Integrated application of a commercial biostimulant improves growth, root development, and biomass accumulation in *Coffea arabica* seedlings

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Abstract

The use of plant biostimulants has emerged as a promising strategy to improve seedling quality and sustainability in coffee production systems. This study evaluated the effects of the integrated application of a commercial biostimulant on the growth and biomass accumulation of *Coffea arabica* seedlings under nursery conditions. The experiment was conducted in a randomized complete block design with two treatments, control and biostimulant application, and twenty replications. The commercial product Enervig Leg® was applied during seed treatment (6 mL kg^{-1} of seeds) and by foliar spraying (150 mL per 200 L of water) during the early vegetative stage. Growth and biomass variables were evaluated at 180 days after emergence. Biostimulant-treated seedlings showed significant increases in plant height, stem diameter, shoot dry mass, and root dry mass compared to the control treatment. Shoot dry mass increased by 37.7%, while root dry mass increased by 104.5% in treated plants. The treatment also promoted greater root system development and more balanced biomass allocation between shoot and root components. The low coefficients of variation observed for all variables indicated high experimental precision and uniformity of responses under nursery conditions. The results demonstrate that the integrated use of plant biostimulants can improve seedling vigor and structural development in *Coffea arabica*, representing a promising strategy to optimize nursery management and support sustainable coffee production systems.

Keywords: plant growth regulators, inthernursery production, seed treatment, foliar fertilization, root architecture.

Aplicação integrada de um bioestimulante comercial melhora o crescimento, o desenvolvimento radicular e o acúmulo de biomassa em mudas de *Coffea arabica*

Resumo

O uso de bioestimulantes vegetais tem se destacado como uma estratégia promissora para melhorar a qualidade de mudas e a sustentabilidade dos sistemas de produção cafeeira. Objetivou-se avaliar os efeitos da aplicação integrada de um bioestimulante comercial sobre o crescimento e o acúmulo de biomassa de mudas de *Coffea arabica* em condições de viveiro. O experimento foi conduzido em delineamento em blocos casualizados, com dois tratamentos, testemunha e aplicação de bioestimulante, e vinte repetições. O produto comercial Enervig Leg® foi aplicado no tratamento de sementes, na dose de 6 mL kg^{-1} de sementes, e via pulverização foliar, na dose de 150 mL por 200 L de água, durante a fase inicial de desenvolvimento vegetativo. As variáveis de crescimento e biomassa foram avaliadas aos 180 dias após a emergência. As mudas tratadas com bioestimulante apresentaram aumentos significativos na altura de plantas, diâmetro do caule, massa seca da parte aérea e massa seca das raízes em comparação à testemunha. A massa seca da parte aérea aumentou 37,7%, enquanto a massa

seca radicular apresentou incremento de 104,5% nas plantas tratadas. O tratamento também promoveu maior desenvolvimento do sistema radicular e melhor distribuição de biomassa entre parte aérea e raízes. Os baixos coeficientes de variação observados indicaram elevada precisão experimental e uniformidade das respostas em condições de viveiro. Os resultados demonstram que o uso integrado de bioestimulantes pode melhorar o vigor e o desenvolvimento estrutural de mudas de *Coffea arabica*, representando uma estratégia promissora para otimizar o manejo de viveiros e contribuir para sistemas de produção cafeeira mais sustentáveis.

Palavras-chave: reguladores vegetais, produção de mudas, tratamento de sementes, fertilização foliar, arquitetura radicular

1. Introduction

Coffee cultivation plays a fundamental role in global agriculture due to its economic and social importance, especially in tropical countries. Among cultivated species, *Coffea arabica* L. is the most commercially important because of its superior beverage quality and high market value (Davis et al., 2012). Brazil is the world's largest coffee producer and exporter, and the establishment of vigorous and uniform seedlings is considered one of the main factors determining crop establishment and long-term productivity (DaMatta et al., 2007). In commercial coffee production systems, seedling quality directly influences field survival, vegetative growth, and the productive potential of plantations.

The production of high-quality coffee seedlings depends on several factors, including genetic material, substrate composition, irrigation management, environmental conditions, and nutrient availability. However, coffee seedlings commonly exhibit slow initial growth under nursery conditions, which may compromise uniformity and reduce seedling vigor (Medina et al., 2023). In this context, strategies capable of stimulating vegetative growth and improving root system development are essential to optimize nursery management and increase seedling quality.

In recent years, plant biostimulants have received increasing attention as sustainable tools to improve plant growth and crop performance. According to du Jardin (2015), plant biostimulants are substances or microorganisms capable of stimulating natural plant processes, enhancing nutrient uptake, stress tolerance, and crop quality regardless of their direct nutrient content. These products may contain plant growth regulators, amino acids, organic compounds, and micronutrients that influence plant metabolism and development (Backer et al., 2018; Rouphael; Colla, 2020).

Among the compounds commonly present in commercial biostimulants, auxins, cytokinins, and gibberellins are recognized for their role in plant growth regulation. Auxins are directly associated with root initiation and elongation, cytokinins are related to cell division and shoot development, and gibberellins promote stem elongation and vegetative growth (Davies, 2010; Taiz et al., 2017). In addition, micronutrients such as zinc, manganese, iron, and copper participate in several metabolic pathways related to enzymatic activation and plant structural development.

Previous studies have demonstrated positive effects of biostimulants on seedling growth and biomass accumulation in several crops, including coffee (Medina et al., 2023; Souza et al., 2025). Enhanced root development is particularly important in coffee seedlings because root architecture directly affects water and nutrient uptake and contributes to successful establishment after transplanting (Marschner, 2012). However, despite the increasing use of these products in commercial nurseries, there is still limited information regarding the integrated application of biostimulants through seed treatment and foliar spraying during coffee seedling production under nursery conditions.

Therefore, we hypothesized that the integrated application of a commercial biostimulant containing plant growth regulators could promote improvements in vegetative growth, root development, and biomass accumulation in *Coffea arabica* seedlings. Thus, the objective of this study was to evaluate the effects of integrated seed and foliar application of a commercial biostimulant on the growth and biomass accumulation of coffee seedlings produced under nursery conditions.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted in a commercial coffee seedling nursery located in the rural area of the

municipality of Boa Esperança, Minas Gerais, Brazil (21°05′35.96″ S and 45°35′12.09″ W; 819 m altitude). The study was carried out from June to November 2023 under typical nursery conditions used for commercial coffee seedling production. Environmental conditions were partially controlled using shade structures with approximately 50% light reduction, aiming to provide uniform light distribution and minimize environmental variability during seedling development. Irrigation was performed twice daily using a sprinkler system in order to maintain adequate substrate moisture and avoid water deficit throughout the experimental period.

2.2 Plant material and substrate preparation

Seeds of *C. arabica* L. cultivar Catucaí 24137 were used in the experiment due to their wide adoption in Brazilian coffee production systems. The seeds were sown in polyethylene bags measuring 11 × 22 cm, commonly used for coffee seedling production under nursery conditions. The substrate used for seedling development consisted of a mixture of soil, cattle manure, and mineral fertilizer. The substrate preparation involved mixing five 50-kg bags of soil with one 50-kg bag of cattle manure, followed by the addition of 4 kg of single superphosphate containing 18% P_2O_5. The components were thoroughly homogenized before filling the containers to ensure uniform nutrient distribution and adequate physical conditions for seedling growth and root development.

2.3 Experimental design and treatments

The experiment was conducted using a randomized complete block design to minimize the effects of environmental heterogeneity within the nursery. Two treatments were evaluated: a control treatment without biostimulant application and a treatment with integrated application of the commercial biostimulant Enervig Leg®. Twenty replications were used, totaling forty experimental units. Each experimental unit consisted of ten plants. To minimize border effects and increase experimental reliability, only the six central plants of each experimental unit were used for evaluations.

The commercial biostimulant Enervig Leg® contains micronutrients, including Cu, Mn, Fe, and Zn, associated with plant growth regulators, namely 90 mg L^{-1} of indolebutyric acid (IBA), 100 mg L^{-1} of gibberellic acid (GA3), and 1,536 mg L^{-1} of kinetin. The biostimulant was applied at two stages of seedling development. The first application was performed during seed treatment at a dose of 6 mL kg^{-1} of seeds. The second application was carried out through foliar spraying at a dose of 150 mL per 200 L of water during the “orelha de onça” stage, which corresponds to an early vegetative development phase in coffee seedlings.

2.4 Crop management practices

All seedlings were maintained under uniform nursery management conditions throughout the experimental period. Irrigation was performed twice daily using a sprinkler irrigation system in order to maintain adequate substrate moisture and avoid water deficit during seedling development. Light conditions were controlled using shade cloth providing approximately 50% light reduction, installed at a height of approximately 2 m above the seedlings. This shading level is commonly adopted in commercial coffee nurseries to reduce excessive solar radiation and promote balanced vegetative growth. Thinning was performed when seedlings reached the “orelha de onça” stage, leaving only the most vigorous seedling in each container. This practice was adopted to ensure greater uniformity among experimental units and reduce competition for water, nutrients, and space within the containers. All management practices were equally applied to all experimental units during the experimental period.

2.5 Growth measurements and data collection

Growth evaluations were performed at 180 days after seedling emergence, corresponding to an advanced stage of nursery development. Morphological and biomass-related variables were measured to assess seedling growth and structural development. Stem diameter was measured in millimeters using a digital caliper. Plant height was measured in centimeters using a measuring tape, considering the distance between the stem base and the apical meristem. Root length was measured in centimeters after carefully removing the seedlings from the containers and fully extending the root system to avoid deformation during measurement. For biomass determination, the six central plants from each experimental unit were collected and separated into shoot and root fractions. The samples were placed in paper bags and dried in a forced-air circulation oven at 70 °C for 48 h until constant

weight was achieved. Subsequently, shoot dry mass and root dry mass were determined using a precision analytical balance.

2.6 Statistical analysis

The data obtained for all evaluated variables were subjected to analysis of variance (ANOVA) to determine the effects of the treatments on coffee seedling growth and biomass accumulation. When significant differences were detected, treatment means were compared using *Student's t-test* at the 5% probability level. The assumptions of normality and homogeneity of variances were verified before the analyses. Results were expressed as mean values ± standard error. In addition, coefficients of variation (CV) were calculated to evaluate experimental precision and data uniformity. All statistical analyses were performed using SISVAR® software, following the procedures described by Ferreira (2011).

3. Results

The integrated application of the commercial biostimulant significantly affected the growth and biomass accumulation of *C. arabica* seedlings produced under nursery conditions (Table 1; Figure 1).

Table 1. Analysis of variance summary and descriptive statistics for growth and biomass variables of *Coffea arabica* seedlings subjected to integrated biostimulant application under nursery conditions.

Source of variation	df	Plant height	Stem diameter	Root length	Shoot dry mass	Root dry mass
Treatment	1	0.0239*	0.0155**	0.0002**	0.0006**	0.0008**
Replication	19	0.4234ns	0.2745ns	0.3298ns	0.3743ns	0.3456ns
Error	19	—	—	—	—	—
Total	39	—	—	—	—	—
CV (%)	—	2.35	1.97	1.77	2.69	1.55
Overall mean	—	9.34 cm	1.95 mm	27.50 cm	8.19 g	2.01 g

Note: Values represent p-values obtained from analysis of variance (ANOVA). ns = non-significant; * significant at $p < 0.05$; ** significant at $p < 0.01$. CV = coefficient of variation. Source: Authors, 2026.

Analysis of variance revealed significant treatment effects for plant height ($p = 0.0239$), stem diameter ($p = 0.0155$), root length ($p = 0.0002$), shoot dry mass (p = 0.0006), and root dry mass ($p = 0.0008$). The coefficients of variation ranged from 1.55% to 2.69%, indicating high experimental precision and uniformity among experimental units (Figure 1a). Biostimulant-treated seedlings exhibited significantly greater vegetative growth compared to untreated seedlings (Figure 1b). Plant height increased from 7.23 cm in the control treatment to 11.44 cm in treated seedlings, corresponding to an increase of 58.2%. Similarly, stem diameter increased from 1.67 mm to 2.22 mm, representing an increase of 32.9%. Root development was markedly influenced by biostimulant application. Root length increased from 18.0 cm in untreated seedlings to 37.0 cm in treated plants, corresponding to an increase of 105.6% (Figure 1b). Biomass accumulation was also positively affected by the treatments (Figure 1c). The experimental design and integrated application strategy adopted in this study are illustrated in Figure 1a, including seed treatment, foliar application, and the growth and biomass evaluations performed at 180 days after emergence.

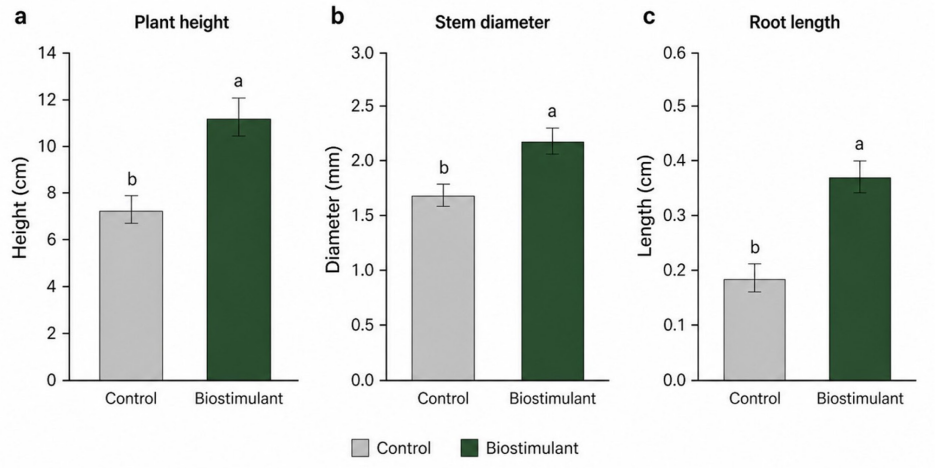

Figure 1. Multipanel overview of the experimental design and the effects of integrated biostimulant application on the growth and biomass accumulation of *Coffea arabica* seedlings produced under nursery conditions. (**a**) Experimental design overview, including plant material, experimental setup, integrated seed and foliar application of Enervig Leg®, and evaluation procedures. (**b**) Effects of biostimulant application on plant height, stem diameter, and root length at 180 days after emergence. (**c**) Effects of biostimulant application on shoot dry mass and root dry mass. Different letters indicate significant differences between treatments according to *Student's t-test* at the 5% probability level. Source: Authors, 2026.

Shoot dry mass increased from 6.89 g in the control treatment to 9.49 g in treated seedlings, representing an increase of 37.7%. Root dry mass increased from 1.32 g to 2.70 g after biostimulant application, corresponding to an increase of 104.5%. For all evaluated variables, seedlings treated with Enervig Leg® differed significantly from the control treatment according to *Student's t-test* at the 5% probability level (Table 2; Figure 2). Overall, the integrated application of the commercial biostimulant promoted consistent increases in vegetative growth, root development, and biomass accumulation in *C. arabica* seedlings under nursery conditions.

Table 2. Mean values of growth and biomass variables of *Coffea arabica* seedlings subjected to integrated biostimulant application under nursery conditions at 180 days after emergence.

Treatment	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Shoot dry mass (g)	Root dry mass (g)
Control	7.23 b	1.67 b	18.0 b	6.89 b	1.32 b
Enervig Leg®	11.44 a	2.22 a	37.0 a	9.49 a	2.70 a
Increase (%)	58.2	32.9	105.6	37.7	104.5
CV (%)	2.35	1.97	1.77	2.69	1.55

Note: Means followed by different letters within the same column differ significantly according to *Student's t-test* at the 5% probability level. CV = coefficient of variation. Source: Authors, 2026.

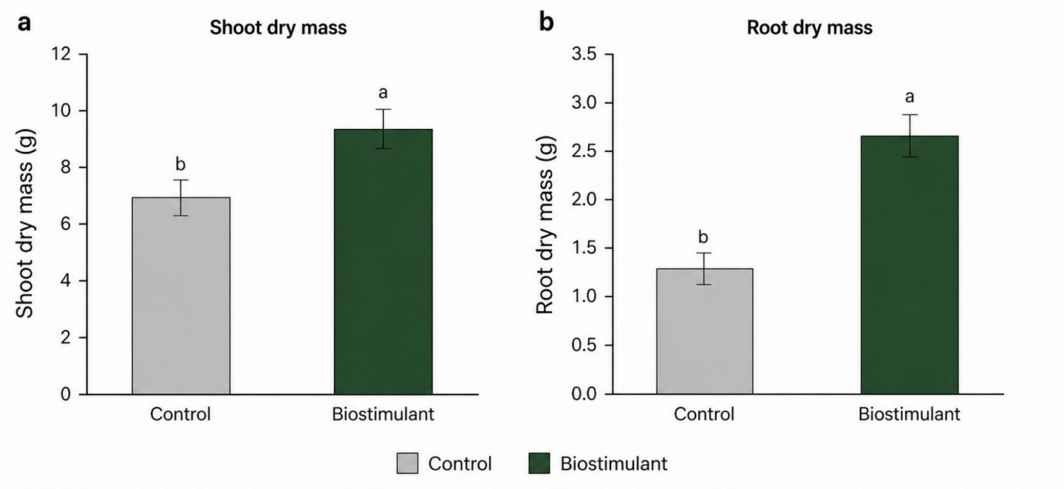

Figure 2. Shoot dry mass (**a**) and root dry mass (**b**) of *Coffea arabica* seedlings treated with the commercial biostimulant Enervig Leg®. Bars represent mean ± standard error. Different lowercase letters indicate significant differences between the control and biostimulant treatments according to the *Student's t-test* ($p \leq 0.05$).

4. Discussion

The results obtained in this study demonstrate that the integrated application of the commercial biostimulant Enervig Leg® promoted significant improvements in the vegetative growth and biomass accumulation of *C. arabica* seedlings produced under nursery conditions. Positive responses were observed for all evaluated variables, including plant height, stem diameter, root length, shoot dry mass, and root dry mass, indicating that the use of biostimulants may contribute to the production of more vigorous and structurally developed coffee seedlings.

The increase observed in plant height and stem diameter suggests that biostimulant application favored vegetative development during the seedling formation stage. These responses may be associated with the presence of plant growth regulators in the commercial formulation, especially gibberellic acid, kinetin, and indolebutyric acid, which are commonly related to cell elongation, cell division, and plant tissue differentiation (Taiz et al., 2017; Davies, 2010). Similar responses have recently been reported for coffee seedlings produced under nursery conditions following the application of bioproducts and plant biostimulants (Medina et al., 2023).

Root development was one of the variables most strongly affected by the treatments. The expressive increase in root length and root dry mass indicates that the integrated seed and foliar application strategy promoted greater root system expansion and biomass allocation to belowground structures. According to Taiz et al. (2017), auxin-related compounds may stimulate root initiation and elongation, contributing to improved root architecture during early plant development. Enhanced root systems are particularly important for coffee seedlings because they may increase the capacity for water and nutrient absorption after transplanting under field conditions (Marschner, 2012). Recent studies with humic substance-based biostimulants have also demonstrated significant increases in root and shoot development of *C. arabica* seedlings under nursery conditions, reinforcing the potential of these products for improving seedling quality and vigor (Souza et al., 2025).

The increase in shoot and root dry mass observed in treated seedlings also indicates that the biostimulant promoted greater biomass accumulation during nursery development. Similar positive effects of commercial biostimulants on biomass production have been described in several crops, including coffee seedlings grown under nursery conditions (Medina et al., 2023; Souza et al., 2025). In the present study, the integrated application strategy may have contributed to continuous stimulation of seedling development throughout different growth stages.

The low coefficients of variation observed for all evaluated variables indicate high experimental precision and uniformity of responses under nursery conditions. This reduced variability may be associated with the standardized management practices adopted during the experiment, including homogeneous substrate preparation, controlled irrigation, shade management, and the evaluation of only the central plants within each experimental unit. Similar low variability has been reported in nursery experiments conducted under controlled environmental conditions (Bergo et al., 2002).

Although the present study demonstrated positive responses in growth and biomass variables, no physiological

or biochemical measurements were performed. Therefore, interpretations regarding metabolic efficiency, hormonal balance, or photosynthetic performance should be considered only as potential explanations for the observed responses rather than confirmed physiological mechanisms. Future studies evaluating gas exchange, chlorophyll content, nutrient uptake, and enzymatic activity may contribute to a better understanding of the mechanisms involved in the responses of coffee seedlings to biostimulant application.

Recent advances have reinforced the importance of plant biostimulants as sustainable tools capable of improving crop performance and supporting environmentally friendly agricultural systems (Nephali et al., 2024). In addition, plant-based biostimulants applied during seed treatment have increasingly been investigated as sustainable alternatives capable of improving seedling vigor and crop establishment (Wazeer et al., 2024). These findings support the results observed in the present study and reinforce the relevance of integrated biostimulant management strategies during coffee seedling production.

The novelty of this study is associated with the integrated application strategy combining seed treatment and foliar spraying of a commercial biostimulant during coffee seedling production under nursery conditions. Although previous studies have evaluated isolated applications of plant growth regulators and biostimulants in coffee crops, information regarding integrated management strategies during the seedling phase remains limited. The results obtained here reinforce the potential use of commercial biostimulants as a practical tool to improve seedling quality and optimize nursery management in coffee production systems.

5. Conclusions

The integrated application of the commercial biostimulant Enervig Leg® promoted significant improvements in the growth and biomass accumulation of *Coffea arabica* seedlings produced under nursery conditions. Biostimulant-treated seedlings exhibited greater plant height, stem diameter, root development, shoot dry mass, and root dry mass compared to untreated seedlings. The combined use of seed treatment and foliar application contributed to the production of more vigorous seedlings with greater biomass accumulation and improved root system development. These results demonstrate the potential of commercial biostimulants as an important management strategy for optimizing coffee seedling production in commercial nurseries. Further studies evaluating physiological and biochemical responses are recommended to better understand the mechanisms associated with the effects of integrated biostimulant application on coffee seedling development.

6. Authors' Contributions

Elias Rodrigues Cunha: conceptualization, methodology, investigation, data collection, formal analysis, writing – original draft preparation. *Edimar Agnaldo Moreira*: supervision, methodology, validation, data interpretation, writing – review and editing.

7. Conflicts of Interest

The authors declare no conflicts of interest.

8. Ethics Approval

Not applicable. This study did not involve humans or live vertebrate animals.

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