



Application of *Lavandula angustifolia* Essential Oil Enhances Sustainable Baby Lettuce Cultivation

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Abstract

Chemical fertilizers improve crop yields but poses serious environmental concerns. Biostimulants offer a sustainable alternative by promoting plant growth and reducing the need for synthetic inputs. Essential oils, rich in bioactive compounds have been explored as natural biostimulants, and the effects on plant growth are not fully understood. This study aimed to evaluate the dose-dependent effects of *Lavandula angustifolia* essential oil on growth, pigments, and mineral–nitrate balance in baby leaf lettuce (*Lactuca sativa* L.), to assess its potential as a natural and sustainable biostimulant. Baby leaf lettuce plants were foliar sprayed with *L. angustifolia* essential oil at 0.5%, 1.0%, and 2.0% concentrations and compared to water and an emulsifier (polysorbate 80) used as controls. The effects of the treatments were evaluated by measuring biometric parameters, biomass, pigment content, leaf mineral composition, and nitrate levels. Treatment with 0.5% and 1.0% essential oil significantly increased leaf area, specific leaf area ($p < 0.01$), biomass (leaf and root fresh and dry weight) ($p < 0.05$), chlorophyll and carotenoid content ($p < 0.001$). At 2.0%, a significant decrease in all these parameters was observed. The foliar mineral composition was significantly affected by the essential oil treatment, with notable changes in N, P, Ca^{2+} , and Mg^{2+} concentrations, as well as an approximately 30% reduction in nitrate content when lavender essential oil was applied. Foliar application of *L. angustifolia* essential oil at low concentrations (0.5–1.0%) enhances growth, pigment accumulation, and mineral uptake while decreasing nitrate content in baby leaf lettuce, indicating its potential as a sustainable biostimulant for leafy crops.

Keywords Aromatic plants · Bioactivator · Nitrate content · Plant extract

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Introduction

As the global population continues to expand, there is an increasing imperative for the adoption of sustainable agricultural production systems. Reliable projections indicate that global demand for food will rise by 35–56% between 2005 and 2050, with demand for crops increasing by 100–110% (Tilman et al. 2011). The use of chemical fertilizers has been demonstrated to enhance global food production, thereby resulting in a substantial increase in crop productivity. The global agricultural system is increasingly reliant on chemical fertilizers, and it is anticipated that global consumption will increase, primarily due to increased demand in developing countries. Nevertheless, the extensive utilization of chemical fertilizers poses a significant threat to ecosystem health (Hedlund et al. 2020), with deleterious effects on soil and water systems, microbial life, other organisms, and human health (Baweja et al. 2020). For this reason, agriculture requires sustainable alternatives to promote plant growth, development, and stress resistance.

Biostimulants support sustainable farming by reducing chemical inputs, lowering environmental impact, protecting ecosystems, and ensuring quality food. Their use is consistent with the objectives of the European Green Deal and Sustainable Development Goal 12, particularly by reducing nutrient losses, promoting environmentally friendly agricultural practices, and supporting responsible production and consumption (United Nations 2017). According to the current conceptual plant biostimulants enhance plant performance primarily by activating intrinsic physiological and metabolic processes—such as nutrient uptake and use efficiency, metabolic regulation, and adaptive responses to abiotic constraints—without acting directly as nutrients or pesticides (Yakhin et al. 2017). Within this framework, genuine biostimulant activity must be clearly distinguished from plant responses primarily driven by stress or hormesis. A true biostimulant effect is defined by the enhancement of plant physiological efficiency, nutrient use efficiency, or developmental performance under non-stress conditions. This enhancement occurs through the modulation of endogenous metabolic and signaling pathways, rather than through growth stimulation resulting from stress-induced compensatory mechanisms or the activation of defence responses.

A wide range of substances has been reported to exert biostimulant effects, including seaweed extracts, amino acids and peptides, humic substances, organic acids, fermentation-derived products, and plant-derived extracts such as essential oils (Canellas et al. 2015; Colla et al. 2015; Parađiković et al. 2019; Popko et al. 2018; Stirk et al. 2020). However, in the case of plant extracts and secondary metabolites, it is particularly important to distinguish true

biostimulant activity—defined as the promotion of plant performance and resilience through physiological regulation—from elicitor effects or mild stress responses that activate defence-related signalling pathways.

Essential oils (EOs) are a mixture of volatile, organic compounds originating from a single botanical source that contribute to the flavour and fragrance of a plant (Jain et al. 2022; Tisserand and Young 2013). EOs contain a wide range of biologically active compounds, such as alkaloids, tannins, steroids, glycosides, resins, phenols, volatile oils, and flavonoids (Turek and Stintzing 2013). Furthermore, several studies have shown multiple biological activities of EOs such as antioxidant, antimicrobial, anti-inflammatory, cytotoxic, allelopathic and insecticidal (Bolouri et al. 2022; Hammer et al. 2002; Lamiri et al. 2001; Schelz et al. 2006). Consequently, EOs have attracted increasing interest in agricultural applications, particularly for the control of fungal, bacterial, and postharvest pathogens (Raveau et al. 2020). In contrast, their effects on plant growth and development are less clearly defined and appear to depend strongly on dose, mode of application, and crop species. Although the effects on plant growth are still partially explored (Ritota et al. 2025; Kapnisis et al. 2022), some studies reported positive outcomes: *Lavandula angustifolia* Miller EO (LEO) prolonged potato storage by reversibly inhibiting sprouting and showing antimicrobial activity (Gómez-Castillo et al. 2013; Vokou et al. 1993); rosemary EO stimulates tomato growth by increasing root fresh weight and nutrient uptake and use efficiency, thereby reducing fertilizer requirements (Souri and Bakhtiarizade 2019); and a rosemary–eucalyptus EO mix enhanced tomato height and yield without altering quality (Chrysargyris et al. 2020). In particular, LEO is mainly composed of linalool and linalyl acetate, and contains more than 100 compounds, including monoterpenes, sesquiterpenes, and esters (Lawrence 2001). Conversely, at low and carefully managed doses, EOs may act as elicitors or modulators of plant metabolism, promoting growth and adaptive capacity without causing overt stress (Licata et al. 2022). At higher concentrations, some compounds may have inhibitory effects, potentially leading to reduced germination or plants growth. In this context, low-dose EO applications have been associated with transient and controlled modulation of reactive oxygen species (ROS) signalling, which plays a recognized role in regulating growth, development, and stress acclimation, rather than constituting a damaging oxidative stress per se (Chen and Yang 2020; Oğuz et al. 2023). Such responses are consistent with a regulatory or elicitor-like mechanism and should be clearly distinguished from hormesis-driven stress induction, even though these phenomena may partially overlap under specific experimental conditions (Stebbing 1982).

Lettuce (*Lactuca sativa* L.) baby leaf is the most widely consumed and traded leafy green worldwide, characterized by rapid growth, a short harvest cycle, and relatively low cultivation costs. The global market value of this crop is estimated at \$2.93 billion annually, with a projected growth rate of 2.52% per year (Gargaro et al. 2023). A major concern, as with many leafy vegetables, is nitrate accumulation in the leaves, which poses risks to both product quality and consumer safety (Bian et al. 2020). Essential oil (EO) compounds are known to interact with plant physiological processes, exerting either promotive or toxic effects depending on the applied concentration (De Almeida et al. 2010). Accordingly, lavender essential oil (LEO) was investigated as a hybrid input, with particular emphasis on its biostimulant-like potential to modulate plant nutrition and metabolism at low concentrations, while clearly distinguishing these effects from stress- or hormesis-driven responses.

The present study was therefore designed to evaluate the biostimulant-like effects of LEO on the growth and development of *Lactuca sativa* L. under greenhouse conditions, focusing on leaf quality parameters, including pigment composition, mineral content, and nitrate levels, with the aim of exploring its potential as a sustainable input for leafy vegetable production.

Materials and Methods

Lavender Crop Cultivation, Essential Oil Production, Extraction and Characterization

Lavender plants were cultivated in a dynamic agrivoltaic system located in the marginal rural area of San Severo (Foggia, Apulia, Italy; 41°34'58.90" N; 15°28'33.85" E), a semi-arid Mediterranean site. Before transplanting, the soil was plowed (40 cm) and harrowed twice. Plants were transplanted in continuous rows spaced 1 × 1 m apart in May 2022 and managed with standard agronomic practices for aromatic crops (fertilization, drip irrigation, and weed control).

Fresh aerial parts (2 kg) of *Lavandula angustifolia* L. cv. Royal Purple were manually harvested at balsamic stage. *Lavandula angustifolia* essential oil (LEO) extraction was performed by hydrodistillation using a Clevenger-type apparatus for 2 h, employing 7 L of distilled water per kilogram of plant material, in accordance with the European Pharmacopoeia. Two hydrodistillations were performed. At the end of the extraction process, the LEO accumulated on the surface of the aqueous phase (hydrolate) was collected. The entire distillate was transferred to a glass separating funnel and allowed to stand for four hours to achieve complete separation of the LEO from the hydrolate. A total of

20 mL of LEO was obtained, corresponding to an EO yield of 1.1%. The extracted LEOs were stored in hermetically sealed dark glass containers at a temperature of 5–6 °C.

GC–MS Analysis of LEO

The volatile profile of LEO was determined by gas chromatography–mass spectrometry (GC–MS). Prior to analysis, the oil was diluted 1:100 (v/v) in hexane; 1 µL was injected into an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA, USA) coupled to a 5977A mass-selective detector (MSD). Separation was achieved on an HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.5 µm film; J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a constant flow of 1.25 mL·min⁻¹. Injections were performed in splitless mode with the inlet set at 250 °C. The oven temperature was programmed from 60 °C to 250 °C at 3 °C·min⁻¹, with a final isothermal hold of 10 min. Mass spectra were recorded under electron-impact ionization (70 eV) over the m/z range 15–550 at 2.9 scans·s⁻¹. Compounds were identified by matching spectra with the NIST11 library (match factor > 90%) and, when available, by comparison of retention times and spectra with authentic standards. Linear retention indices (RIs) were calculated according to van den Dool and Kratz (1963) using a homologous series of n-alkanes (C7–C30; Sigma-Aldrich, Milan, Italy) analyzed under identical conditions. Relative abundances are expressed as percentages of GC peak areas.

Plant Material, Experimental Setup and Treatment

The experiment was conducted in a Mediterranean greenhouse at the University of Sassari (Ottava, Sassari, Sardinia, Italy 40°46'47"N, 8°29'45"E). During the experiment (8 May–11 June 2024), the mean minimum and maximum air temperatures inside the greenhouse were 14.0 °C and 35.8 °C, respectively, while relative humidity ranged from 25 to 91%.

L. sativa *acephala* var. “bionda riccia”, cultivated under a baby leaf production system, was selected for its short cycle and commercial importance. Seeds (Ingengoli® seed company) were manually broadcast-sown at a density of 1.5 g m⁻² and grown in a pot (40 × 20 × 16 cm using a Vigor Plant®—Dorater as substrate) (pH of 5.8–6.8, EC of 0.25–0.35 dS m⁻¹). Polysorbate 80 (P80) was used as an emulsifier to solubilize the LEO in water, at a concentration corresponding to 15% of the essential oil volume (v/v). The experimental treatments (Supplementary Table S1) were control (water only), P80 (water + polysorbate), *Lavandula angustifolia* essential oil (LEO) at doses 0.5%, 1.0% and 2.0% (v/v) (LEO 0.5%, LEO 1.0% and LEO 2.0% respectively). The selected concentrations were based on a

previous experiment conducted with the same EO, in which 4.0% induced marked phytotoxic effects (Piga et al. 2025); therefore, lower concentrations (0.5–2.0%) were tested. The treatments were applied using Gloria® Pro 5 Oil-proof version in order to micro-spray the test solution uniformly on the leaf surface, applying 5 mL of solution per pot. Plants were treated at the stage of 2 true leaves (stage ≈12 of the BBCH phenological scale) (Meier 2001). The experiment was repeated twice, within the same experimental period and under the same environmental conditions, as two independent experiments, using a randomized block design with three replications for each treatment.

Plant Biometric and Biomass Parameters

Plant growth and development were monitored weekly and biometric and biomass parameters were recorded at harvest according to the BBCH phenological stage (≈ BBCH 19) (Meier 2001) on five representative plants per pot. For all biometric and biomass measurements, five representative plants were selected per pot. The following biometric parameters were recorded: number of leaves (LN), plant height in cm (PH), the collar diameter in cm (CD), root length in cm (RL) and mean leaf area (MLA). The leaf area in cm² (LA), including the total number of leaves per plant and its ratio of the leaf area over the number of leaves per plant was measured considering five representative plants per pot, using a planimeter (LI-3100c area meter, Li-Cor, Lincoln, NE, USA).

The following biomass indices were assessed: leaf fresh weight (LFW) and root fresh weight (RFW), expressed in g; leaf dry weight (LDW) and root dry weight (RDW), expressed in mg and specific leaf area expressed in cm² g⁻¹ (SLA) was calculated as the ratio between leaf area (LA) and leaf dry weight (LDW). Dry weight was measured after drying the plant samples for 72 h at 60 °C. Five plants per replicate were used for all above-described measurements.

Estimated Leaf Chlorophyll Content, Carotenoid, Anthocyanin, Flavonoids

Chlorophylls and carotenoids were extracted from 100 mg of fresh leaves using 99.9% methanol at a 1:100 w/v ratio as solvent. Samples were incubated in methanol at –20 °C for two consecutive 24 h extraction cycles with fresh solvent, and the extracts were combined. After extraction, absorbance was measured at 665.2 nm, 652.4 nm, and 470 nm using an Agilent Technology Cary 60 UV–Vis spectrophotometer (Macías-Sánchez et al. 2009). Chlorophyll a (c_a), chlorophyll b (c_b) and carotenoids (c_{c+x}) were calculated using the equations of Lichtenthaler and Buschmann (2001):

$$c_a = 16.72A_{\{665.2\}} - 9.16A_{\{652.4\}} \quad (\mu\text{g mL}^{-1}) \quad (1)$$

$$c_b = 34.09A_{\{652.4\}} - 15.28A_{\{665.2\}} \quad (\mu\text{g mL}^{-1}) \quad (2)$$

$$c_{c+x} = \frac{(1000A_{\{470\}} - 1.63c_a - 104.96c_b)}{221} \quad (\mu\text{g mL}^{-1}) \quad (3)$$

Anthocyanins (Anth) and flavonols (Flav) were extracted as described for chlorophylls using methanol acidified with 80:17.7:2.3 v/v/v mixture of MeOH: dH₂O: 37.0% HCl (0.1 mL mg⁻¹ FW) as a solvent (Hradzina et al. 1982). The absorbance was read at 360 nm for Fl and 530 nm for An. Concentrations were expressed as mg cyanidin-3-glucoside per g of FW using a calibration curve with cyanidin-3-galactoside chloride (Merck KGaA, Darmstadt, Germany). Fl was determined at 360 nm with a calibration curve from quercetin-3-glucoside and expressed as mg quercetin-3-glucoside per g FW.

Chemical Analysis of Lettuce Leaves

Anion and cation contents were determined by ion-exchange chromatography (Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA). Anions were extracted from samples (0.5 g d.w.) with 50 mL 3.5 mmol L⁻¹ Na₂CO₃ and 1.0 mmol L⁻¹ NaHCO₃, and they were separated and quantified using Dionex equipped with an IonPac AG14 precolumn and an IonPac AS14 column. Cations were analysed after incineration of samples (1.0 g d.w.) at 550 °C and acid digestion in 20 mL of 1.0 mol L⁻¹ HCl in boiling water (99.5±0.5 °C) for 30 min. The resulting solution was filtered, diluted, and quantified using Dionex equipped with an IonPac CG12A guard column and an IonPac CS12A analytical column. The data were expressed as mg kg⁻¹ d.w. (Renna et al. 2014). Total nitrogen and total carbon concentrations were determined using a Leco CN 828 Analyzer (Leco corporation, St. Joseph, Michigan). Protein content was then calculated by multiplying total nitrogen by coefficient 6.25. The data were expressed as % d.w.

Statistical Analysis

The statistical differences in biometric, biomass parameters and mineral content were analyzed with one-way ANOVA and Tukey's test ($p < 0.05$).

Results

Chemical Composition of LEO by GC–MS

GC–MS analysis of LEO detected 32 compounds (Supplementary Table S2), which together accounted for 97.4% of the total oil composition. The oil was dominated by oxygenated monoterpenes, with linalool (36.81%) and linalyl acetate (41.0%) as the principal constituents. This chemical profile is consistent with reports on *Lavandula officinalis* from another Mediterranean area (Morocco), although differences in essential oil composition may be related to genetic variability (Diass et al. 2023). Other monoterpenes were present in smaller proportions, including limonene (2.34%), camphor (1.90%), endo-borneol (1.71%), eucalyptol (1.58%), and β -myrcene (1.49%).

Biometric Parameters and Biomass Production of Plants

Treated and not treated lettuce biometric (plant height, PH; root length, RL; collar diameter, CD; and number of leaves, LN) and biomass data (leaf and root fresh and dry weights, LFW; LDW; RFW; and RDW, respectively) are shown in Table 1.

PH, RL and CD were significantly affected by LEO treatment, while P80 and H₂O did not show significant differences. The results indicate that the treatment with LEO at different concentrations significantly affected the plant biometric parameters. Specifically, PH and CD showed the same trend: in both parameters the control treatments (P80 and H₂O) revealed the highest value compared to the other LEO treatments. RL was significantly influenced by the LEO treatments, revealing a significantly highest RL in LEO 0.5% (7.41 cm), followed by LEO 1.0% (6.87 cm) compared to all the other treatments. In addition, the minimum RL was observed under LEO 2.0% treatment (5.77 cm). No significant differences were found in LN.

Biomass parameters, leaf and root fresh and dry weights (LFW; LDW; RFW and RDW respectively) significantly varied based on LEO treatment (Table 1). Leaf biomass

significantly increased (reaching the peak) under LEO 0.5% in fresh and dry plant material (1.19 g and 42.0 mg respectively). In roots, the highest biomass was reached under LEO 1.0% in both fresh and dry weight (0.099 g and 40.90 mg) whereas LEO 2.0% showed the lowest value (32.20 mg). Moreover, P80 treatment for these parameters showed no significant differences effects compared to the H₂O.

Mean leaf area (MLA) and mean specific leaf area (SLA) were significantly affected by LEO treatments (Fig. 1a and b).

MLA increased with the LEO 0.5% and LEO 1.0% treatments compared to the control, with the highest value at LEO 0.5% (6.44 cm²). Furthermore, MLA decreased at LEO 2.0% (4.94 cm²), although remaining higher than the control (H₂O and P80, respectively). SLA followed a similar trend, reaching the highest value with LEO 1.0% (7915.45 cm²/g) and the lowest score at LEO 2.0% (5451.87 cm²/g). P80 and H₂O controls showed comparable results.

Analysis of Chlorophylls, Carotenoids, Flavonoid Glycosides, and Anthocyanins Content

Generally, chlorophyll a, b and total (Chl a, Chl b, and Chl t, respectively, expressed in $\mu\text{g mL}^{-1}$) contents were all positively influenced by LEO treatments, with the highest increases observed at LEO 0.5% and LEO 1.0% concentrations, followed by a slight decrease at LEO 2.0% (Fig. 2a, b and c).

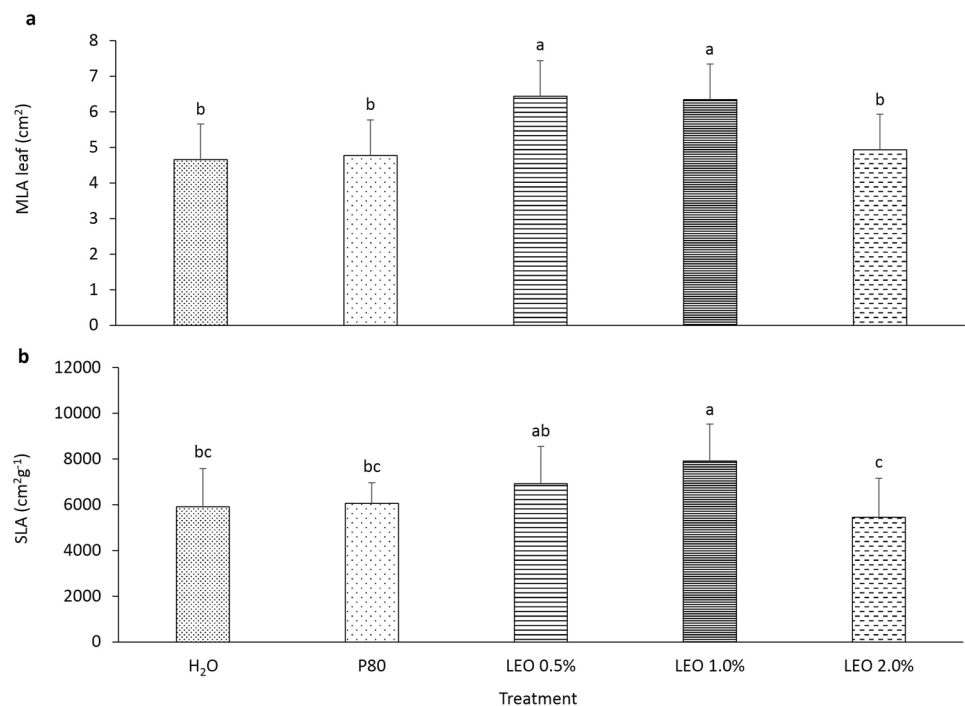
Specifically, the highest Chl a content was observed in the LEO 1.0% treatment (5.83), followed by LEO 0.5% (5.55), both significantly higher than the H₂O treatment (4.64). Regarding Chl b, the highest content was recorded in LEO 0.5% (1.70), which was significantly different from all other treatments. A progressive decrease was observed from LEO 1.0% (1.56) to LEO 2.0% (1.54), both significantly higher than the control (0.92). Similarly, Chl t content was highest in LEO 1.0% (7.40), with no significant difference compared to LEO 0.5% (7.25). Both treatments were significantly higher than LEO 2.0% (6.68) and H₂O (6.13) while P80 showed an intermediate value (6.37).

Table 1 Effects of *Lavandula angustifolia* essential oil (LEO) at 0.5%, 1.0%, and 2.0% on biometric and biomass data of plants

Treatment	PH (cm)	RL (cm)	CD (cm)	LN (unit)	LFW (g)	LDW (mg)	RFW (g)	RDW (mg)
H ₂ O	15.35±1.76 ^a	6.27±1.63 ^{bc}	1.69±0.46 ^a	3.90±0.61	0.93±0.24 ^b	29.0±1.60 ^b	0.076±0.02 ^b	33.70±8.00 ^{ab}
P80	15.26±1.02 ^a	6.15±1.56 ^{bc}	1.68±0.30 ^a	3.90±0.72	0.94±0.31 ^b	30.0±1.00 ^b	0.068±0.03 ^b	37.70±11.10 ^{ab}
LEO 0.5%	13.33±1.37 ^b	7.41±1.62 ^a	1.40±0.30 ^b	4.13±0.68	1.19±0.20 ^a	42.0±2.00 ^a	0.097±0.03 ^a	38.40±14.40 ^{ab}
LEO 1.0%	14.19±1.22 ^b	6.87±1.59 ^{ab}	1.46±0.29 ^{ab}	4.10±0.61	1.05±0.19 ^{ab}	33.0±0.80 ^{ab}	0.099±0.03 ^a	40.90±10.10 ^a
LEO 2.0%	13.70±1.12 ^b	5.77±1.19 ^c	1.43±0.36 ^{ab}	3.80±0.89	1.06±0.10 ^{ab}	36.0±1.40 ^{ab}	0.080±0.03 ^{ab}	32.20±11.60 ^b

Treatments included a water control (H₂O) and polysorbate (P80). Parameters include, plant height (PH, in cm), root length (RL, in cm), collar diameter (CD in cm), number of leaves (LN), leaf fresh weight (LFW, in g), root fresh weight (RFW, in g), leaf dry weight (LDW, in mg) and root dry weight (RDW, in mg). The mean value and the standard deviation (SD) are reported. Data were analysed using one-way ANOVA followed by Tukey's HSD test for multiple comparisons. Different superscript letters within a column indicate significant differences among treatments ($p < 0.05$).

Fig. 1 Effects of *Lavandula angustifolia* essential oil (LEO) at 0.5%, 1.0%, and 2.0% on mean leaf area (MLA) (**a**) and specific leaf area (SLA) (**b**). Treatments included a water control (H₂O) and polysorbate (P80). Data are expressed as means $\pm$ standard deviation (SD). Statistical differences among treatments were assessed using one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.01$). Different letters above bars indicate significant differences



As regards carotenoids (Car), flavonoids-glycoside (Flav), and anthocyanins (Anth), two distinct patterns were observed between the LEO treatments and the H₂O and P80 treatments (Fig. 3a, b and c respectively). While carotenoids showed a significant trend response across treatments, both flavonoids and anthocyanins generally decreased in the LEO treatments compared to H₂O and P80 treatments.

The highest and significant value of Car was observed in LEO 0.5% (2.30) compared to other treatments, followed by a decrease in LEO 1.0% (2.20) and LEO 2.0% (1.97) treatment. P80 showed a value (2.19) like LEO 1.0%, similar to H₂O (2.08) with LEO 2.0%. Flav and Anth showed the same trend. Overall, in both pigments a significant decrease of Flav and Anth was observed as a consequence of LEO treatments at all levels. The two controls showed the highest Flav and Anth values.

Chemical Composition of Lettuce

The composition of lettuce leaves was significantly different based on the LEO treatment administered (Table 2a and 2b and Fig. 4). Table 2a and 2b reports the macro and micro-nutrient elements, respectively, significantly affected by the LEO treatments (ANOVA, $p < 0.05$).

Overall carbon (C) content was quite similar among treatments, and it ranged from a minimum of 37.26% (P80) to a maximum of 38.63% (LEO 2.0%). Protein (Prot) and nitrogen (N) followed a similar trend with a significant increase of Prot and N as consequence of all LEO treatments, with a peak under LEO 0.5% (31.96% and 5.11%

respectively for Prot and N) and a significantly low level under P80 and H₂O. In terms of total and inorganic phosphorus content (P and PO₄³⁻ in g kg⁻¹ DW, respectively) as expected PO₄³⁻ and P revealed comparable trends among treatments. Overall, the highest values were found under LEO 0.5% in both parameters (9.39 and 3.00 g kg⁻¹ DW for PO₄³⁻ and P respectively); the lowest values were found under LEO 1.0% and LEO 2.0% (Table 2a). Potassium, calcium and magnesium (K⁺, Ca²⁺, Mg²⁺) significantly varied based on the given treatment (Table 2b). K⁺ content presented the highest concentration (under controls conditions) (58.50 g kg⁻¹ DW and 56.79 g kg⁻¹ DW for P80 and H₂O respectively); while the lowest value was recorded in LEO 1.0% (47.70 g kg⁻¹ DW). Ca²⁺ levels increased progressively with LEO concentration, reaching the highest value in LEO 2.0% (17.34 g kg⁻¹ DW) and its lowest content under H₂O treatment (5.03 g kg⁻¹ DW). Mg²⁺ also responded significantly to the treatments. LEO 1.0% showed the highest Mg²⁺ content (3.41 g kg⁻¹ DW) while its lowest value was found under H₂O (1.92 g kg⁻¹ DW). Figure 4 reports the nitrate (NO₃⁻) concentrations.

Interestingly, this decreases from the highest values detected under H₂O (1827.86 mg kg⁻¹ FW) and P80 (1900.18 mg kg⁻¹ FW), to the minimum level under LEO 2.0% (1310.99 mg kg⁻¹ DW). Overall, a significant decrease of NO₃⁻ content was observed with the increase of LEO %.

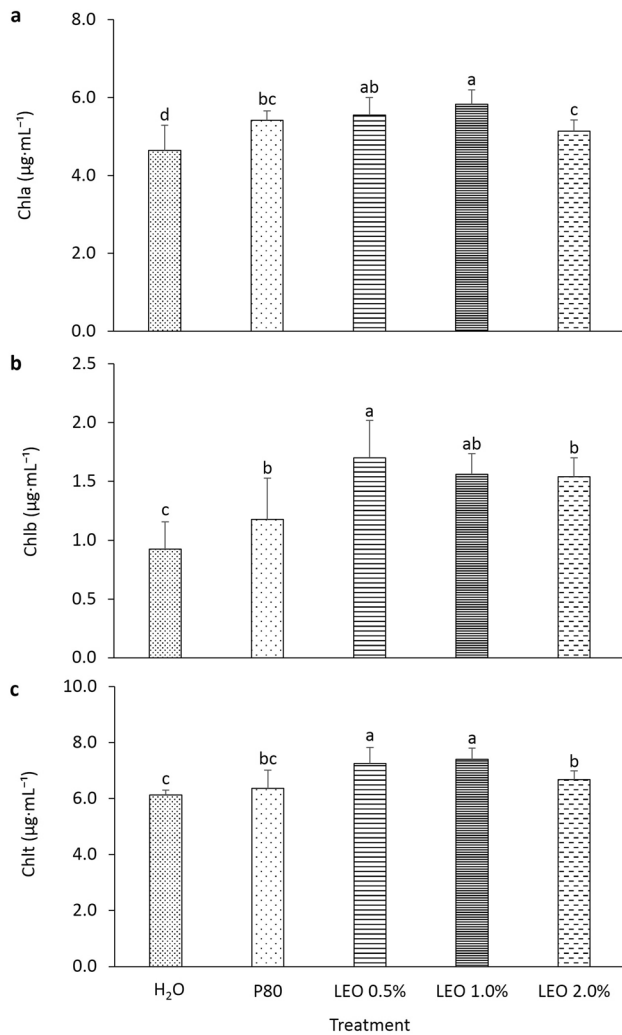


Fig. 2 Effects of *Lavandula angustifolia* essential oil (LEO) on chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chlt) contents are shown in figure **a**, **b**, and **c**, respectively. Treatments included a water control (H₂O), polysorbate (P80) and LEO at 0.5%, 1.0%, and 2.0%. Data are expressed as means ± standard deviation (SD). Statistical differences among treatments were assessed using one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.0001$). Different letters above bars indicate significant differences

Discussion

The present study showed that a single foliar application of LEO significantly impacts growth, biomass, pigments and macro-micronutrient contents of baby lettuce leaves. These results are in agreement with recent studies reporting positive effects of lavender-based formulations and other essential oils on lettuce growth, yield and quality, although responses strongly dependent on formulation, dose and application method (Ritota et al. 2025; Kapnisis et al. 2022). Previous studies highlighted the delicate balance between bio-stimulatory and phytotoxic thresholds of EOs application in plants (De Almeida et al. 2010; Werrie et al.

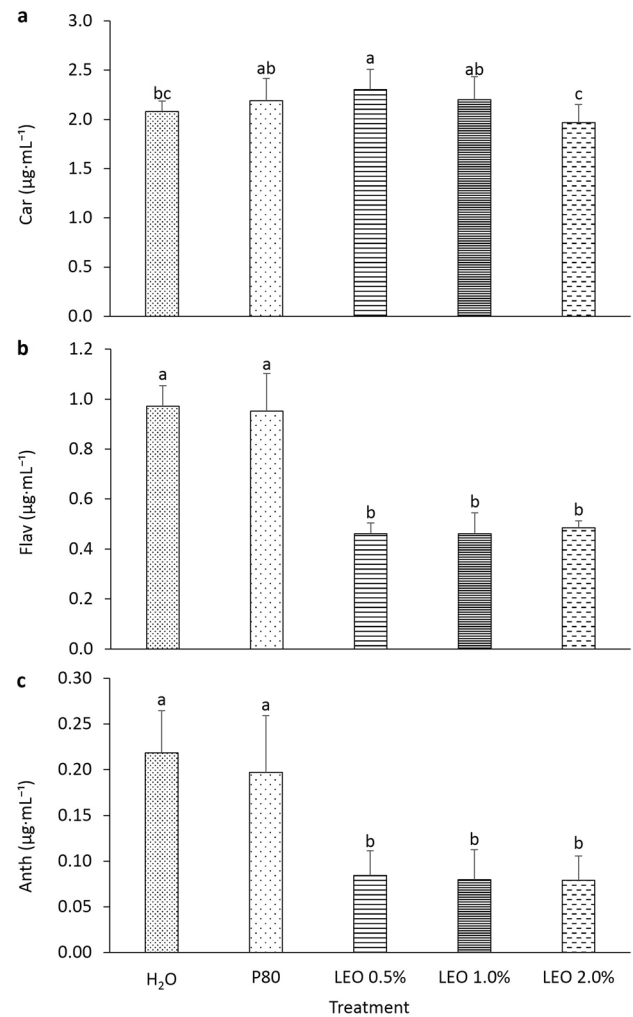


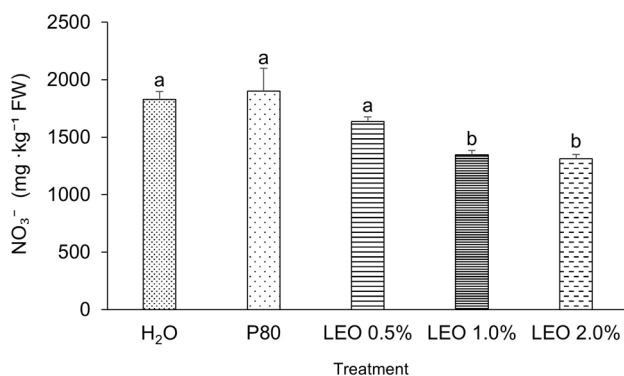
Fig. 3 Effects of *Lavandula angustifolia* essential oil (LEO) on carotenoids (Car), flavonol glycosides (Flav), and anthocyanins (Anth) contents in lettuce leaves are shown in figure **a**, **b**, and **c**, respectively. Treatments included a water control (H₂O) and LEO at 0.5%, 1.0%, and 2.0%. Data are expressed as means ± standard deviation (SD). Statistical differences among treatments were assessed using one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.0001$). Different letters above bars indicate significant differences

2020). In this research, both biometric and biomass parameters were considered. LEO treatments (0.5–2.0%) had a detrimental effect on PH and CD, but positive effects were observed on LFW, RFW, LDW, RDW, as well as on MLA and SLA, with a significant increase of these parameters compared to the controls. That increment acted in a specific range of LEO% (0.5 and 1.0%). Previous studies (Erofeeva 2024; Piga et al. 2025; El-Tazy et al. 2025; Calabrese and Blain 2011) showed that dose-dependent responses are typical of substances inducing stress in plants, such as essential oils rich in monoterpenes, with higher doses shifting energy from growth to defense, and lower doses stimulating both growth and defense metabolism. EO qualitative and quantitative composition, the bioactivity of its constituents, the

Table 2 Effects of *Lavandula angustifolia* essential oil (LEO) at 0.5%, 1.0%, and 2.0% on macro and micronutrient in lettuce leaves; (a) total carbon (C), total protein (Prot), total nitrogen (N), inorganic phosphorus (PO_4^{3-}), total phosphorus (P); (b) total potassium (K^+), total calcium (Ca^{2+}), total magnesium (Mg^{2+}), sodium (Na^+), fluoride (F^-) sulfate (SO_4^{2-}), chloride (Cl^-) and lithium (Li^+)

a								
Treatment	C (% DW)	Prot (% DW)	N (% DW)	PO ₄ ³⁻ (g kg ⁻¹ DW)	P (g kg ⁻¹ DW)			
H ₂ O	37.58±0.11 ^{ab}	23.79±1.88 ^b	3.81±0.30 ^b	8.46±0.24 ^b	2.71±0.08 ^b			
P80	37.26±0.46 ^b	25.79±0.89 ^b	4.13±0.14 ^b	8.66±0.42 ^{ab}	2.77±0.13 ^{ab}			
LEO 0.5%	37.98±0.36 ^{ab}	31.96±0.63 ^a	5.11±0.10 ^a	9.39±0.44 ^a	3.00±0.14 ^a			
LEO 1.0%	37.48±0.59 ^{ab}	31.46±0.04 ^a	5.03±0.01 ^a	8.29±0.09 ^b	2.65±0.03 ^b			
LEO 2.0%	38.63±0.54 ^a	30.94±0.45 ^a	4.95±0.07 ^a	8.24±0.23 ^b	2.64±0.07 ^b			
b								
Treatment	K ⁺ (g kg ⁻¹ DW)	Ca ²⁺ (g kg ⁻¹ DW)	Mg ²⁺ (g kg ⁻¹ DW)	Na ⁺ (g kg ⁻¹ DW)	F ⁻ (g kg ⁻¹ DW)	SO ₄ ²⁻ (g kg ⁻¹ DW)	Cl ⁻ (g kg ⁻¹ DW)	Li ⁺ (g kg ⁻¹ DW)
H ₂ O	56.79±0.41 ^a	5.03±0.47 ^d	1.92±0.19 ^c	3.92±0.16 ^c	0.338±0.010 ^c	6.31±0.32 ^c	53.25±1.02	0.015±0.004 ^{ab}
P80	58.50±2.14 ^a	15.68±0.86 ^{ab}	3.37±0.10 ^a	9.55±0.03 ^a	0.458±0.042 ^b	6.74±0.11 ^c	49.35±1.75	0.005±0.001 ^c
LEO 0.5%	51.31±0.95 ^{bc}	10.19±0.08 ^c	2.31±0.15 ^{bc}	3.74±0.10 ^c	0.525±0.008 ^a	7.85±0.13 ^b	50.32±1.53	0.016±0.002 ^a
LEO 1.0%	47.70±1.27 ^c	14.97±1.09 ^b	3.41±0.12 ^a	7.40±0.29 ^b	0.394±0.012 ^c	9.85±0.58 ^a	52.17±1.39	0.006±0.001 ^c
LEO 2.0%	52.65±1.42 ^b	17.34±1.12 ^a	2.68±0.24 ^b	7.00±0.56 ^b	0.354±0.023 ^c	7.16±0.18 ^{bc}	52.98±5.46	0.010±0.002 ^{bc}

The mean value and the standard deviation (SD) are reported. Different letters show significant differences (LSD test, $p < 0.05$)

**Fig. 4** Nitrate (NO_3^-) content in lettuce treated with different dose of *Lavandula angustifolia* essential oil (LEO). Treatments included a water control (H_2O), polysorbate (P80) and LEO at 0.5%, 1.0%, and 2.0%. Data are expressed as mean±standard deviation (SD). Statistical analysis was performed using one-way ANOVA, followed by Tukey's HSD test ($p < 0.0001$). Different letters above bars indicate significant differences

plant species its developmental stage, the tissue treated, and the application method as well as the number of applications are all factors that can potentially affect the plant response to EOs application.

Low doses of LEO act as biostimulant by enhancing plant growth, physiological and enzymatic activity, nutrient uptake, and protein content, whereas higher doses may exert toxic effects and serve as herbicides at sub-toxic levels. Such low-dose stimuli are hypothesized to promote metabolic adjustments that improve photosynthesis, water balance, and stress tolerance under suboptimal conditions (Agathokleous et al. 2019; Belz and Duke 2017; Brito et al. 2018; Jalal et al. 2021; Mejía-Teniente et al. 2013; Muszyńska et al. 2019; Savvides et al. 2016).

These findings are consistent with previous reports, showing that low to moderate LEO concentrations enhance lettuce baby leaf root length and biomass. At sub-toxic levels, LEO may function as a mild stress elicitor, inducing ROS-mediated signaling that activates antioxidant and defense pathways while promoting growth, a hormetic response previously described for various stressors including herbicides, selenium, nanoparticles, and essential oils (Agathokleous 2018; Belz 2018; Cunha et al. 2024; Sachdev et al. 2021). Such low-dose stimulation can be associated with to improve agronomic performance through enhanced morphological and physiological traits and increased resilience under stress (Chrysargyris et al. 2020; Costa et al. 2024; Huang et al. 2019; Rashad et al. 2022; Şanlı et al. 2019; Vázquez-Hernández et al. 2019). Moreover, plant response to EO is highly dose-dependent, as well as influenced by treatment frequency and volatile composition. Arminante et al. (2006) reported a dose-dependent decrease in root length after application of EOs from some Labiatae, including *L. angustifolia*, on *Raphanus sativus* L., *Lactuca sativa* L., and *Lepidium sativum* L., with promotion of root growth at low monoterpene concentrations. Similarly, short exposure to volatiles from lavender species caused only mild effects on stomatal structure and even biostimulant effects in *Arabidopsis thaliana* under controlled conditions (Schulz et al. 2007). Grønberg Jensen and Ehlers (2009) also observed increased aboveground biomass in thymus species following monoterpene treatment, indicating species-specific responses. Consistently with these findings, all tested concentrations of LEO significantly improved biomass parameters compared to H_2O and P80 controls, with the most pronounced effects observed between 0.5% and 1.0% concentration. Our data clearly show a plant dose dependent response: At 2.0% LEO, reductions in biometric, leaf area,

and biomass parameters suggest the onset of stress conditions possibly associated with impaired carbon balance. High concentrations of monoterpene-rich EOs have been reported to disrupt cuticular waxes, inhibit stomatal regulation, enhance transpiration, reduce chlorophyll content, and impair respiration, ultimately compromising photosynthesis and energy metabolism (Batish et al. 2004; Raveau et al. 2020).

Biomass increases were associated with higher chlorophyll levels at 0.5–1.0% LEO, while 2.0% caused a decline, though still above control. Similarly, Ritota et al. (2025) reported increased fresh yield, leaf area index and SPAD values in lettuce following foliar applications of an aqueous lavender extract, supporting the biostimulant potential of lavender-based treatments, while the dose-dependent response observed in the present study, including the decline at 2.0%, likely reflects the higher biological activity of EO constituents. In addition, Kapnisis et al. (2022) observed increased fresh weight and yield-related morphological traits after a single foliar application of an essential oil-based formulation, with the stimulatory effect being confined to a single application, in agreement with the present study showing growth promotion at low EO input and a decline of positive responses when the EO increased. These findings indicate that low to moderate LEO doses may enhance photosynthetic capacity, whereas higher concentrations impair pigment stability and efficiency, consistent with previous reports on EO dose-dependent effects (Jia et al. 2015; Poonpaiboonpipat et al. 2013; Werrie et al. 2020; Wu et al. 2018). High LEO (2.0%) reduced chlorophyll content, which may reflect alterations in PSII efficiency, electron transport disruption, and RuBisCO inactivation, consistent with ROS-mediated stress signaling (Demmig-Adams and Adams III 2017). In our research the drop in chlorophyll observed under LEO 2.0% reflects the transition from stimulation to inhibition. Yet, since Chl levels remained higher than in H₂O, this may represent a reversible regulatory response rather than permanent damage (Tikkanen et al. 2014).

Carotenoid levels followed a dose-dependent pattern under LEO exposure, increasing at 1.0% and declining at 2.0% and in controls (H₂O and P80), suggesting imbalances in non-photochemical quenching and ROS management. These increase at 1.0% which may reflect reinforcement of photoprotective mechanisms and improved excitation energy dissipation, whereas the decrement of carotenoid at 2.0% suggests that the hormetic window was exceeded, leading to partial loss of photoprotective optimization (Jiao et al. 2024; De Faria et al. 2023). Specifically, low-dose stimulation may promote a more efficient regulation of PSII activity and photoprotective processes, as indicated by the concomitant increase in chlorophylls, carotenoids and non-photochemical quenching, together with improved

antioxidant control (Takahashi and Badger 2011). This coordinated response likely contributes to reduced ROS pressure and lipid peroxidation (Viswanathan et al. 2024). Under these conditions, mild stimulation within the hormetic range may have induced flavonol and anthocyanin synthesis as part of an early antioxidant response, and their utilization may have contributed to redox homeostasis (Agati et al. 2021), so their reduced accumulation at sampling may reflect a diminished need for continued secondary antioxidant investment. This pattern is consistent with hormetic responses that reallocate resources from secondary to primary metabolism, thereby enhancing nitrogen assimilation and protein synthesis (Table 2a) (Akula and Ravishankar 2011; Calabrese and Mattson 2017; Foyer and Noctor 2005; Xu et al. 2022; Zhou et al. 2023). In addition, LEO treatments, significantly increased protein, nitrogen, phosphorus, phosphate content (Table 2a) as well as mineral content (Ca²⁺, Mg²⁺, F⁻, SO₄²⁻) (Table 2b). These results are consistent with previous studies conducted by Ritota et al. (2025) on lavender extracts. Furthermore, potassium content was higher in the H₂O control than in LEO-treated plants, suggesting selective modulation of nutrient uptake by EO components. While the mechanisms remain unclear, natural biostimulants are known to enhance nutrient use efficiency via H⁺-ATPase activation and organic acid release (Boutahiri et al. 2024). The increases in root length, biomass, and chlorophyll observed under LEO treatments align with improved nitrogen assimilation and protein synthesis (Masclaux-Daubresse et al. 2010; Xu et al. 2012), likely reflecting elicitor-like and biostimulant activities of EO compounds (Dias et al. 2024; Sourì and Bakhtiarizade 2019). Polysorbate P80 could have enhanced the mobility of certain cations, including Ca²⁺, Mg²⁺ and Na⁺ compared to the water control. Although P80 does not act as a chelating agents, non-ionic surfactants have been demonstrated to enhance leaf cuticle wettability, droplet retention, and penetration (Fernández et al. 2021; Schönherr 2000), facilitating foliar uptake of Ca²⁺ and other cations (Fernández and Eichert 2009; Fernández et al. 2013; Tredenick et al. 2018; Zhu et al. 2019).

In term of detected anions El-Basheer et al. (2020), reported that some components of EO (i.e. alcohols and esters) oxygenated functional groups can interact with anionic element in solution decreasing their free concentration near the leaf surface. This EO component would induce a reduction of availability of sulfate (SO₄²⁻) and fluoride (F⁻) ions in leaves. In this research study, this trend is more evident in F⁻, where the addition of LEO significantly reduces its content to the water treatment value. Furthermore, the application of LEO reduced nitrate accumulation in leaves. This is a particularly salient agronomic trait, given the health concerns associated with elevated nitrate intake in humans.

The observed nitrate reduction may be associated with improved nitrate reductase activity and/or enhanced nitrogen utilization efficiency under LEO stimulation as observed in other biostimulant studies (Ciriello et al. 2024; Colla et al. 2015; Quille et al. 2022). In addition, LEO-treated plants exhibited lower nitrate levels than H₂O controls, particularly at 1.0% and 2.0% (−26.3% and −28.3%, respectively), remaining well below the limit for greenhouse-grown lettuce established by European Commission Regulation (EU) No 1258/2011. This reduction may be partially associated to the higher calcium concentrations observed. Recent findings highlight the role of calcium signaling, mediated by cyclic nucleotide-gated channels (CNGCs), in regulating nitrate uptake and optimizing nitrogen metabolism under stress conditions (Liu et al. 2021; Ullah and Ali 2025). Similarly, biostimulants such as plant extracts, seaweed extracts and humic substances have shown comparable effects in lettuce, enhancing biomass accumulation, photosynthetic performance, mineral uptake, and metabolic activity (Giordano et al. 2022; Di Mola et al. 2019; Taha et al. 2016; Oliveira et al. 2025). Overall, these findings suggest that LEO enhances both the nutritional quality and safety of lettuce, thereby improving its marketability and consumer acceptance. The increase in essential minerals and photosynthetic pigments, along with the reduction in nitrate content, contributes to higher nutritional value and compliance with food safety standards. Furthermore, its biostimulant effects and natural origin support its use as a sustainable product to increase crop yield within integrated plant management systems, with potential applications in environmentally friendly agricultural systems.

Conclusions

The present study showed that a single foliar application of *Lavandula angustifolia* essential oil (LEO) significantly affected baby leaf lettuce. Low concentrations (0.5–1.0%) enhanced leaf area, biomass, pigments, and mineral uptake by stimulating photosynthesis and hormetic responses, while reducing nitrate through improved nitrogen assimilation. These effects improve nutritional quality and support the role of LEO as a sustainable biostimulant. Conversely, the highest concentration (2.0%) reduced growth and pigments, likely due to monoterpene-induced stress. While this observation may indicate a potential herbicidal effect, it was not directly tested in the present study and remains hypothetical. Overall, LEO represents a promising tool for greenhouse leafy vegetables: low dose enhances yield and quality, while further research is required to evaluate its possible herbicidal activity and to confirm its biostimulant potential across different crops and environments.

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Author Contributions GKP conceptualised the manuscript, the methodology of the experimental trials, conducted the investigation and wrote the original draft of the manuscript. MC analysed all the experimental data, contributed to write and review the original draft. MMG, LF, MG, GG contributed to the investigation, laboratory analysis and review the manuscript. AG, OMP conducted laboratory analysis. SM supervised the experimental trials and the manuscript preparation, acquired the necessary funding and contributed to review the original draft. All authors read and approved the final manuscript.

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Availability of Data and Materials The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary materials.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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