

**Short Communication**

## Effects of Vietnamese Balm (*Elsholtzia ciliata* (Thunb.) Hyland) Essential Oil on Growth Performance of Striped Catfish (*Pangasianodon hypophthalmus*) Juvenile stage

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### Abstract

Vietnamese balm (*Elsholtzia ciliata*) is a prominent herb in Vietnamese traditional medicine. This study investigated the impact of Vietnamese balm leaf essential oils on the growth of striped catfish (*Pangasianodon hypophthalmus*) in the juvenile stage. Essential oils were extracted using steam distillation, following European Pharmacopoeia guidelines. Gas chromatography-mass spectrometry identified the essential oil components. The experiment encompassed three treatments: 0% (control), 3%, and 5% of Vietnamese balm leaf essential oils in a completely randomized design. The results demonstrated a time and dose-dependent modulation of striped catfish growth by the essential oils. Diets enriched with 5% Vietnamese balm leaf essential oils significantly enhanced survival rate and growth, indicating potential as a fish feed additive. The essential oil yield was 0.83% dry weight, with major components including (Z)- $\beta$ -Farnesene (24.02%), neral (15.06%), geranial (15.02%), and  $\beta$ -ocimene (13.61%). Additionally, the study emphasized the growth-promoting effects of herbal extracts on various growth parameters. Further investigations should explore the impact of Vietnamese balm essential oil on catfish immune response and disease resistance, providing a comprehensive understanding of its potential benefits.

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## 1. Introduction

In Vietnam, the aquaculture sector is experiencing rapid growth, evidenced by a significant surge in export turnover, reaching approximately 10 billion USD with a total output of about 8.74 million tons, representing an impressive 96.7% of the estimated target for 2021, according to data from the Center for Informatics and Statistics (VASEP, 2022a). A freshwater catfish of substantial economic importance, *Pangasianodon hypophthalmus*, commonly known as striped catfish, is extensively cultivated in various Mekong Delta provinces (Phan et al., 2009). Over the period from 2017 to 2022, the aquaculture production of striped catfish witnessed a notable increase from 1.2 million tons to 1.7 million tons, accompanied by a corresponding rise in export turnover from 1.78 billion USD to 2.44 billion USD (VASEP, 2022b). This escalation can be attributed to the escalating demand in the market, consequently driving an expansion in the scale and area of striped catfish farming across Vietnam.

One of the obstacles often faced in fish farming is disease attacks. Disease attacks caused by bacteria are an obstacle that often occurs in fish farming (Sudarno et al., 2012). Antibiotics have conventionally played a pivotal role in the aquaculture industry, serving purposes such as growth promotion and disease treatment. However, their extensive and frequent use has raised concerns regarding heightened risks of infectious disease outbreaks and deterioration of water quality (Srinivasan and Ramasamy, 2009; Luu et al., 2021). Consequently, alternative approaches have been developed not only to ensure a safe environment but also to optimize the growth performance of aquaculture animals. Phytochemicals, including quercetin, gallocatechin, flavonoids, tannins, and lecithin, possessing antioxidant, antitoxic, and antibacterial properties, have gained attention as feed additives in aquaculture, being derived from herbal plants (Awad and Awaad, 2017; Marimuthu et al., 2022). These plant-based additives have demonstrated the potential to enhance fish growth, making the use of plant extracts a prominent research focus to bolster toxicity resistance in aquatic products (Luu et al., 2022). The safety margin associated with herbal interventions has contributed to the rising popularity of using herbs to stimulate growth or combat diseases in aquatic animals, predominantly drawing from plants traditionally employed in human medicine (Garcia Beltran et al., 2020). Notable plants such as neem (*Azadirachta indica*), turmeric (*Curcuma longa*), tulsi (*Ocimum sanctum*), and malabar nut (*Adhatoda vasica*) have been extensively studied and applied, displaying effectiveness in improving disease resistance (Wang et al., 2009; Wu et al., 2010). For instance, the supplementation of resveratrol through dietary means to juvenile genetically improved farmed tilapia (GIFT) led to increased quantities of serum and

hepatic IgM. Additionally, critical anti-inflammatory cytokines, interleukin-10 (IL-10), and its inverse inhibitor interferon (IFN- $\gamma$ ), witnessed a notable increase in quantity (Zheng et al., 2017). Similarly, products derived from plants such as Livol (derived from *Terminalia arjuna*, *Solanum nigrum*, *Boerhavia diffusa*, and *Citrullus colocynthis*) and glycyrrhizin (a glycosylated saponin) have demonstrated their efficacy in improving growth performance and immune status, respectively (Vaseeharan and Thaya, 2014).

Among these plant-based interventions, Vietnamese balm (*Elsholtzia ciliata* (Thunb.) Hyland), belonging to the Lamiaceae family and native to Asia, holds promise due to its wide distribution across many countries (Guo et al., 2012). Vietnamese balm essential oils (EC EOs) have garnered attention owing to their therapeutic properties, encompassing antibacterial, anti-inflammatory, antioxidant, antiviral, and anticancer attributes (Luu et al., 2023). Extensive analysis through hydro-distillation and gas chromatography-mass spectrometry (GC-MS) has identified 572 volatile compounds from 21 species of *Elsholtzia* (Guo et al., 2012). Notable constituents include  $\alpha$ -pinene,  $\beta$ -pinene, acetophenone, caryophyllene oxide, carvacrol, and benzaldehyde (Luu et al., 2023). Consequently, EC EOs present potential as supplements to enhance fish growth. However, research pertaining to striped catfish at the juvenile stage remains scarce. This study was designed to investigate the effects of varying concentrations of EC EOs supplementation on the growth performance and survival rate of striped catfish at the juvenile stage.

## 2. Materials and Methods

### 2.1 Materials

#### 2.1.1 Plant

*Elsholtzia ciliata* (Thunb.) Hyland leaves were collected in the morning of July (temperature range, 25 - 28°C) from fresh plants growing in Long an province, Vietnam (Figure 1). The samples were air-dried for 5 days and weighed for essential oil extraction.

#### 2.1.2 Fish

A total of 750 striped catfish juvenile stages (40-45 days old) with a weight of 3-5 g were provided by the National Center for Freshwater Aquatic Breeds of Southern Vietnam under the Aquaculture Research Institute 2, An Thai Trung Commune, Cai Be, Tien Giang. Fish with natural bright colors, healthy activities, no deformities, and no signs of disease were selected for the experiment. Each experimental group was conducted with three replicates. All tanks are covered with plastic nets to reduce light intensity and prevent striped catfish from escaping.





**Figure 1.** *Elsholtzia ciliata* (Thunb.) Hyland in nursery garden (A) and collected leaves from fresh plant (B)

### 2.1.3 Ethical approval

This study's animal experiments did not involve any endangered or protected species. All animal experiments were conducted under the guidelines of the Department of Animal Health (TCVN 8710-15:2015, as described in TCVN 8710:2019). Also, this project considered all rules and regulations in conformity with the European Union directive for the protection of experimental animals (2010/63/EU).

## 2.2 Method

### 2.2.1 Oil analyses and quantification

Essential oils were extracted from the air-dried and ground plants (300 g each) using steam distillation for three hours in a Clevenger-type apparatus as recommended by European Pharmacopoeia (ver. 8.2, Monograph 2.8.12). The obtained essential oils were dried over anhydrous sodium sulfate, rotary vacuum evaporation removed the solvent, and stored at 4°C in the dark until further analysis. Measurements were carried out in three replications (Luu *et al.*, 2023).

Gas chromatography-mass spectrometry (GC-MS) was performed using an Agilent Technologies HP 6890N/HP 5973 MSD gas chromatography system with a capillary column DB5 - MS as the stationary phase. The inner diameter of the column was 30 cm × 0.25 mm × 0.25 μm. The sample injected into the detector was 0.1 μL. For the vaporization of aromatic components,

the column furnace temperature was set at 50 °C for 1 minute, followed by a gradual increase of 10°C/min to 250°C, where it was kept for 20 minutes. The sample injection chamber temperature was maintained at 250°C, and helium was used as the carrier gas (Alomar *et al.*, 2022). The identification of essential oil constituents was done by comparing the mass spectra with the Wiley and NIST library database, as well as the retention indices and previous literature (Belhacehemi *et al.*, 2022).

### 2.2.2 Growth experiment

The fish were acclimatized under laboratory conditions for a period of 15 days. Following this, they were kept in a composite tank with a capacity of 90 liters (75 × 45 × 45 cm) and fed three times per day with a formula diet at a rate of 3 - 4% of their body weight per day.

The feed used in the experiment was 32% protein industrial feed (Grobest, Vietnam), with a size of 2 mm per tablet. The essential oils for each treatment were added to the feed by diluting them with 10 mL of DMSO (Merck, Germany), followed by 10 mL of water. The mixture was then sprayed and mixed until the extract was fully absorbed into the feed and allowed to dry naturally for a period of 4 hours. Afterward, the food pellets were coated with ink oil and allowed to dry naturally for 8 hours at room temperature. The pellets were stored at 4°C throughout the experiment (Luu *et*

al., 2022).

For the plant extract-based diet feeding trial, the fish were randomly divided into 12 tanks with three treatments (every treatment/3 tank) (control (using basal feed), Ec 3%, Ec 5%). Each treatment was fed three times per day for two weeks at a rate of 3 - 4% of their body weight (7 am, 12 pm, and 4 pm) (Luu et al., 2022). Each tank, with a capacity of 90 L, contained 50 fish and operated on a 12-hour light cycle. After a 30-minute meal, uneaten feed (including waste feed) was collected and dried at 60°C until a stable weight was reached. Next, the weight difference between the fed amount and the uneaten feed was calculated. These values were also used to calculate feed conversion ratios (FCR).

Water replacement was conducted only in control at a level of 10% every two days. Water temperature, dissolved oxygen, and pH were monitored daily and maintained throughout the experimental period at  $30 \pm 2^\circ\text{C}$ ,  $5.7 \pm 0.01$  mg/L, and  $7.5 \pm 0.02$ , respectively. Temperature was measured using a thermometer, whereas pH and DO were measured using a portable pH meter and DO meter.

### 2.2.3 Sample collection and analysis parameters

Fish weight and length are measured before being placed into tanks (initial parameters). In addition, at the end of the experiment, which lasted 35 days, fish in each aquarium were weighted and counted to analyze growth indices (final parameters). Growth parameters such as final length and weight, net gain in length and weight, percentage final weight, daily weight and length gain, and specific growth rate (SGR), FCR, and FCE were measured using the following formulas (Tok et al., 2017).

Survival rate (%) = Final number of fish/initial number of fish x 100.....(i)

Weight gain (WG) = Final weight - initial .....(ii)

Percent weight gain (%WG) = (Final weight - initial weight) ÷ initial weight x 100.....(iii)

Percentage specific growth rate (SGR) = (Loge final body weight - loge initial body weight) ÷ Culture days x 100.....(iv)

Daily weight gain (DWG) = (Final weight - initial weight) ÷ Days of culture.....(v)

Length gain (LG) = Final length - initial .....(vi)

Percentage length gain (%WG) = (Final length - initial length) ÷ initial length x 100.....(vii)

Percentage specific growth rate (%SGRL) = (Loge final body length - loge initial body length) ÷ Culture days x 100.....(viii)

Daily length gain (DLG) = (Final length - initial length) ÷ Days of culture.....(ix)

Feed conversion ratio (FCR) = Dry feed fed ÷ live weight gain.....(x)

Feed efficiency (FCE) = Live weight gain ÷ dry feed fed.....(xi)

## 2.4 Analysis Data

The data of growth parameters of striped catfish at each time point were tested. The one-way ANOVA with a significant level of 5% ( $p < 0.05$ ) was used to compare the mean values at each time point. These analyses were performed on the SPSS statistical software to test the significant difference at probability  $P < 0.05$ . All data were presented as mean ± standard deviation (SD).

## 3. Results and Discussion

### 3.1 Essential Oil Yields and Chemical Composition of *Esholtzia ciliata* Essential Oils

The yield of *E. ciliata* EOs was 0.83% (w/w). The chemical components of EOs from *E. ciliata* by GC-MS are indicated in Table 1. Ec EOs contained a complex mixture consisting mainly of monoterpenes and sesquiterpenes. The essential oil contained higher amounts of monoterpenoids and sesquiterpenoids (Table 1). The major compounds of Ec EOs were (Z)-β-Farnesene (24.02%), followed by neral (15.06%), geranial (15.02%), and β-ocimene (13.61%) (Table 1).

### 3.2 Effect of *Elshotzia ciliata* Essential Oils in Growth Performance of Striped Catfish Juvenile Stage

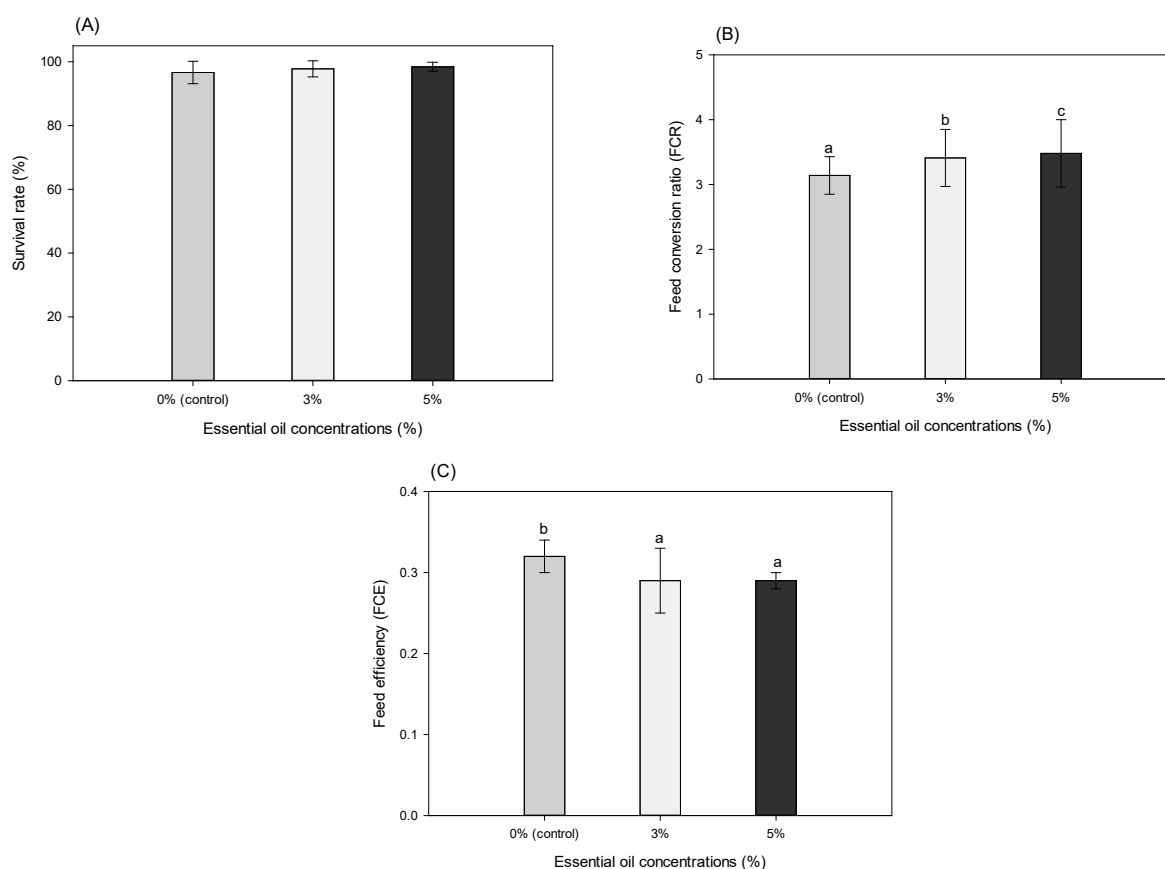
#### 3.2.1 Survival rate, feed conversion ratio and feed efficiency

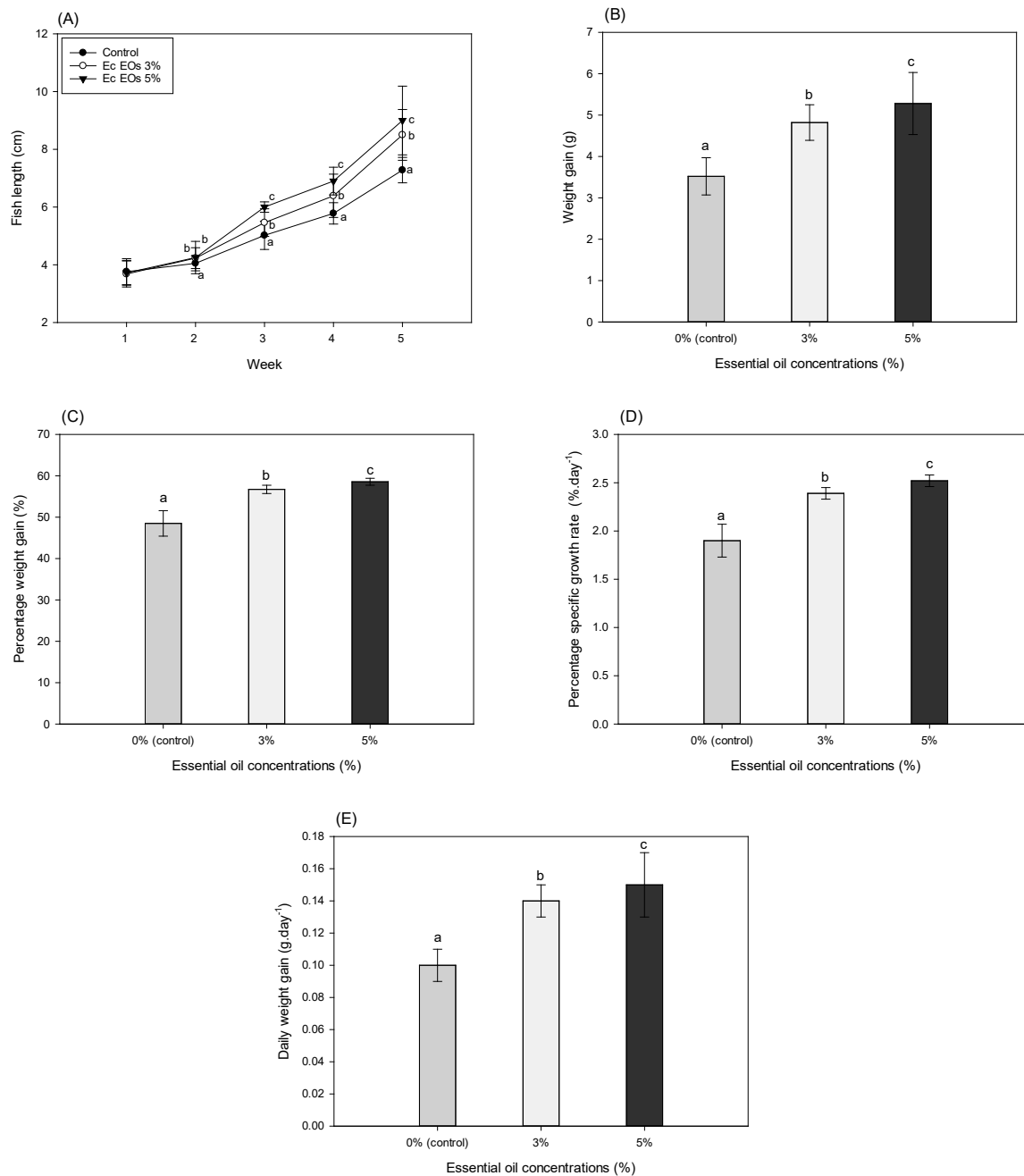
When evaluating fish growth performance, it is crucial to consider survival rate, feed conversion ratio, and feed efficiency (Abd El-Hack et al., 2022). The survival rate indicates the ability of fish to survive in response to a feed supplemented with other ingredients (Raa, 2000). In contrast, the feed conversion ratio measures the efficiency of converting feed into fish biomass. The results of the survival rate, feed conversion ratio, and feed efficiency of striped catfish after 35 days of culturing are shown in Figure 2.

The survival rate of fish ranged from approximately 96.44% to 97.78%, and there was no significant difference between the treatments ( $p > 0.05$ )

**Table 1.** Yields of essential oils and identification result of chemical components of EC EOs by GC-MS

No.	Compounds	Area (%)
1	1-Octen-3-ol	4.20 ± 0.05
2	6-Methyl-5-hepten-2-one	1.00 ± 0.04
3	β-Myrcene	0.20 ± 0.02
4	3-Octanol	0.27 ± 0.03
5	D-Limonene	1.22 ± 0.50
6	β-ocimene	13.61 ± 0.45
7	Acetophenone	2.11 ± 0.33
8	Linalool	0.23 ± 0.05
9	Isogeranial	0.94 ± 0.06
10	Nerol	1.15 ± 0.24
11	Neral	15.06 ± 0.76
12	Geraniol	0.52 ± 0.08
13	Geranial	15.02 ± 0.20
14	(Z)-Geranic acid. methyl ester	0.14 ± 0.03
15	(E)-Geranic acid. methyl ester	0.30 ± 0.01
16	Caryophyllene	9.59 ± 0.10
17	(E)-α-Bergamotene	0.26 ± 0.04
18	(Z)-β-Farnesene	24.02 ± 0.80
19	Humulene	1.33 ± 0.18
20	Germacrene D	2.13 ± 0.15
21	δ-Cadinene	0.14 ± 0.01
22	Nerolidol	0.69 ± 0.08
23	Caryophyllene oxide	0.30 ± 0.04
24	α-Cadinol	0.20 ± 0.07
Total		94.63 ± 0.16
Essential oil yields (%)		0.83 ± 0.17

**Figure 2.** Effects of dietary EC EOs supplement at different concentrations on (A) survival rate; (B) feed conversion ratio (FCR); (C) feed efficiency (FCE). Values are mean ± SD, different letters indicate significant differences between treatments (p < 0.05).



**Figure 3.** Effects of dietary EC EOs supplement at different concentrations on (A) average weight; (B) weight gain (WG); (C) percentage weight gain (%WG); (D) percentage specific growth rate (%SGR); (E) daily weight gain (DWG). Values are mean  $\pm$  SD, different letters indicate significant differences between treatments ( $p < 0.05$ ).

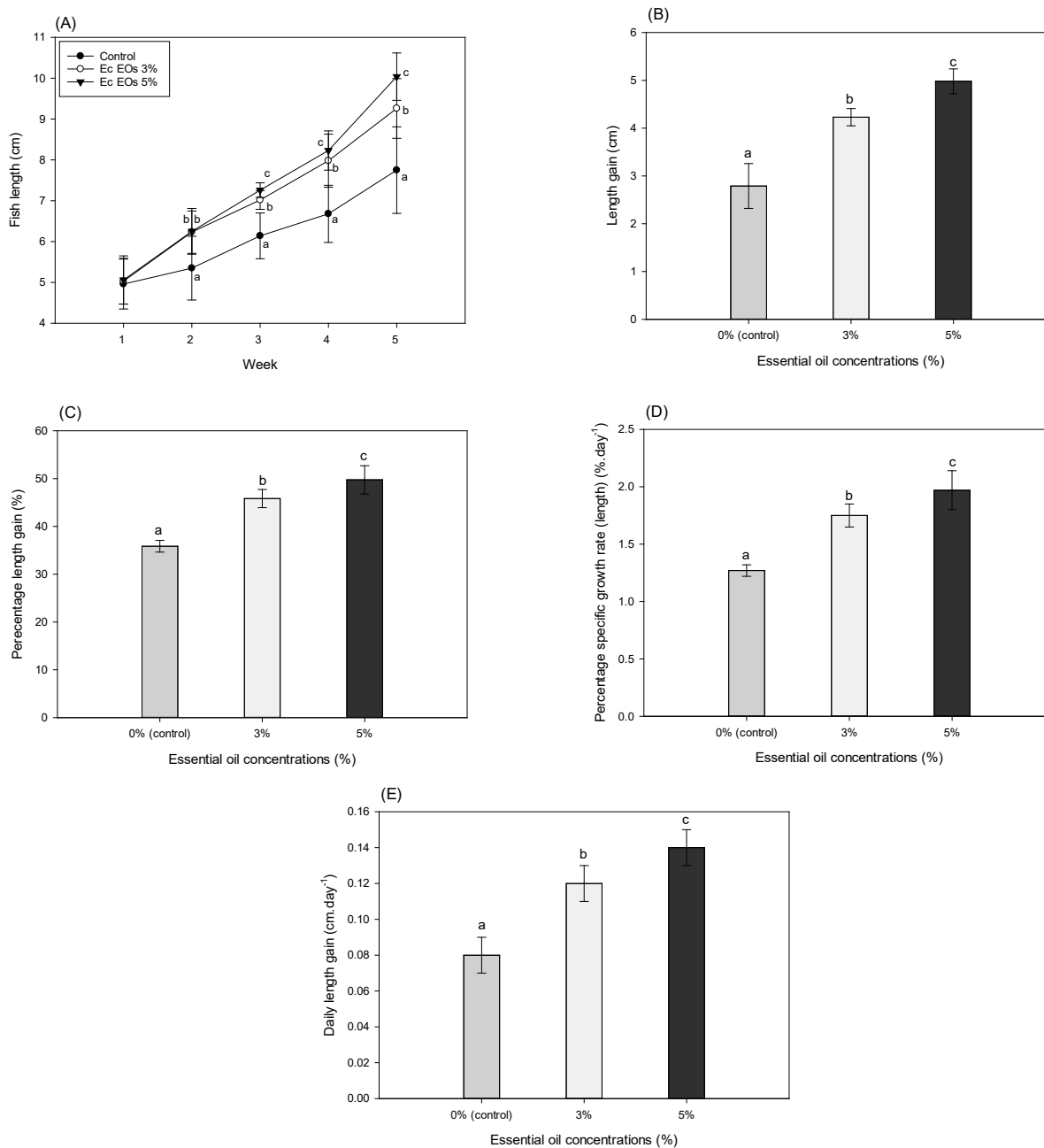
(Figure 2A). Experimental results showed that there was a statistical difference in the FCR values of the different treatments when feeding fish with supplemented EC EOs (Figure 2B, C). The lowest FCR value ( $3.14 \pm 0.29$ ) was observed in the control treatment, and the highest FCR value ( $3.48 \pm 0.52$ ) was observed in the treatment with EC EOs 5% (Figure 2B). In the present study, the high FCR value was due to the fact that the use of fish feed in the treatments supplemented with EC EOs was less efficient than the control, with fewer nutrients extracted

from the feed and turned into meat. The feed conversion efficiency did not change significantly between the different treatments, showing the highest FCE value ( $0.32 \pm 0.02$ ) in the control treatment and the FCE value fluctuating around 0.29 for the treatment of adding EC EOs to fish feed (Figure 2C).

### 3.2.2 Fish weight

Fish weight is essential for evaluating fish growth performance (Shekarabi et al., 2020). This data directly





**Figure 4.** Effects of dietary ECEO supplement at different concentrations on (A) average length; (B) length gain (LG); (C) percentage length gain (%LG); (D) percentage specific growth rate (%SGRL); (E) daily length gain (DLG). Values are mean  $\pm$  SD, different letters indicate significant differences between treatments ( $p < 0.05$ ).

indicates fish growth and the potential impact of EC EOs added to the feed. Results of the effect of EC EOs supplemented diets on striped catfish growth indices (weight) after 35 days are given in Figure 3.

At the beginning of the experiment, the fish weight ranged from  $3.68 \pm 0.45$  g to  $3.76 \pm 0.45$  g, with no statistically significant difference ( $p > 0.05$ ) (Figure 3A). After 35 days of the experiment, striped catfish in the treatments supplemented with Ec EOs (3% and 5%) had an average weight gain of  $4.82 \pm 0.43$  g and  $5.28 \pm 0.75$  g, respectively, which was significantly different

from fish in the control treatment ( $3.52 \pm 0.45$  g) ( $p < 0.05$ ) (Figure 3B). The specific growth rate (SRG) and daily weight gain (DWG) of fish in the treatments supplemented with EC EOs were  $2.39 \pm 0.06$  to  $2.52 \pm 0.06$  %.day<sup>-1</sup>;  $0.14 \pm 0.01$  to  $0.15 \pm 0.01$  g.day<sup>-1</sup>, respectively, both of which were higher than the control ( $0.10 \pm 0.01$  g.day<sup>-1</sup>) with statistical significance ( $p < 0.05$ ) (Figure 3C, D, E). Fish fed with 5% Ec EOs had the highest growth indices (weight).

### 3.2.3 Fish length

In addition to fish weight, another crucial criterion

for evaluating fish growth performance is measuring fish length (Cottrell et al., 2020). Fish length parameters can assess the fish growths and efficiency of EC EOs supplementation in fish feed. Results of the effect of EC EOs supplemented diets on striped catfish growth indices (length) after 35 days are given in Figure 4.

At the start of the experiment, the length of the fish ranged from  $4.96 \pm 0.61$  to  $5.06 \pm 0.59$  cm, and there was no significant difference among the groups ( $p > 0.05$ ) (Figure 4A). After 35 days, in addition to weight gain, the treatments showed differences in length growth, which is indicative of an increase in volume. The highest final length was observed in the treatment supplemented with 5% EC EOs ( $10.04 \pm 0.58$  cm) (Figure 4B). In the treatments with EC EOs, length gain ranged from 4.23 to 4.98 cm, with a specific growth rate ( $SRG_L$ ) of 1.75 to  $1.97\% \cdot \text{day}^{-1}$  and a daily length gain (DLG) reached 0.12 to  $0.14 \text{ cm} \cdot \text{day}^{-1}$ . These growth rates were significantly different from those in the control treatment ( $p < 0.05$ ) (Figure 4C, D, E).

### 3.3 Discussion

EC EOs have been extensively researched in content, composition, and uses. In this study, the EOs yields of *E. ciliata* grown in Vietnam were 0.83 (%) higher than the study of Pingzhao et al. (2016) (0.03%). In addition, this set of components is quite similar to those found by Nguyen et al. (2011) found that the main constituents were neral (15.2–20.5%), geranial (19.5–26.5%), limonene (10.9–14.2%), and (Z)- $\beta$ -farnesene (10.8–11.7%). Similarly, EC Eos in Le et al. (2022) contains 41 identified compounds, accounting for 98.% of the oil's chemical composition. The predominant components were found to be citral (40.2%),  $\beta$ -(E)-ocimene (14.0%), linalool (8.3%), 1-octen-3-ol (7.1%) and  $\beta$ -(E)-farnesene (6.2%). (Le et al., 2022).

Functional and nutritional supplements such as EOs and balanced diets can stimulate immune responses in fish. In this study, the immunomodulatory effects of EC EOs were evaluated by examining alterations in growth parameters. The supplementation of EC EOs to the fish feed did not influence the survival rate of the experimental catfish. The survival rate observed in fish supplemented with Ec EOs at different concentrations was higher than that reported by Pham et al. (2018) when GroBiotic®-A was added to catfish feed (72.9% to 83.3%) (Pham et al., 2018). All growth parameters in the two treatment groups (supplemented with EC EOs) are higher than the control and statically different. Furthermore, the growth results were lower compared to the study by Bui and Nguyen (2020) involving inulin supplementation in the striped catfish diet, where DWG

ranged from 0.45 to 0.70 (Bui and Nguyen, 2020). Comparatively, the addition of EC EOs resulted in lower DWG ( $0.20 - 0.25 \text{ g} \cdot \text{day}^{-1}$  compared to  $0.14 - 0.15 \text{ g} \cdot \text{day}^{-1}$ ) than a study by Ho et al. (2020) where organic minerals were added to the feed for mass growth, potentially due to differences in the experimental fish stages (Ho et al., 2020).

Numerous herbs are recognized for their growth-promoting effects in aquatic animals, attributed to their efficient feed conversion, enhanced protein metabolism, and energy retention. Many plants encompass a spectrum of active components, including alkaloids, steroids, phenols, tannins, terpenoids, saponins, glycosides, flavonoids, and polysaccharides, among others (Sivaram et al., 2004; Harikrishnan et al., 2011). Our findings revealed a gradual increase in daily length growth in treatments supplemented with EC EOs, significantly surpassing the control group. This suggests that EC EOs affected not only the weight but also the length of the striped catfish. During the transition from fry growth to the juvenile stage, fish length increases rapidly, and the growth rates of weight and length are positively correlated. In addition, adding plant essential oils to food will increase the digestibility of nutrients in food (Zeng et al., 2015) and stimulate the fish's pancreas to secrete enzymes such as protease and lipase, increasing the ability to absorb nutrients (Imani et al., 2017; Ghafarifarsani et al., 2022) this lead to the FCR of fish in treatment supplemented with EC EOs was lower than the control. Therefore, in the same experiment, a rapid increase in weight growth rate would correspondingly boost the length growth rate. *E. ciliata* is known to contain various chemical components, including essential oil, elsholtzia ketone, flavonoids, steroids, and triterpenes (Kassuya et al., 2005; Luu et al., 2023). Given the diverse compounds and secondary metabolites present, EC EOs have been reported to exhibit immunomodulatory activities (Kassuya et al., 2005; Jantan et al., 2014). Our results demonstrate that fish in treatments fed with supplemented essential oil exhibited a higher growth rate than the control treatment. For example, Abdel-Hakim et al. (2010) demonstrated that adding garlic to tilapia feed during the rearing period (22 weeks) enhanced survival, growth, feed conversion, and protein utilization efficiency (Abdel-Hakim et al., 2010). Additionally, the inclusion of herbs like *Sesbania grandiflora*, *Moringa oleifera*, *Plectranthus amboinicus*, and *Ocimum basilicum* in the feed promoted growth in *Oreochromis mossambicus* (Lee and Gao, 2012). Recently, Luu et al. (2022) established that adding garlic and Vietnamese balm to the feed improved the growth parameters of striped catfish at the juvenile stage compared to the control treatment (Luu et al., 2022).

### 4. Conclusion



Fresh leaves of *E. ciliata* were hydro-distilled to yield essential oil of 0.83% dry weight, respectively. GC-MS analyses revealed that EC EOs had a complex mixture of compounds that consisted of monoterpenes and sesquiterpenes. (Z)- $\beta$ -Farnesene (24.02%), followed by neral (15.06%), geranial (15.02%), and  $\beta$ -ocimene (13.61%) were accounted as the major components of EC EOs. Based on surveying the effect of adding Ec EOs to the striped catfish diet, some conclusions were drawn. The addition of herbal extracts to the feed increased growth parameters such as WG, DWG, SGR, %WG, LG, DLG, SGR<sub>L</sub>, %LG, and FCR in the juvenile stage. The treatment with 5% EC EOs has the potential to be used as a feed supplement to support fish growth with the best growth parameters and has a statistical difference compared to the remaining treatments ( $p < 0.05$ ). However, there is no statistical difference between Ec 3% and Ec 5% in the feed efficiency criterion.

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### Authors' Contributions

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### Conflict of Interest

The authors declare that they have no competing interests.

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