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Biostimulant Effects of Silicon-Rich Horsetail Extract on Morphological and Growth Characteristics of *Zataria multiflora*

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Abstract

Thyme is a unique plant with beneficial biological properties and is widely used in the healthcare and treatment sectors. This study was conducted to investigate the effect of horsetail extract on the morphological and growth characteristics of *Zataria multiflora* (ZM). For this purpose, foliar treatments with horsetail extract at concentrations of 0%, 0.5%, 1%, and 2% were applied to plants at the 7–9 leaf stage. The experiment followed a randomized complete block design with three replications. Evaluated traits included plant height, number of leaves per plant, number of sub-branches, leaf area index, plant fresh weight, plant dry weight, antioxidant activity, and essential oil composition. The results showed that the application of silicon-rich horsetail extract enhanced both growth and phytochemical traits. The 2% horsetail extract treatment significantly increased plant height, leaf length, leaf width, fresh weight, and dry weight. The highest antioxidant enzyme activity was observed under the 2% extract treatment. Additionally, essential oil content increased with higher extract concentrations. The main essential oil components identified were carvacrol (33.75–39.4%), thymol (12.81–14.35%), linalool (7.12–10.22%), and *p*-cymene (7.45–8.38%). The maximum levels of carvacrol (39.4%) and thymol (14.84%) were achieved at 1% and 2% extract concentrations, showing significant differences compared to the control group. Horsetail extract, rich in silicon, acts as a biostimulant and natural fertilizer, potentially improving the yield and essential oil production of *Z. multiflora*, particularly under organic cultivation systems.

1. Introduction

Zataria multiflora Boiss. (Shirazi thyme, ZM) is native to central and southern Iran, Pakistan, and Afghanistan. It is characterized by oval leaves and dense, white, hairy, rounded buds along the leaf margins [1, 2]. Beyond its

culinary use, the aerial parts of ZM are widely employed in traditional medicine for their antiseptic, analgesic, antiparasitic, and antidiarrheal properties. Additionally, Shirazi thyme has been used to treat respiratory conditions, premature labor pain, gastrointestinal disorders, fever, lacerations, musculoskeletal pain, headache, vomiting, and colds [3, 4]. Recent pharmacological research supports the therapeutic efficacy of ZM, reporting analgesic, spasmolytic, and anti-inflammatory effects. Currently, pharmaceutical formulations such as syrups, soft capsules, and vaginal creams derived from ZM are commercially available for various therapeutic applications. Despite these advances, ZM remains integral to traditional medicine [5, 6].

Growing interest in environmentally sustainable agricultural practices has prompted the exploration of natural, multifunctional bio-products such as plant extracts for crop protection, growth enhancement, and biostimulation. Plant extracts exhibit diverse biological activities, including antifungal, antimicrobial, antiparasitic, antioxidant, and anti-inflammatory properties, offering potential as eco-friendly inputs for sustainable agriculture [7, 8].

The aerial parts of horsetail (*Equisetum arvense* L.) represent a notable natural source of silicon (Si). This herbaceous, perennial species of the Equisetaceae family contains more than 25% silica by dry weight (DW) [9]. Silicon is recognized as a multifunctional element in plant biology, contributing to improved growth, yield, photosynthetic efficiency, nitrogen fixation, and enhanced tolerance to biotic and abiotic stresses [10, 11]. The beneficial effects of silicon supplementation have been demonstrated in several species, including *Coriandrum sativum* [12], *Triticum aestivum* [13], *Phoenix dactylifera* [14], *Brassica napus* [15], and *Echinacea purpurea* [16].

Silicon plays various roles in enhancing the physiological and metabolic characteristics of plants. The horsetail plant (*Equisetum arvense*) is a natural source rich in silicon, which has been recognized in numerous scientific studies as an effective source of this element. However, studies directly investigating the effect of horsetail extract on plant growth are limited. Most research on silicon in plants primarily focuses on examining its role in improving plant resistance to environmental stresses or enhancing structural characteristics, without addressing the direct impact of silicon-containing plant extracts. Given the potential of horsetail extract as a natural source of bioavailable silicon, further investigation may provide valuable insights into its role in enhancing the growth and physiological traits of medicinal plants. Therefore, the present study aimed to evaluate the effects of horsetail extract on the morphological and growth characteristics of Shirazi thyme.

2. Materials and Methods

2.1 *Cultivation of plants and experimental design microstructure*

This study was conducted during 2024 and 2025 at the Agricultural Department of the Research Institute of Karaj University to evaluate the effects of horsetail extract on *Zataria multiflora* (Shirazi thyme) under pot culture conditions (Figure 1). The experiment was arranged in a randomized complete block design (RCBD) with four treatments and three replications. The authors confirm that all necessary permits for sample collection and experimental procedures were obtained, and the study adhered to the IUCN Policy Statement. Treatments included a control (sprayed with distilled water) and foliar applications of horsetail extract at concentrations of 0.5%, 1%, and 2%. Plants were grown in 25 cm diameter plastic pots filled with a sterilized growing medium composed of garden soil, sand, and vermicompost in a 1:1:1 ratio. Prior to sowing, the potting mix was thoroughly sterilized. Seeds were sown directly into each pot (3-5 seeds per pot), and seedlings were later thinned to retain one vigorous plant per pot. Pots were placed outdoors under natural sunlight (at least 6 h of direct light per day), and irrigation was applied regularly based on environmental conditions and plant needs. A balanced NPK fertilizer was also

applied in liquid form to provide base nutrition. Foliar spraying of horsetail extract began at the 6-8 leaf stage (approximately 30-35 days after emergence) and was repeated every 15 days for a total of three applications. Routine maintenance practices, including manual weeding and pest control, were performed uniformly across all pots. Morphological and phytochemical traits were evaluated at the full flowering stage, when the plants reached physiological maturity and peak essential oil production.

Figure 1. Different stages of seedling growth from germination to transplantation into pots. These stages include development of the first true leaves, early vegetative growth, and final transfer to a pot

2.2 *Extraction of horsetail and silicon quantification*

Aerial parts of *Equisetum arvense* were harvested from Chalus County (Mazandaran, Iran) in June 2024 and shade-dried at 20 ± 1°C. After grinding, phytochemical extraction was performed *via* maceration in 55% ethanol for 24 h with agitation. The filtrate was concentrated under reduced pressure and stored at 4 °C. Working concentrations (0.5%, 1%, and 2% w/v) were freshly prepared with distilled water for foliar application. Given that ethanol does not effectively extract inorganic silicon, acid digestion (HNO_3:HCl, 3:1) followed by ICP-OES analysis was employed to quantify silicon content in the plant material. The corresponding silicon concentrations in the prepared extracts were confirmed to be proportional to extract strength, indicating a direct relationship between applied extract volume and bioavailable silicon levels potentially contributing to the physiological enhancements observed in *Zataria multiflora* [17].

2.3 *Assessment of agro-morphological characteristics and productivity*

Four subshrubs per treatment were randomly selected 140 days after sowing for agro-morphological assessment. Measurements were conducted during the early flowering stage, characterized by visible floral bud formation prior to full anthesis, to capture the physiological transition into reproductive growth. The recorded traits included plant height, number of leaves per plant, number of sub-branches, and leaf area index (LAI). To determine LAI, a sample of fully expanded mature leaves from each plant was selected, and individual leaf area was measured using a digital leaf area meter (Model LICOR-3100, LI-COR Inc., Lincoln, NE, USA). LAI was then calculated by dividing the total one-sided leaf area (cm^2) by the corresponding ground area occupied by the plant (cm^2). Subsequently, aerial parts were harvested and gently rinsed with distilled water to eliminate surface debris. The washed fresh weight (WFW) was immediately recorded using a precision digital scale. Samples were then shade-dried at ambient temperature until a constant weight was achieved, after which the DW was measured for biomass analysis [11].

2.4 *Measurement of antioxidant enzyme and oxidant*

Antioxidant enzyme activities were determined following established protocols. Catalase (CAT) activity was measured based on the method of Aebi (1984), by monitoring the decomposition rate of hydrogen peroxide at 240 nm in a reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. One unit of CAT activity was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute under assay conditions [18].

Superoxide dismutase (SOD) activity was quantified according to the protocol of Giannopolitis and Ries (1977), which is based on the inhibition of nitro blue tetrazolium (NBT) photoreduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction [19].

Malondialdehyde (MDA) content, a marker of lipid peroxidation, was determined using the thiobarbituric acid (TBA) reaction method described by Heath and Packer (1968). Fresh tissue was homogenized in 0.1% trichloroacetic acid (TCA), and the supernatant was mixed with TBA reagent. Absorbance was measured at 532 nm, with correction at 600 nm for non-specific turbidity [20].

Hydrogen peroxide (H_2O_2) concentration was assessed using the method developed by Alexieva et al. (2001). Leaf samples were homogenized in 0.1% TCA and centrifuged at 11,000 × g for 15 minutes. The supernatant was reacted with potassium iodide (KI) and absorbance was recorded at 390 nm. H_2O_2 content was calculated using a standard curve [21].

All spectrophotometric measurements were performed using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan), and enzyme activities were expressed on a fresh weight basis (units per gram FW).

2.5 *Essential oil analysis by GC and GC–MS*

The chemical composition of the essential oils was analyzed using both Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC–MS) techniques, in order to identify and quantify volatile constituents.

Essential oil samples were injected into a Trace GC Ultra (ThermoQuest-Finnigan, USA) gas chromatograph equipped with a flame ionization detector (FID) and coupled with a quadrupole mass spectrometer (MS). The GC was fitted with a non-polar DB-5 fused-silica capillary column (30 m × 0.20 mm i.d., film thickness 0.20 µm, Agilent Technologies). The oven temperature program was as follows: an initial temperature of 55 °C was held for 2 minutes, followed by a linear increase of 5 °C/min up to 240 °C, where it was held for 10 minutes. The injector and detector (FID) temperatures were maintained at 240 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.1 mL/min. A 1 µL volume of each essential oil sample was injected using a split ratio of 1:10.

MS detection was performed in electron ionization (EI) mode at an energy level of 70 eV, covering a mass scan range of m/z 40–400. The identification of essential oil constituents was carried out based on a combination of analytical approaches. These included comparison of the obtained mass spectra with those in the NIST (National Institute of Standards and Technology) and Wiley spectral libraries, calculation and comparison of retention indices (RI) using a homologous series of *n*-alkanes (C8–C24) analyzed under identical chromatographic conditions, and verification through comparison with published literature data. Only those compounds that met both spectral and chromatographic criteria were reported as identified constituents of the essential oil. The relative concentrations of identified components were calculated as percentages based on peak area normalization without the use of correction factors or response coefficients. Each sample was analyzed in duplicate to ensure reproducibility, and results were expressed as mean relative percentages of individual constituents [22].

2.6 *Statistical analysis*

Data from both years were pooled for analysis. Normality was assessed using SPSS v25. Data were analyzed via ANOVA, and means were compared using the least significant difference (LSD) test. A significance level of 0.05 was considered as significant.

3. Results and Discussion

3.1 *Morphological traits*

The analysis of variance conducted on the data indicated that the effect of different concentrations of horsetail extract on the morphological and functional characteristics evaluated in this study was significant at the 5% level ($P < 0.05$) (Figure 2A). The average plant height increased significantly with higher extract concentrations. The tallest plants (52 cm) were observed at the 2% extract level, while the shortest (41 cm) were recorded in the control group. Application of the 2% extract resulted in a 26.31% increase in plant height compared to the control (Figure 2B).

Application of 1% and 2% extract concentrations also led to a notable increase in leaf length, measuring 13.86 mm and 12.16 mm, respectively, compared to the control (Figure 3). Additionally, the treatments improved leaf width, with the 2% extract showing the highest increase of 6.53 mm, representing a 27.38% improvement over the control (Figure 2B).

The findings further indicated that foliar application of horsetail extract at 0.5%, 1%, and 2% concentrations increased plant fresh weight by 9.73%, 19.94%, and 22.14%, respectively, compared to the control. The 2% extract treatment produced the highest DW of 59.25 grams, reflecting a 32.24% increase over the control. Moreover, analysis of bract length revealed that the 2% concentration resulted in the longest bracts at 3.15 cm, representing a 19.09% increase compared to the control. It is noteworthy that bract length and width were not significantly affected by extract concentration ($p > 0.05$) (Figure 2B).

3.2 *Effects of horsetail extract concentrations on oxidative stress markers and antioxidant enzyme activities*

The analysis of the effects of different concentrations of horsetail extract revealed that foliar application at 1% concentration significantly reduced MDA levels compared to the control group. Statistical results showed that both MDA and hydrogen peroxide (H_2O_2) levels were lower in all extract-treated groups than in the control; however, these reductions were not statistically significant.

The findings demonstrated that increasing the extract concentration to 1% and 2% resulted in a significant increase in the activities of CAT, peroxidase (POX), and SOD compared to the control group ($p \leq 0.05$). No significant difference was observed between the 0.5% concentration and the control group. The highest antioxidant enzyme activity was recorded in plants treated with 2% horsetail extract ($p \leq 0.05$). Notably, antioxidant enzyme levels in the 1% treatment were also significantly higher than in the control group (Figure 3). The presence of silicon compounds in the horsetail extract contributed to a reduction in oxidative stress markers (MDA and H_2O_2) and enhanced the activity of antioxidant enzymes (CAT, POX, and SOD) in *Zataria multiflora*.

A

concentration(%)	Plant height	Leaf length	Leaf width	Percent of dry weight	Fresh weight	dry weight	Bract length	Bract width
0	41.22	10.08	4.85	69.73	141.39	44.52	2.92	1.74
0.5	44.76	11.41	5.79	84.27	155.74	46.8	3.01	1.82
1	48.65	13.84	6.4	103.95	164.37	52.18	3.11	1.86
2	52.5	12.19	6.53	99.24	171.56	59.25	3.15	1.85

B

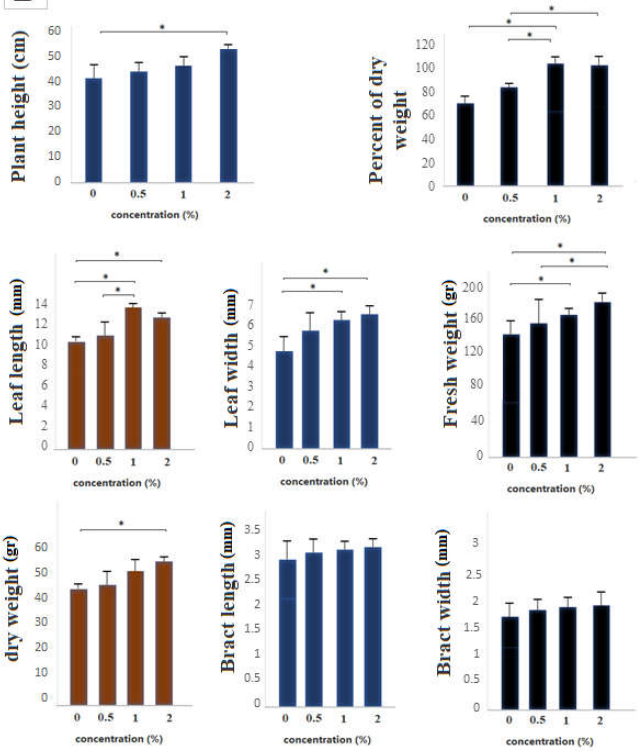

Figure 2. The effect of different extract concentrations on the morphological and functional characteristics A) Mean of morphological traits in the studied groups and B) Charts. * and ** indicate differences from their respective controls at $P < 0.05$ and $P < 0.01$ respectively. Data represent mean ± SD from three independent experiments.

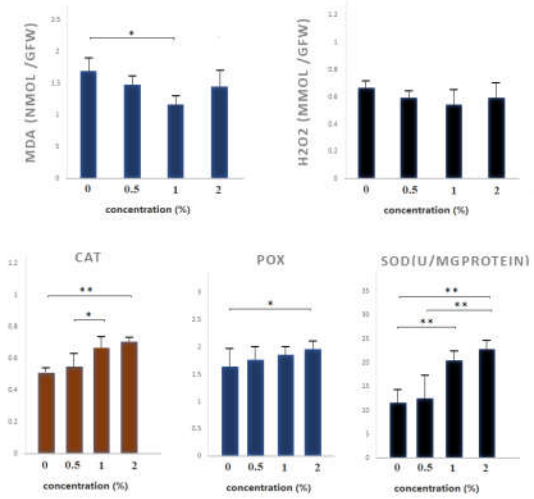

Figure 3. Antioxidant defense results

3.3 *Content and composition of essential oils*

The results of this experiment indicated that foliar application of horsetail extract had a significant effect on the essential oil content of Shirazi thyme ($P < 0.05$). All concentrations of horsetail extract used in this study led to an increase in essential oil production in the treated plants. Among the 37 identified compounds, the application of horsetail extract significantly increased the levels of *p*-Cymene, *trans*-Linalool oxide, Linalool, Dodecane, Thymol, and Carvacrol. The highest essential oil content was observed in plants treated with 2% horsetail extract (Table 2). Essential oil yield was significantly influenced by foliar application of horsetail extract ($P < 0.05$), with increasing extract concentrations resulting in higher yields.

The GC–MS analysis detected 37 compounds in the essential oil extracted from the vegetative parts of Shirazi thyme treated with horsetail extract. The analysis revealed the major compounds and their structures, identified according to their specific peak values, as illustrated in Figure 4. The detailed composition of essential oil compounds is presented in Table 1.

Table 1. Amount of essential oil in 2% horsetail extract

Composition	RI	Extract concentration (%)				p-value
		Control	0.5	1	2	
α-Thujene	930	0	0	0.17	0	0.74
α -Pinene	938	0.94	0.79	0.91	1.06	0.09
β-Pinene	979	0	0.15	0	0	0.18
n-Octanone-3	986	1.29	1.84	0.72	1.25	0.22
β-Myrcene	991	1.01	0.93	1.09	0.83	0.55
Decane	1003	2.38	2.04	2.15	2.2	0.26
α-Terpinene	1017	0.44	0.73	0.19	0.52	0.37
p-Cymene	1027	7.45	8.84	8.19	8.38	0.04*
γ-Terpinene	1060	2.81	3.33	2.11	1.28	0.21
cis-4-thujano	1069	0	1.33	0.46	0	0.37
Linalool oxide cis	1078	0.6	0.55	0.59	0.74	0.15
Linalool oxide trans	1091	0.54	0.62	1.48	0.41	0.03*
Linalool	1099	7.12	9.13	8.96	10.22	0.008*
Terpineneol-1	1137	0.41	1.15	0.61	0.78	0.14
α-Terpineol	1191	1.49	0.47	0	0.52	0.52
Dodecane	1201	0.95	1.39	1.74	1.85	0.02*
Thymol methyl ether	1236	1.18	0	0	0.64	0.33
Carvacrol methyl ether	1248	2.82	2.05	2.4	2.53	0.41
Linalyl acetate	1256	0	0.22	0	0.65	0.64
Bornyl acetate	1284	0	0	0.21	0	--
Thymol	1295	13.17	12.96	12.81	14.35	0.006*
Carvacrol	1302	33.75	34.86	39.4	38.17	0.01*
p-Thymol	1335	0	0.61	0.28	0.92	0.52
Thymol acetate	1357	0.93	1.24	0	0	0.13
Carvacrol acetate	1374	1.19	1.94	0.47	0.32	0.08
Tetradecane	1418	0	0	0.17	0	--
β-Caryophyllene	1421	1.87	1.85	1.55	1.76	0.16
Aromadendrene	1442	0.62	1.15	1.18	1.24	0.11
α-Humulene	1462	1.43	1.98	0.77	0.43	0.07
1*H*-Cycloprop [*e*] azulene	1503	0.58	0.92	1.19	0.33	0.29
Spathulenol	1574	0.98	0.59	0.51	0.55	0.74
Caryophyllene oxide	1582	0.89	0.53	0.43	0.48	0.41
Hexadecane	1602	0.96	0	0.86	0	0.35
Isospathulenol	1636	0	0.25	0.83	0	0.36
Valencene	1672	0.78	0	0	0.12	0.71
Benzoic acid	1753	0.52	0.4	0.72	0.83	0.29
Phthalic acid	2529	2.73	2.81	1.92	1.84	0.11
Total		93.35	94.27	95.14	95.94	0.24
Essential oil content (%)		0.29	0.33	0.48	0.51	

The results showed that Carvacrol (33.75–39.4%), Thymol (12.81–14.35%), Linalool (7.12–10.22%), and *p*-Cymene (7.45–8.38%) were the predominant components across all samples. Notably, the highest Carvacrol content (39.4%) and Thymol content (14.84%) were obtained with foliar application of 1% and 2% horsetail extract, respectively, showing significant differences compared to the control (Table 1).

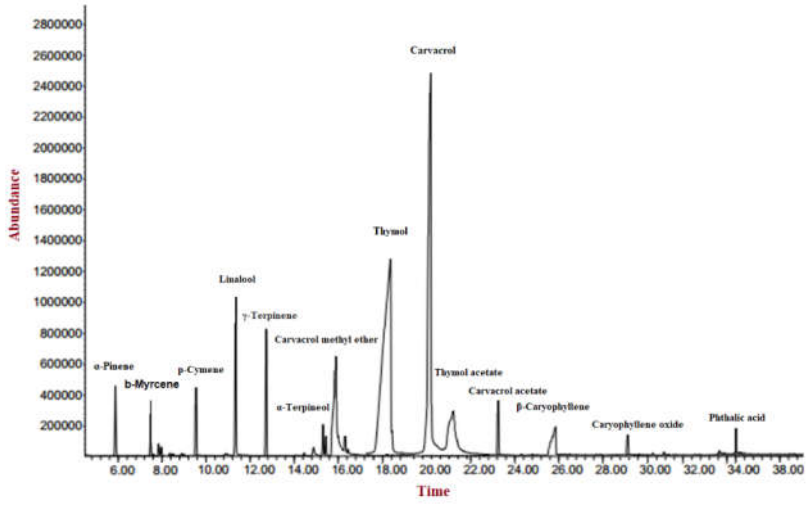

Figure 4. The GC/MS chromatogram of *Zataria multiflora* essential oils

Given the diverse culinary and medicinal applications of *Thymus* (Shirazi thyme), this study investigated the use of horsetail extract as a plant biostimulant to evaluate its effects on growth, performance, and morphological characteristics. The morphological traits of *Zataria multiflora* were assessed under four different concentrations of horsetail extract. The results demonstrated that foliar application of horsetail extract at concentrations of 0.5%, 1%, and 2% significantly improved the plant's morphological characteristics compared to the control group, with the greatest effect observed at the 2% concentration.

Horsetail extract is rich in silicon, and numerous studies have highlighted the beneficial impacts of silicon on the growth, performance, physiology, and metabolism of various plant species. Silicon has been shown to enhance root and shoot length, DW, and leaf area [13, 23]. In a published study, the effects of foliar application of horsetail extract at concentrations of 0.5%, 1%, and 2% on basil were evaluated. The results showed that the application of this extract led to significant increases in plant height, leaf number, number of lateral branches, LAI fresh and DW, as well as phenolic content, flavonoids, anthocyanins, and antioxidant activity. Notably, the 2% concentration had the most significant impact on improving these traits [24]. Several investigations have focused on both the direct and indirect effects of silicon compounds on morphological changes and physiological functions in plants [25]. Silicon influences plant growth by affecting cellular pathways. Recent research suggests that the increase in fresh weight observed in plants treated with silica nanoparticles may be due to enhanced photosynthesis, transpiration, and chlorophyll content [26].

In the present study, antioxidant enzyme activities were evaluated under different concentrations of horsetail extract. The results indicated that exposure to the extract led to a significant reduction in MDA concentration. Statistical analysis showed that both MDA and hydrogen peroxide (H_2O_2) levels were lower in all extract-treated groups compared to the control; however, these reductions were not statistically significant relative to the control. Furthermore, the findings revealed that increasing extract concentration resulted in a significant increase in the activities of CAT, POX, and SOD compared to the control group. This outcome is likely attributed to the silicon

content of the extract. In this context, it has been reported that silicon in horsetail extract protected purple coneflower plants against salt stress by reducing MDA and H_2O_2 levels, indicating reduced oxidative damage and lipid peroxidation. The interaction between salinity and silicon on the caffeic acid content of roots was significant at the 5% level, suggesting that silicon can mitigate the negative effects of salt stress on purple coneflower [15]. Earlier studies have demonstrated that silicon nanoparticles enhance protein levels, free amino acids, and nutrient uptake, in addition to increasing the activity of SOD and POX [26]. Silicon exhibits a strong affinity for oxygen, which explains why it predominantly occurs as silica (SiO_2) in natural environments [27].

Research has shown that silicon can influence molecular pathways to increase the activities of antioxidant enzymes such as SOD, CAT, and POX under environmental stress conditions [28, 29]. Another study on tomatoes under salt stress reported that optimal concentrations of silicon reduced MDA, H_2O_2, and antioxidant enzyme activities, indicating decreased oxidative damage [30]. Silicon plays an important role in enhancing sustainable plant growth, strengthening defense systems, and mitigating and managing environmental stresses [31]. In another study on oilseed plants, silicon application reduced MDA and H_2O_2 concentrations in leaves under salt stress, indicating decreased lipid peroxidation and oxidative damage [32].

Although the increase in reactive oxygen species (ROS) during stressful conditions is considered a potential threat to cells, these molecules also function as crucial messengers that activate plant stress responses and defense mechanisms. Consequently, ROS serve a dual role, acting both as indicators of stress and as secondary messengers in stress-related signaling pathways [33, 34, 35]. It should be noted that chemical compounds play a vital role in the cellular and biochemical functions of plants, and increasing antioxidant enzyme activities while reducing oxidants is generally beneficial to the plant [36].

In the present study, evaluation of essential oil content and composition showed that key compounds such as carvacrol and thymol increased significantly following foliar application of silicon-rich horsetail extract. These two compounds possess significant antimicrobial and anticancer activities, making them highly important in biomedical research [37,38,39]. Previous research indicates that silicon enhances essential oil content. Silicon is crucial in stimulating metabolite production by inducing various transcriptional modifications [40]. It enhances essential oil content by improving cell growth traits, ion uptake, and the density and size of essential oil glands in the leaves [41].

One of the limitations of the present study was the limited number of directly related investigations. Environmental variables such as temperature, humidity, light intensity, and soil conditions were controlled during the experiment; however, their fluctuations in natural field conditions could influence the efficacy of horsetail extract and silicon bioavailability. Although no direct research has been conducted on the effects of horsetail extract on antioxidant enzyme activity in *Zataria multiflora*, existing studies suggest that silicon in plant extracts may play an important role in reducing oxidative stress and enhancing antioxidant enzyme activity. These findings can serve as a foundation for future research in this area.

4. Conclusion

The use of horsetail extract as a foliar spray, rich in silicon and acting as a biostimulant, shows significant potential in enhancing growth characteristics and essential oil yield in *Thymus*, while also impacting the composition of essential oils. The increase in phenolic compound levels observed at different concentrations of the extract suggests an improvement in antioxidant capacity and a stronger antioxidant defense system. Consequently, employing

silicon-rich horsetail extract as a foliar treatment can be considered a promising approach for boosting the yield and productivity of medicinal plants, especially in organic essential oil cultivation. Additionally, the application of this plant extract in agriculture presents an effective method for improving plant performance, mitigating environmental stresses, and enhancing the quality of agricultural products.

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Conflicts of Interest

The author declares that there are no conflicts of interest regarding this article.

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