Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Methyl jasmonate effects on *Lactuca serriola* L.: Antioxidant defense and bioactive compound changes

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Article Info

Article history:

Received: June 29, 2024
Accepted: September 09, 2024
Published: September 30, 2024

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Abstract

The effect of methyl jasmonate (MeJA) foliar spray on the activity of antioxidant enzymes—Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX), and Guaiacol peroxidase (GPX)—along with assessments of total phenolic and flavonoid contents and antioxidant activity (IC50), was examined in Prickly lettuce (*Lactuca serriola* L.). The study involved treating plants with three MeJA solutions (0, 200, and 400 µM) and harvesting samples at four distinct time intervals. Varied MeJA concentrations and time intervals resulted in a substantial increase in the activity of all the antioxidant enzymes investigated in this study. Both concentration levels and time courses exhibited progressive outcomes. Moreover, MeJA treatment led to elevated levels of total phenolic and flavonoid contents, reaching peaks of 17.02 (mg GAL/g DW) and 8.3 (mg QUE/g DW), respectively, particularly in response to the 400 µM concentration. However, the total flavonoid content did not show any significant variation between the two concentrations. Based on the half-maximal inhibitory concentration (IC50) values, the antioxidant activity in MeJA-treated plants was found to be lower compared to the controls. However, our findings suggest that, under specific conditions discussed in this study, MeJA has the potential to enhance the nutritional value of *L. serriola*.

Keywords: Antioxidant enzymes, Prickly lettuce, Total flavonoid, Total phenol

1. Introduction

Plants are recognized as vital sources of bioactive compounds with a wide range of biological effects, such as anticancer, antioxidant, antimicrobial, and anti-inflammatory properties [1]. These compounds can be stimulated by plant hormones, including the widely occurring jasmonic acid (JA) and its volatile derivative, methyl jasmonate (MeJA), which are derived from linolenic acid. These hormones perform pivotal roles in regulating diverse plant processes, including defense responses, flowering, and senescence [2]. While they are well known for initiating defense reactions against herbivores and pathogens by modulating defense-related genes under stress, they also activate secondary metabolic pathways, stimulating the accumulation of defense compounds.

Generally, when plants are subjected to stressors like MeJA as an elicitor, they stimulate molecular signal transduction and gene expression regulation, leading to the accumulation of secondary metabolites and phytochemicals by activating metabolic pathways, elevating their nutraceutical attributes. As a result, presence of compounds like terpenoids and phenolics enhances antioxidant activity and disease-preventive potential and through their synergistic interactions [3].

The application of exogenous MeJA induces reactive oxygen species (ROS) generation and modulates defense responses via increased antioxidant enzyme activity [4]. While ROS, detrimental at high levels, requires a delicate balance to prevent oxidative damage while retaining the beneficial effects of ROS. Plants employ enzymatic (e.g., SOD, CAT, GPX and APX) and non-enzymatic (e.g. ascorbate, glutathione, carotenoids, tocopherols, phenolics) antioxidant systems to scavenge or detoxify excess ROS.

Prickly lettuce (*Lactuca serriola* L.), an herbaceous plant from the Asteraceae family, is closely related to and

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.9.23

likely the ancestor of cultivated lettuce (*Lactuca sativa* L.). *L. serriola,* which is rich in lactucarium, is a source of essential minerals, vitamins, natural antioxidants, flavonoids, and phenolics, serving various human nutritional and medicinal purposes, including its use as a sedative, expectorant, disinfectant, and diuretic. Pharmacological evaluations of the plant extract have revealed anti-inflammatory, anti-carcinogenic, analgesic, and antioxidant properties. These advantageous effects are attributed to the significant phenolic content, which facilitates the efficient neutralization of free radicals. The plant's substantial antioxidant capacity suggests the potential to enhance its culinary and health benefits by elevating its flavonoid and phenolic contents [5]. Under different stress conditions, several secondary metabolites, including MeJA and JA, undergo changes through the octadecanoid pathway, reinforcing the plant's defense mechanisms.

MeJA treatments have been demonstrated to elevate CAT and APX enzyme activity in two plum cultivars. Applying exogenous MeJA on romaine lettuce (*L. sativa* L.) increased total phenolic compounds, antioxidant capacity, and PAL enzyme activity up to 8 days post-treatment [6].

Based on previous research, we hypothesize that the nutritional attributes of *L. serriola* could be enhanced through the application of various concentrations of exogenous MeJA at different time intervals post-treatment. Hence, the objective of this study was to assess whether these treatments can improve the antioxidant properties (total phenol, total flavonoid, and antioxidant enzyme activity) and antioxidant activity of *L. serriola*. This is the first time such an investigation has been conducted on *L. serriola*.

2. Materials and methods

2.1. Plant material

L. serriola seeds were collected from Karaj, Iran (35°46′ N, 51°00′ E). To create working stock and eliminate environmental effects, a single seed from the collection was grown in the research greenhouse of the Iranian Biological Research Center (IBRC). Plants were initiated by seeding the working stock collections in plastic pots filled with potting media (peat moss, cocopeat and perlite) and maintained at temperatures of 26°C during the day and 22°C at night (with fluctuations of ±3°C). Adequate irrigation was provided as needed, accompanied by weekly fertilizer application.

For the MeJA treatment, plants were sprayed with three different concentrations (0, 200 and 400 µM) of MeJA solutions, dissolved in 0.2% ethanol, covering all aboveground parts until runoff. This MeJA application occurred two weeks before the onset of bolting, with intervals of one week. Leaves were collected for biochemical analysis on days 1, 2, 4, and 6 following the final MeJA application.

2.2. Phytochemical factors' assessment

2.2.1. Extraction of plant material

The methanolic extract for determining total polyphenol and flavonoid content in *L. serriola* leaves was prepared following [7]. Freeze-dried sample (1g) was powdered with liquid nitrogen, mixed with 10 mL of 80% methanol, and vortexed. The extract underwent two 15-minute sonication cycles at ambient temperature, then centrifuged at 8000× g for 10 min to collect the supernatants for analysis.

2.2.2. Total phenol content

To assess total phenolic content(TPC), sample extracts (40 µl) were mixed with Folin-Ciocalteu's reagent (120 µl) and distilled water (40 µl) in a tube. After 5 min at room temperature, sodium carbonate (100 µl, 20% w/v) was added, and tubes were incubated in darkness at 25°C for 1 hour. The absorbance was assessed at 750 nm utilizing a microplate reader (Eon) against a blank. TPC was quantified as mg of gallic acid equivalents (GAE) g^{-1} DW [8].

2.2.3. Total flavonoid content

The content of total flavonoid (TFC) was determined according to the Chang et al. (2002) [9] method. 30 µL of plant extract was mixed with 30 µL of 5% sodium nitrite, 60 µL of 10% aluminum chloride solution, and 180 µL of 1 mol L−1 potassium acetate solution., absorbance at 380 nm was measured after 15 min with a microplate reader. TFC was determined using a calibration curve of quercetin, and findings were presented as milligrams of quercetin equivalent (QUE) per gram of dry weight.

2.2.4. Antioxidant activity

Following the method by Jadid et al. (2017)[10], methanol extracts from control and treated samples were assessed for DPPH radical scavenging activity. Different extract concentrations (75, 150, 300, and 400 mg L^{-1}) were added to 150 µL of DPPH solution, homogenized, and the mixture was then allowed to incubate for 30 minutes at 25°C, after which absorbance was assessed at 517 nm by a microplate reader. A standard curve was generated using varying concentrations of ascorbic acid. The calculation of radical scavenging activity (RSA) was performed utilizing the formula :

$$\text{RSA} \quad (\%) \quad = \quad \frac{\text{Abs} \quad \text{Blank–Abs} \quad \text{Sample}}{\text{Abs Blank} \times 100}$$

The absorbance of the DPPH solutions with and without extract are denoted as Abs Sample and Abs Blank, respectively. The antioxidant activity(IC50) of the extract was determined based on the IC50 values.

2.3. Antioxidant enzyme activity

2.3.1. Enzyme extraction

To prepare samples for antioxidant enzyme activity measurements, the frozen samples were subjected to liquid nitrogen grinding at a dosage of 500 mg each. The powdered samples were combined with 2 mL of extraction solution (0.5% Tris-HCL, pH 8.0 and 0.05% polyvinyl pyrrolidone). The extract was centrifuged at 13,000 rpm for 20 min at 4°C. The obtained supernatant was utilized to assess the activity of antioxidant enzymes [11].

2.3.2. Catalase activity

Quantitative measurement of catalase activity was performed using the spectrophotometric approach at 240 nm introduced by Aebi (1984) [12]. The reaction mixture contained 3 µL of the extract followed by the addition of 285 µL of phosphate buffer (pH 7.0) and 6 µL of 3% hydrogen peroxide (H_2O_2). The degree of H_2O_2 decomposition within 1 min served as the basis for determining CAT activity.

2.3.3. Guaiacol peroxidase activity

To assess GPX enzyme activity, we employed the method outlined by MacAdam et al. (1992) [13]. For the test, 9.4 µL of extract was mixed with 285 µL phosphate solution (pH 7.0), 4.7 µL H_2O_2 (3%) and 4.7 µL guaiacol, in microplate reader wells. The plate then was briefly shaken. The alterations in absorbance at 436 nm were monitored for a duration of 1 minute and GPX activity was determined as ΔOD g^{-1} FW min^{-1}.

2.3.4. Ascorbate peroxidase activity

The measurement of APX activity followed the methodology outlined by Nakano and Asada (1987)[14] and was conducted using a spectrophotometer at 290 nm. To carry out the assay, 14 µL of extract was mixed with 282 µLof 50 mM phosphate buffer (pH 7.0), 2.8 µL of H_2O_2 (5%), and 1.4 µL ascorbic acid (50 µM) in tubes. After incubation at 25°C for 1 min, the APX activity was assessed by monitoring the reduction in ascorbate content over a period of 1 minute.

2.3.5. Superoxide dismutase activity

For assessment of SOD activity, 10 µL of extract was combined with 290 µL of reaction buffer, consisting of 50 mM phosphate buffer (pH 7), 0.1 M EDTA, 13 mM L-methionine, 4 µM riboflavin and 75 µM NBT in a 96-well plate. Following homogenization, the plates were exposed to light for 15 min, and then the absorbance was assessed at 560 nm. SOD activity was quantified by its ability to reduce NBT photoreduction by 50%, with one unit of SOD activity defined as such [15].

2.3.6. Experimental design

Statistical data analysis was carried out utilizing SAS 9.4. The experimental design followed a randomized complete design with a factorial setup and each experiment was replicated three times. The least significant difference (LSD) method was employed to identify differences among all sample means (with a significance level of $p < 0.01$). Data correlation was performed using the R package to assess relationships between variables.

3. Results

3.1. Antioxidant enzyme activity

The activity levels of the antioxidant enzymes CAT, SOD, GPX, and APX in response to foliar application of MeJA at different concentrations and durations were measured. The interaction between different concentrations of MeJA and various time courses was statistically significant ($P < 0.01$).The activity of all enzymes was significantly modified by MeJA treatment compared with control samples ($P \leq 0.01$). SOD activity in the treated samples exhibited a notable increase ($P \leq 0.01$) compared to the control samples. Among the concentrations tested, the highest activity was observed in the 400 µM MeJA treatment after 2 days, reaching 17.23 U g^{-1} FW, while the lowest activity was recorded in the 200 µM treatment after 6 days, measuring 11.55 U g^{-1} FW. Notably, on days 4 and 6, a significant decline in SOD activity was observed in comparison to day 2, although it remained higher than that of the control samples (Fig. 1A).

The activity of the CAT enzyme increased significantly ($P \leq 0.01$) across various MeJA concentrations and exposure times, except for the 6-day interval following treatment with 200 µM of MeJA. However, even in this case, CAT activity remained consistent with day 4 levels and was higher than that of the control samples. The highest CAT activity was observed at 6 and 4 days after treatment with 400 µM of MeJA, while the lowest activity was recorded 1 day after treatment with 200 µM of MeJA (Fig. 1B).

MeJA significantly increased APX activity at all durations ($P \leq 0.01$), with both concentrations positively influencing APX activity compared to controls (Fig. 1C). The highest APX activity occurred after treatment with 400 µM MeJA on days 4 and 2, measuring 4.43 U g^{-1} FW and 4.13 U g^{-1} FW, respectively. MeJA consistently enhanced APX enzyme activity across all time intervals ($p < 0.001$), with the 400 µM treatment inducing a more pronounced elevation compared to 200 µM.

The activity of the GPX enzyme demonstrated a significantly greater level in samples treated with 400 µM MeJA compared to the control samples. Peak activity was recorded on day 2 and day 6 after treatment, reaching values of 0.65 and 0.64 U g^{-1} FW, respectively (Fig. 1D). Plants exposed to 200 µM MeJA exhibited a noteworthy rise in GPX activity as early as 2 days post-MeJA application, with a mean value of 0.38 U g^{-1} FW. The enzyme activity remained relatively stable across different durations at the same concentration of MeJA compared to untreated samples. Both 200 µM and 400 µM concentrations of MeJA have favorable effects on all antioxidant enzyme activities and phytochemical compound levels.

3.2. Total phenol content

The total content of phenolic compounds was evaluated in *L. serriola* subjected to 200 and 400 µ M MeJA treatments. The total phenol content rose significantly for both MeJA concentrations across most time intervals ($p < 0.001$), with the 400 µ M treatment causing a notably greater increase compared to the 200 µ M treatment. The highest total phenolic content was observed in the 400 µ M treatment on days 4 and 6 after application (17.02 and 16.75 mg GAL g^{-1} DW, respectively), while the lowest content was found in the 200 µ M of MeJA treatment after the first day (11.29 mg GAL g^{-1} DW) (Fig. 2).

3.3. Total flavonoid content

The application of methyl jasmonate resulted in an

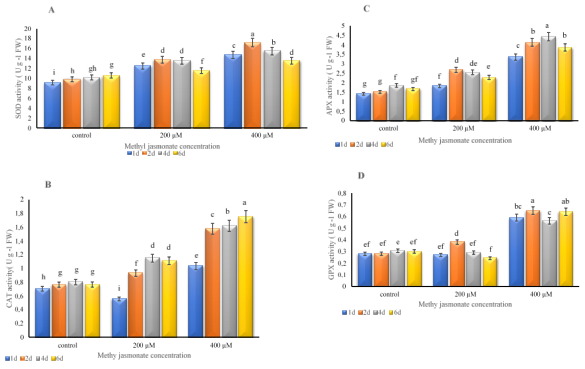

Fig. 1. The impact of varied concentrations of methyl jasmonate over various durations of exposure on the activity of antioxidant enzymes in leaf samples of *Lactuca serriola*, (A) SOD, (B) CAT, (C) APX and (D) GPX. ($P<0.01$). Different letters in columns indicate significant differences between values.

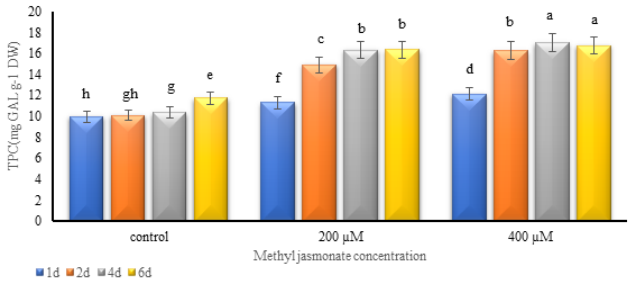

Fig. 2. The impact of varied concentrations of methyl jasmonate over various durations of exposure on total content of phenolic compounds (TPC) (mg Gallic acid per gram of dry weight) in leaf samples of *Lactuca serriola.* (P<0.01). Different letters in columns indicate significant differences between values.

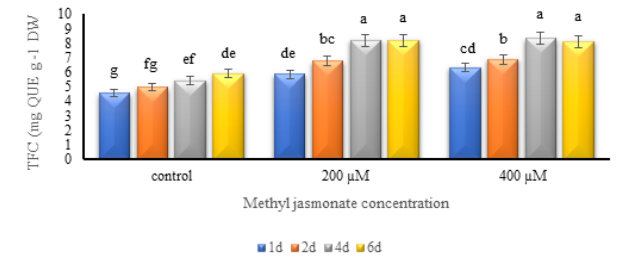

Fig. 3. The impact of varied concentrations of methyl jasmonate over various durations of exposure on total flavonoid content (TFC) (mg Quercetin/g of dry weight in leaf samples of *Lactuca serriola*. (P<0.05). Different letters in columns indicate significant differences between values.

increase in total flavonoid content at both concentrations compared to the control samples ($p < 0.05$). The peak value was attained at 400 µ M, measuring 8.31 mg QUE/g of DW 4 days after the treatment, whereas the lowest amount was registered at 200 µM after just 1 day of treatment (5.86 mg QUE g^{-1} DW). No further significant changes in total flavonoid content occurred at either concentration on days 4 and 6 (Fig. 3).

3.4. IC50 value

The application of MeJA treatments resulted in a reduction of IC50 values when compared to the control group ($p < 0.05$), indicating an enhanced antioxidant activity (Fig. 4). Notably, the 400 µM concentration exhibited a lower IC50 value than the 200 µM concentration, implying a heightened antioxidant potential. Within the control group, a steady significant decrease in IC50 values was observed over the duration of the experiment. The day 6 and 4 values following the treatment with 400 µM reflected the lowest IC50 values, indicative of the highest antioxidant activity (4.06 and 4.21 µg ASC mL^{-1}, respectively). In contrast, the highest IC50 value was observed on day 1 with the 200 µM treatment (6.95 µg ASC mL^{-1}). No significant change in IC50 values occurred between days 4 and 6.

3.5. Correlation analysis

The correlation analysis of the studied parameters is illustrated in Fig. 5. A very strong positive correlation was observed between TFC and TPC, with darker colors indicating stronger correlations. Additionally, a strong negative correlation was evident between IC50 and both TPC and TFC.

4. Discussion

4.1. The methyl jasmonate impact on antioxidant enzyme activity

In this study, foliar application of MeJA markedly elevated the activity of antioxidant enzymes (CAT, SOD, APX, and GPX) in *L. serriola* plants compared to controls. Elevated SOD activity in response to MeJA suggests enhanced superoxide scavenging. MeJA induces H2O2 production, neutralized by CAT, GPX, or APX reactions, driven by elevated H2O2 levels. CAT efficiently breaks down H2O2 without energy consumption, reducing levels swiftly [16]. Increased CAT activity indicates MeJA enhances H2O2 decomposition, possibly activating defense mechanisms against oxidative stress. The significant rise in GPX and APX activities highlights MeJA's role in enhancing peroxide-detoxifying enzyme activity, crucial for

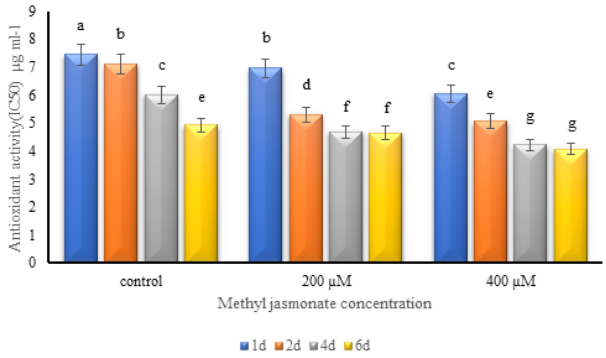

Fig. 4. The impact of varied concentrations of methyl jasmonate over various durations of exposure on antioxidant activity (IC50) (µg ASC/mL) in leaf samples of *Lactuca serriola.* (P<0.05). Different letters in columns indicate significant differences between values.

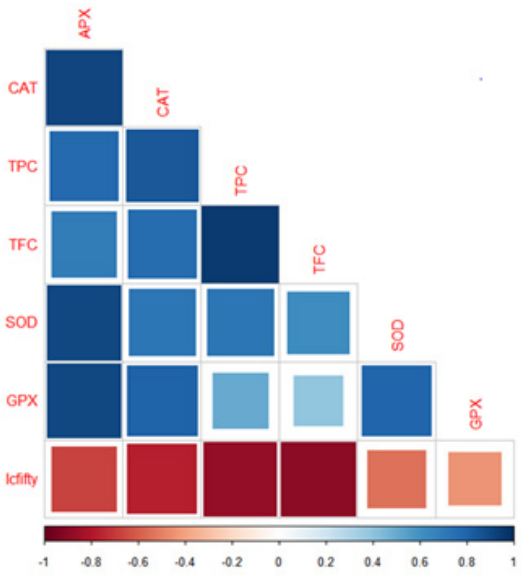

Fig. 5. The correlation analysis of the studied parameters. CAT: catalase, SOD: superoxide dismutase, GPX: Guaiacol peroxidase, APX: ascorbate peroxidase, TPC: Total phenolic contents, TFC: Total flavonoid contents, IC50: antioxidant capacity.

cellular redox balance and protecting plants from oxidative damage.

Efficient antioxidant enzyme function is closely linked to stress tolerance, with increased enzyme activity and metabolite levels observed under different environmental stresses. Heightened antioxidant enzyme activity has been reported following the foliar application of MeJA to *Arabidopsis thaliana* for a duration of 7 days[17]. Serna-Escolano et al. (2019)[18] demonstrated that preharvest MeJA treatments on lemon fruits led to notable increases in CAT and APX enzyme activities across various concentrations.

4.2. The effect of methyl jasmonate application on total phenol and flavonoid content

In this study, total phenol and flavonoid content was significantly increased in response to MeJA application ($p < 0.001$ and $p < 0.05$ respectively). The application of methyl jasmonate triggers a significant increase in the total phenol and flavonoid content in plants, indicating the stimulation of secondary metabolites with antioxidant properties. These newly synthesized compounds serve as potent scavengers of free radicals, effectively boosting overall antioxidant capacity[19]. The increase in phenolic content resulting from MeJA treatment can be ascribed to its function as an elicitor, activating the phenylpropanoid pathway. This pathway, known for its inducible defense response, leads to the accumulation of phenolic compounds, thereby enriching the potential health benefits through consumption. Additionally, the absence of an increase in total phenolic content after day 4 of MeJA application could be partly attributed to the utilization of phenolic compounds in the lignification process, as suggested by Kim et al. (2007)[6]. Furthermore, the elevation in phenolics and flavonoids observed in plants treated with preharvest MeJA is linked to the modulation of diverse physiological and metabolic processes[3]. These alterations not only reinforce the plants' antioxidant potential but also positively impact the nutritional value of the crops[20].

Jasmonates, acting as phytohormones mediating stress responses, have displayed their capacity to enhance the production of phenolic compounds across a range of plant species. Our findings align with the results of Beak et al. (2021) [7], which reported that application of 0.25 mM methyl jasmonate on “Kumato” tomato increased the total phenol and flavonoid content significantly compared to untreated samples. A similar increase was observed following the application of 100 µM of MeJA to two grape cultivars exposed to boron toxicity[21]. However, these findings are in contrast with the conclusions of Yamamoto et al. (2020) [22], who observed a notable decrease in flavonoids in citrus following MeJA treatment. This suggests that MeJA might exhibit distinct regulatory effects on bioactive substances in diverse crops, warranting further investigation. Therefore, it is crucial for the food industry to promote the content of phenolic compounds and flavonoids in fruits and vegetables through preharvest and postharvest treatments, as this has the potential to protect human health [3].

4.3. The effect of methyl jasmonate application on IC50 values

Antioxidant activity was evaluated via the DPPH method, known for its sensitivity in measuring antioxidant efficacy. IC50 values, indicating the concentration to scavenge 50% of free radicals, were determined for each MeJA concentration over varied time periods. A reverse correlation between IC50 values and antioxidant activity was observed, signifying that greater antioxidant activity corresponds to lower IC50 values [23]. The enhanced antioxidant activity was observed in this research as a result of MeJA application. Bioactive substances in plants, including phenolic compounds, terpenoids, and carotenoids, exhibit a range of antioxidant capacities. Nevertheless, their efficacy as antioxidants varies depending on their unique structures and prevalence within the plant [6].

The heightened antioxidant capacity in samples treated with MeJA in this investigation might be attributed to the increased abundance of phenolic and flavonoid compounds [24]. The significant inverse relationship between IC50 values, and total phenolic and flavonoid compounds (Fig. 5) suggests that the boost in antioxidant activity is linked to the elevated concentrations of total phenolic and flavonoid compounds[25]. Our findings are consistent with the increased antioxidant activity observed in peppermint plants treated with 75 µM MeJA[6].

6. Conclusion

This study investigated the impact of exogenous MeJA foliar application on *L. serriola* antioxidant enzyme activity, total phenol and flavonoid content, and antioxidant activity. Results show enhanced enzyme activity, increased phytochemical levels, and improved antioxidant capacity at MeJA concentrations of 200 µM and 400 µM. These findings enhance our understanding of MeJA's potential in enhancing plant health and antioxidant defense mechanisms. Further research is needed to explore MeJA's broader implications in diverse plant species and elucidate its molecular mechanisms.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

This study was sponsored by Urmia University and the University of Tehran. Moreover, this study was supported by the RUDN University Strategic Academic Leadership Program. We would like to express our sincere gratitude to Dr. Justin Parker for proofreading the manuscript.

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