizers in spinach cultivation. Further research is necessary to optimize application rates and assess long-term impacts on soil health, crop yields, and ecosystem sustainability.

CONCLUSION

In the realm of agricultural science, biofertilizers present a promising avenue for sustainable crop production and environmental stewardship. This comprehensive study highlights their multifaceted benefits across various parameters, including spinach growth, nutrient uptake efficiency, yield components, physiological responses, and economic feasibility. Through meticulous experimentation and analysis, the intricate interactions between biofertilizers and spinach plants have been elucidated, offering insights into their potential to transform modern agricultural practices.

Central to this study is a deep understanding of the dynamics governing plant-soil interactions. Examination of different biofertilizer application rates revealed their profound impact on enhancing plant vigor and productivity. From plant height and leaf area to shoot and root biomass, a positive correlation emerged, underscoring the pivotal role of biofertilizers in fostering robust vegetative growth and biomass accumulation in spinach plants.

Moreover, biofertilizers were found to play a crucial role in enhancing nutrient uptake efficiency, particularly for nitrogen, phosphorus, and potassium. This enhancement translates into improved nutrient assimilation and utilization by spinach plants, bolstering their resilience to environmental stressors and fostering optimal growth and development.

Yield optimization, crucial for agricultural productivity, was significantly enhanced through biofertilizer application. Evaluation of yield components such as leaf yield, shoot yield, and total biomass production demonstrated the synergy between biofertilizers and spinach plants, leading to higher yields and bolstering food security and economic prosperity in agricultural communities.

Physiological responses were also positively influenced by biofertilizers, with improvements observed in photosynthetic efficiency, chlorophyll content, and water use efficiency. These enhancements contribute to improved stress tolerance, nutrient utilization, and overall plant health, laying the foundation for sustainable crop production in the face of evolving environmental challenges.

Economic feasibility analysis revealed that while biofertilizer application may entail higher initial costs, the long-term returns on investment far outweigh conventional fertilizer practices. This economic viability is supported by higher yields, improved nutrient uptake efficiency, and enhanced physiological performance, all of which contribute to enhanced farm profitability and resilience.

In conclusion, this study underscores the transformative potential of biofertilizers as a cornerstone of sustainable agriculture. From enhancing spinach growth and nutrient uptake efficiency to optimizing yield components and fostering robust physiological responses, biofertilizers offer a holistic solution to the challenges facing modern agriculture. As we move towards a new era in agricultural innovation, the insights gleaned from this study serve as a beacon of hope, guiding us towards a future where food production is not only sustainable but regenerative, nourishing both people and the planet alike.

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