Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 06, Issue: 01 (JAN-FEB, 2026)
Available online at http://www.agriarticles.com

Integration of Biochar and Endophytic Microbial Consortia in Soilless Regenerative Systems for Sustainable *Lilium* Cultivation under Protected Conditions

*Rupali Koshti[1], Jadhav Amol[2] and Anu Seng Chaupoo[3]
[1]PhD Scholar, Department of Horticulture, Jawaharlal Nehru Krishi Vishwavidhalaya, Jabalpur, Madhya Pradesh
[2]PhD Scholar, Department of Floriculsture and landscaping, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
[3]Teaching Assistant, Department of Horticulture, Faculty of Agricultural Sciences, Rajiv Gandhi University, Rono Hills, Doimukh, Arunachal Pradesh, 791112
Corresponding Author's email: www.rkkoshti16@gmail.com

Soilless substrate culture under protected conditions is widely used in cut-flower production systems, especially for high-value bulbous ornamentals like *Lilium*. While this approach allows precise control over water and nutrients, it typically depends on intensive chemical fertilizers and biologically inert media, which limits sustainability. Regenerative amendments such as biochar and plant-associated endophytic microbes have shown promise in improving substrate function, nutrient retention, microbial activity and plant performance in soil systems. However, their integrated use in soilless protected floriculture remains under-explored. Here, we synthesize existing evidence and propose a comprehensive experimental framework to evaluate the combined effects of biochar and endophytic microbial consortia on growth, physiological performance, flowering, vase life, substrate functional traits and substrate microbiome dynamics of *Lilium* grown in soilless media. We hypothesize that biochar improves substrate physicochemical properties and serves as a habitat for beneficial microbes, while endophytes enhance nutrient uptake and stress resilience, leading to synergistic improvements in plant growth and flower quality. The proposed study integrates agronomic, biochemical, microbiological and molecular analyses to provide mechanistic insights and practical recommendations for sustainable, low-input soilless floriculture.

Keywords: *Lilium*, biochar, endophytes, soilless cultivation, substrate microbiome, protected cultivation, vase life, regenerative horticulture.

Introduction

Cut-flower production is one of the fastest-growing segments of high-value horticulture worldwide (ISHS, 2015). Among ornamentals, lilies (*Lilium* spp.) are particularly prized for their large, colorful flowers, long stems and commercial demand in domestic and export markets. Protected cultivation using polyhouses or greenhouses and soilless substrates has enabled growers to produce high-quality cut lilies with reduced soil-borne disease, superior control over water and nutrient supply and consistent yields (ISHS, 2015). However, soilless substrates such as peat, coir, perlite or rockwool are biologically inert and often require frequent applications of soluble fertilizers to meet plant nutritional demands, leading to environmental concerns such as nutrient leaching and energy-intensive production systems (Hussain *et al*., 2018; Zulfiqar *et al*., 2022).

Regenerative agriculture offers tools to improve sustainability by enhancing biological function, nutrient cycling and resource-use efficiency. Biochar, a carbon-rich material from pyrolyzed biomass, has attracted considerable interest because it can improve substrate properties such as porosity, water-holding capacity and cation exchange capacity (CEC), while serving as a refuge for microbes (Zulfiqar *et al*., 2022). Endophytic microbes - bacteria and fungi that colonize internal plant tissues without causing disease - have been documented to enhance plant growth, nutrient uptake, stress tolerance and disease resistance in various horticultural crops (Ali *et al*., 2024; Khan *et al*., 2020). Combining these two regenerative inputs may create synergistic effects in substrate biology and plant performance.

Despite promising evidence from soil-based systems and agronomic crops, the combined application of biochar and endophytic microbial consortia in soilless protected floriculture systems (especially *Lilium*) has not been sufficiently studied. Understanding how these amendments interact in soilless substrates to influence nutrient dynamics, microbial community structure, plant physiology, flowering and vase life could enable a shift toward low-input, biologically active production systems that reduce fertilizer dependence and improve product quality.

This article reviews current knowledge on biochar and endophyte functions relevant to soilless systems, highlights research gaps and proposes a comprehensive experimental design to evaluate the integrated approach in *Lilium*. The outcomes can inform sustainable floriculture practices and contribute to circular, regenerative horticultural systems.

Literature Review

Biochar Properties and Mechanisms in Substrate Systems

Biochar is produced by thermal decomposition (pyrolysis) of organic biomass under limited oxygen conditions. Its structure typically features a high specific surface area, extensive porosity, and a range of functional groups that confer nutrient-binding capacity (CEC), making it an effective soil amendment in conventional cropping systems (Zulfiqar *et al*., 2022). The benefits of biochar include increased water-holding capacity, improved aeration, moderated pH, enhanced nutrient retention and provision of microhabitats for microorganisms (Zulfiqar *et al*., 2022; Dispenza *et al*., 2016).

In horticultural substrates, especially peat-based mixes, biochar can:

- Increase physical stability and reduce bulk density;
- Improve water retention and reduce irrigation frequency;
- Buffer pH and nutrient availability;
- Serve as a porous habitat for microbial colonization;
- Reduce fertilizer leaching by adsorbing cations and anions (Dispenza *et al*., 2016; Escuer *et al*., 2021; Zulfiqar *et al*., 2022).

However, not all biochars are equal. Their effects depend on feedstock type, pyrolysis temperature and post-production conditioning. For example, hardwood biochars produced at moderate temperatures (~500–600°C) generally possess balanced porosity and stability, while low-temperature or high-ash biochars can release salts or phytotoxic compounds that adversely affect seed germination or early plant growth if not properly washed or aged (Escuer *et al*., 2021). Therefore, characterization of biochar (pH, electrical conductivity [EC], volatile organic compounds, CEC) and appropriate pre-conditioning are critical steps before incorporation into horticultural mixes.

Several studies in ornamentals and seedlings suggest that biochar, when blended at low to moderate rates with peat or coir, can improve seedling vigor and reduce reliance on peat, offering environmental benefits by decreasing extraction of peatlands (Dispenza *et al*., 2016; Escuer *et al*., 2021). In soilless floriculture, limited but emerging evidence indicates potential for improved nutrient retention and physical properties that merit further exploration.

Endophytes in *Lilium* and Ornamentals

Endophytic microorganisms (bacteria and fungi) inhabit plant internal tissues without causing disease and are known to confer multiple benefits to the host plant, such as:

- Production of phytohormones (e.g., indole-3-acetic acid [IAA]) that promote growth;
- Solubilization of phosphate and other nutrients;

- Production of siderophores that enhance iron acquisition;
- Antagonism toward pathogens via antimicrobial compounds;
- Modulation of host stress responses (Ali *et al*., 2024; Khan *et al*., 2020).

In studies on *Lilium*, several endophytic strains - particularly *Bacillus* species like *Bacillus velezensis* - have been isolated from bulbs and shoots and shown to produce PGP traits and antifungal activity against common pathogens (Khan *et al*., 2020). These findings suggest that carefully selected or designed endophytic consortia can improve lilies' vegetative performance, flowering attributes, and possibly reduce disease pressure under greenhouse conditions.

The benefits of endophytes have also been documented in other ornamentals and horticultural crops, where inoculation enhanced nutrient uptake, stress tolerance (e.g., drought, salinity) and overall plant vigour (Ali *et al*., 2024). However, the establishment of introduced endophytes in soilless systems remains challenging due to the lack of native microbial communities and limited habitat, making substrate carriers or supportive amendments necessary to enhance survival and function.

Biochar-Microbe Interactions and Synergies

The combination of biochar and microbial inoculants has attracted attention in agronomy and remediation studies. Biochar's porous structure provides refuge from predation and desiccation for microbes, and its complex surface chemistry can adsorb organic compounds, creating microhabitats where microbes can establish and remain active (Li *et al*., 2023; Hussain *et al*., 2018; Zulfiqar *et al*., 2022). In such systems, biochar can act as a carrier for beneficial microbes, improving their persistence, colonization of plant roots and functional contributions to nutrient cycling.

In soil and soil-like systems, combined biochar-microbe treatments often show greater positive effects on plant growth and soil health indicators (e.g., microbial biomass, enzyme activity) than either amendment alone (Li *et al*., 2023; Hussain *et al*., 2018). For example, biochar combined with nitrogen-fixing bacteria increased plant biomass more than biochar or inoculant alone, likely due to enhanced microbial survival and nutrient provision (Li *et al*., 2023). These synergistic interactions are promising for horticultural substrates but require validation, particularly in substrate contexts where organic matter is minimal and turnover is rapid.

(https://etia-group.com/biochar/)

Soilless Cultivation of *Lilium* and Horticultural Substrate Dynamics

Soilless cultivation - using inert or partially inert substrates with controlled fertigation - is standard for many ornamentals, including lilies (ISHS, 2015; Nikrazm and Alizadesh, 2011). Optimal substrates balance air-filled porosity and water-holding capacity, ensuring oxygen availability at the root zone while maintaining adequate moisture. Nutrient solutions must match crop demands throughout phenological stages, with electrical conductivity (EC) and ion ratios tailored to bulbous plants' unique nutrient requirements.

High fertilizer input is common in soilless systems because nutrients are easily washed out of porous substrates by frequent irrigations. This not only raises production costs but also leads to nutrient losses to the environment. Introducing amendments that increase substrate CEC, reduce leaching and support biologically active nutrient cycling could reduce fertilizer needs without compromising plant performance. Biochar - modifying substrate physical and chemical properties - and microbial inoculants - enhancing nutrient uptake and internal plant physiology - represent regenerative options that may improve sustainability and product quality in protected cultivation.

Objectives and Hypotheses

Objectives

The main objective is to evaluate the effects of biochar incorporation and endophytic microbial consortia inoculation on soilless *Lilium* cultivation in protected conditions, specifically:

1. To quantify plant growth and flowering responses (height, number of leaves, bud length/diameter, flower diameter, spike length) to biochar and endophyte treatments.
2. To measure physiological status (chlorophyll content, photosynthetic performance) and tissue nutrient concentrations (N, P, K).
3. To determine vase life and postharvest quality (days to wilt, water uptake, microbial load in vase solutions).
4. To assess substrate functional changes (pH, EC, water retention, microbial biomass, enzyme activities).
5. To profile substrate and root microbiomes (community composition and diversity) and track introduced endophyte persistence.
6. To evaluate whether the integrated approach allows reduction of fertilizer inputs while maintaining or improving quality.

Hypotheses

H1: Biochar amendment improves substrate physical and chemical properties, leading to enhanced plant growth and nutrient retention compared to control.

H2: Endophytic microbial inoculation enhances nutrient uptake and plant physiological performance, thereby improving flowering and vase qualities.

H3: Combined biochar + endophyte treatments exhibit synergistic effects, producing greater improvements than single amendments.

H4: Biochar serves as a carrier that increases the persistence and efficacy of inoculated endophytes in soilless substrates.

Materials and Methods

Experimental Site and Conditions

The experiment will be conducted in a controlled greenhouse/polyhouse. Environmental variables (temperature, relative humidity, light intensity) will be monitored. A randomized complete block design (RCBD) with four treatments - Control, Biochar (B), Endophyte (E), Biochar + Endophyte (B+E) - will be implemented with four replicates.

Plant Material and Cultivar

Uniform bulbs of a commercial *Lilium* cultivar (e.g., *Lilium asiatic hybrid 'Eyeliner'*) will be selected. Bulbs will be surface-sterilized before planting to minimize initial pathogen load.

Substrate and Amendments

Control substrate: commercial peat/coir/perlite mix.

Biochar: hardwood biochar pyrolyzed at 500–600°C, sieved (<2 mm), washed/aged and characterized (pH, EC, CEC).

Endophytic consortium: strains (e.g., *Bacillus velezensis*) selected based on PGP traits and absence of pathogenicity; prepared in standardized INOCulum.

Treatments:

- T1: Control
- T2: Control + Biochar (7.5% v/v)
- T3: Control + Endophyte
- T4: Control + Biochar + Endophyte

Fertilization and Irrigation

Standard nutrient solution optimized for *Lilium* growth will be applied uniformly, with leachate collected for nutrient loss analysis. Sub-treatments with reduced fertilization levels (75%, 50%) can also be included.

Measurements and Data Collection

Measurements include growth parameters (height, leaves), flowering traits, chlorophyll content (SPAD), tissue nutrients, substrate physical-chemical traits, microbial biomass and enzyme activities, vase life assays, and microbiome profiling (16S/ITS amplicon sequencing).

Statistical Analysis

Data will be analyzed using mixed-model ANOVA, repeated measures where appropriate, and multivariate analyses for microbiome data

Expected Results and Discussion

Substrate Physical-Chemical Improvements

We expect biochar amendment to increase water retention, porosity and nutrient adsorption, resulting in lower leachate loss and more stable EC and pH compared to control (Dispenza *et al*., 2016; Escuer *et al*., 2021; Zulfiqar *et al*., 2022). These changes should benefit bulb establishment and early vegetative growth by providing a more balanced substrate environment.

Microbial Establishment and Persistence

Biochar's porous matrix is predicted to support higher microbial biomass and activity by sheltering microbes from desiccation and predation (Li *et al*., 2023). Endophyte inoculation is expected to increase colonization of root and bulb tissues, with biochar aiding persistence and efficacy.

Plant Growth and Flower Quality

Based on evidence of PGP effects of endophytes and biochar's influence on nutrient retention and water relations, integrated treatments should enhance vegetative growth, flowering timing and floral attributes (bud diameter, spike length) compared to controls. Endophytic inoculants' production of phytohormones and nutrient solubilization may further improve plant physiology (Khan *et al*., 2020; Ali *et al*., 2024).

Vase Life and Postharvest Performance

Positive preharvest nutrition and reduced plant stress are associated with improved vase life (Mishra *et al*., 2015). We anticipate that plants from biochar + endophyte treatments will show longer vase life and reduced microbial loads in vase solutions due to healthier xylem function and possible antagonistic effects of endophytes against spoilage organisms.

Microbiome Dynamics

Sequencing is expected to reveal distinct microbial communities in amended substrates, with higher alpha diversity and enrichment of beneficial taxa in biochar + endophyte treatments. Network analyses may identify key taxa correlated with positive agronomic outcomes.

Conclusion

Integrating biochar and endophytic microbial consortia into soilless substrates for *Lilium* cultivation under protected conditions represents a promising regenerative approach that could enhance growth, nutrient use efficiency, substrate function and product quality while reducing environmental impacts of intensive fertilizer use. The proposed experimental framework combines agronomic, physiological and microbiological analyses to generate mechanistic insights and actionable recommendations for growers.

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