



The effect of silver nanoparticles on the physico-biochemical characteristics and essential oil performance of peppermint (*Mentha piperita* L.)

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Original Article

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KEYWORDS

Antioxidant system,
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EXTENDED ABSTRACT

Introduction: Peppermint (*Mentha piperita* L.) is an economically and medicinally significant plant valued for its essential oil. Silver nanoparticles (AgNPs) act as potent elicitors, triggering defense responses that include the production of reactive oxygen species (ROS) and the accumulation of secondary metabolites. However, the impact of AgNPs on peppermint's physico-biochemical traits and essential oil performance remains underexplored.

Aims: This study aimed to evaluate the effects of AgNPs on various physico-biochemical characteristics and essential oil performance in peppermint. The analyzed parameters, including H₂O₂, soluble proteins, phenols, flavonoids, antioxidant enzymes (SOD, CAT, APX), and photosynthetic pigments (chlorophyll and carotenoids), were examined to determine the optimal concentrations for enhanced metabolic activity.

Materials and methods: Rhizomes were planted in pots and grown under controlled greenhouse conditions, including a daytime temperature of 25°C, a photoperiod of 16 h of light and 8 h of darkness, and a relative humidity of 60. At the ten-leaf stage, plants underwent foliar spraying with 0 (control), 1, and 2 mM AgNPs. The AgNPs solution was prepared, characterized via UV-Vis spectroscopy, and SEM (20 nm quasi-spherical particles). A completely randomized design with three replicates was used to assess parameters including H₂O₂ content, soluble proteins, phenols, flavonoids, antioxidant enzymes (SOD, CAT, APX), photosynthetic pigments (chlorophyll, carotenoids), and essential oil performance. Statistical analysis was performed using ANOVA and LSD tests (p<0.05).

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Results: AgNPs significantly elevated all measured parameters compared to the control in a generally concentration-dependent manner: Treatment with 1 and 2 mM AgNPs increased H₂O₂ content by 2- and 3-fold, respectively. Treatment with 2 mM AgNPs resulted in a 2.4-fold increase in protein content. 1 and 2 mM AgNPs resulted in a 2.8- and 2.6-fold increase in leaf phenolic content, respectively. Concentrations of 1 and 2 mM AgNPs resulted in a 1.8- and 2.6-fold increase in leaf flavonoid content, respectively. Treatment with 2 mM AgNPs resulted in a 3.3-fold increase in SOD activity. Treatment with 2 mM AgNPs had a significant positive effect (about a 2.2-fold) on CAT enzyme activity. However, treatment with 1 mM AgNPs failed to affect CAT enzyme activity. AgNPs 1 and 2 mM increased APX enzyme activity by 1.7 and 2.5 times, respectively. Treatment with 1 and 2 mM AgNPs increased total chlorophyll concentration by 2.4- and 3-fold, respectively. 1 and 2 mM AgNPs increased carotenoid concentration by 1.8- and 3.5-fold, respectively. Treatment with 1 and 2 mM increased essential oil yield by 2.2- and 2.3-fold, respectively.

Discussion: AgNPs significantly increased the level of H₂O₂, soluble proteins, phenols, flavonoids, antioxidant enzymes (SOD, CAT, APX), chlorophyll, carotenoids, and essential oil yield. Although the mechanisms of plant response to AgNP elicitors are not well understood, we proposed that the improvement in peppermint's physico-biochemical traits in our study was due to the following series of mechanisms: AgNPs induce ROS (e.g., H₂O₂) generation through NADPH oxidase activation and electron leakage from organelles. This oxidative stress activates MAPK cascades and calcium signaling, which upregulate transcription factors (*MYB*, *bHLH*). These transcription factors subsequently enhance the expression of phenylpropanoid pathway enzymes (e.g., PAL) and terpenoid biosynthesis genes, leading to increased essential oil biosynthesis and accumulation. AgNP elicitors also improve antioxidant enzyme activity (SOD, CAT, APX) to mitigate ROS damage. In addition to the enzymatic antioxidant system, AgNPs enhance components of the non-enzymatic antioxidant system, including phenols and phenoloids. Furthermore, these nanoparticles upregulate key chlorophyll biosynthesis genes (*HEMA1*, *CHLH*), while suppress degradation-related genes (*NYC1*, *PAO*), resulting in elevated photosynthetic pigment concentrations.

Conclusion: Foliar application of 2 mM AgNPs significantly enhances peppermint's antioxidant capacity, photosynthetic efficiency, and essential oil biosynthesis by modulating ROS-mediated signaling. Therefore, this concentration is recommended for maximizing peppermint's medicinal and economic potential. Future studies should explore broader concentration ranges and early gene expression responses (6–12 h post-treatment).



تاثیر نانوذرات نقره بر صفات فیزیکیوشیمیایی و عملکرد اسانس گیاه نعناع فلفلی (*Mentha piperita* L.)

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چکیده	واژگان کلیدی
<p>هدف: نعناع فلفلی (<i>Mentha piperita</i> L.) یکی از گیاهان مهم از نظر اقتصادی و دارویی است که به واسطه پتانسیل خود در حوزه دارو و درمان، سبب جلب توجه محققان به استفاده از ایستورها برای افزایش اسانس و زیست توده آن شده است. بنابراین، این مطالعه با هدف تعیین اثر نانوذرات نقره بر صفات فیزیکیوشیمیایی و عملکرد اسانس گیاه نعناع فلفلی انجام شد. مواد و روش‌ها: تعداد پنج ریزوم در در عمق ۵ سانتی متری گلدان‌های پلاستیکی کاشته شدند. با استقرار کامل گیاه در مرحله ده برگی، محلول-پاشی با غلظت‌های ۰ (شاهد)، ۱ و ۲ میلی مولار نانوذرات نقره انجام شد. تاثیر نانوذرات نقره بر صفات فیزیکیوشیمیایی و عملکرد اسانس در قالب طرح کاملاً تصادفی با سه تکرار تجزیه و تحلیل شد. نتایج: نتایج نشان داد که نانوذرات نقره، به ویژه با افزایش غلظت از ۱ به ۲ میلی مولار، باعث افزایش صفات فیزیکیوشیمیایی و عملکرد اسانس نعناع فلفلی نسبت به شاهد شدند. غلظت ۲ میلی مولار نانوذره نقره به عنوان موثرین تیمار سبب افزایش محتوای H_2O_2، پروتئین، فنول، فلاونوئیدها، فعالیت آنزیم‌های آنتی‌اکسیدانت SOD، CAT، و APX شد. در محتوای رنگیزه‌های کلروفیل و کارتنوئیدها و عملکرد اسانس نیز چنین افزایشی مشاهده شد. نتیجه‌گیری: با توجه به آثار نانوذرات نقره بر صفات فیزیکیوشیمیایی و بیوشیمیایی نعناع فلفلی، استفاده از غلظت ۲ میلی مولار نانوذره نقره می‌تواند باعث افزایش رنگیزه‌های فتوسنتزی، بهبود محتوای اسانس و تقویت سیستم آنتی‌اکسیدانتی آنزیمی و غیر آنزیمی گیاه شود.</p>	<p>اسانس، سیستم آنتی-اکسیدانت، نانوذرات، نعناع فلفلی</p> <p>تاریخ دریافت: ۱۴۰۳/۱۲/۲۰</p> <p>تاریخ بازنگری: ۱۴۰۴/۰۵/۰۴</p> <p>تاریخ پذیرش: ۱۴۰۴/۰۷/۰۸</p>