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RESEARCH COMMUNICATION

Biotechnology

Impact of *Gliricidia sepium* mulch enriched with native Sri Lankan bacterial strains on cinnamon (*Cinnamomum verum*)

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Abstract: The integration of organic mulches with beneficial microorganisms offers a sustainable strategy for enhancing soil fertility and plant productivity. This study evaluated the effects of *Gliricidia sepium* mulch enriched with two native bacterial strains (I-I and I-II) from Sri Lanka on the vegetative growth, essential oil yield, and soil nutrient status of *Cinnamomum verum* variety *Sri Gemunu* under greenhouse conditions. A 12-month experiment was conducted in a completely randomized design with three treatments: control (mulch only), bacterial strain I-I enriched mulch (G I-I), and bacterial strain I-II enriched mulch (G I-II). Vegetative growth parameters, essential oil yield, and soil nutrient levels were assessed. Both bacterial treatments significantly improved plant height, stem circumference, leaf number, and root length compared to the control, with G I-II exhibiting significant performance in most growth metrics. Soil analysis indicated significantly elevated phosphorus levels in both inoculated treatments, while nitrogen levels increased only in the G I-I treatment. These findings highlight the potential of plant growth promoting bacteria-enriched *Gliricidia sepium* mulch to enhance vegetative growth and soil fertility in cinnamon cultivation, suggesting potential gains in overall oil productivity, supporting its relevance in sustainable cinnamon production systems.

Keywords: Bacterial strains, *Cinnamomum verum*, *Gliricidia sepium* mulch, soil nutrient content, vegetative growth.

INTRODUCTION

Sustainable agricultural practices are increasingly being explored to enhance crop productivity while minimizing environmental degradation. Organic mulching has gained attention due to its ability to improve soil fertility, moisture retention, and microbial activity (Rossi et al., 2024). Among various organic mulches, *Gliricidia sepium* has been widely recognized for its nitrogen-fixing ability and as an effective soil enhancer by contributing to increase total soil carbon, nitrogen, available phosphorus, pH balance, cation exchange capacity, and organic matter (Alamu et al., 2023). However, there are no reports on enhancing the efficacy of *Gliricidia sepium* mulch through microbial inoculation to improve nutrient availability for plant growth.

The introduction of plant growth promoting bacterial strains helps protect plants from abiotic stresses and biotic stresses, while enhancing plant growth through various direct and indirect mechanisms, including nitrogen fixation, phosphorus solubilization, production of phytohormones, and competing for space on plant roots

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(De Souza et al., 2015). The incorporation of beneficial bacterial strains in mulch treatments has been reported to enhance soil microbial diversity and nutrient cycling. Recently, Ebrahimi et al. (2023) explored the effect of combined application of mycorrhiza fungi, growth-promoting bacteria, and mulch of plant residues on the enhancement of plant growth and essential oil yield of the medicinal plant anise (*Pimpinella anisum* L.).

True cinnamon (*Cinnamomum verum*) is the most valuable spice crop cultivated in Sri Lanka for export income (Sri Lanka Export Development Board, 2023). The variety *Sri Gemunu* has gained prominence due to its high essential oil content and superior growth characteristics (Department of Cinnamon Development, 2024). Enhancing its growth through organic methods, such as *Gliricidia sepium* mulch enriched with plant growth-promoting bacteria, could be suggested as an innovative alternative to heavy use of synthetic fertilizers in cinnamon cultivation.

This study investigates the effects of *Gliricidia sepium* mulch enriched with bacterial strains I-I and I-II on the growth, essential oil yield, and soil nutrient composition of *Cinnamomum verum* variety *Sri Gemunu*. The research aims to introduce a novel approach for cinnamon fertilizer use, contributing to sustainable cinnamon cultivation.

MATERIALS AND METHODS

Bacterial strains

Sri Lankan bacterial strains (I-I and I-II) available in the collection at the Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, were used for the experiment. Of these, the *16s rRNA* (GenBank: PV174539 and PV174565) and *Gyrase A* (GenBank: PV253967 and PV253968) gene sequences are available. According to Sandamini et al. (2025), these strains were partially identified in a previous study: strains I-I and I-II belonged to the genus *Bacillus* based on the *gyr*A sequencing having over 99 % BLAST identity with matches identified by others as *Bacillus cereus*.

Experimental setup and treatment application

The study was carried out at the greenhouse of the Faculty of Agriculture, University of Ruhuna, Mapalana, Sri Lanka, for 12 months. The experiment followed a completely randomized design (CRD) with five replicates per treatment.

Each experimental unit consisted of a UV treated polyethylene grow bag from the local market (60.96 × 60.96 cm), each filled with 20 kg of growth substrate, composed of an equal mixture of topsoil, cow dung, coir dust, and soil (1:1:1:1). Four-month-old vegetative-propagated *Cinnamomum verum* (variety *Sri Gemunu*) plants from National Cinnamon Research and Training Centre, Palolpitiya, Sri Lanka, were used as planting material. The experiment included three treatments: a control (C) with only *Gliricidia sepium* mulch, *Gliricidia sepium* mulch inoculated with strain I-I (G I-I), and *Gliricidia sepium* mulch inoculated with strain I-II (G I-II). The strains I–I and I–II were preserved in glycerol stocks at –20°C in the bacterial collection at the Faculty of Agriculture, University of Ruhuna. Bacterial strains were cultured in LB broth (10 g/L tryptone, 10 g/L NaCl, and 5 g/L yeast extract) and incubated overnight on a shaker at 250 rpm. The culture was diluted with sterile distilled water to a final concentration of 10^9 CFU mL^{-1}. These bacterial suspensions were applied to fresh *Gliricidia sepium* mulch (250 g) every 14 days, using 200 mL per pot per application.

Collection of vegetative data

The following vegetative growth parameters were evaluated at the 12th month of plant establishment: Plant height, stem circumference (at 10 cm above ground level), leaf count per plant, internodal length, root length, and chlorophyll content index (CCI) using a CCM-200 Plus Chlorophyll Content Meter (Opti-Sciences).

Leaf essential oil extraction and measuring oil yield

After assessing plant growth, mature, healthy leaves free from disease were collected and air-dried in a shaded environment at room temperature for two weeks. Two replicates of essential oil were extracted through hydro-distillation following a standardized procedure. Dried leaves (80 g) were distilled in 250 mL of distilled water for four hours. The total oil yield was measured and expressed in microliters per gram (μL/g) of dried leaf material.

Soil nutrient analysis

The total nitrogen (N) and available phosphorus (P) of composite samples prepared by soil collected from the different layers of the pot were analyzed, as described by Sparks (1996).

Data analysis

Plant growth parameters, and soil nutrients were analyzed through one-way ANOVA. Treatment means were compared with the Dunnett test at a statistical significance level of $p < 0.05$ using only *Gliricidia sepium* mulch treatment as a reference. IBM SPSS Statistics (Version 25) was used for the analysis.

RESULTS AND DISCUSSION

Vegetative growth performance

The results indicate that both treatments of *Gliricidia sepium* mulch inoculated with bacterial strains I-I and I-II produced significant improvements in several vegetative growth parameters compared to the control treatment, which is only *Gliricidia sepium* mulch (Table 1). G I-II treatments resulted in significantly greater plant height and root length compared to the control. In terms of stem circumference and number of leaves per plant, both G I-I and G I-II exhibited a significant increase over the control. However, no significant differences were found in the internodal length and CCI. Overall, Dunnett's test results demonstrate that the G I-I and G I-II significantly enhanced vegetative growth parameters of *C. verum* compared to the control, with G I-II generally outperforming G I-I, particularly in plant height, leaf number, and root length.

These findings are in accordance with inoculation of these strains to rice, which produced significant results over negative control (Chathurika et al., 2024). The improved growth of cinnamon under bacteria-enriched *Gliricidia sepium* mulch must be linked to the plant growth-promoting traits of strains I-I and I-II, including phosphate solubilization and IAA production (Chathurika et al., 2024; Sandamini et al., 2025). The significant increase in the soil nitrogen nutrient by bacterial strain I-I treatment suggests the efficient decomposition of *Gliricidia sepium* mulch due to the known acid forming ability of I-I (unpublished data). Although strain I-I is not proven for the possession of *Nif* genes based on our unpublished data, it may promote the nitrogen fixing bacteria in the environment by making nutrient available. IAA production is suggested as a strategy attributed to the two strains to enhance root development and nutrient uptake according to previous reports (Çakmakçı et al., 2023; De Souza et al., 2015).

Table 1: Effect of treatments on the vegetative growth of *Cinnamomum verum* at the 12th month of plant establishment.

Treatment	Plant height (cm)	Stem circumference (cm)	Number of leaves per plant	Internodal length (cm)	Chlorophyll content index (CCI)	Root length (cm)
C	68.20 ± 1.32	2.18 ± 0.08	124.60 ± 11.37	4.18 ± 0.14	48.41 ± 4.48	77.00 ± 2.14
G I-I	88.40 ± 6.93	2.84 ± 0.25*	168.00 ± 8.29*	4.28 ± 0.25	52.81 ± 2.44	96.00 ± 6.59
G I-II	90.00 ± 10.00*	2.81 ± 0.12*	190.40 ± 17.77**	4.00 ± 0.12	52.93 ± 1.94	102.40 ± 9.39*

The mean value of five replicates ±SE. Asterisks represent the statistical significance of the difference between the mean values of each treatment and its associated control based on Dunnett's test. The significance levels: $^{**}0.001 < p < 0.01$; $^{*}0.01 < p < 0.05$.

Essential leaf oil yield

There was no significant difference in oil yield extracted from the leaves of *C.verum* among three treatments. The *Gliricidia sepium* mulch was inoculated with strain I-I, yielded 21.34 ± 0.82 µL/g, the *Gliricidia sepium* mulch inoculated with strain I-II, yielded 16.41 ± 1.09 µL/g while control produced 18.75 ± 2.53 µL/g of essential oil. Although essential oil concentration (%) was not significantly affected by either bacterial treatment, the enhanced vegetative biomass observed in inoculated plants suggests a potential increase in total essential oil yield per plant due to greater leaf biomass. PGPB inoculation generally enhances plant growth and secondary metabolite production, although responses may vary depending on bacterial strain and plant species (Banchio *et al.*, 2010; Çakmakçı *et al.*, 2023).

Figure 1: Comparison of vegetative growth parameters of *C. verum* at the 12th month of plant establishment; C: *Gliricidia sepium* mulch, G I-I: *Gliricidia sepium* mulch inoculated with bacterial strain I-I, and G I-II: *Gliricidia sepium* mulch inoculated with bacterial strain I-II (G I-II). (a) Aboveground plant growth, (b) Root system development. Scale bars represent 30 cm.

Available phosphorus and total nitrogen content of soil

Table 2: Effect of treatments on the available phosphorus and total nitrogen in pots after 12 months under greenhouse conditions.

Treatment	Available phosphorus (ppm)	Total nitrogen (%)
C	0.3125 ± 0.0025	0.2832 ± 0.0032
G I-I	0.3617 ± 0.0017**	0.3438 ± 0.0038**
G I-II	0.3443 ± 0.0043*	0.2705 ± 0.0005

Values represent the mean of two replicates ± SE. Asterisks indicate statistical significance of differences between each treatment and the control, as determined by Dunnett's test. Significance levels: **0.001 < p < 0.01; *0.01 < p < 0.05.

Table 2 summarizes the effects of the two bacteria-inoculated mulch treatments (I–I and I–II) compared with the control on soil nutrient status after 12 months under greenhouse conditions. Both inoculated mulch treatments resulted in significantly higher available phosphorus concentrations relative to the control, indicating enhanced phosphorus availability in the soil. In contrast, a significant increase in total nitrogen content was observed only in the G I–I treatment, whereas G I–II did not differ significantly from the control. These findings suggest strain-specific effects on soil nutrient dynamics, with both strains contributing to phosphorus enrichment, while only strain I–I enhanced soil nitrogen levels.

This study explores an underutilized approach in cinnamon cultivation by inoculating nitrogen-rich *Gliricidia sepium* mulch with plant growth-promoting bacteria, rather than applying bacteria directly to soil or foliage. This strategy was associated with improved soil nutrient status and supported vegetative growth, likely reflecting effects of *Bacillus* strains I-I and I-II-mediated nutrient mineralization during mulch decomposition. The nutrient-rich *Gliricidia* biomass provides a substrate that may facilitate these outcomes, consistent with previous reports of enhanced cinnamon growth with mycorrhiza inoculation (Priya *et al*., 2026).

The bacterial strain-enriched mulch demonstrated potential to enhance vegetative growth and soil nutrient content under greenhouse conditions. Future research should validate these findings under field conditions, assess nutrient cycling and microbial community dynamics, examine essential oil composition, and explore practical mulch application strategies, including cost–benefit analyses, before suggesting it as a sustainable agriculture strategy.

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