

Growth Regulation and Residue Safety of 6-Benzyladenine in Vietnamese Balm (*Elsholtzia ciliata* (Thunb.) Hyl.): A Stage-Specific and Data-Driven Assessment

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Research Article

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Abstract

Elsholtzia ciliata is an aromatic medicinal species valued for its essential oils, yet strategies to optimize biomass production, leaf development, and biochemical performance remain underexplored. Current knowledge lacks an integrated evaluation of how exogenous 6-benzyladenine (6-BA) modulates vegetative morphology, photosynthetic physiology, and consumer safety across horticultural time scales. In this study, growth dynamics were quantified over eight weeks, and controlled foliar 6-BA applications were performed at two developmental stages (week 4 and week 6), followed by high-resolution phenotyping, biochemical assays, and residue analysis. Machine-learning models (XGBoost, random forest, elastic net, linear regression) were trained to predict growth and evaluate feature contributions through SHAP analysis. The results showed significant time-dependent increases in vegetative biomass, with fresh biomass rising from 0.7 to 40.2 g plant⁻¹ between week 1 and week 8. Exogenous 6-BA significantly enhanced leaf number, leaf area, and biomass, with optimal responses at 15 ppm and week-4 spraying. XGBoost achieved superior predictive accuracy (R^2 up to 0.94), revealing dose, timing, and post-spray duration as key determinants. Residue and dietary risk assessments indicated rapid 6-BA dissipation and negligible chronic risk across age groups. These findings establish a quantitative framework for growth stage-specific regulation of *E. ciliata* and support 6-BA as a safe and effective horticultural enhancer. Future studies should incorporate multi-environment validations and metabolomic profiling to improve translational applicability in commercial cultivation systems.

1. Introduction

Vietnamese balm (*Elsholtzia ciliata* (Thunb.) Hyland) is an aromatic herb of the mint family (Lamiaceae) that holds significant medicinal and economic value in Asia (Wang et al 2022). It has long been utilized in traditional medicine for its warm, spicy properties and is reputed to treat ailments ranging from the common cold to inflammation and allergies (Kim et al 2011; Pudziuelyte et al 2020). Modern pharmacological reviews note that *E. ciliata* contains a rich array of phytochemicals, including flavonoids, terpenoids, ... that confer antioxidant, anti-inflammatory, antimicrobial, and antiviral activities (Pudziuelyte et al 2020; Wang et al 2022). The plant's essential oil, extracted primarily from its leaves, is especially prized for its therapeutic properties, such as antioxidant, antibacterial and anticancer effects (Khang et al 2023). Consequently, there is growing demand for *E. ciliata* as a raw material in the medicinal herb and essential oil industries, intensifying interest in cultivation practices that can maximize leaf biomass and essential oil yield. However, traditional cultivation remains rudimentary in many areas, and producers often rely on empirical use of growth substances (Tho et al 2024), underscoring the need for scientific strategies to enhance yield and quality sustainably.

One promising approach to improve the productivity of aromatic herbs is the application of plant growth regulators, particularly cytokinins (Prins et al 2010; Farhadi and Moghaddam 2023). Cytokinins are a class of phytohormones that stimulate cell division and influence numerous developmental processes in plants (Svolacchia and Sabatini 2023). Exogenous cytokinin application has a pronounced impact on shoot branching, leaf expansion, and delay of leaf senescence. By releasing axillary buds from apical

dominance, cytokinins promote the formation of additional shoots and leaves (Mok 2019; Xu et al 2020; Sosnowski et al 2023). In aromatic and medicinal plants, these effects can translate into greater sites for essential oil biosynthesis. For example, cytokinin treatments have been shown to increase the number and size of leaves as well as the density of glandular trichomes, thereby boosting essential oil accumulation in several herb species (Sota et al 2019; Khetsa et al 2021). Early studies attributed such enhancements to cytokinin-driven metabolic changes that stimulate monoterpene synthesis, enriching the essential oil profile (El-keltawi and Croteau 1987; Danova et al 2018; Camas-Reyes et al 2022; Norouzi et al 2025). These findings provide a strong theoretical basis for using cytokinins to intensify both growth and secondary metabolite production in *E. ciliata* and related plants. Among cytokinin analogues, 6-benzyladenine (6-BA) is particularly potent and widely used in horticulture. Foliar application of 6-BA has been reported to improve plant growth and yield in diverse crops by promoting cell proliferation and morphogenesis (Gulzar et al 2024; Zhang et al 2025; Bai et al 2026). In aromatic herbs, 6-BA is gaining attention as a tool to increase vegetative biomass and essential oil output (Sudriá et al 2001; Aminifard et al 2020; Tho et al 2026). Preliminary trials on *E. ciliata* hinted that appropriate doses of 6-BA could markedly increase leaf chlorophyll content and essential oil percentage relative to untreated controls (Tho et al 2026). Despite these positive outcomes, achieving the optimal growth regulator regimen remains challenging. In practice, farmers cultivating leafy medicinal plants often resort to high, empirical doses of plant growth regulators to accelerate growth, which may not maximize efficacy and could lead to excessive residue accumulation. This underscores the need for a data-driven approach to determine the optimal concentration and timing of 6-BA application that will maximize horticultural benefits for *E. ciliata*.

Although the growth-promoting effects of cytokinins like 6-BA are established, recent studies have not fully addressed several critical questions in the context of *E. ciliata* cultivation. Most research in the past few years has focused on either morphological or biochemical endpoints in isolation (Tho et al 2024; Tho et al 2026). For example, identifying a single best dose for biomass or oil yield without examining the dynamic growth trajectory of the plant over time. Little is known about how *E. ciliata*'s vegetative growth rate progresses weekly, or how the timing of 6-BA application in mid or late vegetative stage might differentially influence outcomes. In addition, conventional analyses used in prior works cannot easily capture the complex, non-linear interactions between treatment factors (dose, timing, duration after treatment) and multiple growth traits. There is a paucity of research leveraging modern machine learning techniques to model and predict plant growth responses to plant growth regulator treatments. Advanced algorithms can handle complex multivariate patterns and have demonstrated superior accuracy in agronomic trait prediction (Xu et al 2022; Azrai et al 2024; Javed and Murad 2024). Furthermore, the emergence of explainable AI methods (notably SHapley Additive exPlanations, SHAP) enables interpretation of machine learning models by quantifying each factor's contribution to the prediction. To date, such interpretable machine learning approaches have not been applied to elucidate how dosage, application timing, and growth interval collectively shape the developmental outcomes in some plant species (Wei et al 2022; Abekoon et al 2025; Wang et al 2025). Without these tools, researchers and growers may miss subtle but important insights that could inform optimal cultivation

practices. Furthermore, a critical gap lies in the safety and regulatory aspect of using growth enhancers on edible or medicinal plants. *E. ciliata* leaves are not only distilled for essential oil but are also sometimes used directly in foods or herbal preparations (Pudziuelyte et al 2017; Khang et al 2023; Tho et al 2024; Tho et al 2026), meaning any chemical residues could be ingested by consumers. Surprisingly, recent literature lacks an integrated evaluation of whether applying 6-BA at effective growth-promoting levels leaves behind residues at harvest and if so, whether those residues pose any dietary risk. Regulatory agencies have set maximum residue limits (MRLs) for 6-BA on certain food crops and require risk assessments to ensure consumer safety. In the European Union, for example, the use of 6-BA as a plant growth regulator is accompanied by strict evaluation of chronic dietary exposure to verify that the chronic risk quotient (RQ) remains well below 1 for all consumer age groups (European Food Safety Authority et al 2020). The absence of residue and risk analysis in agronomic studies of 6-BA could become a barrier to practical adoption, especially for medicinal herbs destined for human consumption. Therefore, it is scientifically and societally important to fill this gap by determining the residue dissipation dynamics of 6-BA in *E. ciliata* and assessing whether its use can be considered safe under realistic consumption scenarios.

In light of the above, the present study was designed to provide a comprehensive evaluation of how exogenous 6-BA influences the growth and quality of *E. ciliata* across developmental stages, using state-of-the-art analytical tools. Specifically, this study aims to: (1) characterize the vegetative growth dynamics of *E. ciliata* over time and quantify how key morphological and biomass traits change weekly under controlled conditions; (2) determine the effects of foliar 6-BA application at two different growth stages (mid-vegetative vs. late-vegetative) and at varying concentrations on plant architecture, biomass allocation, and leaf physiological parameters; (3) develop predictive models using machine learning to capture the relationships between 6-BA treatment factors and growth outcomes, and apply SHAP analysis to interpret the relative importance of dosage, timing, and post-treatment duration for each trait; and (4) evaluate the fate of 6-BA residues on treated plants and perform a probabilistic dietary risk assessment using Monte Carlo simulations to ensure that the enhanced growth regime does not pose undue health risks to consumers. This study aims to elucidate how and why 6-BA can be used to safely boost the growth, leaf productivity, and biochemical performance of *E. ciliata*, ultimately guiding improved horticultural management for this valuable species.

2. Materials and methods

2.1. Materials

Seeds of *E. ciliata* were obtained from Huong Nong Seed Company Limited, Vietnam. The 6-BA used in the experiment was procured from Merck (Germany) with analytical-grade purity.

2.2. Experimental site and laboratory facilities

The assessment of 6-BA effects on the growth of *E. ciliata* was conducted at a horticultural nursery located in Can Giuoc District, Long An Province, Vietnam (10°36'21.4" N, 106°36'49.4" E). All parameters

were measured in horticultural nursery. Fresh biomass and dry biomass were carried out in the Teaching Methods and Plant Physiology Laboratory at Ho Chi Minh City University of Education. In addition, essential oil extraction procedures were performed at the Research Institute of Biotechnology and Environment, Nong Lam University, Ho Chi Minh City.

Soil analysis at the nursery site revealed low proportions of coarse sand (1.3%) and fine sand (1.0%), with a notably high clay content of 52.9%. The soil exhibited moderate fertility, indicated by an organic matter content of 2.06%. The measured pH was 5.16, classifying the soil as slightly acidic. Electrical conductivity was recorded at 188.00 $\mu\text{S}/\text{cm}$. The light intensity at the nursery ranged from approximately 35,528 to 41,957 lux. Ambient temperature fluctuated between 27.0°C and 29.5°C, while relative humidity varied from 77% to 84%.

2.3. Experimental desgin

The growth and development of *E. ciliata* were monitored under natural conditions from seedling stage (Fig. 1). Seeds were sown directly into grey soil at a nursery site, with seedlings evaluated beginning at the two-leaf stage. Prior to sowing, the soil was supplemented once with 400 kg/ha of Agrimartin (BioPellets) organic fertilizer (Uni-Farm, Vietnam). Plants were irrigated twice daily (morning and afternoon) using a sprinkler system comprising three emitters per 15 m² plot. Cultivation adhered to organic standards, with no application of synthetic fertilizers or chemical pesticides. The experimental layout followed a Randomized Complete Block Design (RCBD), consisting of four replicates with 25 plants per replicate. Plant spacing was maintained at 20 cm, and the total cultivated area was 45 m².

Based on preliminary growth assessments identifying key vegetative milestones, foliar applications of 6-BA were performed at two distinct developmental stages as the rapid vegetative growth phase (week 4) and the maturation phase (week 6). For both stages, plants were treated with a single foliar spray of 6-BA at concentrations of 0 ppm (distilled water control), 5 ppm, 15 ppm, or 25 ppm. Applications were conducted at 17:00 h to minimize photodegradation, with a spray volume of 0.5 L per treatment. Irrigation resumed two hours post-application. Each treatment was implemented under RCBD conditions with four replicates per stage.

2.4. Monitored growth parameters

To evaluate the morphological responses of *E. ciliata* to treatment conditions, a series of growth parameters were measured weekly from week 1 to week 8. Measurements were conducted weekly from week 1 to week 8, covering key traits related to plant architecture, foliar development, root morphology, and biomass accumulation (Table 1). The measurement methods and reference sources are detailed in Table 1. Each experiment included four replicates with 25 plants per replicate.

Table 1
Growth parameters and measurement methods for *Elsholtzia ciliata*

Parameter	Unit	Description
Plant height	cm	Distance from cotyledonary scar to apex of main stem
Stem diameter	cm	Measured just below the first two true leaves using calipers
Number of primary branches	count	Count of first-order branches
Number of leaves	count	Total number of leaves per plant
Leaf length & width	cm	Measured at the largest point on mature leaves
Leaf area	cm ²	Calculated from scanned images using Fiji software
Number of roots	count	Count of roots per plant
Root length	cm	Length of the longest root
Fresh and dry biomass	g	Fresh weight measured post-harvest. For the dry biomass, the plant was then wrapped in a clean paper bag, labelled, and oven-dried (Memmert, China) at 65 °C until the biomass remain unchanged

2.5. Leaf biochemical characterization and photosynthetic activity assessment

2.5.1. Photosynthetic activity

Photosynthetic intensity was evaluated by measuring oxygen exchange. Leaf segments ($\leq 10 \text{ cm}^2$) were excised using a specialized cutter and placed in an LD₂ gas-exchange chamber (Hansatech Instruments, UK) for a 10-minute measurement interval. Oxygen evolution and consumption were monitored with an oxygen electrode under controlled light (150 $\mu\text{mol photons/m}^2/\text{s}$ from a white LED) and dark conditions, respectively. The chamber temperature was maintained at $27 \pm 0.2^\circ\text{C}$. Data collected between the 4th and 7th minute were used to compute PI (expressed as $\mu\text{mol O}_2/\text{dm}^2/\text{h}$) based on oxygen concentration changes recorded in real-time (Kanechi et al 1998; Tho et al 2024).

2.5.2. Chlorophyll determination

Fresh leaf samples (0.02 g, n = 10) were ground in 5 mL of chilled 80% acetone solution. The resulting homogenate was centrifuged at 5000 rpm for 5 minutes at 4°C. The absorbance of the supernatant was measured at wavelengths of 475, 645, and 663 nm using a spectrophotometer, based on the method described by Lichtenthaler (1987). Total chlorophyll (chlorophyll a + b) was calculated and expressed in milligrams per gram of fresh weight (mg/g FW).

2.5.3. Quantification of soluble sugars

For soluble sugar analysis, 0.1 g of fresh leaf tissue ($n = 10$) was placed in a 1.5 mL microcentrifuge tube containing 1 mL of distilled water. After homogenization, samples were heated in a boiling water bath for 10 minutes, cooled to room temperature, and centrifuged at 4000 rpm for 10 minutes. The sugar content in the supernatant was determined using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, China), and absorbance was read at 620 nm. Results were reported in mg/g FW.

2.5.4. Essential oil extraction

Essential oils were isolated from 150 g of dried aerial parts ($n = 10$) that had been shade-dried under well-ventilated conditions for two days. Extraction was carried out via hydrodistillation using a Clevenger-type apparatus (Dolphin Labware, India). The collected oil was dried using anhydrous sodium sulfate (Na_2SO_4), then sealed in airtight containers and stored at -0.5°C prior to further analysis, as per the methodology of Khang et al (2023).

2.6. Residue quantification and dietary risk assessment of 6-BA in *elsholtzia ciliata* leaves

To evaluate 6-BA residue levels in *E. ciliata*, leaf samples were harvested at 1, 2, 3, and 4 weeks following foliar application. Each analytical replicate consisted of 10.00 ± 0.05 g of fresh tissue placed into a 50 mL centrifuge tube. Extraction was initiated by adding 10 mL of acetonitrile containing 1% (v/v) acetic acid, followed by 2 minutes of homogenization. Subsequently, 4 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate were introduced to promote dehydration, and the samples were vortexed for 1 minute before centrifugation at 4000 rpm for 3 minutes.

A 2.0 mL aliquot of the clear supernatant was transferred into a dispersive solid-phase extraction tube pre-packed with 25 mg of C18 and 150 mg of anhydrous magnesium sulfate. The extract was vortexed for 1 minute, centrifuged at 16,000 rpm for 5 minutes, and the final supernatant was filtered through a $0.22\ \mu\text{m}$ organic membrane prior to high-performance liquid chromatography (HPLC) analysis. This sample preparation protocol was adapted from Zhang et al (2022) with optimizations applied to the HPLC parameters for this study.

Chromatographic separation was performed using a Shimadzu LC-20AD HPLC system (Japan), equipped with a CNWSIL C18 analytical column ($4.6 \times 250\ \text{mm}$, $5\ \mu\text{m}$). The mobile phase consisted of solvent A (5 mmol/L ammonium acetate with 0.05% formic acid) and solvent B (HPLC-grade acetonitrile), delivered at a flow rate of 0.5 mL/min under isocratic conditions (25% B, 0.01–30 min). The injection volume was set at 5 μL . Detection was carried out using a UV detector (SPD-20A, Shimadzu). A standard 6-BA solution was prepared at 100 mg/L in methanol and stored at -4°C . Calibration standards were made by serial dilution in acetonitrile to final concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 $\text{mg}\cdot\text{L}^{-1}$. Quantification was performed using an external standard method.

To evaluate potential dietary exposure, a residue risk assessment was conducted following the methodology described by Dong et al (2017). The estimated daily intake (EDI) and risk quotient (RQ) for adult consumers were calculated using Eqs. (1) and (2).

$$EDI = \frac{F \times STMR}{BW} \quad (1)$$

$$RQ = \frac{EDI}{ADI} \quad (2)$$

Where EDI is the estimated daily intake (mg/kg body weight), F is the average daily consumption of the crop (kg/day), STMR refers to the supervised trials median residue level (mg/kg), BW is the average body weight (kg), ADI is the acceptable daily intake (mg/kg body weight), and RQ represents the risk quotient, used to assess the safety of dietary exposure.

A probabilistic Monte Carlo simulation was conducted to characterize variability and uncertainty in chronic dietary exposure to 6-BA across different age groups. For each age group, 10,000 iterations were performed. The resulting RQ distributions were summarized for each age group by their mean, median, and 95th percentile values, and the proportion of simulations with $RQ \geq 1$ was used as an indicator of potential chronic health concern. Graphical outputs, including probability density curves and cumulative distribution plots, were used to compare RQ profiles across age groups and to visualize the upper-tail behavior of exposure. All Monte Carlo simulations and statistical analyses were performed using RStudio (version 4.5.1) with mc2d and @riskR packages for random sampling and probabilistic risk assessment.

2.7. Machine learning-based approached

Machine learning analyses were performed to model plant growth responses of *E. ciliata* to exogenous 6-BA application. Four target traits were considered as number of leaves, leaf area, leaf fresh biomass, and leaf dry biomass. The input predictors included 6-BA concentration (ppm), time after spray (weeks), and spray timing (week 4 or week 6). All computations were conducted in R (v4.5.1) using the xgboost, caret, tidyverse, and SHAPforxgboost packages.

Input variables were inspected for missing values, type consistency, and distributional characteristics. Spray timing was converted to a factor, while 6-BA concentration and time after spray were retained as numeric variables. Four supervised regression algorithms were benchmarked: linear regression, elastic net regression, random forest, and extreme gradient boosting (XGBoost). Model performance was assessed via repeated cross-validation using the coefficient of determination (R^2), root mean squared error (RMSE), and mean absolute error (MAE) as primary metrics. The model yielding the best validation performance across all traits was selected for downstream analyses.

To quantify predictor contributions and capture nonlinear effects, SHAP (SHapley Additive exPlanations) values were computed for the final XGBoost models. SHAP summary plots and SHAP dependence plots

were generated to visualize global and feature-specific effects, respectively. The final XGBoost models were used to explore the response surfaces of each trait across the experimental input space without retraining. A structured grid search was performed over 6-BA concentration and time after spray with spray timing evaluated separately for Week 4 and Week 6 application. For each trait, the maximum predicted response and the corresponding input conditions were identified. Biologically meaningful optimal ranges were defined as combinations yielding $\geq 95\%$ of the maximum predicted trait value.

2.8. Data analysis

Plant performance was evaluated through the relative growth rate (RGR), which served as the basis for forecasting the temporal trajectory of *E. ciliata*. The RGR was determined for each plant and growth interval following Eq. (3), where (X_1) and (X_2) denote the fresh biomass at times (t_1) and (t_2) (week).

$$\text{Relative growth rate} = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (3)$$

Differences among measured traits were statistically assessed using a three-way analysis of variance (ANOVA), which enabled simultaneous evaluation of the main effects and interaction effects among experimental factors. The three factors included in the model were BA concentration, spray timing, and time after spray, and each trait was analyzed independently. Prior to analysis, the assumptions of ANOVA, including normality of residuals, homogeneity of variances, and independence of observations, were evaluated through diagnostic residual plots and Shapiro–Wilk and Levene’s tests.

3. Results

3.1. Vegetative growth dynamics and biomass accumulation

Time exerted a significant effect on vegetative growth and biomass accumulation of *E. ciliata* from week 1 to week 8 (Fig. 1). Z-score clustering of 17 morphological and biomass traits revealed a consistent temporal progression, with the lowest standardized values detected at weeks 1–2 and the highest values detected at weeks 7–8 (Fig. 1A). Hierarchical clustering separated traits into two major clusters as (i) leaf- and biomass-related variables (including leaf fresh biomass, stem fresh biomass, root dry biomass, and total dry biomass) and (ii) architectural variables (including number of branches, number of leaves, and trunk diameter) (Fig. 1A). Relative growth rate varied significantly across weeks, with a maximum value of 1.18 at week 4 and subsequent declines to 0.05 at week 7 and 0.02 at week 8 (Fig. 1B). RGR at week 4 was 6.9-fold higher than at week 3 and 59-fold higher than at week 8 (Fig. 1B). In addition, fresh biomass increased from 0.7 g plant⁻¹ at week 1 to 40.2 g plant⁻¹ at week 8 (Fig. 1D). Biomass at week 8 was 10.6-fold higher than at week 3 and 53.6-fold higher than at week 1 (Fig. 1D). Time-series imaging documented progressive increases in plant height, branching, and leaf area from weeks 1–6 and the presence of terminal flowering structures at week 8 (Fig. 1C–E).

3.2. Morphological responses to 6-BA treatment

Exogenous 6-BA application significantly affected morphological and biomass of *E. ciliata* traits at both spray timings. Hierarchical Z-score heatmaps showed distinct response profiles for spray at week 4 (Fig. 2A) and week 6 (Fig. 2B). For spray at week 4, higher 6-BA concentrations were associated with increased plant height, number of leaves, and fresh biomass, while root-associated biomass traits showed comparatively lower Z-scores. For spray at week 6, leaf morphological traits (leaf area, leaf width, and leaf length) exhibited the highest Z-scores at 15 ppm. Analysis of variance showed significant treatment effects on eleven traits ($p < 0.05$), with the strongest significance observed for plant height ($-\log_{10}p = 66.2$), trunk diameter ($-\log_{10}p = 60.4$), number of leaves ($-\log_{10}p = 55.7$), and fresh biomass ($-\log_{10}p = 42.3$) (Fig. 2C). Effect size analysis identified the largest partial η^2 values for leaf length (0.92), plant height (0.89), number of leaves (0.87), and number of roots (0.85) (Fig. 2D).

3.3. Predictive modeling and feature attribution

XGBoost yielded the highest cross-validated predictive performance for all growth traits, outperforming linear regression, elastic net regression, and random forest (Table 2). For number of leaves, XGB achieved an R^2 of 0.91, an RMSE of 15.14 leaves, and an MAE of 10.44 leaves, compared to random forest ($R^2 = 0.71$) and linear methods ($R^2 = 0.65$) (Table 2). For leaf area, XGB achieved an R^2 of 0.94, an RMSE of 5.07 cm², and an MAE of 3.80 cm², relative to random forest ($R^2 = 0.74$) and linear methods ($R^2 = 0.46$). For leaf fresh biomass, XGB produced an R^2 of 0.76, an RMSE of 0.98 g, and an MAE of 0.77 g, outperforming all other models ($R^2 \leq 0.62$). For leaf dry biomass, XGB produced an R^2 of 0.74, an RMSE of 0.14 g, and an MAE of 0.11 g.

Table 2
Cross-validated predictive performance of four regression models for plant growth traits

Target Trait	Model	R ²	RMSE	MAE
Number of leaves	Linear regression	0.65	30.81	24.98
	Elastic net regression	0.65	30.81	24.97
	Random forest	0.71	27.97	21.58
	Extreme gradient boosting	0.91	15.14	10.44
Leaf area (cm ²)	Linear regression	0.46	15.91	13.57
	Elastic net regression	0.46	15.91	13.57
	Random forest	0.74	11.05	9.08
	Extreme gradient boosting	0.94	5.07	3.80
Leaf fresh biomass (g)	Linear regression	0.62	1.22	0.96
	Elastic net regression	0.62	1.22	0.96
	Random forest	0.60	1.24	1.00
	Extreme gradient boosting	0.76	0.98	0.77
Leaf dry biomass (g)	Linear regression	0.62	0.17	0.14
	Elastic net regression	0.62	0.17	0.14
	Random forest	0.59	0.17	0.14
	Extreme gradient boosting	0.74	0.14	0.11

Observed–predicted comparisons indicated close agreement for all traits, with point clouds distributed along the 1:1 identity line (Fig. 3A). The narrow vertical dispersion observed for leaf area and leaf dry biomass was consistent with higher R² values. Residuals were symmetrically distributed around zero for all traits, with no visible heteroscedasticity across predicted values (Fig. 3B). Residual ranges were – 50 to + 20 leaves for number of leaves, – 30 to + 20 cm² for leaf area, – 1.0 to + 0.6 g for leaf fresh biomass, and – 0.1 to + 0.1 g for leaf dry biomass.

SHAP analysis quantified the relative contribution of the three predictors (6-BA concentration, time after spray, and spray timing) for each trait (Fig. 3C). For number of leaves, the highest mean absolute SHAP value was attributed to 6-BA concentration (25.25 units), followed by time after spray (27.33 units) and spray timing (21.01 units). For leaf area, time after spray exhibited the highest mean absolute SHAP contribution (27.33 cm²), followed by 6-BA concentration (25.25 cm²) and spray timing (21.01 cm²). For leaf fresh biomass, time after spray exhibited the highest mean absolute SHAP contribution (1.19 g), followed by spray timing (0.84 g) and 6-BA concentration (0.58 g). For leaf dry biomass, time after spray

yielded the highest mean absolute SHAP contribution (0.17 g), followed by spray timing (0.12 g) and 6-BA concentration (0.08 g).

SHAP dependence analyses showed structured responses to increasing 6-BA concentration for all traits (Fig. 3D). For number of leaves, SHAP values increased from – 50 to + 15 as 6-BA increased from 0 to 25 ppm. For leaf area, SHAP values increased from – 30 to + 7 cm² across the same 6-BA gradient. For leaf fresh biomass, SHAP values increased from – 1.0 to + 0.2 g, and for leaf dry biomass from – 0.1 to + 0.05 g. For all traits, dependence plots exhibited minimal within-dose variation because 6-BA concentration was discretely applied at four levels (0, 5, 15, 25 ppm), resulting in four corresponding SHAP clusters.

3.4. Leaf growth and biochemical responses

The application of 6-BA resulted in measurable changes in *E. ciliata* growth traits and leaf biochemical attributes under different spray timings (Fig. 5). Spatial response profiling showed that the highest predicted values for number of leaves, leaf area, leaf fresh biomass, and leaf dry biomass occurred at 15 ppm 6-BA and 4 weeks after spray for both spray timings (Fig. 5A). The peak predicted leaf number was 297.2 plants under week 4 and 280.2 plants under week 6, while the peak predicted leaf dry biomass reached 1.50 g and 1.49 g under week 4 and week 6, respectively. Observed trait measurements showed significant differences between spray timings (Fig. 5B). Week 4 plants exhibited greater leaf number (median 298 vs. 260, $p < 0.001$), greater leaf area (median 94.7 cm² vs. 80.0 cm², $p < 0.001$), and greater leaf dry biomass (median 1.50 g vs. 1.36 g, $p < 0.001$) than week 6 plants. Week 4 and week 6 plants did not differ significantly in leaf fresh biomass (median 16.1 g vs. 15.9 g, $p > 0.05$). In addition, leaf biochemical traits also differed by spray timing (Fig. 5C). Week 4 plants showed higher photosynthesis intensity (median 31.8 $\mu\text{mol O}_2 \text{ dm}^{-2} \text{ h}^{-1}$ vs. 29.8 $\mu\text{mol O}_2 \text{ dm}^{-2} \text{ h}^{-1}$, $p < 0.001$) and higher soluble sugar content (median 21.3 mg g⁻¹ FW vs. 18.0 mg g⁻¹ FW, $p < 0.01$) than week 6 plants. Chlorophyll content was also higher under week 4 (median 22.9 mg g⁻¹ FW vs. 18.0 mg g⁻¹ FW, $p < 0.001$). Essential oil yield differed between timings (median 2.28% vs. 2.05%, $p < 0.01$).

3.5. Residues and dietary risk assessment of 6-BA in *Elsholtzia ciliata*

Foliar application of 6-BA at 15 ppm resulted in detectable residues in *E. ciliata* at 1 week after spraying, whereas no residues were detected at 2–4 weeks. The mean residue concentration at 1 week was $0.1531 \pm 0.0278 \text{ mg.kg}^{-1}$ (Table 3). In addition, chronic dietary exposure estimates were calculated for ten age groups based on residue levels, vegetable consumption, and body weight parameters. EDI values ranged from 1.51 $\mu\text{g.kg}^{-1}.\text{bw.day}^{-1}$ in the 2–3 year age group to 0.70 $\mu\text{g.kg}^{-1}.\text{bw.day}^{-1}$ in individuals aged ≥ 70 years (Table 4). Corresponding chronic RQ ranged from 0.151 to 0.070 (Table 3). Monte Carlo simulation (10,000 iterations) further quantified probabilistic variation in RQ distributions across age groups. Mean RQ values ranged from 0.153 in the 2–3 year age group to 0.070 in the ≥ 70 year group (Table 5). Median RQ values ranged from 0.151 to 0.069 across the same age categories (Table 4). The

95th percentile RQ ranged from 0.216 (2–3 years) to 0.100 (≥ 70 years), and no iteration produced an RQ ≥ 1 for any age group (Fig. 6).

Table 3
Residues of 6-BA detected in *Elsholtzia ciliata* after foliar spraying at 15 ppm

Chemical	Spraying dose (ppm)	Time after spraying (week)	Residues (mg.kg ⁻¹)	MRL (mg.kg ⁻¹)
6-BA	15	1	0.1531 $\pm$ 0.0278	0.2
		2	-	
		3	-	
		4	-	
Notes: “-” indicates that not detected				

Table 4
Chronic dietary risk assessment of 6-BA residues in *Elsholtzia ciliata*

Age group (years)	Body weight (kg)	Vegetable consumption (g.day ⁻¹)	EDI ($\mu\text{g.kg}^{-1}\text{bw.day}^{-1}$)	RQ
2–3	12.7	125.1	1.51	0.151
4–6	16.5	162.8	1.51	0.151
7–10	22.3	206.5	1.42	0.142
11–13	34.05	235.85	1.06	0.106
14–17	45.95	255.35	0.85	0.085
18–29	55.25	286.05	0.79	0.079
30–44	60.3	297.15	0.75	0.075
45–59	60.05	304.95	0.78	0.078
60–69	57.95	278.75	0.74	0.074
≥ 70	54.75	248.90	0.70	0.070

Table 5
Probabilistic risk characterization of 6-BA exposure from *Elsholtzia ciliata* in different age groups by Monte Carlo simulation with 10,000 iterations

Age group (years)	Mean RQ	Median RQ	90th percentile RQ	95th percentile RQ	99th percentile RQ	P(RQ ≥ 1)
2–3	0.153	0.151	0.201	0.216	0.245	< 1×10 ⁻⁴
4–6	0.152	0.150	0.200	0.214	0.243	< 1×10 ⁻⁴
7–10	0.142	0.140	0.188	0.201	0.227	< 1×10 ⁻⁴
11–13	0.107	0.106	0.141	0.152	0.170	< 1×10 ⁻⁴
14–17	0.086	0.085	0.113	0.121	0.136	< 1×10 ⁻⁴
18–29	0.080	0.079	0.105	0.113	0.127	< 1×10 ⁻⁴
30–44	0.076	0.076	0.100	0.107	0.122	< 1×10 ⁻⁴
45–59	0.078	0.077	0.103	0.111	0.125	< 1×10 ⁻⁴
60–69	0.075	0.074	0.098	0.105	0.119	< 1×10 ⁻⁴
≥ 70	0.070	0.069	0.093	0.100	0.113	< 1×10 ⁻⁴

4. Discussion

The results demonstrate a clear temporal pattern in the vegetative growth of *E. ciliata*. All measured morphological and biomass traits increased progressively from week 1 to week 8, with distinct clustering of early vs. late growth stages. In the initial weeks (1–2), plants exhibited minimal size, whereas by weeks 7–8, trait values were at their maximum. This progression was accompanied by a sharp peak in RGR at mid-development as RGR reached 1.18 at week 4, then dramatically declined to near-zero by weeks 7–8. Such a trend indicates an early exponential growth phase transitioning to a plateau as the plants approached maturity. By week 8, *E. ciliata* had initiated flowering, which likely diverted resources from vegetative expansion to reproductive structures, thereby slowing biomass accumulation. This aligns with the general growth phenology of annual herbs, wherein maximal growth occurs prior to flowering, followed by a tapering off once reproductive development commences (Pearson and D’Orangeville 2022). The drop in RGR from its peak to week 8 underscores how markedly growth efficiency can fall once the plant’s developmental program shifts towards flowering. This finding

is consistent with the notion that, in many species, most biomass is accumulated early in the season when conditions favor vegetative growth (Malhi et al 2006; van Heerden et al 2010; Wang et al 2020).

Exogenous application of the cytokinin 6-BA had pronounced effects on *E. ciliata* morphology and biomass production. These findings align with the broader literature on cytokinin-mediated growth regulation. Exogenous 6-BA has consistently been shown to promote shoot elongation, stimulate leaf initiation, and delay senescence across a range of plant species. Yu et al (2023) demonstrated that foliar 6-BA application in maize enhanced chlorophyll retention, extended functional leaf area, and ultimately increased grain yield. Similarly, Panozzo et al (2025) reported that cytokinin application at wheat flowering increased canopy greenness and CO₂ assimilation by approximately 15%, though additional or later applications did not improve outcomes further. These studies underscore the importance of application timing in maximizing cytokinin efficacy. In our study, it can be seen that the hormone's influence was dose-dependent and also interacted with the timing of application (spray at week 4 vs. week 6). Hierarchical clustering of standardized trait values revealed that higher 6-BA concentrations tended to promote above-ground growth more than below-ground growth, especially when applied at the younger stage. For plants sprayed at week 4, increasing 6-BA dose (up to 15 ppm) led to taller plants with more numerous leaves and greater fresh weight also the root and shoot-related parameters. To explain this trend, it can be said that 6-BA preferentially stimulates shoot development, a classic cytokinin effect, potentially at the expense of root growth (Howell et al 2003; Müller and Leyser 2011; Kurepa and Smalle 2022; Sardoei et al 2024). Indeed, cytokinins are known to favor shoot organogenesis and can inhibit root elongation by altering the auxin-to-cytokinin balance in tissues (Schaller et al 2015; Ćosić and Raspor 2022). In our study, traits like branch number and trunk diameter clustered separately from leaf and biomass metrics, indicating that structural architecture may respond differently to 6-BA than foliar growth does. When 6-BA was applied later (week 6), the most notable improvements were in leaf size (area, length, width), with an optimal response observed at 15 ppm. Doses beyond 15 ppm did not further increase leaf size substantially, hinting at a saturation effect or slight phytotoxicity at the highest concentration which were observed in previous studies (Tho et al 2024; Tho et al 2026). Notably, analysis of variance confirmed significant treatment effects on a majority of traits, with the strongest effects on plant height, trunk diameter, leaf number, and total fresh biomass. These extremely low p-values reflect a robust, highly reproducible influence of 6-BA on *E. ciliata* growth. Additionally, the large effect sizes indicate that a substantial proportion of the variation in those traits was attributable to the hormone treatment. In practical terms, 6-BA application at moderate concentration caused *E. ciliata* to develop a significantly larger canopy, more leaves of larger size on taller stems, relative to untreated controls, especially when applied at the mid-vegetative stage.

The use of machine learning provided further insight into growth dynamics. Of the models tested, the gradient-boosted tree model (XGBoost) achieved the highest predictive performance for all growth traits. This superiority of XGBoost is in line with other recent studies employing ensemble learning for plant trait prediction. In a study on tomato fruit quality, XGBoost consistently outperformed neural networks, achieving $R^2 = 0.98$ for soluble solids content (M'hamdi et al 2024). The strength of XGBoost lies in capturing non-linear interactions and hierarchical feature effects, which simpler models or un-tuned

forests may miss. In our case, the agreement between observed and predicted values and the homogeneously distributed residuals suggest that the model effectively captured the key drivers of *E. ciliata* growth under 6-BA treatment. It is noteworthy that the three input features, 6-BA concentration, time after spray, and spray timing, sufficed to predict the measured outcomes with high accuracy. This implies these factors were indeed the primary determinants of the variation seen in leaf and biomass traits, with relatively little random noise or unmodeled influence. However, for traits like leaf fresh and dry biomass, the R^2 values were slightly lower (0.74–0.76). This could be due to individual plant differences or micro-environmental factors not captured in the model. Furthermore, interpretation of the XGBoost model via SHAP sheds light on the relative importance of each factor and their mechanistic roles. The SHAP feature importance showed that time after spray was the most influential predictor for traits related to size and mass (leaf area, fresh and dry weight), whereas 6-BA concentration was comparably or most influential for the number of leaves. Specifically, for leaf area, time after spray contributed the largest share of predictive power, underscoring that the duration of hormone action (or simply the growth time since application) is critical for leaf expansion. In contrast, leaf count was driven strongly by hormone concentration, reflecting that producing new leaves is highly dependent on cytokinin dose with higher 6-BA levels likely broke apical dominance and triggered more bud outgrowth, yielding a greater leaf count which was consistent with previous studies (Howell et al 2003; Benedetto et al 2013; Mok 2019; Svolacchia and Sabatini 2023). Meanwhile, spray timing (week 4 vs week 6) also had non-negligible contributions, indicating that inherent developmental stage differences between the two application timings affected outcomes. The SHAP dependence plots further revealed clear positive dose-response relationships as 6-BA concentration increased from 0 to 25 ppm, SHAP values rose steadily for all traits. For instance, the model attributes roughly 40–50 more leaves to a 25 ppm treatment compared to 0 ppm, and about + 7 cm² in leaf area, all else being equal. These patterns confirm that 6-BA's effect is monotonic and beneficial up to the highest tested dose, though the marginal gains diminished at the upper end. Interestingly, the SHAP clusters correspond to the discrete hormone levels, with little within-level variance which show a reflection of our experimental design (only four set doses) and an assurance that the model is not overfitting noise but truly learning the stepwise effect of each dose.

The interaction between 6-BA application timing and dosage had pronounced effects on both the morphological and biochemical traits of *Elsholtzia ciliata*. Two-factor response surface models identified 15 ppm 6-BA as the optimal condition for enhancing vegetative performance, irrespective of whether the spray occurred at week 4 or week 6. Experimental data confirmed this modeling prediction: plants treated at week 4 exhibited significantly higher leaf number, leaf area, and leaf dry biomass compared to those treated at week 6. The earlier application provided a longer window for developmental responses to manifest, contributing to superior structural development and photosynthetic efficiency. Notably, while fresh biomass remained similar across treatments, the week-4 application yielded greater dry biomass, indicating a higher proportion of structural compounds and reduced moisture content in leaves. This disparity suggests that plants treated later, closer to flowering onset, produced less mature or thinner leaves, which had less time to accumulate dry matter. Conversely, the earlier 6-BA application likely

extended the vegetative phase and enabled leaves to fully develop, contributing to increased dry mass and potentially improving post-harvest processing efficiency. Biochemical assessments further reinforced the morphological advantages of earlier hormone application. Week-4 treated plants exhibited significantly higher photosynthetic oxygen evolution, chlorophyll content, and soluble sugar concentrations than their week-6 counterparts. These enhancements align with the well-established role of cytokinins in delaying senescence, maintaining chlorophyll integrity, and prolonging the functional lifespan of leaves (Zwack and Rashotte 2013; Dani et al 2016; Hönig et al 2018). Increased chlorophyll likely facilitated enhanced light capture and carbon fixation, while elevated sugar levels indicate increased assimilate production and mobilization (Wang et al 2009; Thompson et al 2017; Jha 2019; Qin et al 2025). The accumulation of sugars could result from cytokinin-induced stimulation of source activity (photosynthesis) and sink strength (active growth tissues) (Roitsch and Ehneß 2000; Werner et al 2008), leading to an overall increase in carbon allocation to leaves.

The improvement in physiological traits translated into modest but significant gains in essential oil content, a key quality attribute for *E. ciliata*. Plants sprayed at week 4 produced higher essential oil concentrations than those treated at week 6. It is plausible that robust early growth promoted by timely cytokinin application created favorable conditions such as increased leaf surface area and prolonged metabolic activity for essential oil biosynthesis. Similar observations have been reported in other species. For instance, mulberry (*Morus alba*) plants treated with 6-BA not only demonstrated improved vegetative growth but also showed elevated flavonoid content (Zhang et al 2022), suggesting that exogenous cytokinin can concurrently enhance primary and secondary metabolism. In the present study, earlier treatment during the active vegetative phase of *E. ciliata* produced greater morphological and biochemical benefits than a late-stage application, reinforcing the timing-sensitive nature of cytokinin responsiveness. Mechanistically, the superior outcomes of week-4 6-BA application likely stem from the hormone's influence on shoot meristem activity and chloroplast maintenance. By promoting cell division and inhibiting senescence-related pathways, 6-BA extended the vegetative growth window and improved leaf quality (Zhang et al 2023; Zhang et al 2024). The delayed chlorophyll degradation observed in week-4 plants supports this explanation, as cytokinins are known to suppress senescence-associated genes and enhance the expression of chlorophyll biosynthesis enzymes (Liu et al 2016; Hu et al 2021; Glanz-Ildan et al 2022). The elevated sugar content may further reflect enhanced sink–source dynamics, whereby increased leaf area and activity enabled greater carbohydrate accumulation and distribution to developing tissues (Watt et al 2013; Osorio et al 2014). The slight increase in essential oil concentration may represent a secondary effect of prolonged vegetative metabolism or increased energy availability for secondary metabolite biosynthesis.

The residue and safety assessment of 6-BA residues in *E. ciliata* provides crucial context for practical application. Foliar spraying at 15 ppm left a detectable residue of 0.153 mg·kg⁻¹ in the herb one week after treatment, but by two weeks post-spray the compound was no longer detectable in plant tissues. This rapid dissipation is encouraging, indicating that 6-BA is either metabolized by the plant or degrades to below detectable levels within a short period. Many cytokinin compounds, including 6-BA, are known to have relatively short half-lives in plants and are often considered low-risk in terms of persistence

(Armaković et al 2025). To evaluate consumer safety, chronic dietary exposure was estimated for various age groups using the residue data (Boobis et al 2008; Nougadère et al 2012; Winter 2015). The resulting exposure levels (EDI) were extremely low on the order of 1 µg of 6-BA per kg body weight per day for the most exposed group (toddlers). The corresponding chronic RQ were between 0.070 and 0.151 across age groups, with even the 95th percentile of exposure not exceeding 0.22. An RQ below 1.0 indicates that the exposure is below the established safe intake threshold (Zhang et al 2022). In fact, our RQs are well below 1, suggesting a wide margin of safety. These findings are in accordance with other recent risk assessments of 6-BA on edible crops. For instance, a study on mulberry leaves (which are used in herbal teas and foods) found that after applying 30 mg.L⁻¹ 6-BA, the residual hormone posed an “extremely low” dietary risk, with no health concerns identified (Zhang et al 2022). Our Monte Carlo simulation, which did not produce any scenario with RQ ≥ 1, reinforces the conclusion that consuming *E. ciliata* treated with 6-BA at the tested dose is unlikely to pose any appreciable risk to human health. It is worth noting that no acute toxic effects were observed and chronic exposure over a lifetime at these levels would still be far below harmful levels. Additionally, the absence of residues after 2–4 weeks implies that a short pre-harvest interval (perhaps 1–2 weeks) would be sufficient to ensure the herb is free of 6-BA, which is a practical guideline for farmers. In summary, the application of 6-BA, at least at 15 ppm, is not only effective in stimulating growth but also safe for end consumers under the conditions studied.

5. Conclusion

The present study elucidated the vegetative growth dynamics of *E. ciliata* and demonstrated that exogenous 6-BA application constitutes an effective means of modulating architectural traits, biomass allocation, and leaf biochemical performance. High-resolution phenotyping combined with machine learning enabled the development of accurate predictive models and quantitative feature attribution, thereby providing mechanistic insight into dose, timing, and post-spray progression effects. Integration of physiological measurements and essential oil yield further highlighted the agronomic relevance of optimal treatment windows, particularly the superiority of week-4 spraying for enhancing photosynthetic capacity, soluble sugar accumulation, and chlorophyll content. Collectively, these findings provide a structured framework for growth stage-specific regulation in *E. ciliata*, representing a novel contribution to both horticultural management and data-driven plant modulation strategies. Importantly, dietary risk assessment confirmed that 6-BA residues dissipated rapidly and did not pose a chronic hazard across consumer age groups, underscoring the practical applicability and food safety compatibility of the proposed approach.

Despite these advances, the study was constrained by the controlled environmental conditions, limited concentration range, and reliance on a single germplasm source, which may not capture genotype-by-environment interactions or broader horticultural variability. Biochemical profiling focused on a restricted set of traits and did not include targeted metabolomics to resolve downstream biochemical pathways or essential oil chemotypes. Future research should expand to multi-environment field trials, diverse

genotypes, and finer temporal sampling of 6-BA signal transduction to elucidate underlying regulatory networks. Multi-omic integration, including transcriptomics, metabolomics, and hormonal profiling, would further resolve mechanistic causality, while techno-economic assessments are warranted to evaluate scalability in commercial production systems. Together, these directions would strengthen translational relevance and deepen biological understanding without diminishing the robust contributions established in the current work.

Declarations

Declaration of competing interest

The authors declare no conflict of interest.

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Author Contribution

Luu Tang Phuc Khang : Roles/Writing - Original draft, Formal analysis, Data curation, Methodology, Investigation, Writing, Review & Editing, Resources, Conceptualization. Nguyen Xuan Tong: Roles/Writing - Original draft, Formal analysis, Data curation, Methodology, Investigation, Writing, Review & Editing.

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Data Availability

Data will be made on request

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Figures

Figures

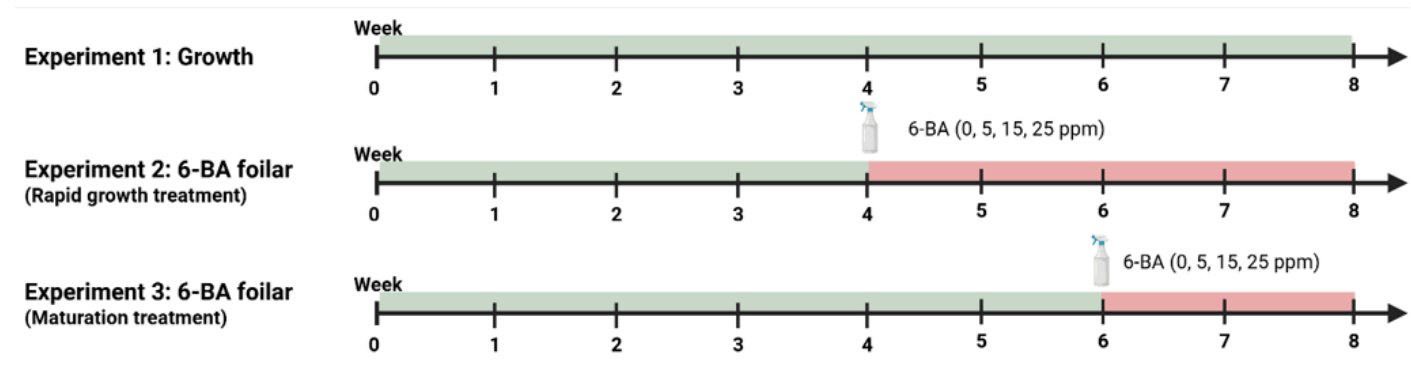


Figure 1

Timeline of growth and 6-BA foliar application in *Elsholtzia ciliata* experiments

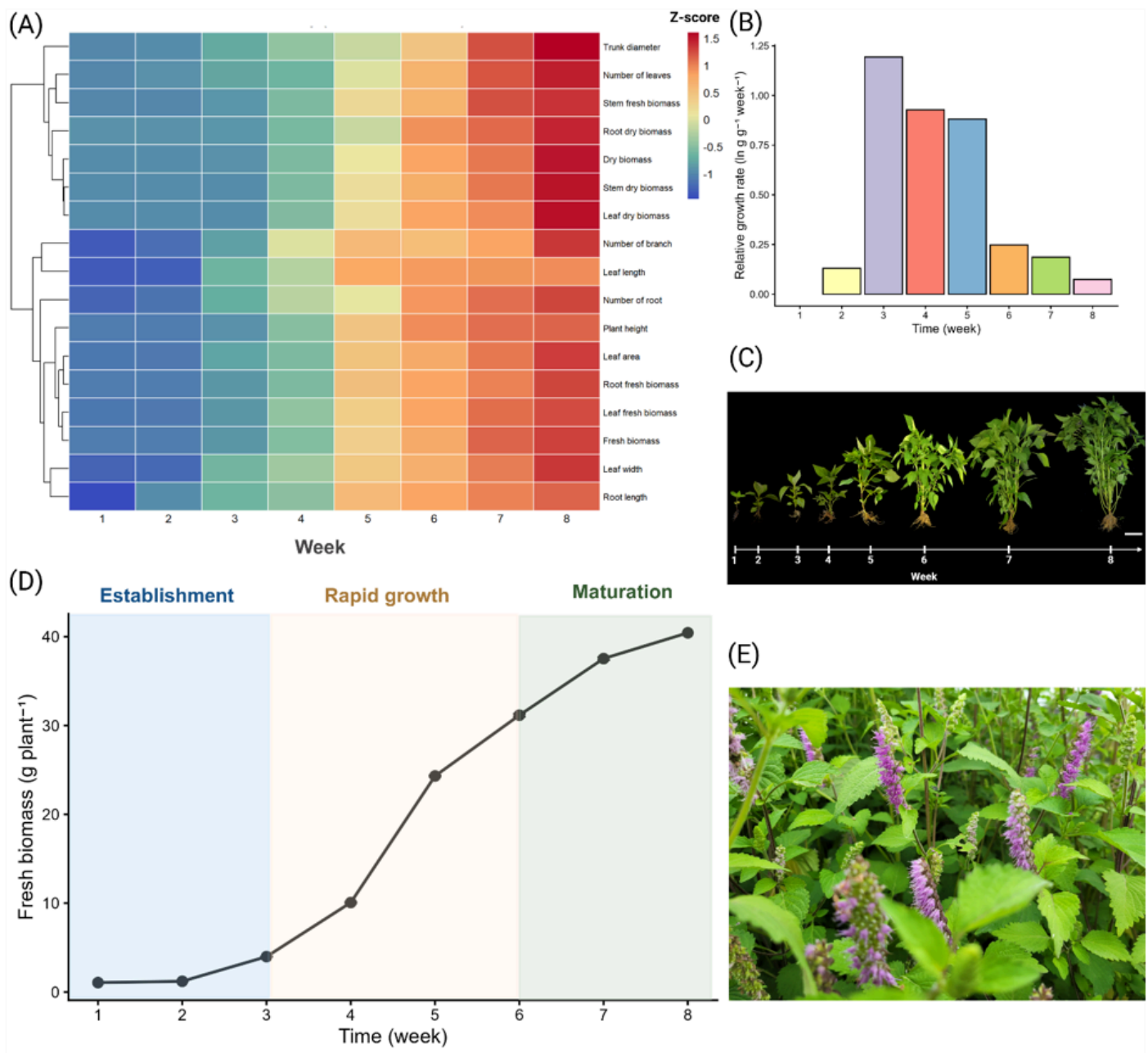


Figure 2

Growth and biomass dynamics of *Elsholtzia ciliata* over eight weeks. (A) Z-score heatmap and hierarchical clustering of 17 morphological and biomass traits (weeks 1–8). (B) Relative growth rate (ln g g⁻¹ week⁻¹). (C) Representative whole-plant images (weeks 1–8). (D) Growth curve based on fresh biomass (g plant⁻¹) across weeks. (E) Terminal inflorescences visible at week 8.

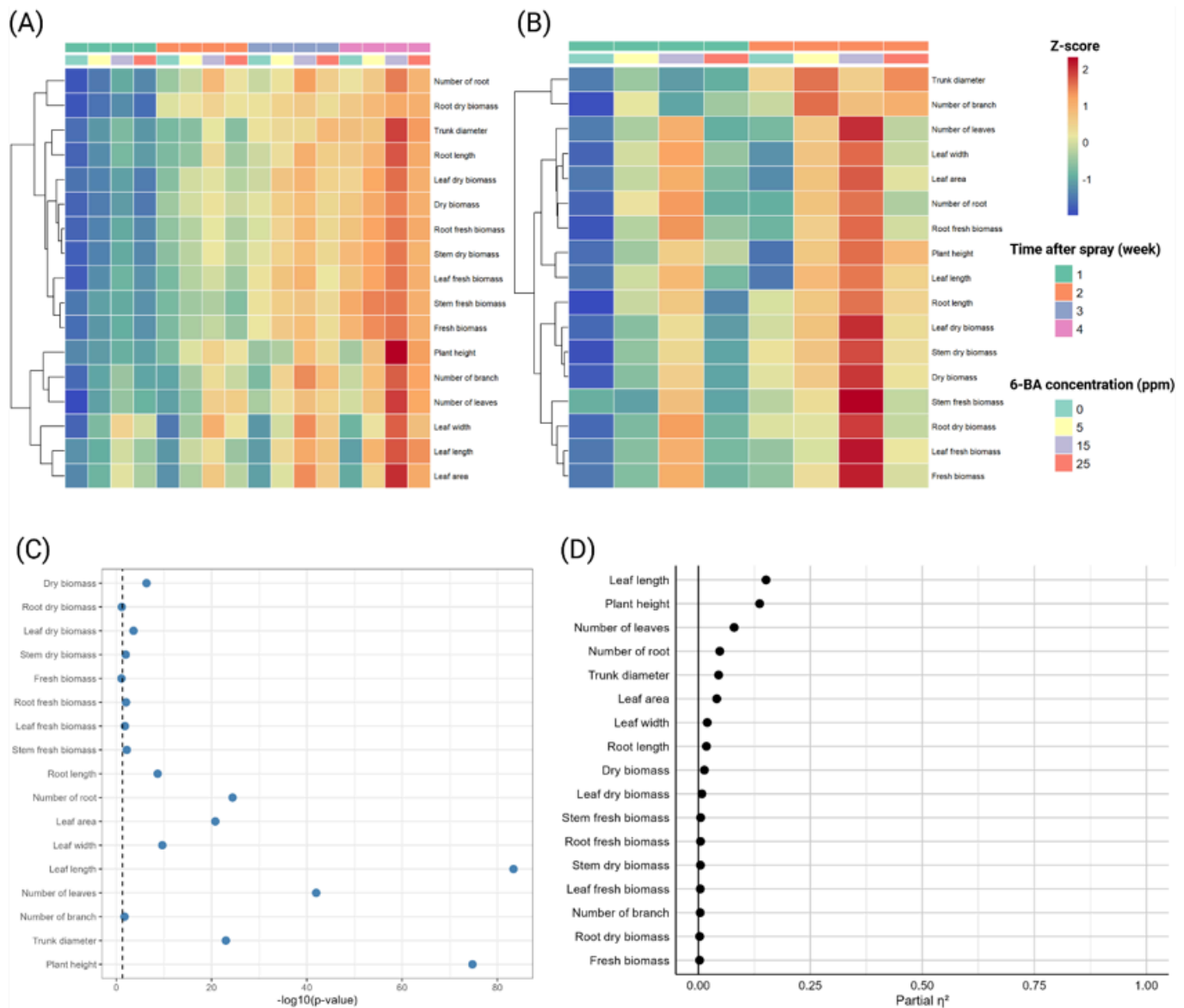


Figure 3

Multivariate and univariate responses of morphological traits to spray week, time after spray, and 6-BA concentration based on three-way ANOVA. (A) Z-score heatmap for spray week 4. (B) Z-score heatmap for spray week 6. (C) $-\log_{10}(p\text{-values})$ from three-way ANOVA showing significance of main effects, two-way interactions, and the three-way interaction (dashed line indicates $p = 0.05$). (D) Partial η^2 values from three-way ANOVA quantifying effect size contributions of main effects and interaction terms. Traits include plant height, trunk diameter, branch number, leaf number, leaf dimensions, root dimensions, and biomass traits.

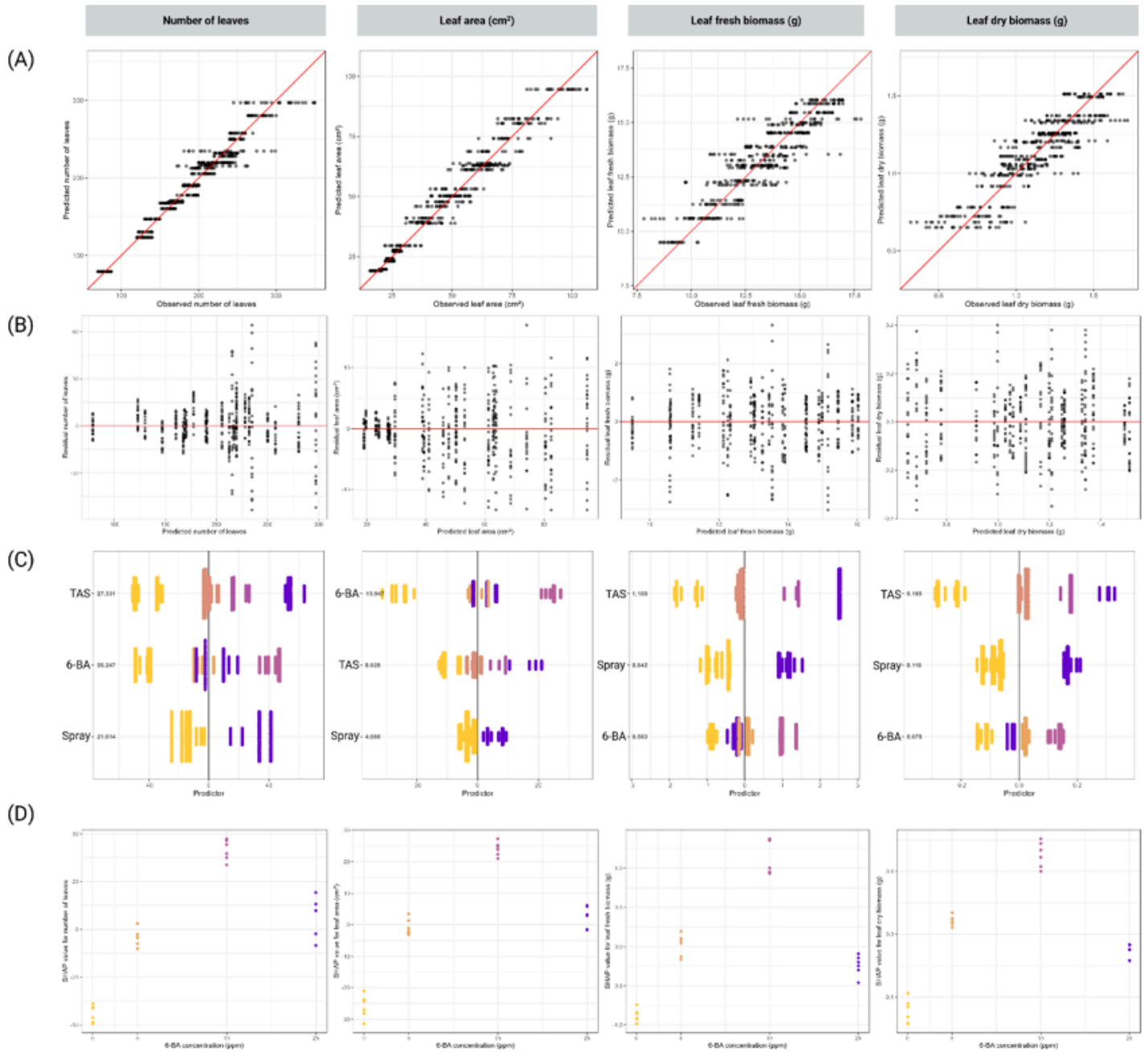


Figure 4

Predictive performance and model interpretation for plant growth traits using extreme gradient boosting (XGB). (A) Observed vs. predicted values with 1:1 identity lines for number of leaves, leaf area (cm²), leaf fresh biomass (g), and leaf dry biomass (g). (B) Residual diagnostics showing residuals (observed – predicted) vs. predicted values with horizontal zero-reference lines. (C) SHAP summary (beeswarm) plots showing the distribution of SHAP values for each predictor and trait, ordered by mean absolute SHAP contribution. (D) SHAP dependence of 6-BA concentration (ppm) for each trait, showing the functional contribution of 6-BA to model output.

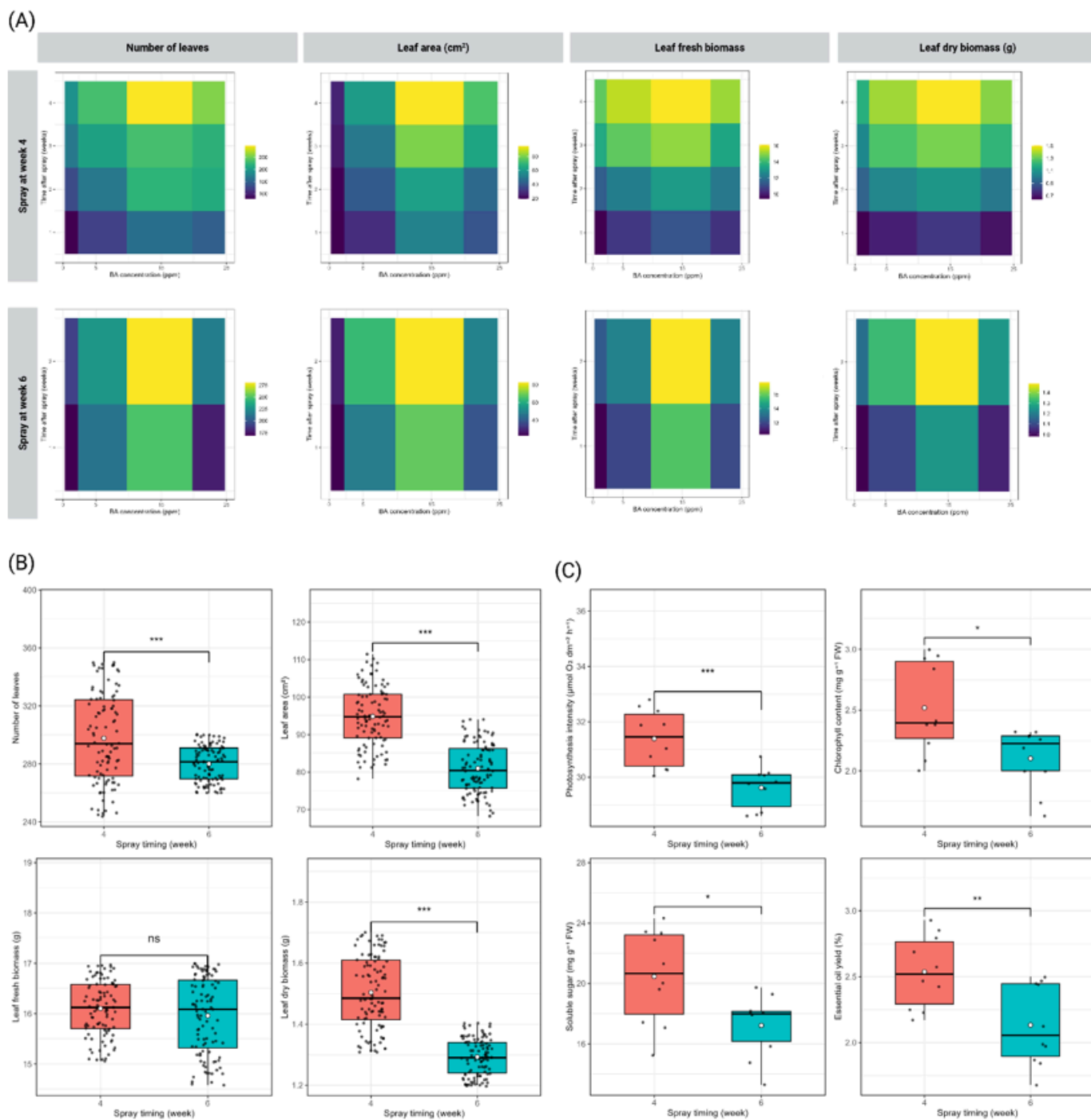


Figure 5

Growth and biochemical responses to 6-BA under two spray timings. (A) Model-predicted response surfaces for number of leaves, leaf area, leaf fresh biomass, and leaf dry biomass as functions of 6-BA concentration and time after spray for week 4 and week 6 spray timings. (B) Observed growth traits compared between week 4 and week 6 spray timings: number of leaves, leaf area, leaf fresh biomass, and leaf dry biomass. (C) Observed biochemical traits compared between week 4 and week 6 spray timings: photosynthesis intensity, chlorophyll content, soluble sugar content, and essential oil yield. ($p < 0.05$, $*p < 0.01$, $**p < 0.001$; ns = not significant).

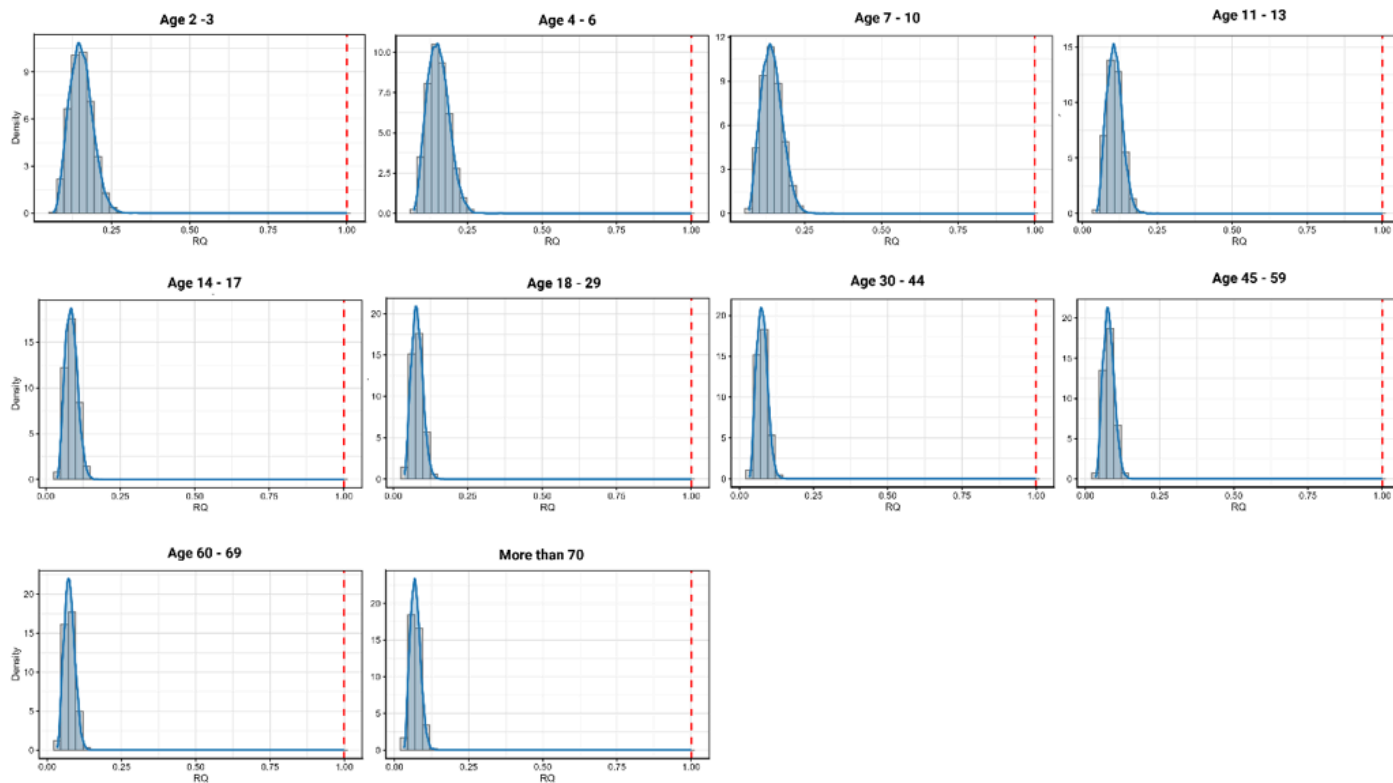


Figure 6

Probability density distributions of chronic dietary risk quotients for 6-BA exposure from *Elsholtzia ciliata* across ten age groups generated by Monte Carlo simulation (10,000 iterations). Dashed red lines indicate the risk threshold of RQ = 1.