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Evaluation of different combinations of biostimulants in the development of lettuce seedlings

Avaliação de diferentes combinações de bioestimulantes no desenvolvimento da alface (Lactuca sativa L.)

Evaluación de diferentes combinaciones de bioestimulantes en el desarrollo de la lechuga (Lactuca sativa L.)

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Abstract:
Lettuce is one of the most economically and socially important leafy vegetables in Brazil, and the quality of the seedlings produced is a determining factor for the success of the crop. Therefore, this study aimed to evaluate the effect of different combinations of biostimulants on the development of lettuce seedlings of the Jade cultivar. The experiment was conducted in a protected environment at the University Farm of the UniSALESIANO University Center, in Lins-SP, in a completely randomized design with six treatments and five replications: T1 (control); T2 (Radicor, 3 mL L^{-1}); T3 (Radicor + Trichodermaiz, 1 g L^{-1}); T4 (Radicor + Trichodermaiz + Dunna, 2 mL L^{-1}); T5 (Radicor + Trichodermaiz + Elmo, 2 mL L^{-1}); T6 (Radicor + Trichodermaiz + Elmo + Dunna). Forty-three days after transplanting, the following were evaluated: root length (RL), total length (TL), shoot fresh weight (SFW), root fresh weight (RFW), number of leaves (NL), shoot length (SL), stem diameter (SD), and aerial part diameter (APD). The data were subjected to analysis of variance, and the means were compared using Tukey's test at a 5% probability level, with the aid of the SISVAR software. It was concluded that treatment T6 provided the greatest root length (49.92 cm), differing significantly from the other treatments. For root fresh weight, T4 and T6 recorded the highest values (32.00 g), being superior to the others. Treatment T3 resulted in the greatest total plant length (73.48 cm), and T4 presented the largest stem diameter (1.97 cm). Combinations containing Dunna — formulated with *Bacillus aryabhattai*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* — had a positive effect on root development, while the presence of *Trichoderma harzianum* (Trichodermaiz) was crucial for shoot growth. The results indicate that microbial biostimulation via soil application promotes differential responses in lettuce seedlings, particularly in the root system.

Keywords: seedling production; growth-promoting rhizobacteria; biostimulation; *Trichoderma harzianum*; bio-inputs.

Resumo:
A alface é uma das hortaliças folhosas de maior relevância econômica e social no Brasil, e a qualidade das mudas produzidas constitui um fator determinante para o sucesso da cultura. Sendo assim, o trabalho teve como objetivo avaliar o efeito de diferentes combinações de bioestimulantes no desenvolvimento de mudas de alface cultivar Jade. O experimento foi conduzido em ambiente protegido na Fazenda Escola do Centro Universitário UniSALESIANO, em Lins-SP, em delineamento inteiramente casualizado com seis tratamentos e cinco repetições: T1 (Testemunha); T2 (Radicor, 3 mL L^{-1}); T3 (Radicor + Trichodermaiz, 1 g L^{-1}); T4 (Radicor + Trichodermaiz + Dunna, 2 mL L^{-1}); T5 (Radicor + Trichodermaiz + Elmo, 2 mL L^{-1}); T6 (Radicor + Trichodermaiz + Elmo + Dunna). Aos 43 dias após o transplantio, na colheita foram avaliados: comprimento da raiz (CR), comprimento total (CT), massa fresca da parte aérea (MFPA), massa fresca das raízes (MFR), número de folhas (NF), comprimento da parte aérea (CPA), diâmetro do caule (DC) e diâmetro da parte aérea (DPA). Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey a 5% de probabilidade, com auxílio do software SISVAR. Concluiu-se que o tratamento T6 proporcionou o maior comprimento radicular (49,92 cm), diferindo significativamente dos demais tratamentos. Para a massa fresca das raízes, T4 e T6 registraram

os maiores valores (32,00 g), sendo superiores aos demais. O tratamento T3 resultou no maior comprimento total de planta (73,48 cm) e T4 apresentou o maior diâmetro de caule (1,97 cm). As combinações contendo Dunna — formulado à base de *Bacillus aryabhattai*, *Bacillus amyloliquefaciens* e *Bacillus subtilis* — exerceram efeito positivo sobre o desenvolvimento radicular, ao passo que a presença de *Trichoderma harzianum* (Trichodermaiz) foi determinante para o crescimento da parte aérea. Os resultados indicam que a bioestimulação microbiana via aplicação ao solo promove respostas diferenciais em alface, com destaque para o sistema radicular.

Palavras-chave: produção de mudas; rizobactérias promotoras do crescimento; bioestimulação; *Trichoderma harzianum*; bioinsumos.

Resumen:
La lechuga es una de las hortalizas de hoja de mayor relevancia económica y social en Brasil, y la calidad de las plántulas producidas constituye un factor determinante para el éxito de la cultura. Siendo así, el trabajo tuvo como objetivo evaluar el efecto de diferentes combinaciones de bioestimulantes en el desarrollo de plántulas de lechuga cultivar Jade. El experimento fue conducido en ambiente protegido en la Hacienda Escuela del Centro Universitario UniSALESIANO, en Lins-SP, en diseño completamente casualizado con seis tratamientos y cinco repeticiones: T1 (Testemunha); T2 (Radicor, 3 mL L−1); T3 (Radicor + Trichodermaiz, 1 g L−1); T4 (Radicor + Trichodermaiz + Dunna, 2 mL L−1); T5 (Radicor + Trichodermaiz + Elmo, 2 mL L−1); T6 (Radicor + Trichodermaiz + Elmo + Dunna). A los 43 días después del trasplante, se evaluaron la longitud de la raíz (CR), la longitud total (CT), la masa fresca de la parte aérea (MFPA), la masa fresca de las raíces (MFR), el número de hojas (NF), la longitud de la parte aérea (CPA), el diámetro del caule (DC) y el diámetro de la parte aérea (DPA), los datos fueron sometidos al análisis de varianza y los promedios comparados por el test de Tukey al 5% de probabilidad, con auxilio del software SISVAR. Se concluyó que el tratamiento T6 proporcionó la mayor longitud radicular (49,92 cm), con diferencia significativa de los demás tratamientos. Para la masa fresca de las raíces, T4 y T6 registraron los mayores valores (32,00 g), siendo superiores a los demás. El tratamiento T3 resultó en la mayor longitud total de planta (73,48 cm) y T4 presentó el mayor diámetro de caule (1,97 cm). Las combinaciones que contienen Dunna — formulado a base de *Bacillus aryabhattai*, *Bacillus amyloliquefaciens* y *Bacillus subtilis*— ejerceron un efecto positivo sobre el desarrollo radicular, mientras que la presencia de *Trichoderma harzianum* (Trichodermaiz) fue determinante para el crecimiento de la parte aérea. Los resultados indican que la bioestimulación microbiana vía aplicación al suelo promueve respuestas diferenciales en lechuga, con destaque para el sistema radicular.

Palabras clave: producción de plántulas; rizobacterias promotoras del crecimiento; bioestimulación; *Trichoderma harzianum*; bioinsumos.

1 Introduction

The lettuce (*Lactuca sativa L.*), belonging to the Asteraceae family, stands out among the leafy vegetables most produced and consumed worldwide. Rich in macro and micronutrients essential to human food, the culture has high economic and food importance, being widely consumed in the form of salads and other fresh foods (Santos-Naressi *et al.*, 2022).

Among the vegetables of greatest relevance for the Brazilian population's food, lettuce has a broad productive expression.The state of São Paulo stands out as a national leader in the production of this crop, with more than 220,000 tons produced annually, in addition to playing a strategic role in the supply of the Greater São Paulo and other regions of the country (SAA, 2026).Because it is a culture with high nutritional demand, lettuce benefits from soils rich in organic matter to achieve high productivity (Meireles *et al.*, 2017).In addition, by accumulating

large quantity of nutrients in its leaves, it becomes very demanding in cultural treatments, and in order to achieve success in production, it is necessary to adopt adequate management (Nick, 2019).

Given the increased demand for high quality lettuce and productivity, the use of biostimulants has gained prominence in the cultivation of this vegetable (Nogueira *et al.*, 2023).In this context, the application of biostimulants positively interferes in the metabolic dynamics of cultures, promoting physiological adjustments that enhance growth and productivity.As Mattos and Caires (2022), these substances are fundamental to mitigate the negative impacts of abiotic stress, optimizing the utilization of resources by plants.

In addition, the use of biofertilizers has been identified as a sustainable alternative to the excessive use of chemical fertilizers, since these inputs promote microbial activity, increase fertility and soil quality and can contribute to the improvement of soil aggregation, favoring the development of crops (Mahmud *et al.*, 2021). In Brazil, the bioinsumes sector expanded to an annual rate of 22%, quadrupling the world average, and in the 2024/2025 harvest the area treated with these products reached 156 million hectares, with a technological adoption rate of around 26% (Blink/CropLife Brasil, 2025).

Among the most relevant mechanisms associated with microbial biostimulants are bacteria of the genus Bacillus, especially *B. subtilis*, *B. amyloliquefaciens* and *B. aryabhattai*, which actively colonize the rhizosphere and establish biofilms associated with the roots, bringing numerous benefits to the plant, such as a microenvironment of greater moisture and availability of nutrients around the root system, besides stimulating the production of exopolysaccharides that contribute to the aggregation of the soil and protection against water stresses (Nordgaard *et al.*, 2022).

In addition to these characteristics, species of the genus Bacillus are recognized for their ability to produce plant growth-promoting compounds, including indol-3-acetic acid (AIA), an auxin directly related to the development of the root system.The production of this phytormone is associated with cell stretching in the meristematic regions of the roots and with greater formation of lateral roots, increasing the absorption surface and the exploited root volume.In addition, some species of the genus are able to solubilize insoluble inorganic phosphates, releasing phosphorus to the soil solution and expanding their availability to plants (Almeida, 2024).

Similarly, the biostimulant effects observed in growth-promoting bacteria can also be verified in certain beneficial fungi.In this context, the fungus *Trichoderma harzianum* represents another effective agent.In a study conducted with lettuce in a hydroponic system,

Alzyoud *et al.* (2025) found that inoculation with T. harzianum promoted significant increases in the fresh mass of the aerial part, in the leaf area, in the number of leaves and in the chlorophyll content, results attributed by the authors to the ability of this microorganism to produce phytohormones, solubilize micronutrients and induce enzymes related to the primary metabolism of plants.

In addition to growth-promoting microorganisms, other substances of natural origin have also been widely used as biostimulants in agriculture.Among them, humic substances, consisting mainly of humic and ulvic acids, which arouse great agronomic interest due to their ability to stimulate plant development and improve soil conditions.

When mimicking the action of auxin, humic acids induce root growth by stimulating the activity of membrane H+-ATPases, favoring the absorption of ions and cell elongation. They also contribute to the improvement of the physicochemical properties of the soil, increase cationic exchange capacity and stimulate the beneficial microbiota of the rhizosphere (Castro, 2023). When associated with seaweed extracts, rich in betaines, mannitol, polyamines and natural cytokinins, humic substances enhance the biostimulant effect on plant growth and resistance to adverse conditions (El Boukhari *et al.*, 2020; Kocira *et al.*, 2020).

Despite the growing number of studies on biostimulants in horticulture, few verify the effect of combinations of microbial and organomineral products applied via soil in the development of lettuce plants.The central hypothesis of this study was the combination of biostimulants of different categories — organomineral fertilizer based on humic substances, algae extract and amino acids; biofungidide based on *Trichoderma harzianum*; and inoculating agents based on Bacillus spp. — results in superior response to the application of isolated products, especially on variables related to the root system and the structural vigor of plants.

In this sense, the present study aimed to evaluate the effect of different combinations of biostimulants on the growth and development of lettuce seedlings cultivar Jade, by analyzing biometric parameters.

2 Material and methods

2.1 Place of the experiment

The experiment was conducted in a protected environment (house of vegetation with polyethylene cover) at the School Farm of UniSALESIANO University Center, in the municipality of Lins, State of São Paulo (21°42'21,3"S; 49°45'25,3"O; altitude: 390 m).The

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region's climate is classified as Aw (tropical with dry season in winter), according to Köppen-Geiger.The experiment was conducted between April and May 2026.

2.2 Chemical characterization of the soil

Prior to the implementation of the experiment, soil samples were collected in the layer of 0–20 cm and sent to the laboratory for chemical analysis. The results are presented in Table 1. Based on the recommendations of the Bulletin 100 of the Instituto Agronomico de Campinas (Raij *et al.*, 2022), fertilized and limed with simple superphosphate (1.44 kg for 9 m2 area) and urea (0.0495 kg), adapting the soil to the nutritional requirements of the culture. The soil was sieved, homogenized and used to fill the vessels.

Table 1 – Chemical soil analysis (layer 0–20 cm) used in the experiment, Farm Escola UniSALESIANO, Lins-SP, 2026.

Parameter	Unit	Value
Matéria orgânica (MO)	g dm^{-3}	10
pH ($CaCl_2$)	—	5,5
Fósforo (P-resina)	mg dm^{-3}	5
Potássio (K^+)	mmolc dm^{-3}	4.1
Cálcio (Ca^{2+})	mmolc dm^{-3}	19
Magnésio (Mg^{2+})	mmolc dm^{-3}	6
Alumínio (Al^{3+})	mmolc dm^{-3}	<1
H + Al	mmolc dm^{-3}	14
Enxofre (SO_4^{2-})	mg dm^{-3}	8
Soma de bases (SB)	mmolc dm^{-3}	29,1
CTC	mmolc dm^{-3}	43,1
Saturação de bases (V%)	%	67,5

Source: Developed by the authors (2026).

2.3 Experimental design and statistical analysis

The design was completely randomized (DIC), with six treatments and five repetitions, totaling 30 experimental units. Each experimental unit corresponded to a plastic vessel with volumetric capacity of 12 liters (27 × 27 × 21 cm), filled with corrected and fertilized soil. In each vessel, a seedlings of lettuce were transplanted to cultivate Jade (developed by Sakata Seed do Brasil), produced in a certified nursery, 30 days old.

The data obtained were tabulated and subjected to analysis of variance (ANOVA). When significant effect ($p \leq 0.05$) was observed, the means were compared by the Tukey test at 5% probability. All analyses were processed using the SISVAR software version 5.8 (Ferreira, 2019).

2.4 Treatments

Six treatments were evaluated: T1 — absolute test (without application of biostimulants); T2 — Radicor (3 mL L−1); T3 — Radicor (3 mL L−1) + Trichodermaiz (1 g L−1); T4 — Radicor (3 mL L−1) + Trichodermaiz (1 g L−1) + Dunna (2 mL L−1); T5 — Radicor (3 mL L−1) + Trichodermaiz (1 g L−1) + Elmo (2 mL L−1); T6 — Radicor (3 mL L−1) + Trichodermaiz (1 g L−1) + Elmo (2 mL L−1).

The products used belong to the portfolio of Companhia Nitro Química Brasileira (Nitro Agro) and were selected because they represent different functional categories of biostimulants.Table 2 presents the characterization of each product according to information from the official leaflets and technical sheets of the manufacturer.

Table 2 – Characterization of biostimulants used in the experiment, according to official manufacturer information (Nitro Agro).

Name commercial	Manufacturer	Classification	Ingredient(s) Assets(S)	Purpose Agronomics
Radicor	Nitro Agro	Fertilizante organomineral (via solo)	Humic substances (COT: 12% m/m; N: 1% m/m); seaweed extract; amino acids	Improvement of soil health; stimulation of root development; potentiation of nutrient absorption
Trichodermaiz WP PRO	Nitro Agro	Biofungicida (pó molhável)	*Trichoderma harzianum* isolado IB19/17 (mín. 7×10^9 conídios viáveis g^{-1})	Biological control of soil pathogens; indirect promotion of plant growth

Dunna	Nitro Agro	Inoculante microbiano (PGPR)	*Bacillus aryabhattai*, *Bacillus amyloliquefaciens* e *Bacillus subtilis*	Promotion of root growth; formation of biofilm in the rhizosphere; improvement of nutrient absorption and tolerance to abiotic stresses
Elmo (NT 5-24)	Nitro Agro	Nematicida microbiológico (suspensão concentrada)	*Bacillus amyloliquefaciens*, *B. subtilis* (cepas CCT 8100 e ATCC 6051), *B. licheniformis* (ATCC 14580) e *B. megaterium* (CCT 8101) — mín. 1×10^8 UFC mL^{-1} de pc, cada cepa	Controle biológico de Meloidogyne incognita; efeito secundário de promoção do crescimento via colonização radicular

Source: Official leaflets and technical files Nitro Agro (Company Nitro Química Brasileira, 2024/2025). PGPR: plant growth-promoting rhizobacteria; COT: total organic carbon; UFC: colony forming unit; pc: commercial product.

The doses applied were defined according to the recommendations contained in the official leaflets and technical materials made available by the manufacturer (Nitro Agro), seeking to reproduce the conditions normally used by the producers in the field. For Radicor, the dose was 3 mL L−1 of water; for Trichodermaiz WP PRO, 1 g L−1; and for Dunna and Elmo, 2 mL L−1 each.

2.5 Experimental driving

The seedlings were transplanted in the vessels on 04/04/2026.The first application of the products occurred on 08/04/2026, four days after transplantation; subsequent applications were performed at 10 days intervals, on 18/04/2026 and 28/04//2026, totaling three applications throughout the experimental cycle.Irrigation was performed manually, so as to maintain soil moisture within the range appropriate for the development of lettuce.

The applications were performed in the morning (between 7h and 9h), hours of lower temperature and higher relative humidity of the air, conditions that favor the viability of microorganisms and the absorption of organic compounds by the roots. The products were diluted in 1 L of drinking water, and each vessel received 200 mL of the solution via the plant.

2.6 Evaluations

At the end of the experiment, at 43 days after transplantation, the plants were harvested and evaluated for the following variables: (i) root length (CR, cm) — measured with tape measure from the neck to the root apex; (ii) total plant length (CT, cm) — distance from the apex from the root to the highest leaf, measured with trene; (iii) fresh aerial mass (MFPA, g)

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— determined in an analytical scale immediately after the separation of the root system; (iv) fresh root mass (MFR, g) — obtained after washing and surface drying of the roots; (v) number of leaves (NF) — manual count of all expanded leaves; (vi) length of the aerial part (CPA, cm) — distance from the neck to the leaf apex, measured with trene; (vii) stem diameter (DC, cm) — measured with pachymeter in the colle region; (viii) air diameter (DPA, cm) — maximum width of the leaf, measured with treine.

3 Results and discussion

The means obtained for the eight variables evaluated are shown in Table 3. The treatments with biostimulants promoted different responses in the development of lettuce, with significant variation mainly in the variables related to the root system and stem thickening, while other parameters of the aerial part (fresh mass, length, number of leaves and diameter) did not present statistical difference between treatments.

Table 3 – Root length (CR), total length (CT), fresh aerial mass (MFPA), fresh root mass (MFR), number of leaves (NF), length of the aerial part (CPA), stem diameter (DC) and diameter of the aerial part (DPA) of lettuce cv. Jade subjected to different combinations of biostimulants. Lins-SP, 2026.

TRAT	CR (cm)	CT (cm)	MFPA (g)	MFR. (g)	NF	CPA (cm)	DC (cm)	DPA (cm)
T1	35,00 b	60,80 b	157,60 a	18,00 b	23,60 a	25,60 a	1,50 c	33,20 to
T2	33,26 b	59,46 b	210,00 a	18,00 b	25,20 a	26,20 a	1,91 ab	34,00 to
T3	34,88 b	73,48 a	164,00 a	27,00 ab	23,20 a	24,60 a	1,84 ab	34,00 to
T4	33,84 b	65,64 ab	186,40 a	32,00 a	25,40 a	25,80 a	1,97 a	34.60 to
T5	29,96 b	60,50 b	202,80 a	21,00 b	24,20 a	27,40 a	1,84 ab	36.80 to
T6	49,92 a	67,62 ab	166,80 a	32,00 a	23,80 a	25,70 a	1,66 bc	34,20 to
Média geral	36,14	64,58	181,2	25	24,23	26,05	1,78	34.46
C.V.(%)	**14,3**	**7,84**	**18,15**	**22,86**	**11,25**	**9,30**	**8,00**	7,02

Means followed by the same lowercase letter in the column do not differ by the Tukey test ($p \leq 0.05$). T1: witness; T2: Radicor; T3: Radicor + Trichodermaiz; T4: Radicor + Trichodermaiz + Dunna; T5: Radicor + Trichodermaiz + Elmo; T6: Radicor + Trichodermaiz + Elmo + Dunna. Trat.: treatment; CR: root length; CT: total length; MFPA: fresh mass of the aerial part; MFR: fresh mass of the roots; NF: number of leaves; CPA: length of the aerial part; DC: stem diameter; DPA: diameter of the aerial part. CV: coefficient of variation.

Source: Developed by the authors (2026).

The root length (CR) was the variable that presented the greatest statistical difference between treatments. The T6 (Radicor + Trichodermaiz + Elmo + Dunna) recorded an average of 49.92 cm, 42.6% higher than that of the witness (35.00 cm) and statistically different from all other treatments by the Tukey test. It is noteworthy that T5 (Radicor + Trichodermaiz + Elmo), composed by the same T6 base, but without the Dunna inoculator, was the treatment with the lowest root length among those containing biostimulants (29.96 cm), showing that the role of Bacillus spp. present in the Dunna was determinant for the increase observed in T6. This difference indicates that the absence of the Elmo in T4 did not prevent the expression of the radicular effects of Dunna, but that, in T6, the combination with the Elmo species may have created favorable synergistic conditions for the deepening and extension of the root system.

The central mechanism of this effect is related to the ability of the species of Bacillus present in the Dunna to colonize the rhizosphere and secrete bioactive compounds that alter the hormonal balance in the roots. The production of indol-3-acetic acid (IAA) by *B. subtilis* and *B. amyloliquefaciens* is widely documented in the literature and results in greater cell division and elongation in the meristematic region of the roots, stimulating the formation of lateral roots and root roots. Experiment conducted by Nordgaard *et al.* (2022) with *B. subtilis* in roots of *Arabidopsis thaliana* demonstrated rapid adaptation of the microorganism to the root microenvironment, with formation of dense biofilms and expressive increase of root colonization over time. The formation of biofilms is physiologically relevant because the produced exopolysaccharides create a hydrated matrix around the roots, favoring local water availability and nutrient diffusion, besides protecting bacterial cells against temperature fluctuations and competitors (Nordgaard *et al.*, 2022).This biofilm-root interface amplifies the production of phytohormones and enzymes that increase root growth — which would explain, at least in part, the superior behavior of T6.

The increase in the root fresh mass (MFR) reinforces this interpretation. The T4 and T6 treatments presented the highest averages (32.00 g), differing significantly from T1 and T2 (18.00 g) and T5 (21.00 g). The agreement between CR and MFR indicates that the highest length observed in T6 does not result from thinner or less dense roots, but from a genuinely more robust root system — with higher specific surface and presumably greater water and nutrient absorption capacity. Almeida (2024), in a study with crespa lettuce, found that inoculation with *B. subtilis* increased both the fresh mass and root drought, corroborating the hypothesis that species of the genus Bacillus promote a real increase in root biomass, probably associated with the solubilization of inorganic phosphates — a process that increases the

availability of P in the rhizosphere — and the synthesis of siderophores that facilitate the absorption of micronutrients such as iron and zinc, essential to the synthesis of nucleic acids and energy metabolism of root cells.

The absence of significant response in CR for T5 (without Dunna, but with Elmo) indicates that Elmo, although formulated with similar strains of Bacillus spp., did not produce the same effect of promoting root growth when applied alone or in association with Trichodermaiz without Dunna. This result may be related to the composition of specific strains in each product: Elmo is registered as microbiological nematicide (NT 5-24), with strains selected for the control of Meloidogyne incognita, while Dunna was formulated with emphasis on growth promotion. Functional differences between Bacillus strains contained in each product — even belonging to the same gender — determine distinct profiles of production of secondary metabolites, which can explain the distinct responses observed between T5 and T6.

For the total length of the plant (CT), which integrates the growth of the shoot and root, the treatment T3 (Radicor + Trichodermaiz) showed the highest mean (73.48 cm), statistically differing from T1, T2 and T5, but not from T4 and T6. The lower performance of T2 (Radicor alone, 59.46 cm), even lower than the witness (60.80 cm), suggests that the humic substances, the extract of algae and the amino acids of Radicor, when applied alone, did not promote sufficient increase in the overall growth of the plant in the evaluated conditions.

The superiority of T3 in relation to the witness and to T2 and T5 is attributed to the biostimulant effect of *Trichoderma harzianum* (isolated IB19/17), which goes beyond its primary biofunginicidal action. This fungus acts on plant metabolism through the production of phytohormones — such as gibberelins and auxins — and phosphatase and cellulase enzymes that increase the availability of nutrients in the rhizosphere. In lettuce grown in hydroponic system, Alzyoud *et al.* (2025) found that inoculation with T. harzianum significantly increased the fresh mass of the air, the leaf area, the chlorophyll content and the number of leaves, indicating that the fungus stimulates metabolic processes linked to carbon assimilation and vegetative growth. In this sense, the results of the present study are consistent with this line of evidence, revealing that, in the context of the tested combinations, the presence of T. harzianum was the main responsible for the highest TC. The stabilization of the TC response when other ingredients were added (T4, T5 and T6) may indicate that the positive effect of Trichodermaiz on the total length was strong enough to maintain numerically high results, but without statistical difference of the more complete treatments.

Regarding the diameter of the stem (DC), variable associated with structural vigor of the seedlings and the support capacity and translocation of photoassimilates, the T4 treatment

(Radicor + Trichodermaiz + Dunna) presented the highest value (1.97 cm), being the only treatment exclusively classified in the group "a" by the Tukey test, followed by T2 (1.91 cm, ab group) and T3 and T5 (1.84 cm, ab group). The witness (T1) recorded the lowest diameter (1.50 cm, c group), while T6 was in the bc group (1.66 cm), a value lower than that of T4, which deserves analysis.

The performance of T2 (Radicor alone) for DC, superior to the witness and close to the best treatment, indicates that the organomineral fertilizer based on humic substances, algae extract and amino acids exerted considerable influence on the structural development of the stem. Castro (2023) demonstrated that humic substances act on hormonal regulation, especially in the synthesis of cytokinins, hormones associated with cell division and thickening of vascular tissues. The extracts of algae contribute with betaines and polyamines that participate in the synthesis of cell wall proteins, while free amino acids provide readily available nitrogen for the biosynthesis of structural compounds. The association of these three fractions explains the role of Radicor in the favoring of CD.

The superiority of T4 over the other treatments, including T6, which contains the same products more Elmo, suggests that the addition of Elmo may have created a certain functional antagony or competition between the microbial strains, partially compromising the expression of the effect on the stem. This hypothesis is plausible in view of the fact that the Elmo is formulated with selected strains for biocontrol of nematodes, whose mode of action involves the intense colonization of the rhizosphere, possible competition with the strains of Dunna by the root surface. In any case, the T6 CD remained superior to that of the witness, and the difference between T4 and T6 should not be interpreted as a negative effect of the Elmo, but as an indication that the additive between biostimulants is not linear and depends on the compatibility between the microorganisms involved.

The results showed that not all the variables evaluated responded differently to the application of biostimulants.The fresh mass of the aerial part (FMPA), the number of leaves (NF), the length of the aerial part (CPA) and the diameter of the aerial part (APD) did not show statistically significant differences between treatments (Table 3).

From an agronomic point of view, the absence of response of MFPA may be associated with the experimental period of only 43 days after transplantation, an interval that, under commercial cultivation conditions, would correspond to the time of harvest, when the plant has not yet completed its full potential for accumulation of foliar biomass. The biostimulants evaluated, especially microbial inoculators, act primarily on the root system in the early stages of colonization, reflecting the expected aerial part in longer periods of evaluation. It is possible

that, in experiments of longer duration or with more advanced stage harvest, the differences in MFPA become statistically detectable. Furthermore, the edaphic conditions, with V% of 67.5% and P-resin of only 5 mg dm−3, below the appropriate level for lettuce, may have limited the differential expression of biostimulants on the aerial part.

4 Conclusion

The results indicate that the combinations containing the inoculant Dunna, based on *Bacillus aryabhattai*, *B. amyloliquefaciens* and *B. subtilis*, were the most effective for root development, while the presence of *Trichoderma harzianum* (Trichodermaiz) was determinant for the highest total plant growth. New studies with higher number of evaluations throughout the cycle, higher level of phosphorus available in the soil and field conditions are needed to confirm and expand the understanding of the interaction between these biostimulants in lettuce culture.

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