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Influence of plant growth promoting microbial consortia on secondary metabolite accumulation and potential pharmacological properties of brinjal (*Solanum melongena* L.)

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Abstract
The interaction between plants and beneficial soil microorganisms has emerged as a promising strategy to enhance both productivity and phytochemical composition in medicinal and vegetable crops. *Solanum melongena* L. (brinjal or eggplant) is a widely cultivated vegetable known for its high antioxidant potential, attributed to its secondary metabolites such as phenolics, flavonoids, and alkaloids. The present study investigates the influence of a plant growth promoting rhizobacterial (PGPR) consortium composed of *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens* on growth, secondary metabolite biosynthesis, and pharmacological activities of brinjal. The consortium inoculation significantly increased chlorophyll content, plant biomass, and fruit yield, alongside enhancing the accumulation of total phenolics, flavonoids, and anthocyanins. GC-MS profiling revealed elevated levels of nasunin, chlorogenic acid, and solasodine in treated plants. Furthermore, extracts from inoculated plants showed higher antioxidant and antibacterial activities compared to the control. These findings highlight the potential of microbial consortia as bioenhancers for improving the nutritional and therapeutic quality of brinjal.

Keywords: *Solanum melongena*, PGPR consortium, secondary metabolites, antioxidant activity, pharmacological potential

Introduction
The increasing global demand for natural bioactive compounds has shifted focus toward eco-friendly agricultural approaches that promote both yield and metabolite quality. *Solanum melongena* L., commonly known as brinjal or eggplant, is a member of the Solanaceae family and a rich source of secondary metabolites such as polyphenols, flavonoids, anthocyanins, and steroidal alkaloids, which confer antioxidant, antimicrobial, and anti-inflammatory properties. Conventional agricultural inputs often enhance productivity but compromise nutritional quality and soil sustainability. Thus, integrating microbial inoculants capable of promoting plant growth and secondary metabolism has become a crucial step toward sustainable cultivation.

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial microbes that colonize the rhizosphere and stimulate plant growth through mechanisms such as nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone regulation. Several studies have shown that microbial consortia outperform single strains by offering synergistic benefits to plants, including enhanced stress tolerance and metabolite accumulation. However, limited information is available on how microbial consortia affect secondary metabolism and pharmacological traits in *S. melongena*.

This research aims to elucidate the effects of a PGPR consortium on growth performance, biochemical composition, and pharmacological potential of brinjal under controlled greenhouse conditions. Emphasis is placed on changes in phenolic and flavonoid content, as well as *in vitro* antioxidant and antibacterial activities of the resulting fruit extracts.

2. Materials and Methods

2.1 Experimental Site and Design

The experiment was conducted at the Department of Plant and Soil Science, Agriculture and Forestry College, Rampur, Chitwan, Nepal (27.65°N, 84.35°E) during the 2023-24 growing season under greenhouse conditions. The climate of the region is subtropical with an average annual temperature of 24°C and relative humidity around 70%. The soil was loamy with a pH of 6.8 and organic carbon content of 1.2%.

A randomized complete block design (RCBD) with three replicates was used. Treatments included control (uninoculated) and microbial consortia inoculation.

2.2 Microbial Consortium Preparation

The PGPR consortium included *Azospirillum brasilense* (nitrogen fixer), *Bacillus subtilis* (phosphate solubilizer), and *Pseudomonas fluorescens* (siderophore producer). Individual cultures were grown in nutrient broth, mixed in equal proportions (1:1:1 v/v), and adjusted to 10^8 CFU mL^{-1} prior to seed and soil application.

2.3 Planting and Inoculation

Brinjal seeds (cv. Pusa Purple Long) were surface-sterilized and inoculated with the microbial mixture. Seedlings were transplanted after 25 days into pots containing sterilized loam soil and farmyard manure (3:1 ratio). A 20 mL suspension of the consortium was applied near the root zone at 15-day intervals.

2.4 Growth and Yield Parameters

Growth parameters such as plant height, number of leaves, and total biomass were recorded at 30, 60, and 90 days after transplanting (DAT). Fruit yield per plant and total chlorophyll content were determined using standard methods (Arnon, 1949) [1].

2.5 Biochemical Analysis

Total phenolic content (TPC) was measured by the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g FW. Total flavonoid content (TFC) was quantified using the aluminium chloride method (mg quercetin equivalents/g FW). Anthocyanin content was measured spectrophotometrically at 530 nm.

2.6 GC-MS Profiling

Methanolic extracts were subjected to gas chromatography-mass spectrometry (GC-MS) to identify and quantify secondary metabolites. Major compounds were identified based on retention indices and comparison with NIST database spectra.

2.7 Pharmacological Assays

Antioxidant activity was estimated using DPPH radical scavenging assay (IC_{50} values). Antibacterial activity was evaluated using the agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*.

2.8 Statistical Analysis

Data were analyzed using one-way ANOVA (SPSS v25). Mean separation was performed by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

3. Results

3.1 Growth and Yield Enhancement

The microbial consortia significantly improved plant height (22.6%), leaf area (18.5%), and total biomass (27.3%) compared to control. Fruit yield per plant increased by 31.2%. Enhanced chlorophyll content suggested improved photosynthetic efficiency due to microbial inoculation.

3.2 Secondary Metabolite Accumulation

The treated plants showed a 42% increase in total phenolic content, 36% in flavonoids, and 28% in anthocyanin concentration relative to the control (Table 1). GC-MS analysis confirmed elevated levels of nasunin (major anthocyanin), chlorogenic acid, and solasodine (steroidal alkaloid), indicating stimulation of the phenylpropanoid and terpenoid pathways.

Table 1: Influence of microbial consortium on major secondary metabolites in *S. melongena*.

Parameter	Control	PGPR Consortium	% Increase
Total Phenolics (mg GAE/g FW)	2.6 ± 0.12	3.7 ± 0.15	42.3
Total Flavonoids (mg QE/g FW)	1.9 ± 0.08	2.6 ± 0.10	36.8
Anthocyanins (A530 nm units)	0.65 ± 0.03	0.83 ± 0.05	27.7

3.3 Antioxidant and Antibacterial Activities

DPPH assay revealed higher antioxidant activity in treated extracts (IC_{50} = 55.4 µg/mL) than control (IC_{50} = 78.9 µg/mL). Similarly, microbial-treated fruit extracts exhibited enhanced antibacterial zones of inhibition (17-22 mm) against test pathogens, compared to 9-14 mm in control samples.

4. Discussion: The present study clearly demonstrates that inoculation with a plant growth-promoting rhizobacterial (PGPR) consortium consisting of *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens* markedly enhanced growth performance, secondary metabolite accumulation, and pharmacological activities of *Solanum melongena* L. under greenhouse conditions. These results highlight the synergistic influence of multi-strain microbial inoculation on the physiological and biochemical attributes of brinjal, offering valuable insights for sustainable horticultural production systems.

The observed increases in plant height, leaf area, biomass, and fruit yield in consortium-treated plants can be attributed to the combined effects of nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone secretion by the PGPR members. *Azospirillum* species are known for their ability to fix atmospheric nitrogen and produce indole-3-acetic acid (IAA), which stimulates root elongation and lateral branching. *Bacillus subtilis* enhances phosphorus availability by solubilizing insoluble phosphate compounds and producing organic acids, whereas *Pseudomonas fluorescens* contributes siderophores that improve iron uptake and promote rhizospheric health. The interaction among these strains creates a more favorable root environment, leading to improved nutrient absorption, chlorophyll synthesis, and photosynthetic activity.

The significant rise in chlorophyll content (Arnon, 1949) [1] in inoculated plants supports this hypothesis, suggesting that the consortium improved nitrogen assimilation and carbon metabolism. Similar findings were reported by Sinha *et al.* (2016) [6], who observed enhanced growth and fruit yield in eggplant following PGPR and organic manure application. Thus, microbial inoculation not only contributes to improved plant vigor but also reduces dependency on chemical fertilizers, aligning with the goals of sustainable agriculture.

The marked increase in total phenolic, flavonoid, and anthocyanin contents in treated plants suggests that the microbial consortium activated the plant's secondary metabolic machinery. PGPR-mediated stimulation of phenolic metabolism has been associated with the upregulation of the phenylpropanoid pathway, particularly through the activation of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) enzymes. This biochemical shift enhances the biosynthesis of antioxidant compounds that play critical roles in plant defense and stress tolerance.

GC-MS analysis revealed elevated levels of nasunin, chlorogenic acid, and solasodine metabolites known for their potent antioxidant and cytoprotective properties. Nasunin, the primary anthocyanin in brinjal peel, acts as a strong radical scavenger and metal chelator. Its accumulation reflects enhanced anthocyanin biosynthesis triggered by microbial elicitation. Chlorogenic acid, a hydroxycinnamic acid derivative, contributes to anti-inflammatory and antibacterial activity, whereas solasodine, a steroidal alkaloid, exhibits notable pharmacological potential against microbial infections and certain cancer cell lines. The synergistic enhancement of these compounds signifies that the microbial consortium not only promotes growth but also enriches the therapeutic value of the crop.

These observations corroborate earlier reports by Khan *et al.* (2020) [8], who found that *Bacillus subtilis* and *Pseudomonas fluorescens* treatments improved antioxidant metabolism and enzymatic activity in brinjal under abiotic stress. Moreover, Muralidharan and Nair (2018) [7] established that brinjal anthocyanins are sensitive to both environmental and microbial stimuli, supporting the current study's inference that microbial elicitation can be an effective biotechnological tool for secondary metabolite enhancement.

The DPPH radical scavenging assay revealed a significant decrease in IC_{50} values for inoculated plants, confirming elevated antioxidant potential. This improvement can be linked directly to the higher phenolic and flavonoid content. Phenolic compounds act as hydrogen donors and metal ion chelators, effectively neutralizing reactive oxygen species (ROS). The enhanced antioxidant response also reflects improved redox homeostasis within plant tissues, suggesting microbial-induced activation of defense-related pathways (Sharma *et al.*, 2012) [2].

Antibacterial assays further demonstrated that methanolic extracts from consortium-treated fruits inhibited both Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative (*Escherichia coli*) bacteria more effectively than control extracts. The observed zones of inhibition (17-22 mm) indicate a significant increase in bioactive secondary metabolites with antimicrobial properties. Such findings validate the hypothesis that PGPR inoculation can modulate not only the quantitative levels but also the qualitative diversity of pharmacologically active metabolites.

From an agronomic perspective, the use of microbial consortia represents a low-cost, environmentally sustainable practice that enhances both productivity and nutritional quality. The dual benefit improved yield and enriched bioactive profile holds potential for integrating microbial inoculants into large-scale vegetable production systems. Moreover, the enriched secondary metabolite profile of brinjal, especially anthocyanins and phenolics, makes it a promising raw material for the nutraceutical and pharmaceutical industries.

The current study's outcomes align with previous findings emphasizing the multifunctional role of PGPR in improving plant health, yield, and bioactivity (Gupta *et al.*, 2000; Singh *et al.*, 2011) [3, 4]. The observed metabolic and pharmacological enhancements suggest that microbial consortia function as bio-elicitors, stimulating innate plant defense mechanisms and secondary metabolism. These interactions underscore the potential of microbial biotechnology in augmenting the medicinal value of common vegetables.

5. Conclusion

The findings of this study confirm that inoculation of *Solanum melongena* with a PGPR consortium comprising *Azospirillum brasilense*, *Bacillus subtilis*, and *Pseudomonas fluorescens* significantly improves both agronomic performance and bioactive compound accumulation. Treated plants exhibited higher biomass, chlorophyll content, and fruit yield, along with enhanced levels of total phenolics, flavonoids, anthocyanins, and key pharmacological compounds such as nasunin, chlorogenic acid, and solasodine. These biochemical enhancements translated into stronger antioxidant and antibacterial activities, underscoring the crop's improved therapeutic potential.

Collectively, these results demonstrate that PGPR consortia can act as natural bio-stimulants and bio-fortifiers, providing a sustainable and eco-friendly alternative to chemical inputs. The integration of such microbial technologies into brinjal cultivation could enhance the crop's nutraceutical value, contributing to food security and public health. Future research should focus on molecular characterization of biosynthetic pathways, gene expression profiling of key enzymes (e.g., PAL, CHS, DFR), and long-term field trials to validate these findings across diverse agroecological zones.

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