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Effects of foliar application of zinc sulfate on growth, yield and essential oil of Perilla (*Perilla frutescens* L.) and holy basil (*Ocimum sanctum* L.)

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ABSTRACT

This study was conducted on gray soil at the research station of the Faculty of Agronomy, Nong Lam University - Ho Chi Minh City, Viet Nam. The objective of the study was to investigate the effects of foliar zinc sulfate application on perilla and holy basil cultivation. For perilla, several morphological characteristics, including plant height, stem diameter, leaf length, leaf width, and leaf number on the main stem, showed statistically significant differences between the control and the 2.0 g/L $ZnSO_4.7H_2O$ treatment. In contrast, only plant height in holy basil was significantly affected by $ZnSO_4.7H_2O$ spray at 30 days after cutting. Regarding other physiological traits, both perilla and holy basil were able to maintain high levels of chlorophyll, carotenoid, total protein, and essential oil content. These findings suggest that zinc supplementation via foliar nutrition is a promising approach to improve not only agricultural yield but also the economic efficiency of medicinal plant cultivation.

1. INTRODUCTION

Perilla (*Perilla frutescens* (L.) Britt), an edible-medicinal herb widely cultivated in many Asian countries, belongs to the Lamiaceae family, like *Ocimum sanctum* L., commonly known as holy basil. Perilla has been utilized for culinary, flavoring, and medicinal purposes for thousands of years (Mun et al., 2020). With over 100 medically active compounds such as triterpenoids, anthocyanin, and polyphenols have been identified in the perilla plant (Yu et al., 2017), leaves of perilla are rich in bioactive phytochemicals, which contribute to its antioxidant, anti-inflammatory, and anti-allergic properties (Wu et al., 2023). Similarly, holy basil, a perennial herb well-suited to hot and humid climates like Vietnam, produces many active ingredients with antioxidant, anti-allergic, cancer-preventive and hepatoprotective properties (Žabka et al., 2021).

Zinc, an essential micronutrient in plants, plays key roles in modulating the activities of various enzymes and hormones, facilitating metabolic processes of macromolecules, maintaining protein structures, and regulating gene expression (Sheoran et al., 2021). The bioavailability of Zn in soils is often reduced due to the binding of Zn ions to insoluble compounds, low Zn content, high pH, high calcite, and abundance of organic matter, Ca, Mg, Na, HCO_3^-, and PO_4^{3-} (Alloway, 2009). Zinc-linked enzyme activities are crucial for protein synthesis, maintaining cellular membrane integrity, regulating auxin synthesis, and pollen formation (Marschner,

2012). Zinc deficiency can cause plant abnormalities, notably stunted growth, and chlorosis of leaves. Zinc is also essential for gene expression in response to environmental stresses in plants (Cakmak, 2000b). Micronutrient deficiency is a major concern, especially in calcareous and saline soils with high pH (Tavallaliet al., 2010). Zinc deficiency is widespread in sandy soils, peat soils, and soils rich in phosphorus and silicon. Many studies have shown that zinc supplementation, alone or in combination with other biostimulants/ phytohormones/nanoparticles can increase crop yield, photosynthetic activity, nutrient uptake, resistance to adverse environmental conditions, and phenolic compounds and essential oil contents (both quantity and quality) (Vojodi Mehrabani et al., 2017; Al-Zahrani et al., 2022; Ahmad et al., 2023; Nekoukhou et al., 2024).

ZnO nanoparticles (ZnO NP) were sprayed on the leaves of perilla to alleviate the adverse effects of cadmium toxicity (Wang et al., 2023). The results showed that ZnO NP promoted the growth of perilla seedlings, decreased Cd accumulation and increased organic acids (maleic acid, citric acid, and malic acid) and amino acids (glutamate, phenylalanine, and arginine). In a study by Moghimipour et al. (2017), zinc chelate at concentrations of 0, 0.5, 1 and 1.5 g/L and zinc sulphate ($ZnSO_4$) at concentrations of 1 and 1.5 g/L were used to spray on holy basil. The experimental results showed that there was a correlation between the amount of zinc used and the amount of essential oil, specifically, the essential oil content was highest in the treatment of 1.5 g/L zinc chelate and lowest in the control treatment (no spraying) (Moghimipour et al., 2017). However, because there were no statistically significant differences between the 1 and 1.5 g/L zinc chelate treatments and the 1.5 g/L $ZnSO_4$ treatment in terms of the main components of the essential oil and the yield of harvested leaves and stems, the 1.5 g/L $ZnSO_4$ treatment was recommended to achieve higher economic efficiency. On *Pistacia lentiscus* var. chia Duham, foliar application of $ZnSO_4$ increased the content of β-myrcene, germacrene-D and α-pinene in the essential oil (Bayram et al., 2022). In general, the use of foliar fertilizers in general and $ZnSO_4$ in particular significantly increases yield, resistance to insects and diseases, drought tolerance and product quality (Shahrajabian et al., 2022). In Vietnam, relatively few studies have investigated the effects of zinc on medicinal and aromatic plants, including perilla and holy basil. This study aimed to address this gap by determining the effects of foliar zinc sulfate ($ZnSO_4$) application on the growth, biochemical parameters, and essential oil yield of these two plants.

2. MATERIALS AND METHOD

2.1. Materials

Perilla seeds (*Perilla frutescens* (L.) Britt. var. *crispa f. atropurpurea*), characterized by reddish-purple stems, dark purple upper leaves, and light purple undersides, were obtained from Châu Giang Co., Ltd. (purity: 99.1%, 1000-seed weight: 3.35 g). Seeds were sown in a nursery for 30 days and then transplanted to the field when seedlings had 4-6 leaves, reached 4-6 cm in height, and displayed a single, healthy plant per transplanting hole. These seedlings were free of pests and diseases with straight stems. The perilla plantation received fertilizer at a rate of 450 kg/ha urea, 625 kg/ha superphosphate (16% P_2O_5), and 160 kg/ha KCl. Pre-transplanting fertilization included 5 tons/ha manure, 300 kg/ha lime, and 625 kg/ha superphosphate. The remaining fertilizer mixture, consisting of urea and KCl, was divided into three equal side-dressings, each containing one-third of the total nitrogen and potassium. These applications were made at 10, 35, and 55 DAT. Purple basil seedlings (obtained from Sao Viet Seed Company) were raised in trays for 40–45 days before transplanting. Suitable seedlings for transplanting were 4-8 cm tall, possessed 3–4 pairs of true leaves, exhibited an upright and healthy growth pattern, and were free of pests and diseases with no primary branches. The basal fertilizer application in the experiment included 500 kg/ha lime powder, 10 ton/ha composted cow manure, 260 kg/ha ammonium sulfate, 312 kg/ha superphosphate, and 82 kg/ha potassium chloride. Lime powder was applied 10 days before transplanting. The remaining nitrogen, phosphorus, and potassium fertilizers were divided into four applications: all superphosphate and composted cow manure were applied 3 days before mulching (first application); 1/4 of the nitrogen and potassium fertilizers were applied as a top dressing 10 days after transplanting (DAT) (second application); 1/4 of the nitrogen and potassium fertilizers were applied 3 days after the first harvest (third application); and 2/4 of the nitrogen and potassium fertilizers were applied 15 days after the first harvest (fourth application).

Table 1. Climatological conditions in the experimental area

Month	Average temperature (°C)	Rainfall (mm/month)	Average moisture (%)	Sunshine (hour)
Feb.2023	28.2	9.9	71	198.3
Mar.2023	28.3	0.0	73	236.4
Apr.2023	30.4	83.6	76	194.8
May.2023	30.1	37.0	78	182.6

(Source: Vietnam Institute of Meteorology, Hydrology and Climate Change, 2023a, 2023b, 2023c, 2023d)

Generally, the weather is quite favorable for the growth and development of perilla and holy basil. However, due to the low rainfall in February and March, irrigation was required for the plants to grow and develop. Additionally, the cultivated soil was a sandy loam, consisting of 82% sand, 10% silt, and 8% clay. It had a pH of 5.7, 1.21% organic matter, 0.05% total nitrogen (N), 13.1 (mg/100 g) available P_2O_5, 0.031% sulfur (S) and 14.9 mg/kg dry soil Zn (Source: Research Institute for Biotechnology and Environment, Nong Lam University, Viet Nam). Jalil et al. reported that the total Zn^{2+} concentration of soils typically ranges from 3 to 790 mg/kg soil, with an optimal level of 100 mg/kg (Jalil et al., 2023). Our physicochemical analysis revealed that the soil is acidic and deficient in both total nitrogen (N) and zinc. Therefore, the supplementation of additional zinc to plants through foliar application is a suitable approach to promote plant growth.

2.2. Methods

2.2.1. Experimental design

A randomized complete block design (RCBD) with three replicates per treatment was used for both perilla and holy basil (details in Figure 1). Each treatment had a plot size of 8.19 m² and a planting distance of 30 cm (distance between plants in the same row) x 35 cm (distance between rows). $ZnSO_4.7H_2O$ with 98% purity was purchased from VMC Group. Perilla received four sprays of 400 L/ha ZnSO4.7H_2O at 15, 35, 55, and 75 days after transplanting. Holy basil received nSO4.7H_2O four times throughout the season ((10 & 20 days after transplantation (DAT), 10 & 20 days after cutting (DAC)) at the same rate (400 L/ha)

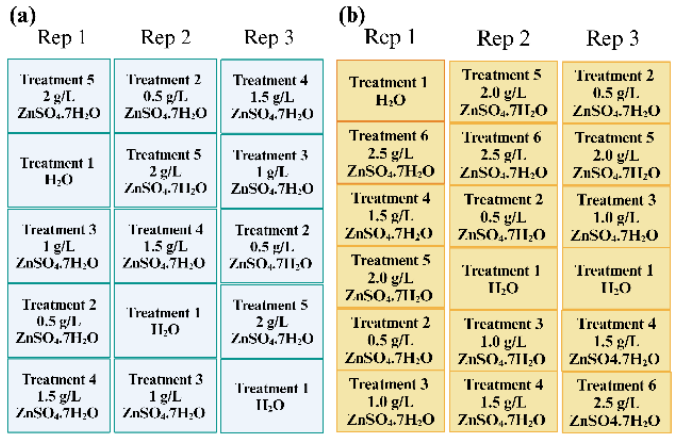

Figure 1. Schematic figure of experimental design for (a) perilla and (b) holy basil

2.2.2. Measurement of growth and development in perilla and holy basil plants

The growth and development of perilla were monitored every 20 days, starting from 20 DAT. Plant height was measured from the base of the main stem to the tip of the highest leaf using a ruler. Main stem diameter was measured at a position 5 cm above the ground using a caliper. The number of true leaves on the main stem was counted. Leaf length and width were measured at 45 days after transplanting on the fifth true leaf from the petiole to the tip and at the widest point, respectively. The average leaf length was then calculated. Finally, all primary branches on the plant were counted. The harvesting time for perilla occurred at 85 DAT.

For holy basil, different measurement schedules were used for the first and second growth cycles. Plant height was measured every 10 days from 20 DAT in the first cycle, from the lowest branching position to the growth tip. In the second cycle, it was measured every 10 DAC, from the lowest branching position to the highest leaf tip. Stem diameter was consistently measured at 5 cm above the ground using a caliper throughout both cycles. The number of leaves on the main stem with visible petioles and blades was counted every 10 days, starting from 20 DAT in the first cycle and 10 DAC in the second cycle. Similarly, the number of primary branches (longer than 1 cm) was counted every 10 days following the same schedule as the leaves. Leaf length and width were measured at 30 DAT in the first harvest and 30 DAC in the second harvest. Measurements were taken on the fifth leaf pair from the top down, from the petiole to the leaf tip for length and at the widest point for width. The harvesting time for holy basil was determined when more than 50% of the plants had bloomed. Specifically, the first harvest occurred between 31.7 and 32.3 DAT. The second harvest was earlier, taking place between 30.6 and 31.7 DAC. The holy basil plants were harvested by cutting them 7-10 cm above the ground with scissors.

2.2.3. Leaf chlorophyll index (SPAD)

Although SPAD, chlorophyll and carotenoid content were measured in holy basil at both the first and second harvests, only data from the second harvest are presented here. This is because there were no significant differences observed between the values from the first harvest and the second harvest. Chlorophyll content was measured using a handheld SPAD-502 Plus chlorophyll meter. For perilla, five random leaves on the target plant were measured, and for holy basil, measurements were taken between the leaves of the fifth leaf pair from the top down at 25 DAT in the first harvest and 25 DAC in the second harvest.

2.2.4. Chlorophyll a, b, and carotenoid content

One gram of perilla leaf was ground with 10 mL of 96% ethanol. The mixture was then simmered at 70°C for 10 minutes, followed by centrifugation at 2500 rpm for 10 minutes. The optical density (OD) of the supernatant was measured at three wavelengths (470 nm, 648 nm, and 664 nm) using a spectrophotometer (UV–2602, USA). The first and second holy basil leaves from the top were collected at harvest time. One gram of leaf tissue was ground with 10 mL of 96% ethanol, centrifuged at 2500 rpm for 10 minutes, and the supernatant was collected. The OD of the supernatant was measured using the same protocol as for perilla.

The chlorophyll a, chlorophyll b and carotenoid contents were determined using the following formulas (Lichtenthaler and Buschman, 2001) (Lichtenthaler& Buschmann, 2001)

$Chl_a = 13.36A_{664} – 5.19A_{648}$

$Chl_b = 27.43A_{648} – 8.12A_{664}$

$Carotenoid = (1000A_{470} – 2.13Chl_a – 97.64Chl_b)/209$

Where: Chl_a: Chlorophyll a; Chl_b: Chlorophyll b; A_{664}: OD value at 664 nm; A_{648}: OD value at 648 nm.

2.2.5. Protein content in leaves

The total protein content of holy basil at both the first and second harvests was measured. However, only data from the second harvest are presented here as there were no significant differences observed between the values from the first harvest and the second harvest. The essential oil and yield represent accumulative data from both the first and the second harvests. Protein content was measured using the same protocol for both perilla and holy basil. One gram of sample was ground in 1 mL of potassium phosphate buffer (0.1 M, pH 7.5) containing 3 mM EDTA and 0.5% PVP. The mixture was centrifuged at 6000 rpm for 20 minutes, and the protein extract was collected. To each test tube containing 0.5 mL of the extract, 1 mL of Bradford reagent was added. The optical density was measured at 595 nm using a spectrophotometer (UV–2602, USA). Protein content was determined by comparing the absorbance with a standard curve of albumin. The results represent the average value of the three measurements.

2.2.6. Perilla and holy basil essential oil

Fresh perilla or holy basil leaves were harvested early in the morning, washed, and cut into small pieces (approximately 3 cm). For both plants, 100 grams of the chopped material was added to 500 ml of water in a flask. The mixture was boiled for 5 hours. The steam, carrying the essential oil, was condensed using a condenser system and collected. A glass tube was then used to extract the essential oil, which was subsequently stored in a bottle at a temperature of 0-4°C.

The essential oil content was determined using the following formula:

Essential oil content (mL/100 g) = Amount of essential oil obtained (mL) / Weight of stem and leaves (100 g) used for distillation x 100 (Pham et al., 2022).

2.2.7. Economic analysis

Total revenue (VND/ha/harvest) = Essential oil yield (L/ha) * Selling price at harvest time (VND)

Total cost (VND/ha/harvest) = General costs (seeds, fertilizers, labor, other materials) + specific costs ($ZnSO_4.7H_2O$)

Profit (VND/ha/harvest) = Total revenue – Total cost

Profit margin (times)= Profit/Total cost

2.2.8. Statistical analysis

Data analysis and data visualization were performed using R 4.4.0. Differences between treatments were tested using the least significant difference (LSD) test at the probability of 0.05.

3. RESULTS AND DISCUSSION

3.1. Results

At 80 DAT, significant differences in plant height were observed between the treatments. The highest plant height was achieved with the highest zinc foliar application (2 g/L $ZnSO_4.7H_2O$), while the control treatment sprayed with water had the lowest plant height. No statistically significant differences in plant height were found among the 0.5 g/L, 1.0 g/L, 1.5 g/L and 2.0 g/L $ZnSO_4.7H_2O$ treatments. Similarly, significant differences in stem diameter, leaf length, and leaf width were observed between the 2 g/L treatment and the control, suggesting positive effects of zinc supplementation on important growth parameters of perilla. However, leaf number and primary branches did not differ significantly among treatments.

Table 2. Effects of $ZnSO_4.7H_2O$ foliar application on plant height, diameter, leaf length and leaf width of perilla

$ZnSO_4.7H_2O$ (g/L)	Plant height (cm) (80 DAT)	Stem diameter (mm) (80 DAT)	Leaf length (cm) (45 DAT)	Leaf width (cm) (45 DAT)	Leaf number (80 DAT)	Primary branches (80 DAT)
0 (control)	138.3^{b}	15.8^{b}	17.0^{b}	8.3^{b}	21.6	32.6
0.5	141.8^{ab}	16.3^{ab}	18.2^{ab}	8.7^{ab}	22.2	33.0
1.0	143.7^{ab}	16.9^{ab}	18.7^{ab}	8.9^{ab}	22.7	33.2
1.5	145.4^{a}	17.6^{ab}	18.8^{ab}	9.1^{ab}	23.1	33.4
2.0	148.0^{a}	18.5^{a}	20.1^{a}	9.7^{a}	23.6	34.4
CV (%)	2.3	4.2	5.3	4.5	9.4	6.7
Fvalue	4.2^{*}	6.6^{*}	3.9^{*}	4.7^{*}	0.4^{ns}	0.3^{ns}

In each column, means followed by the same letter are not significantly different at the 5% level; *: significant at 5% level; ^{ns}: not significant.

Table 3. Effects of $ZnSO_4.7H_2O$ foliar application on plant height, diameter, leaf length and leaf width of holy basil

$ZnSO_4.7H_2O$ (g/L)	Plant height (cm) (30 DAT)	Plant height (cm) (30 DAC)	Stem diameter (mm) (30 DAT)	Stem diameter (mm) (30 DAC)	Leaf length (mm) (30 DAT)	Leaf length (mm) (30 DAC)	Leaf width (mm) (30 DAT)	Leaf width (mm) (30 DAC)
0	41.8	45.4^{b}	5.9	8.2	45.5	44.8	26.6	24.5
0.5	42.8	46.7^{ab}	6.1	8.4	45.6	44.9	26.8	24.9
1.0	43.2	45.9^{ab}	6.1	8.4	47.0	45.9	26.9	25.5
1.5	43.1	47.4^{ab}	6.2	8.5	47.2	46.1	27.5	25.8
2.0	43.7	47.6^{ab}	6.1	8.4	46.5	46.4	27.8	26.0
2.5	44.1	48.3^{a}	6.3	8.4	47.9	46.7	28.3	26.1
CV (%)	7.7	7.4	11.4	8.7	10.2	7.6	9.5	9.7
Fvalue	1.7^{ns}	3.0^{*}	1.4^{ns}	0.8^{ns}	1.3^{ns}	1.6^{ns}	1.9^{ns}	2.0^{ns}

In each column, means followed by the same letter are not significantly different at the 5% level; *: significant at 5% level; ^{ns}: not significant.

In general, no statistically significant differences were found in plant growth parameters, including plant height (measured at 30 DAT), stem diameter (measured at 30 DAT and 30 DAC), leaf length (measured at 30 DAT and 30 DAC), and leaf width (measured at 30 DAT and 30 DAC). However, a significant difference in plant height was observed at 30 DAC between the control and the 2.5 g/L $ZnSO_4.7H_2O$ treatment.

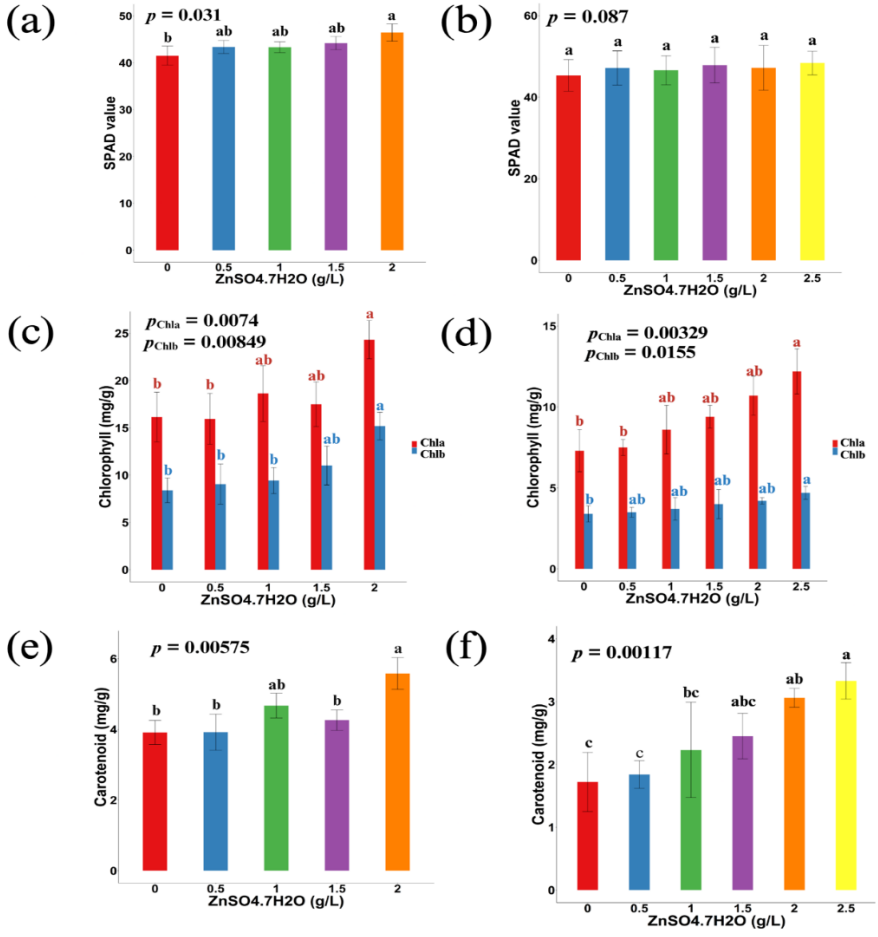

Figure 2. Effects of $ZnSO_4.7H_2O$ foliar spraying on SPAD value (a, b), chlorophyll a, b (c, d) and carotenoid (e, f) of perilla and holy basil.

We found a strong correlation between zinc concentrations and PSAD, chlorophyll, essential oil, total protein, and yield of perilla. Similar findings were found in holy basil. The results of our study aligned with previous studies, indicating the essential roles of zinc supplementation in improving plant growth and its important biochemical features. At elevated concentrations (2-2.5 g/L), $ZnSO_4.7H_2O$ did not demonstrate toxicity in perilla and holy basil.

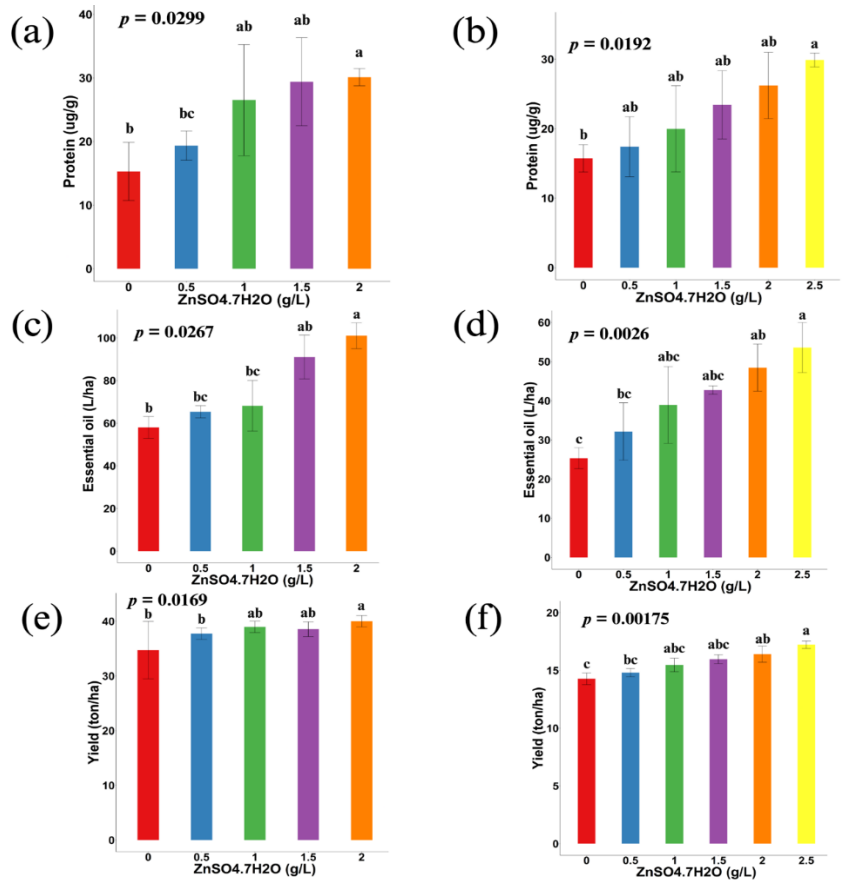

Figure 3. Effects of $ZnSO_4.7H_2O$ foliar spray on total protein (a, b), essential oil content (c, d) and yield (e, f) of perilla and holy basil.

(Note: The total protein content (b) of holy basil at both the first and second harvests was measured. However, only data from the second harvest are presented here, as there were no significant differences observed between the values from the first harvest and the second harvest. The essential oil and yield represent accumulative data from both the first and the second harvests)

Table 4. Economic efficiency of perilla and holy basil crops

$ZnSO_4.7H_2O$ (g/L)	Total revenue (million VND/ha/crop)	Total cost (million VND/ha/crop)	Profit (million VND/ha/crop)	Profit margin (time)
Perilla				
0 (control)	231.96	204.59	27.37	0.13
0.5	261.48	210.44	51.04	0.24
1.0	272.56	219.45	53.11	0.24
1.5	364.12	219.28	144.84	0.66
2.0	404.00	231.11	172.89	0.73
Holy basil				
0 (control)	101.19	85.45	15.74	0.18
0.5	128.44	85.79	42.65	0.5
1.0	155.68	86.27	69.41	0.8
1.5	170.88	86.65	84.24	0.97
2.0	193.74	86.93	106.81	1.23
2.5	214.22	87.34	126.88	1.45

Foliar application of $ZnSO_4 \cdot 7H_2O$ positively affected the protein content, essential oil content, and yield of perilla. These parameters were consistently higher in the treated plants compared to the control plants. Although no significant differences were found between the control, 0.5, 1.0, and 1.5 g/L $ZnSO_4.7H_2O$ treatments, the application of 2.0 g/L $ZnSO_4.7H_2O$ resulted in significantly higher protein, essential oil content, and yield than the control ($p < 0.05$). For holy basil, foliar application of $ZnSO_4.7H_2O$ resulted in dramatic increases in essential oil content and yield at concentrations of 2.0-2.5 g/L compared to the control ($p < 0.01$). Protein content also showed a significant increase, with the highest level observed at 2.5 g/L ZnSO4.7H2O ($p < 0.05$)

Economic analysis of the perilla crop showed that the foliar spraying with 2.5 g/L $ZnSO_4.7H_2O$ had the highest investment cost, while water spraying was the least expensive. However, the use of zinc resulted in higher profits for farmers. This trend was also observed with the holy basil crop, where the highest concentration of $ZnSO_4.7H_2O$ (2.5 g/L) yielded the greatest profit.

3.2. Discussion

The absorption of foliar-applied zinc varies among plants due to differences in leaf waxy layer, chemical composition and structure of cuticles, and density of stomates and trichomes (Sturikova et al., 2018). It also strongly depends on the form of zinc applied. The present study showed the supportive role of $ZnSO_4.7H_2O$ in enhancing growth, photosynthetic pigments, and the essential oil content of perilla and holy basil. An adequate Zn concentration protects plants from photooxidative damage caused by reactive oxygen species (ROS) in chloroplasts (Cakmak, 2000a). The increased plant biomass can be attributed by the protective effect of Zn on the stability of cell membranes (Ghanepour S et al., 2015) and its ability to induce the production of plant growth hormones like auxin (IAA) (Mazaheri Tirani et al., 2019).

Zinc deficiency in human diets is a major burden on human health, particularly in developing countries (Joy et al., 2015). Consequently, increasing the zinc concentration in grains is a global challenge and a key biofortification goal to improve human health (Chen et al., 2017).As soil properties determine the availability of Zn for plant uptake, supplementation of Zn through foliar spray is likely an effective way to supply additional source of Zn to plants, ultimately contributing to improved human health through biofortification. In previous studies, foliar Zn application has been widely employed due to its efficiency (Dimkpa et al., 2013; Ghanepour S et al., 2015; Khan & Siddiqui, 2021; Mazaheri Tirani et al., 2019; Shemi et al., 2021; Song et al., 2021). Another concern is the phytotoxicity caused by elevated Zn concentrations, which may reduce gemination rate, inhibit plant growth, chloroplast function, alter the activity of antioxidant enzymes, and damage cell membrane (García-Gómez et al., 2018). However, in our study, we did not observe any signs of phytotoxicity. Instead, a strong positive correlation between Zn concentration used and plant biomass, biochemical parameters, and essential oils was found in both plants.

4. CONCLUSION

Foliar spraying on perilla at a concentration of 2.0 g/L resulted in the highest plant growth and physiochemical parameters, with the highest chlorophyll index of 46.4 and the highest protein content of 30.1 μg/g. Similarly, foliar application of zinc sulfate on holy basil at 2.5 g/L led to the highest plant growth and biochemical parameters. This application resulted in the highest profit and profit margin of 126.88 million dong and 1.45 times, respectively, after two harvests compared to the control experiment.

Conflict of interest declaration: All authors confirm that they have no conflict of interest.

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