STUDYING AND UTILIZATION OF PLANT GENETIC RESOURCES

Original article
UDC 633.82
DOI: 10.30901/2227-8834-2025-1-17-26

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Effect of zinc and copper on the growth characteristics, yield, essential oil composition, and antimicrobial properties of Thai basil (*Ocimum basilicum* var. *thyrsiflora*) cultivated in Vietnam

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Thai basil (*Ocimum basilicum* var. *thyrsiflora* (L.) Benth.) (Lamiaceae) is widely used for its aromatic leaves and culinary value. This study investigated the effects of zinc (Zn; 0, 10, 20 mg/kg of soil) and copper (Cu; 0, 5, 10 mg/kg of soil), individually and combined, on the growth, yield, essential oil composition, and antimicrobial properties of Thai basil grown in Vietnam. The optimal plant responses, including increased height, lateral branches of the stem number, fresh and dry yield of green mass, and essential oil yield, were achieved at Zn 10 mg/kg and Cu 5 mg/kg. The major essential oil components were linalool (41.64–53.27%), and methyl chavicol (22.45–37.56%), with the highest concentrations recorded at Zn 10 mg/kg and Cu 5 mg/kg. Antimicrobial tests showed strong activity of essential oil against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, with minimum inhibitory concentrations (MIC) between 25 and 100 µg/mL. However, higher levels of Zn and Cu negatively impacted growth, yield, and oil quality. The study highlights the importance of regulating Zn and Cu levels in soil to optimize the growth and essential oil properties of Thai basil. These findings offer valuable guidance for enhancing the agricultural production of Thai basil, balancing both quantity and quality.

Keywords: *Ocimum basilicum* var. *thyrsiflora*, microelements, growth characteristics, essential oil, antimicrobial activity

Acknowledgements: the authors would like to thank Hong Duc University (Vietnam) for their support and facilitation to complete this study.
The authors thank the reviewers for their contribution to the peer review of this work.

For citation: Giang T.V., Huyen T.T., Huyen L.T.T., Lan V.N. Effect of zinc and copper on the growth characteristics, yield, essential oil composition, and antimicrobial properties of Thai basil (*Ocimum basilicum* var. *thyrsiflora*) cultivated in Vietnam. *Proceedings on Applied Botany, Genetics and Breeding*. 2025;186(1):17-26. DOI: 10.30901/2227-8834-2025-1-17-26

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ИЗУЧЕНИЕ И ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ РАСТЕНИЙ

Научная статья
DOI: 10.30901/2227-8834-2025-1-17-26

Влияние цинка и меди на характеристики роста, урожайность, состав эфирного масла и антимикробные свойства тайского базилика (*Ocimum basilicum* var. *thyrsiflora*), выращиваемого во Вьетнаме

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Базилик тайский (*Ocimum basilicum* var. *thyrsiflora* (L.) Benth.) (Lamiaceae) нашел широкое применение благодаря своему химическому составу в парфюмерии, медицине и кулинарии. В данном исследовании изучалось влияние цинка (Zn; 0, 10, 20 мг/кг почвы) и меди (Cu; 0, 5, 10 мг/кг почвы), как по отдельности, так и в комбинации, на рост, урожайность, состав эфирного масла и антимикробные свойства тайского базилика, выращенного во Вьетнаме. Оптимальные параметры растений: увеличение высоты, количества боковых побегов, урожая свежей и сухой зеленой массы, а также выход эфирного масла, были получены при концентрациях Zn 10 мг/кг и Cu 5 мг/кг. Основные компоненты эфирного масла – линалоол (41,64–53,27%) и метилхавикол (22,45–37,56%), максимальные концентрации которых были зафиксированы при тех же дозах цинка и меди. Антимикробные тесты показали высокую активность эфирного масла против *Staphylococcus aureus*, *Escherichia coli* и *Candida albicans*, с минимальными подавляющими концентрациями (MIC) от 25 до 100 мкг/мл. Однако более высокие уровни Zn и Cu отрицательно сказывались на росте, урожайности и качестве эфирного масла. Исследование подчеркивает важность контроля уровней цинка и меди в почве для оптимизации роста и свойств эфирного масла тайского базилика. Полученные результаты могут быть рекомендованы для повышения сельскохозяйственного производства тайского базилика с учетом повышения как урожайности зеленой массы, так и ее качества.

Ключевые слова: *Ocimum basilicum* var. *thyrsiflora*, микроэлементы, характеристики роста, эфирное масло, антимикробная активность

Благодарности: авторы выражают благодарность Университету Хонг Дык (Вьетнам) за поддержку и помощь в осуществлении настоящего исследования.
Авторы благодарят рецензентов за их вклад в экспертную оценку этой работы.

Для цитирования: Гианг Т.В., Хуен Ч.Т., Хуен Л.Т.Т., Лан В.Н. Влияние цинка и меди на характеристики роста, урожайность, состав эфирного масла и антимикробные свойства тайского базилика (*Ocimum basilicum* var. *thyrsiflora*), выращиваемого во Вьетнаме. *Труды по прикладной ботанике, генетике и селекции*. 2025;186(1):17-26. DOI: 10.30901/2227-8834-2025-1-17-26

Introduction

Thai basil, scientifically known as *Ocimum basilicum* var. *thyrsiflora* (L.) Benth., is a unique and aromatic herb that hails from the mint family, Lamiaceae (Sahu et al., 2022). Originating in Southeast Asia, particularly in Thailand, this basil variety is widely cultivated for its distinctive flavor and culinary applications (Sahu et al., 2022). Characterized by its vibrant green, slightly serrated leaves, and strong, sweet fragrance, Thai basil stands out among other basil varieties (Sahu et al., 2022). In cuisine, this basil variety is an essential ingredient, adding a delightful twist to various dishes. It is a key component in traditional curries, stir-fries, and noodle dishes, imparting a unique and aromatic flavor that is both spicy and slightly sweet. The leaves are often used as a garnish, contributing both visual appeal and a burst of fresh aroma to the presented dishes. Beyond its culinary uses, Thai basil is also valued for its potential health benefits. Like other basil varieties, it is rich in essential oils, antioxidants, and nutrients, which are believed to possess anti-inflammatory and antibacterial properties (Avetisyan et al., 2017; Łyczko et al., 2020; Sahu et al., 2022; Sahu et al., 2024). Additionally, the herb is appreciated for its role in traditional medicine, where it is used to address various ailments (Pripdeevech et al., 2010).

Mineral nutrients are pivotal for the growth and development of plants, playing essential roles as key elements in diverse physiological and biochemical processes (Hänsch, Mendel, 2009). Two standout contributors to overall crop health and productivity are zinc (Zn) and copper (Cu) (Mousavi et al., 2013; Kumar et al., 2021). It is important to understand the initial composition of the soil to ensure that plants would receive an adequate supply of these essential nutrients. As an indispensable micronutrient, Zn actively participates in numerous enzymatic reactions crucial for plant metabolism (Mousavi et al., 2013). It plays a central role in nucleic acid and protein synthesis, facilitating cell division and elongation. Additionally, Zn is essential for activating enzymes in photosynthesis, thereby influencing the overall energy conversion efficiency in plants (Mousavi et al., 2013; Prasad et al., 2016). Moreover, it supports the development of a robust root system, enhancing the plant's capacity to absorb water and nutrients from the soil (Mousavi et al., 2013). Cu, another vital micronutrient, is integral to various biochemical processes within plants (Kumar et al., 2021). It acts as a cofactor for numerous enzymes engaged in redox reactions and electron transport chains. Cu's participation in photosynthesis and respiration makes it indispensable for energy metabolism (Kumar et al., 2021). Furthermore, Cu contributes to cell wall lignification, providing structural support to plants and bolstering their resistance to diseases (Adrees et al., 2015; Kumar et al., 2021). Inadequate levels of Zn or Cu can result in diverse growth disorders, adversely affecting plant yield and quality (Adrees et al., 2015; Prasad et al., 2016). Thus, ensuring that plants receive a balanced and sufficient supply of these minerals is crucial for optimal performance.

In recent years, the cultivation of Thai basil has significantly increased in Vietnam, with farmers drawn to its adaptability to various soil types and climates, making it suitable for both lowland and upland areas. Despite its popularity, there is a scarcity of research on the micronutrient requirements of this plant, particularly concerning Zn and Cu. This lack of research underscores the necessity for comprehensive studies to fill this knowledge gap. Hence, the present study investigates the effects of Zn and Cu, individually and in combination, on the growth, yield, essential oil composition, and antimicrobial properties of Thai basil grown in Vietnam.

Materials and methods

Plant materials

The Thai basil (*Ocimum basilicum* var. *thyrsiflora*) seeds were procured from the Vietnam High-tech Plant Seed Center and subsequently cultivated within the net house at Hong Duc University, Thanh Hoa (19°46'16"N, 105°46'47"E), Vietnam. The sowing process commenced in February 2022, during which the ambient temperature averaged 26 ± 2°C, and the relative humidity was maintained at 65 ± 2%. Plastic germination trays served as the chosen containers, and the growth medium employed was a peat-based substrate. During the initial 2-week post-sowing period, the plants underwent a daily watering procedure, which was subsequently adjusted to a weekly schedule. Following a growth duration of 5 weeks, the seedlings underwent transplantation into plastic pots that had been treated with Zn and Cu.

Experimental design and treatments

Plastic pots, measuring 14 cm in diameter and 12 cm in height, were employed in this investigation and filled with soil. The soil samples were obtained from the surface down to a depth of 25 cm at the research farm of Hong Duc University, Thanh Hoa, Vietnam. The trace element composition of the soil was analyzed using atomic absorption spectroscopy (AAS). The physicochemical properties of the soil were determined, with the following results: soil texture – sandy loam; electrical conductivity (EC) – 3.85 dS/m; potential of hydrogen (pH) – 7.23; organic carbon – 0.61%; total nitrogen (N) – 0.08%; phosphorus (P) – 7.54 mg/kg; potassium (K) – 167 mg/kg; iron (Fe) – 11.32 mg/kg; manganese (Mn) – 8.49 mg/kg; copper (Cu) – 0.72 mg/kg, and zinc (Zn) – 0.80 mg/kg.

The experimental design comprised three levels of Zn (0, 10, 20 mg/kg of soil) and three levels of Cu (0, 5, 10 mg/kg soil), administered as $ZnSO_4.2H_2O$ and $CuSO_4.5H_2O$, respectively. A total of nine treatments were implemented, encompassing all possible combinations: (Zn_0Cu_0), (Zn_0Cu_5), (Zn_0Cu_{10}), ($Zn_{10}Cu_0$), ($Zn_{10}Cu_5$), ($Zn_{10}Cu_{10}$), ($Zn_{20}Cu_0$), ($Zn_{20}Cu_5$), and ($Zn_{20}Cu_{10}$), with Zn_0Cu_0 serving as the control. Application of these treatments involved dissolving specified metal salt amounts in 200 mL of distilled water, and uniformly spraying them over the soil surface of each pot to ensure even distribution. Following the treatment application, a two-month incubation period ensued to establish Zn and Cu equilibrium in the pot soil. Subsequently, the seedlings, prepared as described above, were planted in pots treated with Zn and Cu.

The experiments were conducted utilizing a randomized block design with three replications in the net house of Hong Duc University, Thanh Hoa, Vietnam. The plots were irrigated immediately after transplanting and throughout the growing seasons as needed. No pesticides were employed during the experiment, and weed control was carried out manually. Fertilization with essential nutrient elements (N–P–K, urea, triple superphosphate, and potassium sulfate) was applied on the basis of on soil test results to optimize conditions for plant growth. After 12 weeks, Thai basil plants were harvested by cutting them at 5 cm above the soil surface.

Measurement of some morphophysiological traits

Various parameters relevant to plant growth, such as plant height and the number of lateral branches on the stems per plant, were observed. Plant height was measured using a ruler and expressed in cm. The study also investigated factors affecting yield, including both fresh and dry produce, and examined pigment content (SPAD). Total fresh yield was documented and presented in g/plant, and the total dry yield of green mass was measured and reported in g/plant after storing harvested material for 72 h in an oven set at 72°C. Additionally, assimilatory pigment content was evaluated using a non-destructive portable chlorophyll content meter (SPAD 502, Minolta, Japan), which measured the optical absorbance of chlorophyll, with readings recorded in SPAD units.

Essential oil extraction

A total of 200 g of dried aerial parts of Thai basil underwent hydrodistillation for 4 h utilizing a Clevenger-type apparatus, following established procedures (Giang et al., 2023). The essential oil extraction process was reiterated three times to ensure reproducibility. Subsequently, the collected essential oil underwent drying over anhydrous sulfate and was stored at 4°C in preparation for analysis. The essential oil yield was calculated and expressed as a percentage (v/w).

Essential oil analysis

The chemical compositions of the essential oil extracted from Thai basil were analyzed using gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS), following established protocols (Giang et al., 2023). The GC–FID analyses were conducted on an Agilent Technologies HP 7890A Plus gas chromatograph, equipped with a flame ionization detector and an HP-5MS column (30 m × 0.25 mm i. d., film thickness 0.25 μm). The initial oven temperature was set at 60°C for 2 min, followed by an increase to 220°C at a rate of 4°C/min. The injector and detector temperatures were maintained at 250°C and 260°C, respectively. Helium served as the carrier gas with a flow rate of 1 mL/min.

For GC–MS analyses, an Agilent Technologies HP 7890A Plus gas chromatograph, coupled with an HP 5973 MSD mass spectrometer, was employed. The same capillary column and chromatographic conditions from the GC–FID analyses were applied. Mass spectra were collected within the 35–350 amu range at a rate of 1 scan/s, utilizing ionizing electron energy of 70 eV and an emission current of 40 mA. Helium gas served as the carrier gas at a flow rate of 1 mL/min, and the transfer line temperature was set at 260°C.

The identification of essential oil components relied on GC retention time, compared with known compounds, and a comparison of mass spectra with those in the computer database and published spectra (Adams, 2007; NIST, 2018). The percentage composition of components was determined by normalizing the peak area without applying correction factors.

Antimicrobial assay

To evaluate the antimicrobial effectiveness of Thai basil essential oil, three strains of microorganisms were subjected to testing, encompassing one Gram-positive bacteria strain (*Staphylococcus aureus* ATCC 25923), one Gram-negative bacteria strain (*Escherichia coli* ATCC 25922), and one yeast strain (*Candida albicans* ATCC 10231). The minimum inhibitory concentration (MIC) was determined using the previously established broth microdilution susceptibility method (Giang et al., 2023). Gram-positive bacteria were cultured in Mueller-Hinton broth (MHB), while yeast strains were cultivated in Sabouraud broth (SB). The essential oil was dissolved in 1% dimethylsulfoxide (DMSO) and serially diluted in a 96-well microtiter plate. Overnight cultures of each strain were prepared, with a final concentration adjusted to $5{\times}10^5$ CFU/mL for bacteria and $1{\times}10^3$ CFU/mL for yeast in each well. The microorganisms were then incubated for 24 h at 37°C and 30°C for bacteria and yeast, respectively. Gentamicin and nystatin served as standards for bacteria and yeast, respectively. The MIC was defined as the lowest concentration of essential oil that inhibited visible growth of the microorganism.

Statistical analysis

The results were expressed as means and standard errors. The statistical analysis to ascertain significant differences among treatments included the comparison of means through an analysis of variance (ANOVA). Those analyses were performed using the SPSS™ software on a personal computer (SPSS Inc., IL, USA). Subsequent comparisons of treatment means were executed using the least significant difference test, with a significance level set at $p < 0.05$.

Results and discussion

Growth and morphophysiological characteristics

The concentrations of trace elements, such as Zn and Cu, in soils play a crucial role in promoting healthy plant growth and development (Hänsch, Mendel, 2009). Plants require a well-balanced combination of essential nutrients to ensure normal growth and achieve optimal yields. The impact of various treatments involving Zn, Cu, and their combinations on the growth and morphophysiological parameters of Thai basil is detailed in Table 1.

The study revealed that the applied treatments significantly ($p < 0.05$) influenced several key parameters, including plant height, number of lateral branches on the stems per plant, fresh yield, dry yield of green mass, and SPAD, compared to the control treatment. It was noted that plants treated with different concentrations of Zn and Cu exhibited enhanced growth and morphophysiological parameters compared to the control (Ghorbanpour et al., 2016; Lajayer et al., 2017). However, as the concentrations of Zn and Cu increased in various treatments, the vegetative growth parameters gradually declined (see Table 1). The most favorable results in terms of growth and morphophysiological parameters in Thai basil plants were observed with the $Zn_{10}Cu_5$ treatment. Similar positive effects of low Zn and Cu levels have been reported in other plant species, such as *Mentha pulegium* (Lajayer et al., 2017), *Matricaria chamomilla* (Grejtovský et al., 2006), and *Ocimum basilicum* (Ghorbanpour et al., 2016). The application of Zn and Cu at low doses is believed to enhance plant growth by meeting nutritional requirements, improving the efficiency of macro- and microelement absorption, activating enzymes, and facilitating elongation and cell proliferation (Hänsch, Mendel, 2009; Adrees et al., 2015; Prasad et al., 2016).

In this study, the lowest values for growth and morphophysiological parameters in Thai basil plants were recorded when the highest concentrations of Zn and Cu ($Zn_{20}Cu_{10}$) were applied (see Table 1). These values were significantly lower ($p < 0.05$) than those of the control treatment. The decrease in the mentioned growth parameters can be attributed to the accumulation of toxic levels of Cu and Zn in plant tissues, disrupting essential nutrient uptake and transport (Mir et al., 2021; Kaur, Garg, 2021). This interference subsequently affects the mineral nutrition composition of plants, chlorophyll content, photosynthetic rate, and root growth.

Essential oil yield

The essential oil yield of Thai basil, subjected to varying concentrations of Zn and Cu, is illustrated in Figure 1. Our

Table 1. The effect of zinc (Zn) and copper (Cu) on morphological and physiological characteristics of Thai basil (*Ocimum basilicum* var. *thyrsiflora*)[a]

Таблица 1. Влияние цинка (Zn) и меди (Cu) на морфологические и физиологические характеристики тайского базилика (*Ocimum basilicum* var. *thyrsiflora*)[a]

Treatments	Plant height (cm)	Lateral branches of the stems (number per plant)	Fresh yield (g/plant)	Dry yield (g/plant)	SPAD
Zn_0Cu_0	53.03 ± 1.05 c	15.19 ± 0.74 c	62.24 ± 1.01 c	11.04 ± 0.33 c	34.87 ± 0.39 c
Zn_0Cu_5	53.14 ± 1.14 c	14.92 ± 0.57 c	60.75 ± 1.48 c	10.57 ± 0.36 c	34.62 ± 0.41 c
Zn_0Cu_{10}	51.69 ± 1.54 c	14.4 5± 0.63 c	60.22 ± 1.07 c	10.42 ± 0.40 c	34.31 ± 0.45 c
$Zn_{10}Cu_0$	63.95 ± 1.41 a	20.15 ± 0.66 ab	75.81 ± 1.64 ab	13.45 ± 0.28 ab	43.18 ± 0.43 ab
$Zn_{10}Cu_5$	64.47 ± 1.22 a	22.27 ± 0.83 a	79.26 ± 1.41 a	14.28 ± 0.42 a	45.26 ± 0.27 a
$Zn_{10}Cu_{10}$	58.04 ± 0.86 b	18.46 ± 0.70 b	71.84 ± 1.50 b	12.82 ± 0.37 b	40.39 ± 0.31 b
$Zn_{20}Cu_0$	50.89 ± 1.61 c	14.31 ± 0.75 c	58.37 ± 1.04 c	10.27 ± 0.51 c	35.08 ± 0.44 c
$Zn_{20}Cu_5$	52.26 ± 1.64 c	15.48 ± 0.87 c	61.28 ± 1.27 c	10.95 ± 0.29 c	35.21 ± 0.58 c
$Zn_{20}Cu_{10}$	45.27 ± 1.01 d	11.24 ± 0.62 d	51.31 ± 1.17 d	8.30 ± 0.33 d	30.15 ± 0.27 d

Note: [a] – results are means of three measurements ± standard errors. Within each column, means with the same lower-case letters are not statistically different at $p < 0.05$ according to Tukey's test

Примечание: [a] – результаты представлены как средние значения трех измерений ± стандартные ошибки. В пределах каждого столбца средние значения, обозначенные одинаковыми строчными буквами, статистически не различаются при $p < 0,05$ по критерию Тьюки

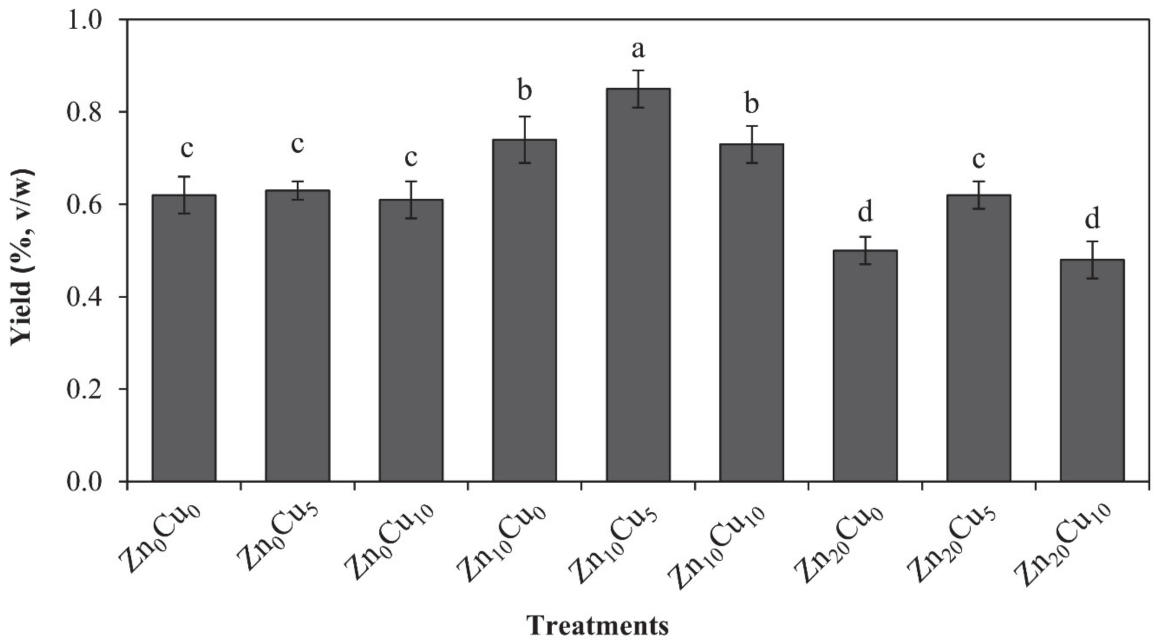

Fig. 1. The effect of zinc (Zn) and copper (Cu) on essential oil yield (%, v/w) of Thai basil (*Ocimum basilicum* var. *thyrsiflora*). Results are means of three measurements ± standard errors. Means with the same lower-case letters are not statistically different at $p < 0.05$ according to Tukey's test

Рис. 1. Влияние цинка (Zn) и меди (Cu) на выход эфирного масла (%, v/w) тайского базилика (*Ocimum basilicum* var. *thyrsiflora*). Результаты представлены как средние значения трех измерений ± стандартные ошибки. Средние значения, обозначенные одинаковыми строчными буквами, статистически не различаются при $p < 0,05$ по критерию Тьюки findings revealed a significant impact ($p < 0.05$) of Zn and Cu treatments on the essential oil yield of Thai basil. Remarkably, the $Zn_{10}Cu_5$ treatment yielded the highest essential oil, showcasing its efficacy in enhancing aromatic compound production. Conversely, Thai basil treated with high concentrations of Zn and Cu ($Zn_{20}Cu_0$ and $Zn_{20}Cu_{10}$) exhibited the lowest essential oil yields, even falling below those of the control group. This pattern aligns with previous studies on herbs like *Ocimum basilicum* (Ghorbanpour et al., 2016) and *Matricaria recutita* (Jeshni et al., 2017), indicating a consistent response to Zn and Cu treatments across different plant species. The observed outcomes can be attributed to the indirect influence of Zn on terpenoid biosynthesis, primarily by augmenting photosynthesis. Furthermore, the potential stimulation of auxin biosynthesis and increased cell division due to Zn application may contribute to the expansion of leaf area and enhanced plant photosynthesis (Mousavi et al., 2013). Similarly, Cu plays a crucial role in enzymatic activities associated with the synthesis of secondary metabolites (Kumar et al., 2021; Kumar et al., 2022). Optimal levels of Cu support the proper functioning of enzymes involved in essential oil formation. However, it is crucial to note that while Zn and Cu are necessary, an excess of Zn and Cu can have adverse effects on plant health, leading to oxidative stress and a decrease in essential oil production (Mir et al., 2021; Kaur, Garg, 2021).

Chemical compositions of the essential oil

The data concerning the chemical profile of Thai basil essential oil under various treatments are presented in Table 2. A total of 25 essential oil constituents were identified, representing 95.01%–97.44% of all constituents in the essential oil. The analysis revealed that Thai basil essential oil primarily consisted of oxygenated monoterpenes (74.20%–93.21%), followed by sesquiterpenes hydrocarbons (3.40%–17.65%), monoterpene hydrocarbons (0.44%–3.36%), and oxygenated sesquiterpenes (0.20%–0.68%). The principal volatile components identified in Thai basil in this study were linalool and methyl chavicol. Our findings align with the research conducted by A. Avetisyan et al. (2017), where linalool and methyl chavicol were identified as major compounds in Thai basil essential oil from Armenia. Similar results were reported by Ma Xiaojing et al. (2022) and J. Łyczko et al. (2020), who identified oxygenated monoterpenes as major compounds in Thai basil essential oil. Additionally, linalool was reported as a major compound in Thai basil essential oil grown in Egypt, according to literature (Said-Al Ahl et al., 2015). R. Sishu et al. (2010) also reported linalool (42.44%) as the main compound in Thai basil essential oil from Ethiopia.

In this study, linalool ranged from 41.64% to 53.27%, and methyl chavicol varied from 22.45% to 37.56% under different treatments (Fig. 2). The contents of linalool and methyl chavicol in Thai basil essential oil significantly increased in plants treated with $Zn_{10}Cu_0$, $Zn_{10}Cu_5$, and $Zn_{10}Cu_{10}$. A similar beneficial effect of micronutrients on essential oil composition was previously reported in *Mentha spicata* (Pande et al., 2011), *Mentha pulegium* (Lajayer et al., 2017), *Ocimum basilicum* (Ghorbanpour et al., 2016), and *Matricaria chamomilla* (Grejtovský et al., 2006). However, other treatments had no significant effect on linalool and methyl chavicol content. Additionally, the content of linalool and methyl chavicol significantly decreased with the application of high Zn and Cu levels, consistent with previous studies that demonstrated adverse effects of excess Zn and Cu on essential oil composition (Ghorbanpour et al., 2016; Lajayer et al., 2017; Kumar et al., 2022). Other essential oil constituents were altered by the applied treatments without a clear trend.

The composition of essential oils is influenced by a multitude of factors, encompassing genetic traits, evolutionary characteristics, climatic fluctuations, photoperiod, temperature, light exposure, and agronomic considerations such as soil amendments, nutrients, and trace elements (Figueiredo et al., 2008; Barra, 2009; Moghaddam, Mehdizadeh, 2017; Kumar et al., 2022). Numerous research studies have underscored the impact of micronutrients like Zn and Cu on essential oil composition (Ghorbanpour et al., 2016; Jeshni et al., 2017; Moghimipour et al., 2017; Lajayer et al., 2017; Kumar et al., 2022). Optimal levels of Zn and Cu play pivotal roles in plant physiology, participating in crucial processes such as metabolism, photosynthesis, the respiratory electron transport chain, chlorophyll and protein synthesis, auxin synthesis, cell division, and serving as essential components of various enzymes (Hänsch, Mendel, 2009; Adrees et al., 2015; Prasad et al., 2016). Additionally, these micronutrients function as regulatory cofactors and contribute to saccharide metabolism (Hänsch, Mendel, 2009). It is well-established that low-level applications of Zn and Cu can enhance essential plant functions, including photosynthesis, CO_2 fixation, and glucose production (Adrees et al., 2015; Prasad et al., 2016). These processes are particularly noteworthy as they serve as precursors for terpenoid biosynthesis (Kumar et al., 2022). Therefore, maintaining optimal levels of Zn and Cu becomes crucial, as they not only play essential roles in enzymatic activity and metabolic processes related to the terpene biosynthesis pathway but also have the potential to influence essential oil quality positively.

Antimicrobial activity of the essential oil

The antimicrobial effectiveness of Thai basil essential oil under different treatments was assessed against three microorganisms using the broth microdilution method. Table 3 displays the MIC values for the Thai basil essential oil. Results indicate inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, with MIC values ranging from 25 to 100 µg/mL under different treatments. These variations in MIC values may be attributed to differences in the chemical composition content of the essential oils. Previous literature supports the notion that alterations in the chemical composition directly influence the antimicrobial activity of essential oils (Chouhan et al., 2017; Angane et al., 2022). Our findings align with previous studies exploring the antimicrobial properties of essential oils derived from various basil varieties globally (Hussain et al., 2008; Avetisyan et al., 2017). Past research has established that essential oils exhibit potent antimicrobial effects when MIC values are below 100 µg/mL (Oliveira et al., 2022). Notably, the essential oil from Thai basil under various treatments in our study demonstrated robust antimicrobial activity based on this criterion.

The antimicrobial effectiveness of Thai basil essential oil against *S. aureus*, *E. coli*, and *C. albicans* can be attributed to its chemical constituents, primarily linalool and methyl chavicol (Moghaddam et al., 2014; Herman et al., 2016). These compounds, alongside minor components, such as 1,8-cineole, ***β***-elemene, ***α***-humulene, and germacrene D, collectively contribute to the oil's antimicrobial properties (De Lacerda Leite et al., 2021; Mączka et al., 2021). Linalool, a monoterpene alcohol, is renowned for its antimicrobial effects, disrupting microbial cell membranes and interfering with metabolic processes, ultimately leading to cell death against various bacteria and fungi (Herman et al., 2016). Methyl chavicol, or estragole, is another essential compound in Thai basil oil with proven antimicrobial properties, exerting antibacterial and antifungal effects by disrupting cell membranes and in-

Table 2. The effect of zinc (Zn) and copper (Cu) on essential oil composition of Thai basil (*Ocimum basilicum* var. *thyrsiflora*), %

Таблица 2. Влияние цинка (Zn) и меди (Cu) на состав эфирного масла тайского базилика (*Ocimum basilicum* var. *thyrsiflora*), %

Compound[a]	RI_{Exp}[b]	RI_{Lit}[c]	Treatments								
			Zn_0Cu_0	Zn_0Cu_5	Zn_0Cu_{10}	$Zn_{10}Cu_0$	$Zn_{10}Cu_5$	$Zn_{10}Cu_{10}$	$Zn_{20}Cu_0$	$Zn_{20}Cu_5$	$Zn_{20}Cu_{10}$
β-Pinene	984	974	0.27	0.12	0.15	0.15	0.11	0.34	0.22	0.48	0.47
Myrcene	992	988	–	0.27	0.18	–	–	0.41	0.19	0.27	0.34
Limonene	1027	1024	0.41	0.30	0.42	0.11	0.15	0.29	0.38	0.35	0.28
1,8-Cineole	1029	1026	4.42	3.16	4.61	1.07	1.26	1.07	2.97	3.84	4.59
(*E*)-*β*-Ocimene	1048	1044	–	0.15	–	–	0.10	–	0.51	0.29	0.37
Terpinolene	1094	1086	1.72	1.08	0.92	0.18	0.25	1.24	1.67	1.97	1.28
Linalool	1097	1095	46.49	47.17	45.85	52.41	53.27	52.52	46.77	47.31	41.64
Camphor	1144	1141	1.27	0.96	1.18	0.45	0.39	0.82	1.28	0.86	1.79
Borneol	1164	1165	0.68	0.87	0.77	0.22	0.16	0.39	0.94	0.71	0.84
Terpinen-4-ol	1178	1174	0.94	0.74	0.40	0.18	0.13	0.25	0.86	0.93	0.79
α-Terpineol	1188	1186	0.78	0.58	0.60	0.13	0.19	0.21	0.61	0.84	0.91
Methyl chavicol	1201	1195	26.38	26.94	27.17	36.64	37.56	32.05	27.08	22.45	23.26
Geraniol	1252	1249	0.67	0.82	0.95	0.15	0.25	0.37	0.70	0.87	0.38
β-Bourbonene	1384	1387	0.21	–	0.37	0.29	0.37	0.14	–	0.77	–
β-Elemene	1389	1389	3.68	4.19	4.82	1.38	1.20	2.33	1.74	2.83	4.52
α-Guaiene	1439	1437	0.38	0.41	0.26	0.12	0.24	0.27	0.68	0.52	0.83
α-Humulene	1448	1452	0.57	1.67	1.29	0.11	0.18	0.16	2.51	1.59	2.95
Germacrene D	1486	1484	1.46	1.59	4.72	1.13	0.16	0.29	0.84	2.35	3.56
Bicyclogermacrene	1497	1500	1.22	0.11	0.54	0.24	0.10	0.10	1.73	0.54	1.27
(*Z*)-*α*-Bisabolene	1504	1506	0.86	0.74	–	0.16	–	0.34	–	0.88	–
α-Bulnesene	1506	1509	3.17	3.52	0.11	0.97	0.83	0.51	2.89	3.79	3.58
γ-Cadinene	1511	1513	0.13	0.15	0.38	0.25	0.17	0.38	0.68	0.52	0.69
δ-Cadinene	1521	1522	0.29	0.34	0.41	0.12	0.15	0.16	0.53	0.38	0.25
(*E*)-Nerolidol	1564	1561	0.52	0.20	0.36	0.17	0.10	0.24	0.27	0.49	0.31
Spathulenol	1585	1577	–	–	0.25	0.19	0.12	0.13	0.29	–	0.37
Total identified (%)			96.52	96.08	96.71	96.82	97.44	95.01	96.34	95.83	95.27
Monoterpene hydrocarbons			2.40	1.92	1.67	0.44	0.61	2.28	2.97	3.36	2.74
Oxygenated monoterpenes			81.63	81.24	81.53	91.25	93.21	87.68	81.21	77.81	74.20
Sesquiterpene hydrocarbons			11.97	12.72	12.90	4.77	3.40	4.68	11.60	14.17	17.65
Oxygenated sesquiterpenes			0.52	0.20	0.61	0.36	0.22	0.37	0.56	0.49	0.68

Note: [a] – elution order on HP-5MS column; [b] – retention indices on HP-5MS column; [c] – literature retention indices; (–) not identified

Примечание: [a] – порядок элюции на колонке HP-5MS; [b] – индексы удерживания на колонке HP-5MS; [c] – литературные индексы удерживания; (–) не идентифицировано

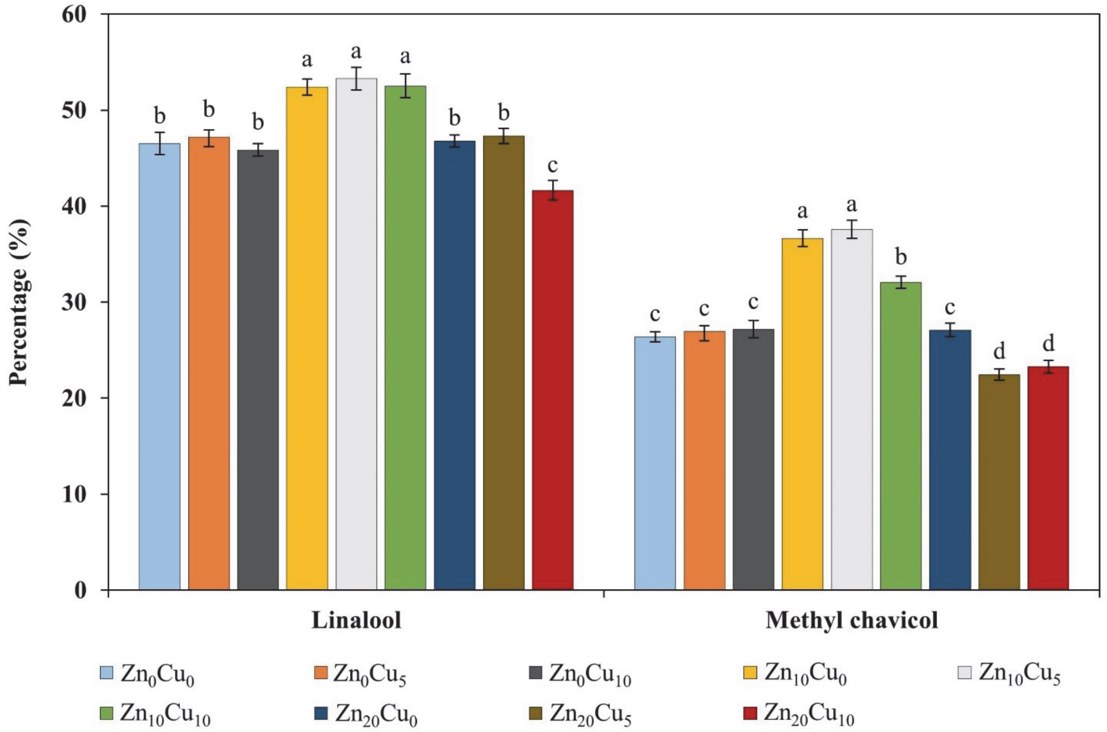

Fig. 2. The effect of zinc (Zn) and copper (Cu) on the content of main compounds (% of the total oil) in Thai basil essential oil (*Ocimum basilicum* var. *thyrsiflora*). Results are means of three measurements ± standard errors. Means with the same lower-case letters are not statistically different at $p < 0.05$ according to Tukey's test

Рис. 2. Влияние цинка (Zn) и меди (Cu) на содержание основных компонентов (% от общего масла) в эфирном масле тайского базилика (*Ocimum basilicum* var. *thyrsiflora*). Результаты представлены как средние значения трех измерений ± стандартные ошибки. Средние значения, обозначенные одинаковыми строчными буквами, статистически не различаются при p < 0,05 по критерию Тьюки

Table 3. Antimicrobial activity of Thai basil essential oil (*Ocimum basilicum* var. *thyrsiflora*) under different zinc (Zn) and copper (Cu) levels, MIC: µg/mL

Таблица 3. Антимикробная активность эфирного масла тайского базилика (*Ocimum basilicum* var. *thyrsiflora*) при различных уровнях цинка (Zn) и меди (Cu), MIC: мкг/мл

Microorganisms	Treatments								
	Zn_0Cu_0	Zn_0Cu_5	Zn_0Cu_{10}	$Zn_{10}Cu_0$	$Zn_{10}Cu_5$	$Zn_{10}Cu_{10}$	$Zn_{20}Cu_0$	$Zn_{20}Cu_5$	$Zn_{20}Cu_{10}$
Staphylococcus aureus	25	100	50	50	25	50	100	50	50
Escherichia coli	50	50	25	25	50	25	50	50	100
Candida albicans	100	50	100	100	50	50	100	50	100

hibiting enzymatic activities (Herman et al., 2016). The minor components, such as 1,8-cineole, *β*-elemene, *α*-humulene, and germacrene D, collectively contribute to the oil's antimicrobial activity by employing various mechanisms, including the disruption of microbial membranes and interference with cellular processes (Angane et al., 2022). The combined action of these chemical constituents results in a broad-spectrum antimicrobial effect against *S. aureus*, *E. coli*, and *C. albicans*. The richness and diversity of compounds in Thai basil essential oil offer a multifaceted approach, providing valuable insights into its potential therapeutic applications as a natural and effective antimicrobial agent.

Conclusions

In conclusion, the results of this study underscore the significance of Zn and Cu concentrations in influencing the characteristics of Thai basil and its essential oil properties. Optimal plant height, lateral branch number, as well as fresh and dry yields, along with essential oil yield, were achieved when Thai basil was exposed to Zn and Cu concentrations of 10 and 5 mg/kg, respectively. The main chemical constituents in the essential oil of Thai basil are linalool (41.64–53.27%) and methyl chavicol (22.45–37.56%). The antimicrobial investigation highlighted the potent activity of Thai basil essential oil against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, with MIC ranging from 25 to 100 µg/mL across all treatments. Nevertheless, elevated concentrations of Zn and Cu resulted in growth inhibition, reduced productivity, and a decline in the quality of Thai basil essential oil. This emphasizes the crucial role of managing soil metal concentrations to optimize the growth and bioactive properties of Thai basil. In general, this study provides valuable insights for cultivating Thai basil to enhance both its quantity and quality.

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Contribution of the authors: the authors contributed equally to this article.
Вклад авторов: все авторы сделали эквивалентный вклад в подготовку публикации.

Conflict of interests: the authors declare no conflicts of interests.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

The article was submitted on 19.10.2024; approved after reviewing on 11.12.2024; accepted for publication on 19.02.2025.
Статья поступила в редакцию 19.10.2024; одобрена после рецензирования 11.12.2024; принята к публикации 19.02.2025.