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Studies on the growth and essential oil accumulation of *Elsholtzia ciliate* (Thunb.) Hyland and the effect of gibberellic acid treatment on growth and essential oil accumulation

Luong Thi Le Tho[1], Luu Tang Phuc Khang[1*], Nguyen Xuan Hieu[1], Dinh Thi Bich Thuy[1], Vo Ngoc Khoi Nguyen[1], Chau Minh Hai Dang[1], Tran Thi Phuong Dung[2]

[1]*Department of Biology, Ho Chi Minh City University of Education, 280 An Duong Vuong Street, Ward 4, District 5, Ho Chi Minh City, Vietnam*

[2]*Department of Primary Education, Ho Chi Minh City University of Education, 280 An Duong Vuong Street, Ward 4, District 5, Ho Chi Minh City, Vietnam*

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Abstract:

Vietnamese balm (*Elsholtzia ciliata* (Thunb.) Hyland) (*E. ciliata*) is known as an aromatic herb and a valuable remedy in oriental medicine thanks to the essential oil contained in the leaves. In plant life, plant growth regulators play an important role in the growth and accumulation of essential oils. This article investigates the effect of gibberellic acid (GA_3) on the growth and essential oil accumulation of *E. ciliata* at the 4-week-old plant stage based on a randomised complete block design with 3 replications. The results showed that the effect of GA_3 (0, 5, 10, 15, 20, and 25 ppm) on growth parameters of *E. ciliata*. GA_3 increases plant height, leaf length, leaf width, whole plant fresh weight, dry weight, nitrate content, and essential oil percentage significantly ($p \leq 0.05$). With an increasing amount of GA_3, all of these parameters increased significantly compared to the control. The treatment with 10 ppm GA_3 resulted in the highest plant height, leaf length, leaf width, and production of fresh weight, dry weights, nitrate content, and more than the control. The highest essential oil yield (1.41%) was obtained by applying 10 ppm GA_3. The results of this study showed that the quantitative and qualitative yield of Vietnamese balm could be increased by treatment with an appropriate amount of gibberellic acid.

Keywords: ***Elsholtzia ciliata*** **(Thunb.) Hyland, essential oil accumulation, gibberellic acid, growth.**

Classification numbers: **3.3, 3.4**

1. Introduction

Vietnam is a country located in an area with a tropical monsoon climate and many geographical regions favourable for plant growth. The diversity of Vietnamese flora is very high, with about 13,000 vascular plants [1]. The Family Lamiaceae (Labiatae), also known as the mint family, is represented by 236 genera and about 7,200 species distributed almost all around the world [2]. Numerous species of the family Lamiaceae, which are rich in volatile oils, are of great importance as medicinal plants and spices.

As people become more and more aware of the effectiveness and benefits of plant aromatics, medicinal plants, and their metabolites in protecting health and beauty, the demand for products of plant origin is constantly increasing. Essential oils (EOs) - secondary metabolites from plants - are widely used in the chemical industry, perfumery, flavouring, and cosmetic industries. Many of their therapeutic properties have been demonstrated in recent years, such as antioxidant, antimicrobial, anti-inflammatory, antiviral, antispasmodic, antiproliferative, and neuroprotective, among others [3]. Nowadays, EOs are likely to be used as safer alternatives to synthetic antibiotics, antifungals, anti-mosquitos, insecticides, and materials used for beauty treatments. With such outstanding values, many plant species containing essential oils, including species belonging to the Lamiaceae family, have become popular crops, and many essential oils are commodities with high economic value.

E. ciliata, a flowering plant belonging to the family Lamiaceae, is a wild plant native to Asia. In traditional medicine, *E. ciliata* has been used for the treatment of headache, fever, diarrhoea, oedema, blood clotting, gastralgia, dysphonia, nephritis, and throat infections [4, 5]. According to scientific literature, *E. ciliata* is a valuable bioactive source of natural antioxidants [6]. *E. ciliata* also possesses anti-inflammatory, antiviral, antibacterial, antioxidant, anticancer, and vasorelaxant effects [6-9]. The chemical composition of *E. ciliata* leaf essential oil is characterised by high amounts of geranial (19.5-26.5%), neral (15.2-20.5%), limonene (10.9-14.2%), and (Z)-p-farnesene (10.8-11.7%) as major constituents [10]. J.H. Jeong, et al. (2005) [11] reported that EOs extracted from

*Corresponding author: Email: ltpkhcmue@gmail.com

E. ciliata inhibit lipid peroxidation. In addition, Y.J. Kim, et al. (2006) [12] showed that a methanol extract *of E. ciliata* showed potent inhibitory activity against pancreatic lipase in a screen of medicinal plants for anti-obesity agents.

Plant growth regulators have immense commercial importance in intensive agriculture and are valuable in the agribusiness of high-value crops for organic cultivation [13]. Efforts to improve EO production by applying plant growth regulators have been suggested [14]. Plant growth regulators have been found to enhance herb and oil yield in some species. Gibberellins (especially gibberellic acid GA_3) are the most critical and valuable compounds for enhancing the productivity of commercial crops. Gibberellins are associated with various growth and development processes such as seed germination, stem and hypocotyl elongation, leaf expansion, floral organ development, flowering time reduction, flower number and size increase, and induction of some hydrolytic enzymes in the aleurone of cereal [15, 16]. In its role in essential oil accumulation, GA_3 has been studied previously; B.R.R. Rao, et al. (1997) [17] sprayed geranium with GA_3 promoted oil yield and prolonged flowering. Reports are available indicating a direct correlation between trichome number and oil production [18]. In parallel, GA_3 also increases leaf area leading to an increase in trichome parameters. The increase in oil accumulation per bed was because of an increase in trichome parameters (density, trichome diameter, total number of trichomes per leaf), leaf area, and oil biogenetic capacity per plant.

There have been much research works on *E. ciliata*. However, these studies focused mainly on extracting and analysing the chemical composition or biological activities of secondary compounds obtained from *E. ciliata* [19, 20]. On the contrary, the number of studies on the effects of phytohormones on the growth and accumulation of essential oil of *E. ciliata* is still limited. Based on the reference information, this study was carried out to evaluate the effects of GA_3 on plant height, leaf length, leaf width, whole plant fresh biomass, dry biomass, nitrate content, and essential oil percentage of *E. ciliata*.

2. Materials and methods

2.1. Plant materials

The seeds of Vietnamese balm (*E. ciliata*) were obtained from Huong Nong Seeds Co., Ltd (Vietnam). *E. ciliata* at the 4-week-old seedling stage.

2.2. Growth conditions

Prior to seed sowing, a homogeneous mixture of soil and organic manure in the ratio of 4:1 was filled in the nursery garden [21]. A uniform basal dose of nitrogen, phosphorus, and potassium was given at a rate of 15, 25, and 25 kg ha^{-1}, respectively. The soil was maintained at proper moisture to ensure better growth of the plants. Relative humidity was set to 60-80% [22]. The seeds were sown directly at a depth of 2 cm in a nursery garden located in Can Giuoc district, Long An province.

2.3. Experimental design

2.3.1. Plant growth assessment experiment

The growth of *E. ciliata* grown from seeds was assessed for 8 weeks (from the time the plant has 2 true leaves to the flowering stage). A randomised complete block design (RCBD) with three replications was applied for the experimental layout. After each week, the plant's growth was recorded for the parameters of plant height, leaf length, leaf width, total plant fresh weight, total plant dry weight, nitrate content, photosynthesis intensity, and respiration rate.

2.3.2. Gibberellic acid experiments

Nursery-raised healthy, vigorously growing 4-week-old seedlings (based on the result of the plant growth assessment experiment) were sprayed once on the foliage of the plants with six levels of GA_3 solution (adjusted to pH 7.0 with NaOH) (0, 5, 10, 15, 20, 25 ppm) (1.5 l) with the help of an atomiser [23]. The spraying volume for each treatment was maintained at 0.5 l. After a lapse of 2 hours following the spraying, regular watering procedures were resumed. Treatments were triplicated in a completely randomised block design three times in factorial experiments. The performance of *E. ciliata* was assessed for the above-mentioned parameters at week 6 (W6) and week 8 (W8) after spraying.

2.3.3. Growth analysis

Growth parameters were measured as follows:

1. Plant height (PH, cm): The plant height was measured from the area where the stem contacts the growing medium to its apex with a ruler.

2. Leaf length (LL, cm): The length of the 4th leaf was measured at its longest size using a technical Palmer ruler (Mitutoyo digital micrometre, Japan).

3. Leaf width (LW, cm): The width of the 4th leaf was measured at its widest size using a technical Palmer ruler (Mitutoyo digital micrometre, Japan).

4. Fresh biomass (FB, g): Randomly chosen plants were taken from each experimental unit. After cleaning them

from dust and separating them from the root system, the total plant fresh weight was recorded with a sensitive scale.

5. Dry biomass (DB, g): After weighting, the plant was wrapped in a clean paper bag, labelled, and oven-dried at 65°C for 48 h to determine the dry weight following the methodology established by M. Corell, et al. (2012) [24].

6. Nitrate content (NC, mg.kg^{-1}/FW): Nitrate content in plants was determined by colour reaction with phenol sulphonic acid solution. The absorbance of the standards and samples was measured with a Shimadzu (Model: 2401) UV-Vis spectrophotometer after setting the blank at 100% transmittance at the wavelength of 410 nm [25].

7. Photosynthesis intensity (PI, µmol O_2/dm^2/h) and respiration rate (RR, µmol O_2/dm^2/h): *E. ciliata* leaves were cut with specialised cutting tools (≤10 cm^2), then placed in the measuring chamber LD_2 (Hansatech, the UK) for 10 minutes. Gas exchange was measured with the instrument's oxygen electrode and was calculated as an increase (when leaves were illuminated with a white LED at 150 µmol/m^2/s) or decrease (when leaves were placed in the dark) of the amount of oxygen in the measuring chamber. The chamber temperature was 27±0.2°C under light-intensity conditions. The photosynthetic intensity and respiratory intensity were calculated at 4 to 7 minutes based on a graph showing the change in oxygen content displayed on the computer [26].

2.3.4. Essential oil contents

Essential oil contents (EO, %) of the air-dried and ground plants (150 g each) were determined by hydrodistillation for 3 h using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored at 4°C in the dark until further analysis [27].

2.3.5. Morphology of trichomes

Leaf samples were transversely cut into thin slices and directly observed for the appearance of hairs under an optical microscope after each week of the growth cycle.

2.4. Data analysis

The data of growth of *E. ciliata* at each week (growth experiment); weeks 6 and 8 (GA_3 treatment experiment); and the data of essential oil contents of the GA_3 treatment experiment at week 8 were tested. One-way ANOVA with a significance level of 5% ($p<0.05$) was used to compare the mean values at each time point. These analyses were performed using SPSS statistical software to test the significant difference at probability $p<0.05$. All data were presented as mean ± standard errors (SE).

3. Results and discussion

3.1. Growth of E. ciliata in nursery garden for 8 weeks

E. ciliata in the nursery garden grew slowly from week 1 to week 4. By week 5, the plants exhibited faster growth, as evidenced by the increase in growth parameters such as plant height (9.10 to 15.58 cm), leaf length (3.34 to 6.94 cm), and leaf width (1.91 to 3.71 cm) (Table 1). The leaves were dark green and deeply lobed, with each axillary bud having 2-4 branches. Stems and roots were sturdy. During weeks 6 and 7, plants continued to grow, showing increased tillering, and flowered at the end of weeks 7 and 8 (Fig. 1). This is consistent with the role of leaves as the organs carrying out the function of photosynthesis and synthesising metabolic compounds for the plant. The rapid growth of leaves before flowering is to create conditions for the plant to accumulate enough energy and achieve physiological maturity, preparing for the flowering stage [28].

Table 1. Growth analysis of *E. ciliata* in the nursery garden.

Parameters		Week							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
PH	cm	1.75±0.14^a	2.81±0.34^b	5.40±0.36^c	9.10±0.44^d	15.58±1.96^e	26.33±1.78^f	28.23±0.70^g	28.34±0.90^g
LL	cm	1.02±0.07^a	1.18±0.10^a	2.80±0.03^b	3.34±0.35^c	6.94±0.56^d	7.02±0.11^d	7.13±0.28^d	7.17±0.09^d
LW	cm	0.72±0.10^a	0.77±0.11^a	1.78±0.03^b	1.91±0.36^b	3.71±0.57^c	3.99±0.11^{cd}	4.07±0.28^d	4.22±0.09^d
FB	g	1.06±0.06^a	1.16±0.06^a	3.45±1.11^b	9.81±0.41^c	25.18±0.43^d	31.95±0.49^e	32.27±0.63^e	32.36±0.45^e
DB	g	0.05±0.01^a	0.06±0.01^a	0.13±0.03^b	0.62±0.08^c	1.31±0.02^d	2.79±0.05^e	2.82±0.02^e	2.83±0.11^e
NC	mg.kg^{-1}/FW	4.75±0.14^a	4.94±0.32^a	12.81±0.59^b	28.61±0.46^c	55.94±0.45^d	58.81±0.58^e	59.10±0.98^{ef}	59.52±0.65^f

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

Fig. 1. Growth of *E. ciliata* in the nursery garden over 8 weeks. Scale bar: 5 cm.

The flowering phase commenced at the end of week 7. At that time, plant growth slowed down, reaching its maximum in plant height (28.34 cm), leaf length (7.17 cm), and leaf width (4.22 cm) due to the vegetative meristem transforming into the floral meristem. Therefore, it was no longer able to lengthen the internodes and create new leaves (Table 1), aiding the plant's transition from the vegetative stage to the flowering stage [28].

During the developmental stages, plants absorb and accumulate nutrients. Nitrate accumulation depends on the plant's requirements during each growth stage. During the vegetative stage, the plant requires a large amount of nitrogen for the development of stems and leaves. As the plant transitions to the reproductive stage, nutrients are prioritised for flowering and fruiting, thereby slowing down or halting growth to concentrate energy on flowering [29]. This was consistent with the increase in FB (3.45 to 31.95 g); DB (0.13 to 2.79 g); nitrate content (12.81 to 58.81 $mg.kg^{-1}$/FW) from weeks 3 to 6. From weeks 6 to 8, the increase in FB (31.95-32.36 g), DB (2.79 to 2.83 g), and nitrate content (58.81 to 59.52 $mg.kg^{-1}$/FW) was slow and showed no significant difference ($p>0.05$) (Table 1).

The photosynthetic intensity gradually increased from week 1 to week 6 (4.95 to 18.06 µmol $O_2/dm^2/h$), peaked at week 6, and then gradually decreased at weeks 7 and 8 (18.06 to 17.93 µmol $O_2/dm^2/h$) (Table 2). Strong photosynthesis during the nutrient stage helps plants to grow quickly, with the energy generated in photosynthesis preferentially used for stem elongation, leaf formation, and branching [30]. Photosynthetic intensity started to decrease slightly from week 7 to week 8 but there is no statistical difference. On the other hand, respiratory intensity increased from week 7 to week 8 (5.77 to 9.84 µmol $O_2/dm^2/h$) to prepare for the flowering stage. In the flowering stage, *E. ciliata* favourably uses a lot of energy from cellular respiration to support flower morphogenesis energy [31].

Table 2. Photosynthetic intensity and respiration rate of *E. ciliata* in the nursery garden.

Parameters		Week							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
PI	µmol$O_2/dm^2/h$	$4.95±0.09^a$	$5.37±0.17^a$	$6.39±0.10^b$	$9.16±0.19^c$	$17.65±0.48^d$	$18.06±0.22^d$	$17.98±0.22^d$	$17.93±0.07^d$
RR	µmol$O_2/dm^2/h$	$22.17±0.50^e$	$20.34±1.04^d$	$20.03±0.75^d$	$11.19±0.66^c$	$5.54±0.41^a$	$5.77±0.26^a$	$5.77±0.19^a$	$9.84±0.35^b$

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

3.2. Morphological changes and accumulation of essential oil in E. ciliata trichomes for 8 weeks

On the cross-section of the leaves, trichomes accumulating essential oil in *E. ciliata* have a 1-cell head and a 2-cell head. At weeks 1 and 2, a new ciliary system formed into undivided cells and there was almost no oil accumulation (Fig. 2A). At week 3, trichomes divided into a 2-cell epithelium (Fig. 2B) and began to accumulate essential oil (Fig. 2D) or did not divide but still accumulated essential oil (Figs. 2C, 2D). By week 4, the accumulation of essential oil in the hair secretion system was enhanced. This transformation was maintained and did not differ from week 4 to week 8 (Fig. 2).

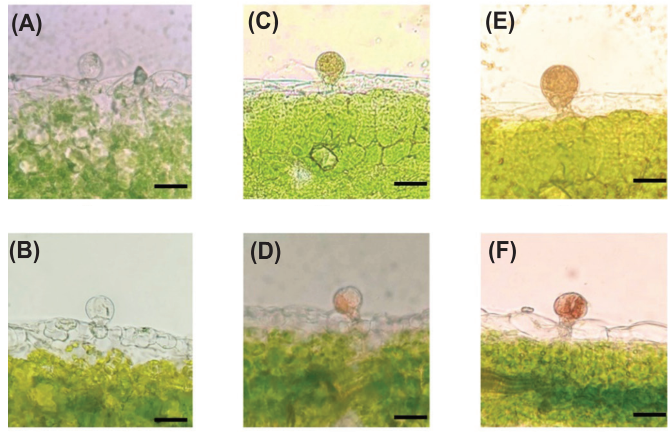

Fig. 2. Feathers in *E. ciliata* 1 cell head has not accumulated essential oil at week 1, 2 (A); the head of 2 cells has not yet accumulated essential oil at week 3 (B); 1 cell head accumulates essential oil at week 3 (C); the head of 2 cells accumulates essential oil at week 3 (D); 1 cell head accumulates essential oils at weeks 4-8 (E); the head of 2 cells accumulates essential oil at weeks 4-8 (F). Scale bar: 10 µm.

3.3. Effect of GA_3 on the growth and accumulation of essential oil in E. ciliata

3.3.1. Plant height

The results in Tables 3 and 4 indicated that spraying GA_3 on *E. ciliata* caused a significant increase in plant height. Increasing GA_3 concentrations from 5 to 10 ppm significantly increased plant height (33.68-45.58 cm at W6; 55.94-77.01 cm at W8). The height of *E. ciliata* was highest (with significant difference) when sprayed with 10 ppm GA_3, reaching 45.58 cm at W6, and 77.01 cm at W8, compared with the control which gave 26.42 cm at W6; and 28.55 at W8.

Table 3. Effect of GA_3 on growth of *E. ciliata* after 2 weeks of treatment.

GA_3 concentrations (ppm)	Parameters					
	PH (g)	*LL (cm)*	*LW (cm)*	*FB (g)*	*DB (g)*	*NC (mg.kg-1/FW)*
0	$26.42±0.84^a$	$7.01±0.10^a$	$3.93±0.18^a$	$29.98±0.98^a$	$2.81±0.04^a$	$58.15±0.43^a$
5	$33.68±0.57^c$	$7.37±0.12^b$	$4.35±0.15^b$	$34.66±1.80^c$	$4.09±0.30^c$	$69.39±0.77^c$
10	$45.58±0.64^f$	$8.73±0.34^d$	$5.50±0.43^d$	$44.02±1.20^e$	$6.39±1.18^e$	$83.39±1.27^e$
15	$44.24±0.48^e$	$8.64±0.46^d$	$5.41±0.34^d$	$43.09±1.56^e$	$6.28±0.32^{de}$	$83.01±1.44^e$
20	$37.90±0.49^d$	$8.64±0.46^d$	$4.72±0.28^c$	$39.81±1.72^d$	$5.73±0.36^d$	$76.41±1.48^d$
25	$29.38±0.57^b$	$7.29±0.13^{ab}$	$4.27±0.10^b$	$31.89±1.54^b$	$3.63±0.07^b$	$65.86±0.91^b$

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

Table 4. Effect of GA_3 on growth of *E. ciliata* after 4 weeks of treatment.

GA_3 concentrations (ppm)	Parameters					
	PH (g)	*LL (cm)*	*LW (cm)*	*FB (g)*	*DB (g)*	*NC (mg.kg-1/FW)*
0	28.55±0.42^{a}	7.17±0.12^{a}	4.30±0.09^{a}	34.47±1.35^{a}	2.96±0.12^{a}	61.25±0.78^{a}
5	55.94±0.48^{c}	8.52±0.41^{b}	5.31±0.34^{b}	49.62±0.67^{c}	7.49±0.22^{c}	95.03±0.67^{c}
10	77.01±0.49^{f}	9.50±0.51^{c}	6.03±0.29^{d}	65.44±1.63^{f}	11.10±0.30^{f}	83.39±1.27^{e}
15	75.05±0.78^{e}	8.81±0.35^{c}	5.79±0.31^{cd}	62.76±2.49^{e}	9.74±0.37^{e}	128.71±1.03^{e}
20	66.89±0.80^{d}	8.64±0.46^{c}	5.51±0.33^{bc}	58.56±1.58^{d}	8.38±0.37^{d}	109.61±0.58^{d}
25	44.45±0.56^{b}	7.52±0.15^{a}	4.46±0.15^{a}	37.15±0.46^{b}	6.13±0.07^{b}	71.09±0.92^{b}

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

3.3.2. Leaf length and leaf width

The data in Tables 3 and 4 indicated that spraying GA_3 on *E. ciliata* caused a considerable increase in the leaf length and leaf width, which reached 7.29-8.73 cm (LL at W6), 4.27-5.50 cm (LW at W6). The highest length and width of the leaves (9.50 cm (LL) and 6.03 cm (LW)) were observed when the plant was sprayed with 10 ppm GA_3 at W8, which differs significantly from all other treatments studied.

3.3.3. Fresh and dry biomass

The results in Tables 3 and 4 showed that the total plant fresh biomass (FB) and dry biomass (DB) were significantly highest (44.02 and 6.39 g (W6); 65.44 and 11.10 g (W8), respectively) when the plants were sprayed with GA_3 at 10 ppm, showing an increase of 47.32%.

3.3.4. Nitrate content

The outcomes in Tables 3 and 4 demonstrate that spraying *E. ciliata* with GA_3 from 5-25 ppm caused significant increase in nitrate content, which ranged from 65.86 to 83.39 $mg.kg^{-1}$/FW at W6, and from 71.09 to 128.71 $mg.kg^{-1}$/FW at W8. The nitrate content had the significantly highest value of 83.39 $mg.kg^{-1}$/FW at W6 with 10 ppm and 128.71 $mg.kg^{-1}$/FW at W8 when plants were treated with 15 ppm GA_3.

3.3.5. Photosynthesis intensity and respiration rate

After 4 weeks of GA_3 treatment, the PIs of all treated plants increased (PI: 19.24-32.49 µmol O_2/dm^2/h at W8) when compared with control (PI: 7.04 µmol O_2/dm^2/h at W8). Plants treated with 10 and 15 ppm GA_3 had increased PIs compared to those treated with 5; 20; and 25 ppm GA_3 and the control. The PI was highest in the 10-ppm GA_3 treatment (29.53 µmol O_2/dm^2/h at W6; 32.49 µmol O_2/dm^2/h at W8) (Table 5, Fig. 3). In contrast, the RIs of the GA_3-treated plants decreased (from 6.94 to 2.90 µmol O_2/dm^2/h at W6; 7.04 to 1.52 µmol O_2/dm^2/h at W8) and were different from that of the control (11.16 µmol O_2/dm^2/h at W6; 9.84 µmol O_2/dm^2/h at W8) (Table 5).

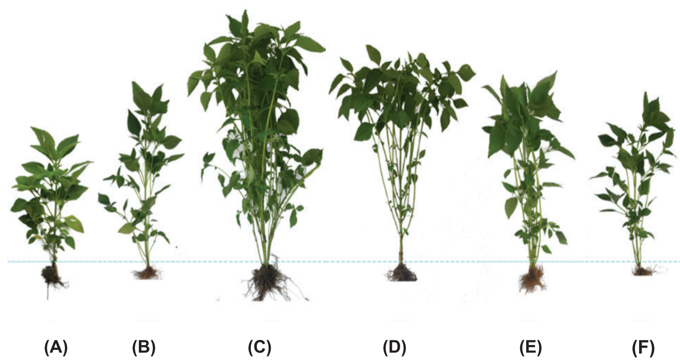

Fig. 3. Effect of GA_3 treatment on the morphology of *E. ciliata* in the nursery garden at week 6: control (A), GA_3 5 ppm (B), GA_3 10 ppm (C), GA_3 15 ppm (D), GA_3 20 ppm (E), GA_3 25 ppm (F). Scale bar: 5 cm.

Table 5. Effect of GA_3 on photosynthetic intensity and respiration rate of *E. ciliata* in the nursery garden.

Parameters		Gibberellic acid concentrations (ppm)					
		0	*5*	*10*	*15*	*20*	*25*
After 2 weeks							
PI	µmol O_2/dm^2/h	9.15±0.51^{a}	19.96±0.43^{b}	29.53±0.48^{d}	27.66±0.93^{c}	20.17±0.72^{b}	19.57±0.15^{b}
RR	µmol O_2/dm^2/h	11.16±0.63^{e}	4.59±0.02^{b}	2.90±0.06^{a}	3.08±0.04^{a}	5.45±0.35^{c}	6.94±0.11^{d}
After 4 weeks							
PI	µmol O_2/dm^2/h	7.04±0.31^{a}	22.09±0.17^{c}	32.49±0.27^{e}	30.01±0.17^{d}	19.36±0.14^{b}	19.24±0.15^{b}
RR	µmol O_2/dm^2/h	9.84±0.35^{e}	3.03±0.07^{c}	1.52±0.05^{a}	2.03±0.07^{b}	6.79±0.04^{d}	7.04±0.16^{d}

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

Gibberellins (especially GA_3) are the most important group of compounds among the known plant growth regulators for enhancing the productivity of commercial crops. However, the high cost and tedious production technology restrict its field application to high-value crops only [32]. As shown in Tables 3 and 4, spraying *E. ciliata* with 10 ppm GA_3 caused a considerable increase in PH, LL, LW, FB, DB, and NC when compared with the control. The increase in plant height under treatments with gibberellic acid in Mentha plants (0.001-0.1 mM GA_3) was also previously reported [33]. The physiological role of gibberellic acid in plants, which involves stimulating and activating the processes of cell division and expansion, can be linked to these results. Additionally, by promoting the synthesis of DNA, RNA, and proteins, gibberellic acid activates many enzymes that are crucial for the growth and development of plants and increases the activity of several biological and physiological processes of the cell [34]. Additionally, gibberellins increase the permeability of cell walls and raise the water content of cells [35]. It is possible to interpret that the gibberellic acid's ability to enhance auxin levels leads to an increase in cell division and cell elongation, resulting in increased plant height [36, 37]. Alternatively, this could be explained by GA_3's capacity to stimulate the synthesis of mRNA for hydrolytic enzymes and by the fact that larger cells eventually result in longer internodes [38]. Bioactive GA promotes growth by opposing the functions of DELLA growth-repressing proteins (DELLAs), members of the GRAS family of transcriptional regulators [39]. The GA pathway is a major regulator of this feed forward loop interconnecting plant growth to nitrate availability. This leads to GA_3 supplementation increasing nitrate content as previously reported by L. Camut, et al. (2021) [40].

3.3.6. Essential oil content

The results obtained in Table 5 reveal that the oil content of *E. ciliata* was positively affected by spraying with 5 to 25 ppm GA_3, reaching values of 1.09 to 1.41%. The oil percentage was highest (1.41%) for plants treated with 10 ppm GA_3. However, between treatments supplemented with GA_3 at concentrations of 10, 15, 20 ppm, there was no statistical difference in essential oil yields (Table 6, Fig. 4). For comparison, the oil content of the control was 0.93%.

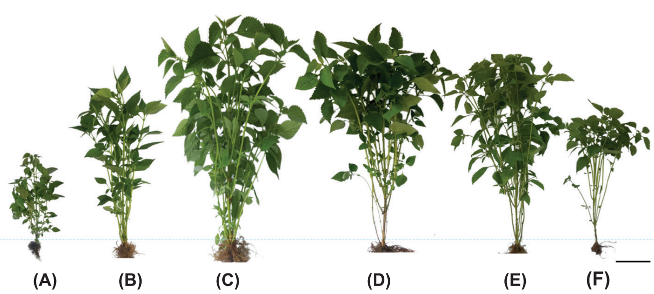

Fig. 4. Effect of GA_3 treatment on the morphology of *E. ciliata* in the nursery garden at week 8: control (A), GA_3 5 ppm (B), GA_3 10 ppm (C), GA_3 15 ppm (D), GA_3 20 ppm (E), GA_3 25 ppm (F). Scale bar: 5 cm.

Table 6. Effect of GA_3 on essential oil content of *E. ciliata* in the nursery garden.

Parameter		Gibberellic acid concentrations (ppm)					
		0	*5*	*10*	*15*	*20*	*25*
EO content	%	0.93 ± 0.04^{b}	1.11 ± 0.02^{c}	1.41 ± 0.02^{e}	1.38 ± 0.03^{de}	1.29 ± 0.04^{d}	1.09 ± 0.06^{c}

Values are mean ±SE, the superscript letters in each row indicate significant differences between weeks ($p<0.05$).

Plant growth regulators have been reported to influence the yield and quality of essential oil in many crops [41]. Growth regulators such as GA_3 can influence essential oil production through effects on plant growth, essential oil biosynthesis, and the number of oil storage structures. On the other hand, the increase in oil content may also be due to their effects on enzymatic pathways of terpenoid biosynthesis [42-44]. GA_3 affects the key enzyme activity of terpenoid biosynthetic pathways (DXS and HMGR) based on decreasing DXS activity in plants. GA_3 treatment caused a decrease in the 1-deoxy-D-xylulose5-phosphate synthase (DXS) activity in male and female *Cannabis sativa* plants [45]. Many reports support a regulatory role of DXS for the production of MEP-derived isoprenoids in plants [46]. J.M. Estevez, et al. (2001) [47] reported that transgene-mediated up or down regulation of DXS levels in *Arabidopsis* was correlated with concomitant changes in the levels of MEP-derived isoprenoid end products. Additionally, D.J. Wilk, et al. (1999) [48] showed that gibberellin works to protect the destruction of chlorophyll by inhibiting the effect of ethylene on chlophyllase transcript accumulation. Similarly, GA_3 also increased oil yield and quality in *Ocimum sanctum* and *Ocimum basilicum* [49]. The result was in conformity with the finding of E.S.M. Rashad, et al. (2009) [50] on *Calendula officinalis* L., A. Dadkhah, et al. (2016) [51] on *Satureja hortensis*, and M.N. Hasan, et al. (2020) [52] on *Rosmarinus officinalis* L.

4. Conclusions

The growth and development of *E. ciliata* (Thunb.) Hyl. can be divided into 3 stages: the slow growth phase (from sowing time to week 4), the fast growth phase (week 4 to week 7), and the flowering phase (stops growing and flowering from week 7 to week 8). The formation of the trichomes of *E. ciliata* starts from the young leaves (1 to 2 weeks old). There are 2 types of hairs, 1-cell hairs and 2-cell hairs, which begin to accumulate essential oil from week 3.

In addition, the results clearly showed that the exogenous application of GA_3 considerably affected the plant growth attributes, oil productivity and plant biomass. Foliar treatment with GA_3 (10 ppm) could be used to promote the growth and accumulation of EOs in Vietnamese balm (*E. ciliata* (Thunb.) Hyl.).

CRediT author statement

Luong Thi Le Tho: Conceptualisation, Writing - Original draft preparation, Literature review; Luu Tang Phuc Khang: Experimental design, Data collection and analysis, Revision; Nguyen Xuan Hieu: Experimental implementation, Data collection; Dinh Thi Bich Thuy: Writing - Original draft preparation, Literature review; Vo Ngoc Khoi Nguyen: Experimental implementation, Data collection; Chau Minh Hai Dang: Experimental implementation, Data collection; Tran Thi Phuong Dung: Conceptualisation, Writing - Original draft preparation, Literature review.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] N.T. Nguyen (2008), *Flora and Species Diversity*, Vietnam National University Publishing House, 146pp (in Vietnamese).

[2] R.M. Harley, S. Atkins, A.L. Budantsev, et al. (2004), "Labiatae", *The Families and Genera of Vascular Plants*, pp.167-282, DOI: 10.1007/978-3-642-18617-2_11.

[3] M.D.J.R. Alanis, J.B. Gonzalez, C.T. Alvarez, et al. (2019), "Chemical composition and biological activities of oregano essential oil and its fractions obtained by vacuum distillation", *Molecules*, **24(10)**, pp.1904-1914, DOI: 10.3390/molecules24101904.

[4] P.M. Zhao, C.X. Liu, D. Lai, et al. (2016), "Analysis of the essential oil of *Elsholtzia ciliate* aerial parts and its insecticidal activities against *Liposcelis bostrychophila*", *Helvetica. Chimica. Acta.*, **99**, pp.90-94, DOI: 10.1002/hlca.201500232.

[5] X. Wang, L. Gong, H. Jiang (2017), "Study on the differences between volatile constituents of the different parts from *Elsholtzia ciliata* by SHS-GC-MS", *American Journal of Analytical Chemistry*, **8(10)**, pp.625-635, DOI: 10.4236/ajac.2017.810045.

[6] M.S. Choi, B. Choi, S.H. Kim, et al. (2015), "Essential oils from the medicinal herbs upregulate dopamine transporter in rat pheochromocytoma cells", *Journal of Medicinal Food*, **18(10)**, pp.1-9, DOI: 10.1089/jmf.2015.3475.

[7] Z. Guo, Z. Liu, X. Wang, et al. (2012), "Elsholtzia: Phytochemistry and biological activities", *Chemistry Central Journal*, **6**, DOI: 10.1186/1752-153X-6-147.

[8] T.P.K. Luu, H.T. Nguyen, V.L. Cao, et al. (2023), "Chemical composition of *Elsholtzia ciliata (Thunb.*) Hyland essential oil in Vietnam with multiple biological utilities: A survey on antioxidant, antimicrobial, anticancer activities", *Academia Journal of Biology*, **45(3)**, pp.99-110, DOI: 10.15625/2615-9023/18230.

[9] L. Pudziuvelyte, V. Jakstas, L. Ivanauskas, et al. (2018), "Different extraction methods for phenolic and volatile compounds recovery from *Elsholtzia ciliata* fresh and dried herbal materials", *Industrial Crops and Products*, **120**, pp.286-294, DOI: 10.1016/j.indcrop.2018.04.069.

[10] X.D. Nguyen, V.H. Le, H.H. Le, et al. (1996), "Composition of the essential oils from the aerial parts of *Elsholtzia ciliata* (Thunb.) Hyland. from Vietnam", *Journal of Essential Oil Research*, **8(1)**, pp.107-109.

[11] J.H. Jeong, H.O. Sohn, H.J. Shin, et al. (2005), "Biological activity of flavor components extracted from *Elsholtzia ciliata* and *Elsholtzia splendens*", *Journal of The Korean Society of Tobacco Science*, **27**, pp.19-30.

[12] Y.J. Kim, B.H. Kim, S.Y. Lee, et al. (2006), "Screening of medicinal plants for development of functional food ingredients with antiobesity", *Journal of The Korean Society for Applied Biological Chemistry*, **49**, pp.221-226.

[13] W. Rademacher (2015), "Plant growth regulators: Backgrounds and uses in plant production", *Journal of Plant Growth Regulation*, **34**, pp.845-872, DOI: 10.1007/s00344-015-9541-6.

[14] M. Moghaddam, L. Mehdizadeh (2017), "Chemistry of essential oils and factors influencing their constituents", *Soft Chemistry and Food Fermentation*, pp.379-419, DOI: 10.1016/B978-0-12-811412-4.00013-8.

[15] S.M. Swain, D.P. Singh (2005), "Tall tales from sly dwarves: Novel functions of gibberellins in plant development", *Trends in Plant Science*, **10(3)**, pp.123-129, DOI: 10.1016/j.tplants.2005.01.007.

[16] A.N.M. Khassawneh, N.S. Karam, R.A. Shibli (2006), "Growth and following of black iris (*Iiris nigricans* Dinsm) following treatment with plant growth regulators", *Scientia Horticulturae*, **107(2)**, pp.187-193, DOI: 10.1016/j.scienta.2005.10.003.

[17] B.R.R. Rao, A.K. Bhattacharya, A.K. Singh, et al. (1997), "Growth, biomass production and essential oil profile of rose-scented geranium (Pelargonium species) as influenced by plant growth regulators", *Pafai J.*, **19**, pp.11-14.

[18] H.K. Lichtenthaler, M. Rohmer, J. Schwender (2006), "Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants", *Physiologia Plantarum*, **101(3)**, pp.643-652, DOI: 10.1111/j.1399-3054.1997.tb01049.x.

[19] D.T. Nguyen, H. Tran, S. Schwaiger, et al. (2021), "Effect of non-volatile constituents of *Elsholtzia ciliata* (Thunb.) hyl. from southern Vietnam on reactive oxygen species and nitric oxide release in macrophages", *Chemistry & Biodiversity*, **18(1)**, DOI: 10.1002/cbdv.202000577.

[20] T.B. Hoang, N. Nguyen (2022), "The antimicrobial activity and chemical composition of *Elsholtzia blanda* (Benth.) Benth. essential oils in Lam Dong province, Vietnam", *Can Tho University Journal of Science*, **14(3)**, pp.72-77, DOI: 10.22144/ctu.jen.2022.044.

[21] M.M.A. Khan, N. Hashmi, Moinuddin, et al. (2014) "Changes in growth, yield, photosynthetic characteristics, enzyme activities and essential oil production of fennel (*Foeniculum vulgare* Mill.*)* under growth regulator treatments", *Journal of Essential Oil Research*, **26(2)**, pp.105-113, DOI: 10.1080/10412905.2013.860408.

[22] N. Lu, E.L. Bernardo, C. Tippayadarapanich, et al. (2017), "Growth and accumulation of secondary metabolites in perilla as affected by photosynthetic photon flux density and electrical conductivity of the nutrient solution", *Frontiers in Plant Science*, **8**, DOI: 10.3389/fpls.2017.00708.

[23] W.A.A.A. Rawi, M.E.A.A. Hadethi, A.A.A. Kareem (2016), "Effect of foliar application of gibberellic acid and seaweed extract spray on growth and leaf mineral content on peach trees", *Iraqi Journal of Agricultural Science*, **47(7)**, pp.98-105.

[24] M. Corell, M.C. Garcia, J.I. Contreras, et al. (2012), "Effect of water stress on *Salvia officinalis* L. bioproductivity and its bioelement concentrations", *Communications in Soil Science and Plant Analysis*, **43(1-2)**, pp.419-425, DOI: 10.1080/00103624.2012.641811.

[25] S. Ramalingam, A. Subramania (2021), "Effective removal of nitrates from the drinking water by chemical and electrochemical methods", *Engineered Science*, **15**, pp.80-88, DOI: 10.30919/es8d456.

[26] P.T. Doan, A.T. Le, N.H. Phan (2020), "Effect of thidiazuron and monochromatic light on shoot development *in vitro* culture of *Polygonum cuspidatum* Sieb et Zucc.", *Science & Technology Development Journal - Natural Sciences*, **4(2)**, pp.468-477 (in Vietnamese).

[27] T.Q.A. Tran, T.T.H. Vo (2022), "Study on the chemical composition and antibacterial properties of Vietnamese balm essential oil (*Elsholtzia ciliata*)", *Journal of Agricultural Science and Technology of the University of Agriculture and Forestry*, **6(1)**, pp.2881-2890 (in Vietnamese).

[28] T.V. Bui (2000), *Plant Physiology. Part II: Development*, Vietnam National University Publishing House, 333pp (in Vietnamese).

[29] A.J. Bloom, F.S. Chapin, H.A. Mooney (1985), "Resource limitation in plants-an economic analogy", *Annual Review of Ecology and Systematics*, **16(1)**, pp.363-392, DOI: 10.1146/annurev.es.16.110185.002051.

[30] B. Marambe, L. Amarasinghe, K. Silva, et al. (2004), "Distribution, biology and management of *Mimosa pipra*", *Sri Lanka Research and Management of Mimosa pigra Canberra*, *Australia: CSIRO Entomology*, pp.85-90.

[31] L. Taiz, E. Zeiger (2002), "Photosynthesis: Physiological and ecological considerations", *Plant Physiology*, **9**, pp.172-174.

[32] S.K. Bose, R.K. Yadav, S. Mishra, et al. (2013), "Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in *Mentha arvensis* L.", *Plant Physiology and Biochemistry*, **66**, pp.150-158, DOI: 10.1016/j.plaphy.2013.02.011.

[33] F. Haider, G.D. Bagchi, A.K. Singh (2009), "Effect of calliterpenone on growth, herb yield and oil quality of *Mentha arvensis*", *International Journal of Integrative Biology*, **7**, pp.53-57.

[34] I.M.A. Alsudays, H.E.S.A. El Sayed (2020), "Influence of ascorbic acid, gibberellic acid and *Moringa oleifera* extract for alleviating salinity stress by enhancing antioxidant enzymatic activity and some physiological studies on two tomato cultivars", *Journal of Applied Life Sciences International*, **23(8)**, pp.39-62, DOI: 10.9734/jalsi/2020/v23i830180.

[35] W. Hopkins, N. Huner (2009), *Introduction to Plant Physiology*, John Willey & Sons, 503pp.

[36] S. Kuraishi, R.M. Muir (1964), "The mechanism of gibberellic action in dwarf pea", *Plant and Cell Physiology*, **5(3)**, pp.259-271, DOI: 10.1093/oxfordjournals.pcp.a079041.

[37] L. Taiz, E. Zeiger (2010), *Plant Physiology*, *5th Edition, Sinauer Associates Inc.*, Publishers Sunderland, Massachusetts USA, 782pp.

[38] N.T. Baliah, P.C. Sheeba, S. Mallika (2018), "Encouraging effect of gibberellic acid on the growth and biochemical characters of green gram (*Vigna radiata* L.)", *Journal of Global Biosciences*, **7**, pp.5522-5529.

[39] J.M. Daviere, P. Achard (2013), "Gibberellin signaling in plants", *Development*, **140(6)**, pp.1147-1151, DOI: 10.1242/dev.087650.

[40] L. Camut, B. Gallova, L. Jilli, et al. (2021), "Nitrate signaling promotes plant growth by upregulating gibberellin biosynthesis and destabilization of della proteins", *Current Biology*, **31(22)**, pp.4971-4982, DOI: 10.1016/j.cub.2021.09.024.

[41] N.S. Sangwan, A.H.A. Farooqi, F. Shabih, et al. (2001), "Regulation of essential oil production in plants", *Plant Growth Regulation*, **34(1)**, pp.3-21, DOI: 10.1023/A:1013386921596.

[42] W.N. Ali, A.O.A. Atrakchii (2022), "Effect of gibberellic, salicylic acids, and NPK fertilizers on growth and chemical constituents of Rosemary plants (*Rosmarinus officinalis* L.)", *Journal of Pharmaceutical Negative Results*, **13(3)**, pp.1842-1850, DOI:10.47750/pnr.2022.13.S03.281.

[43] S. Sharafzadeh, M. Zare (2011), "Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family", *Advances in Environmental Biology*, **5(8)**, pp.2296-2302.

[44] C.L. Prins, I.J. Vieira, S.P. Freitas (2010), "Growth regulators and essential oil production", *Brazilian Journal of Plant Physiology*, **22(2)**, pp.91-102, DOI: 10.1590/S1677-04202010000200003.

[45] H. Mansouri, Z. Asrar, M. Mehrabani (2009), "Effect of gibberellic acid on primary terpenoids and tetrahydrocannabinol in *Cannabis sativa* at flowerin stage", *Journal of Integrative Plant Biology*, **51**, pp.553-561, DOI: 10.1111/j.1744-7909.2009.00833.x.

[46] M.R. Concepcion, A. Boronat (2002), "Elucidation of the methylerythritol phosphate pathway for isoprenoids biosynthesis in bacteria and plastids: A metabolic milestone achieved through genomics", *Plant Physiology*, **130(3)**, pp.1079-1089, DOI: 10.1104/pp.007138.

[47] J.M. Estevez, A. Cantero, A. Reindl, et al. (2001), "1-deoxy-D-Xylulose 5- phosphate synthase, limiting enzyme for plastidic isoprenoid biosynthesis in plants", *Journal of Biological Chemistry*, **276**, pp.22901-22909, DOI: 10.1074/jbc.M100854200.

[48] D.J. Wilk, D. Holland, E.E. Goldschmidt, et al. (1999), "Chlorophyll breakdown by chlorophyllase: Isolation and functional expression of the chlase1 gene from ethylene-treated citrus fruit and its regulation during development", *The Plant Journal*, **20(6)**, pp.653-661, DOI: 10.1046/J.1365-313X.1999.00637.X.

[49] M.N.A. Eid, S.S. Ahmed (1976), "Preliminary studies on the effect of GA_3 and CCC on growth and essential oil content of *Ocimum basilicum* L.", *Egyptian Journal of Horticulture*, **3(1)**, pp.83-87.

[50] E.S.M. Rashad, M.E. Wahed, A.A. Amin (2009), "Effect of sitosterol and gibberellic acid on leaf angle, growth, flowering and biochemical constituents of marigold (*Calendula officinalis* L.)", *Medicinal Aromatic Plant Science Biotechnology*, **3(1)**, pp.21-27.

[51] A. Dadkhah, S. Ghorbanian, G. Rassam (2016), "Foliar application effect of gibberelic acid on growth, volatile oil and nutrients content of *Satureja hortensis* L.", *Zeitschrift für Arznei-& Gewürzpflanzen*, **21(2)**, pp.76-79.

[52] M.N. Hasan, S.M. Sultan (2020), "Effect of Fe and GA_3 on growth and oil characteristics of Rosemary plant (*Rosmarinus officinalis* L.)", *Plant Cell Biotechnology and Molecular Biology*, **21(40)**, pp.55-63.