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Effect of methionine on essential oil accumulation and antioxidant capacity of perilla (*Perilla frutescens* L.)

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Abstract

Perilla (*Perilla frutescens* L.) is a valuable plant known for its essential oil and antioxidant properties. Its essential oil contains bioactive components with antimicrobial, anti-inflammatory and anticancer properties. Additionally, Perilla essential oils act as a natural food preservative, inhibiting the oxidation of lipids and other food constituents. Therefore, enhancing both the yield and quality of essential oil in perilla is imperative. This study aimed to investigate the role of methionine in increasing yield, essential oil content and antioxidant activity in perilla. Perilla plants were subjected to various methionine treatments, including concentrations of 0, 50, 100 and 150 mg L^{-1}, administered either once or twice during the growth. Essential oil content was determined using steam distillation, while antioxidant activity was evaluated using the DPPH scavenging assay and reducing power assay. The findings revealed that supplementing with methionine, especially at a concentration of 150 mg L^{-1} and with a double spray frequency, can augment both yield and essential oil content while boosting antioxidant activity in perilla. These results offer valuable insights for agricultural practices seeking to enhance crop performance and extract essential oils with improved antioxidant properties.

Keywords

accumulation; antioxidant; essential oil; methionine; *Perilla frutescens* L

Introduction

Perilla (*Perilla frutescens* L). is renowned for its abundant reserves of essential oils and potent antioxidant compounds, rendering it a valuable asset in both traditional medicine and the food industry (1). Extracted from perilla, essential oil comprises a range of bioactive components, including perillaldehyde, perilla ketone and rosmarinic acid. Notably, perilla's essential oil exhibits significant pharmacological activities, such as antimicrobial, anti-inflammatory and anticancer properties. Moreover, it serves as a natural food preservative owing to its antioxidant capacity, which effectively prevent the oxidation of lipids and other food constituents (2). Therefore, enhancing the yield and quality of essential oil in perilla remains of paramount importance.

Methionine plays a pivotal role in plant metabolism and growth. Recent studies have suggested that supplementing with methionine can augment the accumulation of essential oils and enhance the antioxidant capacity of plants (3). Serving as a precursor for various secondary metabolites, including phenolic compounds and volatile oils (4), methionine holds significant potential for enhancing the medicinal properties of plants like perilla. This study aims to investigate the impact of methionine on essential oil accumulation and antioxidant capacity in perilla. Bu elucidating the mechanisms underlying the relationship between methionine and these beneficial traits in perilla, this research endeavors to provide valuable insights for optimizing the cultivation and utilization of this remarkable plant.

Materials and Methods

Plant material and methionine treatments

The perilla seeds were sourced from The Southern Seed Company, located in Ho Chi Minh City, Vietnam. Initially, the seeds were sown in seed trays and once they had developed 4 leaves, they were transplanted to the experimental garden for further grown. The soil in the experimental areas possesses specific chemical and physical attributes: a low clay content of only 5.5 %; a predominant sand content of 85.5 % and a moderate silt content of 9 %. Moreover, the soil samples exhibit nitrogen, phosphorus and potassium concentrations of 0.064, 0.016 and 0.039 mg 100 g^{-1} of soil respectively. Daily morning irrigation was conducted until the soil reached optimal moisture level. The fertilizer regimen involved 2 applications: the first occurred on day 25, with a dosage of 50 g of urea per 100 m^2, followed by the second application on day 45, with a dosage of 0.3 kg of NPK per 100 m^2. Methionine solutions at concentrations of 0, 50, 100 and 150 mg L^{-1} were sprayed onto the leaves either once (at 30 days after planting) or twice (at 30 and 50 days after planting).

Determination of essential oil content

Essential oils are extracted using steam distillation, a process conducted at a temperatures nearing 100 °C. During this process, dry steam is passed through the plant material, causing the volatile compounds within the plants to vaporise. Subsequently, the steam, now containing the volatile compounds, is condensed, resulting in the formation of an immiscible liquid phase. This liquid is then separated in a clarifier to isolate the essential oil. Finally, the extracted oil is collected and stored in receivers (5).

Determination of DPPH scavenging assay

The free radical scavenging activity of essential oil was evaluated using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) method . In sample tubes, 3 mL of DPPH solutions were mixed with 100 µL of diluted essential oil, replacing the 100 µL of methanol used in the control (standard tube). The tubes were then left in darkness for 30 min before measuring the absorbance at 517 nm. Antioxidant percentage was calculated using Baliyan's formula (6). The IC_{50} values obtained from the DPPH radical scavenging activity indicate the formulation's ability to neutralize free radicals, with Lower IC_{50} values indicating stronger antioxidant activity.

Determination of reducing power assay

For the reducing power assessment using the FRAP method, essential oil was mixed with 2.5 mL of potassium ferricyanide. After incubating at 50 °C for 20 min, 2.5 mL of trichloroacetic acid was added to the mixture. Following cooled and centrifugation, the absorbance at 700 nm was measured after 5 min (7). The reducing power, evaluated by the IC_{50} values, indicates the formulation's ability to donate electrons and reduce other compounds. Lower IC_{50} values signify stronger reducing power.

Statistical analysis

After conducting 3 replications of the experiment in a randomized block design, the data were analyzed using ANOVA. To determine the differences between means at a 5 % probability level, the Duncan's Multiple Range Test in SPSS 20.0 was utilized. The results were presented as the mean and standard deviation.

Results

Effect of methionine on yield and essential oil content

In terms of yield, it is evident that the use of methionine at a concentration of 150 mg L^{-1} consistently resulted in the highest yield across both single (at 30 days after planting) and double (at 30 and 50 days after planting) spray frequencies. When using a single spray, the control group achieved a yield of 121.33 (g m^{-2}). The highest yield was obtained when using a concentration of 150 mg L^{-1}, with a yield of 350.67 (g m^{-2}) (Table 1, Fig. 1). Regarding the essential oil content, there was no significant difference among the control group and the formulations with methionine concentrations of 50 mg L^{-1}, 100 mg L^{-1}, and 150 mg L^{-1} when using a single spray frequency. The essential oil content ranged from 0.86 to 1.10 (mL g^{-1}), with no significant variations. However, when using a double spray frequency, a notable difference in the essential oil content was observed. The application of methionine at a concentration of 150 mg L^{-1} showed the highest essential oil content of 1.37 (mL g^{-1}), surpassing both the control group and the others (Fig. 1).

Table 1. Effect of methionine on yield and essential oil content at harvest time.

Methionine (mg L^{-1})	Frequency	Yield (g fresh weight m^{-2})	Essential oil content (mL g^{-1} fresh weight)
0	(Control)	121.33 ± 3.21^f	0.86 ± 0.05^c
50	1	251.00 ± 3.61^e	0.89 ± 0.02^c
	2	297.67 ± 2.52^d	0.90 ± 0.02^c
100	1	289.00 ± 8.19^d	0.91 ± 0.02^c
	2	329.67 ± 9.50^c	0.93 ± 0.03^c
150	1	350.67 ± 6.03^b	1.10 ± 0.11^b
	2	441.00 ± 11.53^a	1.37 ± 0.06^a

Values with different letters in a column are significantly different with Duncan's test (p = 0.05).

Antioxidant activity of the essential oil

In terms of DPPH radical scavenging activity, the control group exhibited an IC_{50} value of 8.03, suggesting the lowest antioxidant capacity. The activity slightly increased in the

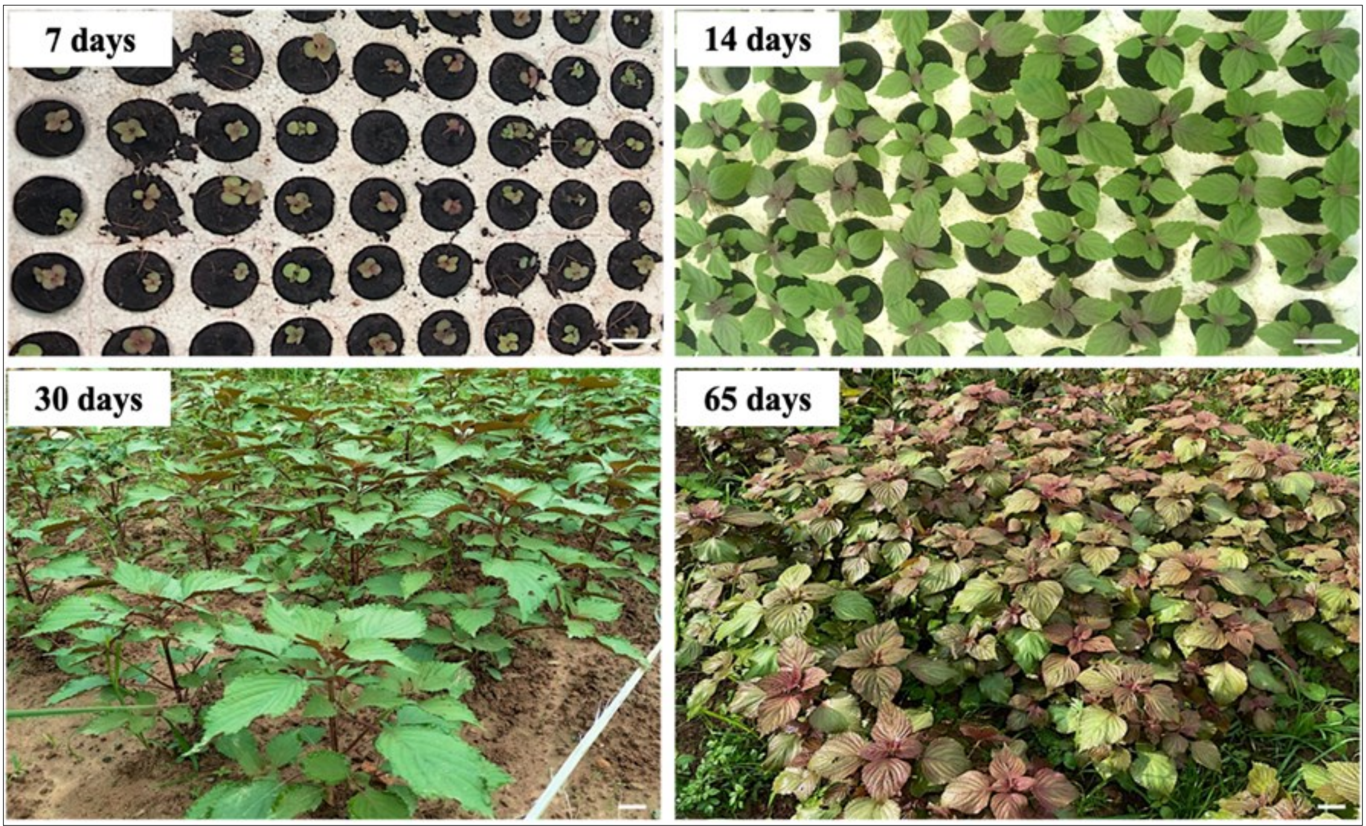

Fig. 1. Effect of 150 mg L^{-1} methionine on the growth of perilla after 7-, 14-, 30- and 65-days planting. Scale bar = 3 cm.

methionine 50 mg L^{-1} treatment and further increased in the methionine 100 mg L^{-1} treatment. The highest DPPH radical scavenging activity was observed in the methionine 150 mg L^{-1} - double spray treatment with an IC_{50} value of 5.93 (Fig. 2). The data provided illustrates the relationship between the concentration of methionine and the reducing power of the solutions, as indicated by the IC_{50} values. Upon analysis, a clear trend emerges: as the concentration of methionine increases, the reducing power tends to increase. Starting with the methionine 50 mg L^{-1} treatment, there is a slight increase in reducing power compared to the control. This trend continues with the Met 100 mg L^{-1} - single spray treatment. The trend reaches its culmination with methionine 150 mg L^{-1}, which

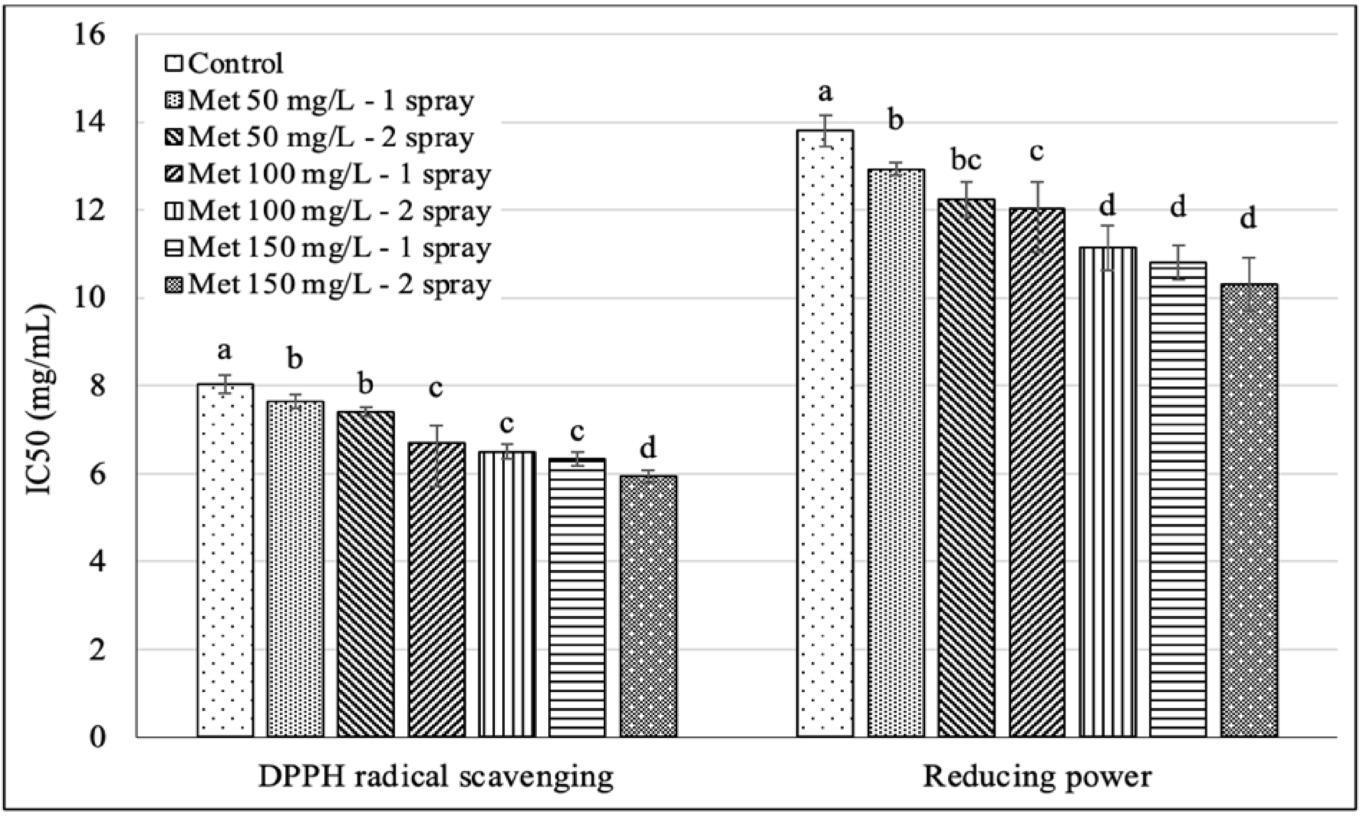

Fig. 2. Effect methionine on antioxidant activity of the essential oil. Values with different letters in column are significantly different with Duncan's test (p=0.05).

exhibits a significant increase in reducing power compared to the control solution (Fig. 2).

Discussion

Methionine serves as a critical amino acid facilitating protein synthesis in plants, thereby playing a pivotal role in the growth, development and metabolism. Supplementing plants with additional methionine provides them with increased resources for protein synthesis, thereby enhancing overall growth and productivity. A study conducted demonstrated that applying methionine at a concentration of 25 mg L^{-1} to cauliflower resulted in increased yield compared to control groups (8). This study similarly found that higher concentrations of methionine, such as 150 mg L^{-1}, consistently led to the highest yields (Table 1, Fig. 1). This suggests that methionine supplementation can augment the plant's physiological processes, leading to improved growth and development, ultimately translating into higher yields. According to one report methionine can enhance the efficiency of nutrient absorption from the soil and facilitate their transport within the plant (9). Additionally, methionine has demonstrated antioxidative properties, enabling plants to mitigate oxidative stress induced by reactive oxygen species (10). Supplementation with methionine enables plants to bolster their antioxidant defense mechanisms, thereby reducing oxidative damage and fostering growth, ultimately leading to increased yields. Furthermore, methionine has been shown to influence the essential oil content in plants. This study observed that methionine supplementation, particularly at concentrations of 150 mg L^{-1}, significantly augmented the essential oil content compared to control groups (Table 1). Methionine acts as a precursor for the biosynthesis of various compounds, including essential oils (11). By providing additional methionine, plants have access to a greater supply of this precursor, which can be utilized for production of essential oil constituents. This heightened availability of precursors can lead to a greater accumulation of essential oils in plant tissues. Additionally, methionine plays a role in activating critical enzymes and metabolic pathways involved in essential oil biosynthesis, such as terpene synthases (12, 13). Research suggests that methionine has the potential to enhance the antioxidant activity of essential oils, crucial for protecting cells from oxidative damage caused by free radicals. Studies on basil have demonstrated that supplemention with methionine can augment the antioxidant capacity of essential oils (14). Similarly, in this study, an increase in antioxidant activity of essential oil was observed when treated with methionine (Fig. 2). Methionine plays a role in the biosynthesis of various antioxidant molecules. By increasing methionine levels, plants have more of this precursor available, leading to an upsurge in the synthesis of antioxidant compounds. These antioxidants, including phenolic compounds and flavonoids like rosmarinic acid, are pivotal in scavenging free radicals and aventing oxidative damage. With a higher concentration of these antioxidants in the essential oil, its overall antioxidant activity is heightened.

Upon analyzing the results, it was concluded that supplementing methionine at a concentration of 150 mg L^{-1} through double spraying in agricultural settings for perilla cultivation would be a practical application of the research findings. This approach aims to boost plant productivity and enhance the accumulation of essential oils. The study unveiled that methionine supplementation positively iinfluenced plant productivity, potentially elevating the overall yield of perilla plants. Additionally, the research indicated that methionine supplementation results in an increased accumulation of essential oils and enhances the antioxidant capacity of the essential oil extracted from perilla. This could prove beneficial for various industries, including fragrance, cosmetics and culinary sectors. Antioxidant-rich essential oils can be utilized in cosmetic and personal care products to counteract oxidative damage to the skin caused by free radicals. They can be included into formulations such as lotions, creams, serums and facial oils to shield against environmental stressors and promote skin health (15). Furthermore, essential oils with antioxidant activity are of interest in the pharmaceutical industry for their potential therapeutic benefits. They can be incorporated into pharmaceutical formulations or used in aromatherapy to bolster overall health and well-being. Essential oils with antioxidant properties may aid in reducing oxidative stress, inflammation and cellular damage associated with various diseases (16).

Conclusion

Supplementing methionine at a concentration of 150 mg L^{-1} through double spraying has shown a positive impact on plant productivity. Furthermore, it has increased essential oil accumulation and boosted the antioxidant capacity of perilla. These results indicate that methionine could serve as a beneficial treatment in agricultural practices aimed at enhancing crop performance, boosting the yield of essential oil-producing plants and improving the antioxidant properties of products.

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Authors' contributions

TTT carried out the experiments and drafted the manuscript. TTT and VHP conceived of the study and participated in manuscript editing, its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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